SELECTION AND EVALUATION OF TEN MEDICINAL PLANTS USED, IN THE VHEMBE DISTRICT, FOR LIFE-THREATENING INFECTIONS

By

SIGIDI MUENDI TSHILILELWA

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS OF PHILOSOPHIAE DOCTOR (PHD) OF SCIENCE DEGREE IN MICROBIOLOGY to the

DEPARTMENT OF MICROBIOLOGY
SCHOOL OF MATHEMATICAL AND NATURAL SCIENCES
UNIVERSITY OF VENDA

Promoter: Prof AN Traoré
Co-Promoters: Prof MP Tshisikhawe
Prof N Potgieter

February 2017
ABSTRACT

Selection and evaluation of ten medicinal plants used, in the Vhembe District, for life-threatening infections

Sigidi MT, Traoré AN, Tshisikhawe MP and Potgieter N

Background

The Vhembe District of the Limpopo Province has a rich tradition in the use of medicinal plants. The use of these plant species, among rural communities has impacted on the development of various traditional medicinal systems. Such development has led to the exploration of different plant species in order to correlate traditional and scientific practices. In the Vhembe District, the vast majority of traditional medicine is indigenous knowledge-based and the pharmacological activities of plants are solely understood by traditional healers. This study investigated the medicinal activities of *Elaedendron transvaalense*, *Cassia abbreviata*, *Ornithogalum ornithogalooides*, *Ochna holstii*, *Lannea edulis*, *Elephantorrhiza elephantina*, *Coccinia rehmannii*, *Jatropha zeyheri*, *Ziziphus mucronata* and *Pterocarpus angolensis*.

Objectives: The general objective was to investigate the anecdotal claims by traditional healers to treat HIV/AIDS by evaluating the in-vitro cytotoxicity, anti-inflammatory, antimicrobial and immunomodulatory potential of the selected medicinal plants.

Methods: Water and ethyl acetate were used as the extraction solvents and the extracts were subjected to phytochemical screening. Determination of radical-scavenging activity of these plants' extracts was carried out using the DPPH assay and reducing-power assay. Different cell lines (RAW 264.7, U937, MeWo, Vero and PBMCs) were treated with various concentrations (50, 100, 125, 250 µg/mL) of *Elaedendron transvaalense*, *Cassia abbreviata*, *Ornithogalum ornithogalooides*, *Ochna holstii*, *Lannea edulis*, *Elephantorrhiza elephantina*, *Coccinia rehmannii*, *Jatropha zeyheri*, *Ziziphus mucronata* and *Pterocarpus angolensis* for anti-inflammation and cytotoxicity testing. The Nitric Oxide Assay was used to determine the anti-inflammatory potential of the plant extracts and the cytotoxicity of the extracts was tested using MTT and XTT assays. The immunomodulatory effects were determined by quantification of cytokine levels (IL-2, IL-6 and TNF-α) using commercial ELISA kits. Susceptibility testing was done using the microbroth dilution and INT assay. Reverse Transcriptase (RT) assay and HIV-p24 ELISA were used for the anti-HIV activity of the
plants. The Alamar Blue Assay was used to test for the anti-mycobacterial activity of *P. angolensis* and *Z. mucronata*, which are the two most used plants from the region.

**Results:** Cytotoxicity of the aqueous extracts was cell specific. The plant extracts exhibited selective toxicity towards the cancer line (U937 and MeWo). *Omnithogalum ornithogaloides*, *E. transvaalense*, *Z. mucronata* and *P. angolensis* exhibited a higher degree of toxicity in U937 cells with a reduction of cell viability by 60%. Furthermore, the adverse toxicity of *O. ornithogaloides* and *E. transvaalense* was observed in Vero cells with reduction of cell viability by 80%. Similar toxic effects were observed in MeWo cells for the same extracts. The ethyl acetate extracts of *C. abbreviata* and *E. elephanta* were the most toxic at 50 and 100 µg/ml with detectable viable cells below 20%. All the extracts proved to possess immunomodulatory activities. The non-infected cells expressed higher levels of IL-2 and *Z. mucronata* stimulated production of IL-2 in HIV-infected cells. IL-6 expression in non-infected cells by *C. rehmannii* was the highest (590 pg/ml) and in HIV-infected cells the expression was suppressed. Cytokine TNF-α was relatively lower in non-infected cells since this cytokine is known to be produced in all stages of HIV. Even though all the plant extracts exhibited no activity against bacterial pathogens, 2 of the extracts (*Z. mucronata* and *P. angolensis*) exhibited strong anti-TB activity. Moreover, the extracts exhibited RT activity with percentage inhibition of 40 and 55% for *C. rehmannii* and *P. angolensis*, respectively. P24 antigen levels were also decreased in the presence of all the tested extracts. *L. edulis* exhibited a decrease of 23 pg/ml in P24 antigen levels.

**Conclusion:** The current work highlighted the biological effects of the 10 medicinal plants. The claims presented by the traditional healers on the efficacy of the selected plants in treatment of HIV/AIDS might be accounted for by the inhibitory activities observed in the reverse transcriptase assay, p24 antigen and also the strong anti-TB effects that possibly combated the opportunistic pathogen. In addition, the plant extracts also possess immunomodulatory effects that possibly boost the immune system to fight off the infections.

**Keywords:** Cytotoxicity, Anti-inflammation, Medicinal plants, Anti-HIV