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In vitro anti-HIV-1 properties of ethnobotanically selected South African plants used in the treatment of sexually transmitted diseases

by

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I declare that the thesis, which I hereby submit for the degree of PhD Medicinal Plant Science (option) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE: ……..

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TABLE OF CONTENTS

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 6

LIST OF TABLES

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

LIST OF ABBREVIATIONS

Abbreviation	Explanation
AIDS:	acquired immunodeficiency syndrome
^{13}C-NMR:	carbon-nuclear magnetic resonance
COSY:	correlated spectroscopy
DNA:	deoxyribonucleic acid
DMSO:	dimethylsulfoxide
DPPH:	1,2 –diphenyl-2-picrylhydrazyl
HIV:	human immunodeficiency virus
HMBC:	heteronuclear multiple bond correlation
HMQC:	heteronuclear multiple quantum correlation
^{1}H-NMR:	proton-nuclear magnetic resonance
LTR:	long terminal repeat
MRNA:	messenger ribonucleic acid
NF-κB:	nuclear factor kappa B
NMR:	nuclear magnetic resonance
NOESY:	nuclear overhauser effect spectroscopy
PBS:	phosphate buffer saline
RT:	reverse transcriptase
STD:	sexually transmitted disease
Tat:	transactivating regulatory protein
TB:	tuberculosis
TLC:	thin layer chromatography
UV:	ultra violet

WHO:	World Health Organization
XTT:	2,3-bis- [2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide

Summary

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Extracts of ten ethnobotanically selected medicinal plants used in the treatment of STD's were investigated for their anti-HIV properties against enzymes and proteins that play a role in the HIV life cycle. The antiviral activity was studied through the luciferase-based assay targeting the HIV promoter activation induced by either the HIV-1 Tat protein or the cellular transcription factor NF-κB, both required for efficient HIV-1 replication. Of the ten plant extracts investigated *Zanthoxylum davyi* and *Elaeodendron transvaalense* showed the most promising results. These extracts also showed specific luciferase inhibitory activity in the Hela-Tet-ON assay and did not show significant toxicity on MT2 cell line. The plant extracts were also tested against some enzymes (glycohydrolase and reverse transcriptase) that play a significant role in the HIV life cycle. *Senna petersiana* and *Terminalia sericea* showed to be potential inhibitors of both glycohydrolase and reverse

transcriptase enzymes.Futher phytochemical studies of *E. transvaalense* have led to the isolation of four known triterpenes [lup-20(30)-ene-3,29-diol, (3α)-(9Cl)] (**1**), [lup-20(29)-ene-30-hydroxy-(9Cl)] (**2**), Ψ – taraxastanonol (**3**), β-sitosterol (**4**) a catechin 4' –*O*- methylepigallocatechin (**5**), the rarely found phenolic derivative, atraric acid (**6**) and the depside, atranorin (**7**). The activities of Compound **6** and **7** were not analyzed further because of the low amount isolated. To evaluate the antiviral activity of the other five isolated compounds, NF-κB, anti-Tat and viral replication assays were performed. Only lup-20(29)-ene-30-hydroxy-(9Cl) (**2)** inhibited NF-κB activity at a low concentration of 10 µg/ml. Lup-20(30)-ene-3,29-diol, (3α)-(9Cl) (**1)** and Ψ – taraxastanonol (**3)** showed anti-NF-κB inhibition at a higher concentration of 50 µg/ml. The activities of the isolated compounds were not significant in other anti-HIV assays. All five isolated compounds were further analyzed for cytotoxicity using the XTT assay on Vero and MCF-7 breast cancer cell lines. Compound **2** demonstrated greater than 50 % growth inhibition at 25 µg/ml. The crude extract and other isolated compounds showed very little or no toxicity at the same concentration. The isolated compounds were also tested in the HIV-reverse transcriptase assay and none of these compounds displayed any RT activity. These results support the ethnomedicinal uses of these plants to some extent.

Keywords: Cytotoxicity; Terpenoid; HIV**;** NF-κB; *Elaeodendron transvaalense*