Antimicrobial activity of compounds isolated from *Lippia javanica* (Burm.f.) Spreng and *Hoslundia opposita* against *Mycobacterium tuberculosis* and HIV-1 Reverse transcriptase

BY

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DECLARATION

The experimental work described in this thesis was conducted in the Department of Plant Science, University of Pretoria and Medical Research Council (MRC) South Africa, Pretoria, from February 2002 to December 2005, under the supervision of Prof. Namrita Lall.

These studies are the result of my own investigation and have not been submitted in any other form to another University.

I declare the above statement to be true

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Silva Fabião Mujovo

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LIST OF ABBREVIATIONS

ABTS: 2, 2′-azino-bis (3-ethylbenzthialzoline-6-sulfonic acid)

AIDS: Acquired immune deficiency syndrome CFU: Colony forming units CD: Circular dichroism

Cosy: Correlated spectroscopy

^{13}C-NMR: Carbon-nuclear magnetic resonance

DEPT: Distortionless enhancement by polarization transfer

DIG-POD: anti-digoxigenine-peroxidase

DIG-dUTP: digoxigenine-deoxyuridine triphosphate

dTT: deoxythymidine triphosphate

DMEM: Dulbecco-modified Eagle's Medium

DMSO: Dimethyl sulphoxide

ds: double-stranded

EDTA: Ethylendiaminotetra acetic acid

ELISA: Enzyme- Linked Immunosorbent Assay

GC: Gas chromatography

GC/ MS: Gas chromatography/ Mass spectra

GP: Glycoprotein

HIV: Human immunodeficiency virus

HMBC: Heteronuclear multiple bond correlation

HMQC: Heteronuclear multiple quantum correlation

^{1}H-NMR: Nuclear magnetic resonance

IN: Integrase

IR: Infra red

MIC: Minimal inhibitory concentration

MRC: Medical Research Council

NOESY: Nuclear overhauser effect spectroscopy

RNA: Ribonuclease

RT: Reverse transcriptase

TLC: Thin layer chromatography

UV: Ultra violet

WHO: World Health Organization

SUMMARY

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Degree: PhD Plant Science

For centuries medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health facilities and services are inadequate. In recent years the use of and search for drugs derived from plants have been accelerated. Ethnopharmacologists, botanists, microbiologists, and natural-product chemists are trying to discover phytochemicals and "leads" which could be developed for the treatment of infectious diseases. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties**.** The evaluation of these plants for biological activity is

necessary, both to substantiate their use by communities, and also to discover possible new drug or herbal preparations.

Twenty five plants selected through ethno-botanical surveys in Mozambique which are used to treat respiratory diseases, wounds, viruses, stomach ailments and etc., were collected and investigated for antimicrobial activity. Acetone extracts of selected plants were tested for antibacterial, antimycobacterial and anti-HIV-1 activity. Antibacterial activity was evaluated using the agar diffusion method. Five Gram-positive (*Bacillus cereus, Bacillus pumilus, Bacillus subtilis, Staphylococcus aureus, Enterococcus faecalis*) and five Gram-negative (*Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens*) bacterial species were used in this study.

The extracts of each plant were tested at concentrations ranging from 0.125 to 5.0 mg/ml. Most of the plant extracts inhibited the growth of the Gram-positive microorganisms. The minimum inhibitory concentration of eight plants (*Cassia abbreviata, Elephanthorrhiza elephantina, Hemizygia bracteosa, Hoslundia opposita, Momordica balsamina, Rhoicissus tomentosa* and *Salvadora australis*) against Gram-positive bacteria was found to be 0.5 mg/ml. Gram-positive bacteria were found to be susceptible to extracts of *Lippia javanica* at concentration of 0.125 mg/ml. Among the 22 acetone extracts tested, two were found to have activity against Gram-negative bacteria at a concentration of 5.0 mg/ml (*Adenia gummifera* and *Momordica balsamina*). *Rhoicissus revoilli* inhibited *E. cloacae*, a Gram-negative strain, at a concentration of 2.5 mg/ml.

To evaluate antimycobacterium activity ten plants species were tested against H37Rv, a drug-sensitive strain of *Mycobacterium tuberculosis* at concentrations ranging from 0.5 to 5.0 mg/ml using BACTEC radiometric method. Four of the plant species tested (C*assia abbreviata, Hemizigya bracteosa, Lippia javanica* and *Melia azedarach*) were observed to be active against the H37Rv. (ATCC 27294) strain of TB at a concentration of 0.5 mg/ml which was the lowest concentration used in this study.

Seventeen plant species, were screened for anti-HIV bioactivity in order to identify their ability to inhibit the enzymes glycohydrolase (α-glucosidase and β- glucuronidase) and eleven species were further tested against Reverse transcriptase. It was found that 8 plant species (*Cassia abbreviata, Elephantorrhiza elephantina*, *Rhoicissus tomentosa, Pseudolachnostylis maprouneifolia, Lippia javanica*, *Litogyne gariepina, Maerua junceae and Momordica balsamina*) showed inhibitory effects against α-glucosidase and β-glucuronidase at a concentration of 200 µg/ml. The results of the tests revealed that the plant extracts of *Melia azedarach* and *Rhoicissus tomentosa* appeared to be active, showing 49 and 40% inhibition of the enzyme activity respectively.

Lippia javanica was found to have the best activity exhibiting a minimum inhibitory concentration of 0.125 mg/ml. The extracts also showed positive activity against *Mycobacterium tuberculosis* at concentration of 0.5 mg/ml and HIV-enzyme glycohydrolase was (α-glucosidase and β-glucuronidase) inhibited by 62 % and 73 % respectively. Considering its medicinal use local for HIV and various infections, it was therefore, selected for identifying its bioactive constituents. In the initial screening of

plants used in Mozambique *Hoslundia opposita* demonstrated good antitubercular activity. It was therefore, selected to identify its bioactive constituents.

A Phytochemical investigation of *L. javanica* led to the isolation of eight compounds, 4-ethyl-nonacosane (**1**), (*E*)-2(3)-tagetenone epoxide (**2**), myrcenone (**3**), piperitenone (**4**), apigenin (**5**), cirsimaritin (**6**), 6-methoxyluteolin 4'-methyl ether (**7**), 6-methoxyluteolin and 3',4',7-trimethyl ether (**8**). Three known compounds, 5,7-dimethoxy-6-methylflavone (**9**), hoslunddiol (**10**) and euscaphic acid (**11**) were isolated from *H. opposita*. This is the first report of compounds (**1**), (**2**), (**5-8**) from *L. javanica* and of compound (**9**) from *H. opposita*. The compounds were tested against *Mycobacterium tuberculosis* and HIV-1 reverse transcriptase for bioactivity. It was found that compounds (**2**), (**4**) and (**9**) inhibited the HIV-1 Reverse transcriptase enzyme by 91%, 53% and 52% respectively at 100 µg/ml. Of all the compounds tested against a drug-sensitive strain of *Mycobacterium tuberculosis*, euscaphic acid (**11**) was found to exhibit a minimum inhibitory concentration of 50 µg/ml against this strain.

The present study has validated scientifically the traditional use of *L. javanica* and *H. opposita* and a few other Mozambican medicinal plants to some extent.