

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

by

Thilivhali Emmanuel Tshikalange

Submitted in partial fulfilment of the requirements for

**DOCTOR OF PHILOSOPHIAE: (OPTION) MEDICINAL PLANT
SCIENCE**

Department of Plant Science

Faculty of Natural and Agricultural Sciences

University of Pretoria

Promoter: Prof JJM Meyer

SEPTEMBER 2007

ACKNOWLEDGEMENTS

I am very grateful to the following individuals and institutions who contributed towards this project.

My supervisor Prof J.J.M. Meyer and Prof N. Lall for all their guidance, suggestions, encouragement and support throughout the course of research.

Dr Ahmed Hussein for all his help and advice with the isolation and identification of compounds.

Dr Mariana van de Venter (Nelson Mandela Metropolitan University) for assistance with the reverse transcriptase assay.

Prof Eduardo Munoz (Spain) for assistance with the anti-HIV assays.

Prof Fredrik Ivars (Sweden) for assistance with NF- κ B assays.

Bridgette and Adrian for toxicity assays.

Dr Josef de Beer for his words of encouragement throughout my studies.

My cousin Alipfali and traditional healers for their help during plant collection.

My family for being there for me (especially my son Wanga and his mother Muhangwi).

All my friends for the moral support they gave me.



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:

DATE:

TABLE OF CONTENTS

Summary: xv

Chapter 1: Introduction

1.1 Background 1

1.2 HIV/AIDS 2

 1.2.1. HIV life cycle 2

 1.2.2 HIV treatment and research on natural products.....5

 1.2.3 Nuclear factor kappa B and viral Tat Trans-activator 8

1.3 Aims and objectives and objectives of the study10

1.4 Plant selection 11

1.5 Scope of thesis 12

1.6 Hypothesis 12

1.7 References 13

Chapter 2: Activity of crude extracts against glycohydrolase and reverse transcriptase enzymes

2.1 Introduction 18

2.2 Material and methods 19

 2.2.1 Plant material 19

 2.2.2 Preparation of extracts 20



2.2.3 Glycohydrolase enzymes	22
2.2.4 HIV reverse transcriptase	23
2.3 Results and discussion	24
2.4 References	28

Chapter 3: NF- κ B, Hela-Tat and cytotoxicity assays on plant extracts

3.1 Introduction	33
3.2 Materials and methods	34
3.2.1 Plant material	34
3.2.2 Cell lines	34
3.2.3 NF- κ B assay	35
3.2.4 Hela Tat luc assay	36
3.2.5 Hela-Tet-OnLuc assay	36
3.2.6 Cytotoxicity assay	37
3.3 Results and discussion	38
3.4 References	45

Chapter 4: Isolation of compounds from *Elaeodendron transvaalense* extracts

4.1 Introduction	47
4.1.1 Plant description	48
4.1.2 Medicinal uses	49
4.1.3 Chemistry	50

4.2 Materials and methods	51
4.2.1 Plant material	51
4.2.2 Preparation of extracts	51
4.2.3 Isolation and identification of compounds	51
4.3 Results and discussion	52
4.3.1 Triterpenoids isolated.....	57
4.3.2 Methylpigallocatechin	69
4.3.3 Phenolic derivative and depside	73
4.4 References	80

Chapter 5: Anti-HIV activity of compounds isolated from *Elaeodendron transvaalense*

5.1 Introduction	85
5.2 Materials and methods	86
5.2.1 C-Med 100 100®	86
5.2.2 Transient transfection and luciferase activity analysis	86
5.2.3 Hela-Tat-Luc assay	87
5.2.4 Anti-HIV-1 replication	88
5.2.4.1 Production of VSV-pseudotyped recombinant viruses	88
5.2.4.2 VSV-pseudotyped HIV-1 infection assay	88
5.2.4.3 HIV reverse transcriptase (RT) assay	89
5.3 Results and discussion	89
5.4 References	93

**Chapter 6: Cytotoxicity of *Elaeodendron transvaalense*
extract and isolated compounds**

6.1 Introduction	96
6.2 Materials and methods	97
6.2.1 Plant material	97
6.2.2 Preparation of the extract	98
6.2.3 Cell culture	98
6.2.4 Toxicity screening (XTT viability assay)	98
6.3 Results and discussion	100
6.4 References	105

Chapter 7: General discussion and conclusions

7.1 Introduction	108
7.2 Activity of crude extracts against reverse transcriptase and glycohydrolase enzymes	109
7.3 NF- κ B, Hela-Tat and cytotoxicity assays on plant extract.....	109
7.4 Isolation of compounds from <i>Elaeodendron transvaalense</i> extract	110
7.5 Anti-HIV activity of pure compounds isolated from <i>Elaeodendron transvaalense</i>	110
7.6 Cytotoxicity of <i>Elaeodendron transvaalense</i> extract and isolated compounds	111
7.7 Conclusion	111

LIST OF FIGURES

Chapter 1

Figure 1.1: Worldwide HIV infection in 2005	3
Figure 1.2: Worldwide HIV prevalence rates in 2005	3
Figure 1.3: The replication cycle of HIV-1	4
Figure 1.4: Anti-HIV constituents obtained from root bark of <i>Zanthoxylum</i> <i>ailanthoides</i>	6
Figure 1.5: The RNA genome of HIV-1	7
Figure 1.6: Nuclear Factor kappa B (NF- κ B) pathway	8
Figure 1.7: A model for regulation of Tat mediated transcriptional activation of the chromatinized HIV LTR promoter	9

Chapter 2

Figure 2.1: Reverse transcriptase colorimetric assay principle	23
---	----

Chapter 3

Figure 3.1: Graph showing the anti-NF- κ B activity of plant extracts at 50 μ g/ml concentration	41
Figure 3.2: Graph showing the anti-Tat activity of plant extracts at 50 μ g/ml concentration	42

Chapter 4

Figure 4.1: Bark and branches of <i>Elaeodendron transvaalense</i>	48
Figure 4.2: Compounds isolated from <i>E. transvaalense</i>	50
Figure 4.3: Column chromatography	53
Figure 4.3: Schematic presentation of isolation steps followed	54
Figure 4.5: Fractions from silica column on TLC pates sprayed with Vanillin reagent. Plate A and B developed with Hexane: ethyl acetate (7:3), Plate C and D fractions developed with hexane: ethyl acetate (1:9)	55
Figure 4.6 The 11 pooled fractions (silica column 1) TLC plates sprayed with Vanillin reagent	56
Figure 4.7: Isolated compounds as seen on TLC plate sprayed with vanillin	56
Figure 4.8: Structures of isolated triterpenes	57
Figure 4.9: ¹ H – NMR spectrum of Compound 1.....	61
Figure 4.10: ¹³ C – NMR spectrum of Compound 1.....	62
Figure 4.11: HMBC spectrum of Compound 1.....	63
Figure 4.12: NOESY spectrum of Compound 1.....	64
Figure 4.13: ¹ H – NMR spectrum of Compound 2	65
Figure 4.14: ¹³ C – NMR spectrum of Compound 2	66
Figure 4.15: HMBC spectrum of Compound 3	67
Figure 4.16: HMQC spectrum of Compound 3	68
Figure 4.17: Structure of Compound 5	69
Figure 4.18: ¹ H – NMR spectrum of Compound 5	71
Figure 4.19: ¹³ C – NMR spectrum of Compound 5	72

Figure 4.20: Structures of Compound 6	73
Figure 4.21: Structures of Compound 7	74
Figure 4.22: ¹ H – NMR spectrum of Compound 6	76
Figure 4.23: ¹³ C – NMR spectrum of Compound 6	77
Figure 4.24: ¹ H – NMR spectrum of Compound 7	78
Figure 4.25: ¹³ C – NMR spectrum of Compound 7	79

Chapter 6

Figure 6.1: Plate design for cytotoxicity assay.....	99
Figure 6.2: Effect of <i>E. transvaalense</i> crude extract and isolated compounds on the growth of the normal Vero cell line	101
Figure 6.3: Effect of <i>E. transvaalense</i> crude extract and isolated compounds on the growth of the MCF-7 cell line	102

LIST OF TABLES

Chapter 2

Table 2.1: Medicinal plants investigated in this study for anti-HIV activity.. 21

Table 2.2: Inhibition of glycohydrolase (percent) in the presence of ten medicinal plant extracts at 200 µg/ml concentration..... 25

Table: 2.3: Effect of plant extracts on the activity of recombinant HIV –1 reverse transcriptase 26

Chapter 3

Table 3.1: Results of anti-HIV evaluations for all plant extracts tested at 50 µg/ml..... 40

Table 3.2: Results of anti-HIV evaluations for plant extracts that showed activity 43

Table 3.3: Cell death (necrosis) percentage at 6, 24 and 32 hour intervals44

Chapter 4

Table 4.1: Other medicinal uses of *E. transvaalense* 49

Table 4.2: ¹H – NMR and ¹³C – NMR data from tritepernoids isolated60

Table 4.3 : ¹H – NMR and ¹³C – NMR data of compound **5** 70



Chapter 5

Table 5.1: Results of anti-HIV evaluations for plant extracts that showed activity	90
---	----

Chapter 6

Table 6.1: IC ₅₀ of the crude extract and isolated compounds from <i>E. transvaalense</i> after 4 days on Vero and breast cancer (MCF-7) cells	103
--	-----

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
^1H -NMR:	proton-nuclear magnetic resonance
LTR:	long terminal repeat
MRNA:	messenger ribonucleic acid
NF- κ B:	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-sulfohenyl]-2H-
tetrazolium-5-carboxanilide

Summary

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

by

Thilivhali Emmanuel Tshikalange

Promoter: Prof J.J. Marion Meyer

Department of Plant Science

Degree: PhD Medicinal Plant Science (option)

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- κ B, 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. Further phytochemical studies of *E. transvaalense* have led to the isolation of four known triterpenes [lup-20(30)-ene-3,29-diol, (3 α)-(9Cl)] (**1**), [lup-20(29)-ene-30-hydroxy-(9Cl)] (**2**), Ψ – taraxastanol (**3**), β -sitosterol (**4**) a catechin 4' –*O*-methyl epigallocatechin (**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- κ B, anti-Tat and viral replication assays were performed. Only lup-20(29)-ene-30-hydroxy-(9Cl) (**2**) inhibited NF- κ B activity at a low concentration of 10 μ g/ml. Lup-20(30)-ene-3,29-diol, (3 α)-(9Cl) (**1**) and Ψ – taraxastanol (**3**) showed anti-NF- κ B inhibition at a higher concentration of 50 μ g/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 μ g/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- κ B; *Elaeodendron transvaalense*