CHAPTER 2:

Activity of crude plant extracts against glycohydrolases and reverse transcriptase

2.1 Introduction

Plant products have attracted attention as possible anti-HIV drugs targeted on the specific steps of the viral life cycle, such as viral attachment and entry as well as on essential enzymes and proteins that play a role during viral genome transcription (Matsuse *et al*., 1999). The approved medications that are currently in use are mainly restricted to target the two viral enzymes, protease and reverse transcriptase (RT). These inhibitors are very expensive and this has led to a global demand for broader, safer and also cheaper medications (Notka *et al*., 2004). A variety of plant extracts have been found to inhibit the reverse transcription process and glycohydrolase enzymes (Collins *et al*., 1997).

Glycohydrolase enzymes are found in the Golgi apparatus of eukaryotic cells. These are responsible for glycosylation of proteins. Inhibition of the

glycohydrolase enzymes has been found to decrease the infection caused by HIV virion, as the HIV glycoproteins are highly glycosylated (Collins *et al*., 1997).

Reverse transcriptase is an enzyme that reads the sequence of HI viral RNA nucleic acids that have entered the host cell and transcribes the sequence into complementary DNA. Without reverse transcriptase, the viral genome cannot be incorporated into the host cell; as a result a virus won't reproduce. Reverse transcriptase is therefore the principal target enzyme of antiretroviral drugs such as Nevarapine and Delavirpine that are used to treat HIV infected patients (De Clercq, 2007; Woradulayapinij *et al*., 2005).

This part of the study focuses on the screening of 10 South African medicinal plants (Table 2.1) associated with the treatment of STD's. Activity of the crude extracts against glycohydrolases and reverse transcriptase will be determined.

2.2 Materials and Methods

2.2.1 Plant Material

All the plant material (roots and stem bark) were collected based on their traditional uses against sexually transmitted diseases (Syphilis, gonorrhea, herpes as well as HIV) and some literature reports on their biological activity as antimicrobial agents. More information on the use of these medicinal plants was gathered through interviews with traditional healers as explained in chapter 1.

The plants investigated (Table 2.1) were collected from Venda in the Limpopo Province (South Africa). Voucher specimens were prepared and identified at the H.G.W.J. Schweikerdt Herbarium, University of Pretoria.

2.2.2 Preparation of extracts

Thirty gram of powdered roots or stem bark were extracted twice for 2 hours with 300 ml of chloroform, acetone, ethyl acetate or water and filtered. The extracts were then concentrated to dryness under reduced pressure and the residue was freshly dissolved in appropriate buffer on each day of experiment for the assays. Depending on the assay, extract that could not dissolve in appropriate buffer were first dissolved in DMSO and later diluted to different concentrations needed for a particular assay. .

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

Species	Family	Part used	Voucher no	Other ethnobotanical information
Anredera cordifolia (Ten.) Steenis	Basellaceae	Stem tubers	Smit 085981	Pain, inflammation (Tornos *et al*., 1999)
Clerodendrum glabrum E. Mey var. glabrum	Lamiaceae	Roots	Van Wyk 51839	Malaria (Clarkson *et al*., 2004)
Elaeodendron transvaalense (Burtt Davy) R.H. Archer	Celastraceae	Stem bark	Tshikalange 092524	Stomach ache, fevers, diarrhoea (Van Wyk *et al*., 1997)
Polianthes tuberosa L	Agavaceae	Roots	E.T 29	Ornamental (Huang *et al*., 2001)
Rauvolfia caffra Sond.	Apocynaceae	Stem bark	Hemm 39291	Diarhoea, abdominal complaints (Palgrave, 1977)
Rotheca myricoides (Hochst.) Vatke	Lamiaceae	Roots	Van Wyk 45727	Malaria (Muregi *et al*., 2007)
Senna occidentalis (L.)	Fabaceae	Roots	Lubbe 075884	Malaria (Tona *et al*., 2004)
Senna petersiana (Bolle) Lock	Fabaceae	Roots	Van Wyk 070978	Fevers, skin infections (Coetzee *et al*., 2000)
Terminalia sericea Burch. ex DC.	Combretaceace	Roots	Van Rensburg 38564	Diabetes, diarrhea (Moshi & Mbwambo, 2005)
Zanthoxylum davyi (I.Verd.) P.G. Waterman	Rutaceae	Roots	Lubbe 078130	Chest pains, wounds, toothache, coughs (Tarus *et al*., 2006)

2.2.3 Glycohydrolase enzymes

To measure the inhibition of the glycohydrolase enzymes, α - glucosidase (Sigma, MO, USA) and β- glucuronidase (Roche Diagnostics, Germany) were used with their corresponding substrates ρ-nitrophenyl-α-D-glucopyranose and ρ-nitrophenyl-β-D-glucuronide in a 96-well microtitre plate in a colorimetric enzyme based assay. The assay was perfomed according to Collins *et al*., (1997). The substrates and enzymes were dissolved in 50 mM morpholinol thanesulfonic acid-NaOH, pH 6.50. The enzyme was calibrated relative to an enzyme concentration of 0.25 µg/ml.

To test enzyme inhibition, each well of the microtitre plate had a reaction volume of 200 µl containing 2mM substrate, 40 mM buffer, enzyme and the plant extracts at 200 µg/ml concentrations. The extracts were allowed to interact with the enzyme for five minutes before the substrate was added. This reaction was allowed to proceed for 15 minutes and terminated with 60 µl of 2 M glycine NaOH, pH 10. The assay was carried out in triplicate. Control 1 only contained the buffer (40 µl) and substrate (20 µl) (no enzyme and extracts were added). For the second control the enzyme, buffer and substrate were added for the reaction to take place. The absorbance was read on a microtitre plate reader at 412 nm. Results were analysed using the formula:

Percent Inhibition = Sample absorbance/Control 1 absorbance X 100

2.2.4 HIV reverse transcriptase (RT) assay

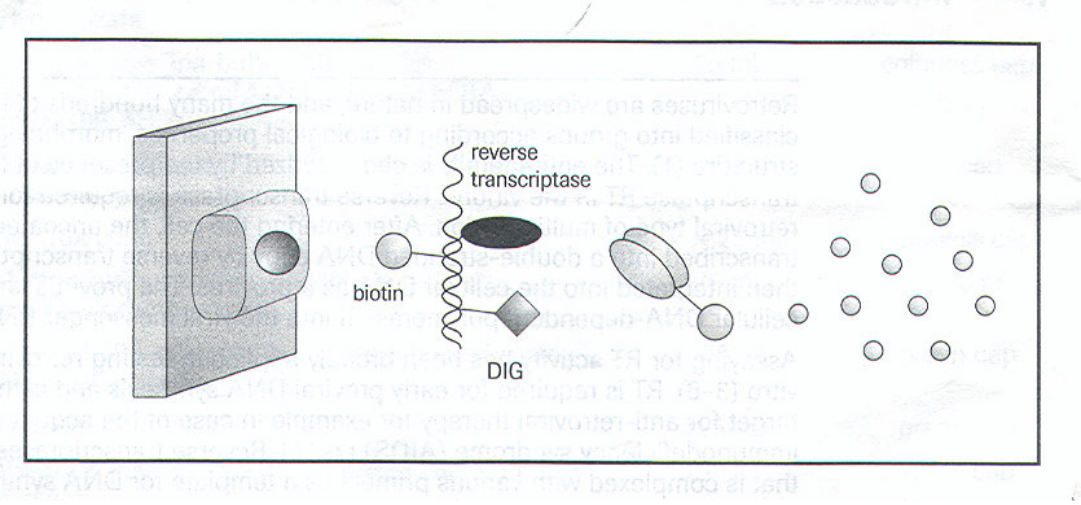

Figure 2.1: Reverse transcriptase colorimetric assay principle (Roche, 2005)

From the 10 plant extracts tested for glycohydrolase enzymes, only the four best plants were selected for RT activity testing. The effects of plant extracts on RT activity *in vitro* were evaluated with recombinant HIV – 1 enzyme, using a non-radioactive HIV–RT colorimetric (Figure 2.1) ELISA kit from Roche, Germany (Ayisi, 2003; Harnett *et al.*, 2005). The concentration of the extracts was 200 ug/ml. Extracts which reduced activity by at least 50 % were considered active (Woradulayapinij *et al.*, 2005). Doxirubicin was used as a positive control at 100 µg/ml. The assay was carried out in triplicate. Control 1 only contained the buffer and reaction mixture (no enzyme and extracts were added). For the second control the enzyme and reaction mixture were added for the reaction to take place. The absorbance was read on a microtitre plate reader at 412 nm and a reference wavelength of 490 nm. Results were analysed using the formula:

Percent Inhibition = Sample absorbance/Control 1 absorbance X 100

2.3 Results and discussion

The results shown in Table 2.1 demostrate the inhibition percentage of plant extracts against glycohydrolase enzymes, *α- glucosidase* and *β- glucuronidase*. The extracts of *Ceodendrum glabrum*, *Polianthes tuberosa* and *Senna occidentalis* showed no inhibition against *α- glucosidase*. The other seven extracts showed inhibition percentage ranging from 4.00 ± 0.01 to 90.00 ± 0.01, with the extracts of *Senna petersiana* (80.00 % inhibition) and *Terminalia sericea* (90.00 % inhibition) being the most active. *Elaeodendron transvaalense* and *Zanthoxylum davyi* showed weaker inhibition (not significant) of 23.00 and 22.00 respectively.

The inhibition percentage of *β- glucuronidase* by the extracts ranged from 1.40 ± 0.01 to 90.00 ± 0.01, with the extract of *P. tuberose* showing no inhibition. The most active extracts against *β- glucuronidase* were *S. petersiana* and *T. sericea* which exhibited 80.00 ± 0.01 and 90.00 ± 0.01 % inhibition respectively. All the extracts were tested at 200 µg/ml to determine their inhibitory activity against each enzyme and 50.00 % inhibition or higher were taken as significant. In this study the most promising inhibition against glycohydrolase enzymes tested was obtained with extracts of *S. petersiana* and *T. sericea*.

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

Plant	Extract	*α- glucosidase*	*β- glucuronidase*
		Inhibition % (S.D)	Inhibition % (S.D)
Anredera cordifolia	70 % Acetone	11.00 ± 0.00	33.00 ± 0.04
Clerodendrum glabrum	Water	ni	4.00 ± 0.01
Elaeodendron transvaalense	Chloroform	23.00 ± 0.02	10.00 ± 0.00
Polianthes tuberosa	70 % Acetone	ni	ni
Rauvolfia caffra	70 % Acetone	10.00 ± 0.00	6.00 ± 0.01
Rotheca myricoides	Water	4.00 ± 0.01	1.40 ± 0.01
Senna occidentalis	70 % Acetone	ni[a]	6.00 ± 0.00
Senna petersiana	70 % Acetone	80.00 ± 0.03	80.00 ± 0.00
Terminalia sericea	Ethyl Acetate	90.00 ± 0.01	90.00 ± 0.01
Zanthoxylum davyi	70 % Acetone	22.00 ± 0.01	14.00 ± 0.10

ni[a] : no inhibition

The *in vitro* inhibitory activities of the extracts against the reverse transcriptase (RT) enzyme are shown in Table 2.3. Only *T. sericea* exhibited the most notable activity of 94 % against RT. *E. transvaalense* (8 % inhibition) and *Zanthoxylum davyi* (20 % inhibition) showed weaker inhibition which was not significant.

Table: 2.3: Effect of plant extracts (200 µg/ml) on the activity of recombinant HIV –1 reverse transcriptase.

Extracts	Inhibition %	Standard deviation
Control 1	100	± 0.02
Control 2	0	± 0.10
E. transvaalense	8.00	± 0.00
S. petersiana	ni*	± 0.00
T. sericea	94.00	± 0.00
Z. davyi	20.00	± 0.02
Doxorubicin (100 µg/ml)	71.00	± 0.17

*ni, no inhibition

The previous reported biological activities (not on HIV) of *T. sericea* were mainly attributed to triterpenoids, saponins and tannins (Steenkamp *et al*., 2004, Bombardelli *et al*., 1974, Fyhrquist *et al*., 2006). Eldeen *et al.* (2006) reported the isolation of the lignan, Anolignan B from *T. sericea* root extract. This compound was first isolated from *Anogeissus acuminata* and was reported as a constituent

acting with Anolignan A to inhibit the enzyme HIV-RT (Eldeen *et al*., 2006).The ethyl acetate extract of *T. sericea* in this study showed the most notable inhibition of reverse transcriptase enzyme and this could possibly be attributed to the abovementioned chemical constituents reported previously. Further phytochemical studies need to be conducted in order to determine the activity of individual compounds against reverse transcriptase enzyme.

2.4 References

AYISI, N.K. & NYADEDZOR, C. 2003. Comparative *in vitro* effects of AZT and extracts of *Ocimum gratissimum*, *Ficus polita*, *Clausena anisata*, *Alchornea cordifolia* and *Elaeophorbia drupifera* against HIV-1 and HIV-2 infections. Antiviral Research 58(1): 25-33.

BOMBARDELLI, E., BONATI. A. & MUSTICH, G. 1974. Triterpenoids of *Terminalia sericea*. Phytochemistry 13: 2559-2562.

CLARKSON, C., MAHARAJ, V.J., CROUCH, N.R., GRACE, O.M., PILLAY, P., MATSABISA, M.G., BHAGWANDIN, N., SMITH, P.J. & FOLB, P.I. 2004. In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. Journal of Ethnopharmacology 92: 177-191.

COLLINS, R.A., NG, T.B., FOUNG, W.P., WAN, C.C. & YEUNG, H.W., 1997. A comparison of human immunodeficiency virus type 1 inhibition by partially purified aqueous extracts of Chinese medicinal herbs. Life Sciences 60: 345-351.

COETZEE, J., MCITEKA, L., MALAN, E. & FERREIRA, D. 2000. Structure and synthesis of the first procassinidin dimers based on epicatechin, and gallo- and epigallo – catechin. Phytochemistry 53:795-804.

DE CLERCQ, E. 2007. Anti-HIV drugs. Verhandelingen - Koninklijke Academie Geneeskdunde van Belgie 69:81-104.

ELDEEN, I.M.S., ELGORASHI, E.E., MULHOLLAND, D.A. & van STADEN, J. 2006. Anolignan B: A bioactive compound from the roots of *Terminalia sericea*. Journal of Ethnopharmacology 103:135-138.

FYHRQUIST, P., MWASUMBI, L., VUORELA, P., VUORELA, H., HILTUNEN, R., MURPHY, C. & ADLERCREUTZ, H. 2006, Preliminary antiproferative effects of some species of *Terminalia*, *Combretum* and *Pteleopsis* collected in Tanzania on some human cancer cell lines. Fitoterapia. 77:356-366.

HARNETT, SM., OOSTHUIZEN, V. & VAN DE VENTER, M. 2005**.** Anti-HIV activities of organic and aqueous extracts of *Sutherlandia frutescens* and *Lobostemon trigonus* . Journal of Ethnopharmacology 96: 113-119.

HUANG, K., MIYAJIMA, I., OKUBO, H., SHEN, T. & HUANG, T. 2001. Flower colours and pigments in hybrid tuberose (*Polianthes*). Scientia Horticulturae 88: 235-241.

MATSUSE, I.T., LIM, Y.A., HATTORI, M., CORREA, M. & GUPTA, M.P., 1999. A search for Anti-viral properties in Panamanian medicinal plants: The effects on HIV and its essential enzymes. Journal of Ethnopharmacology 64: 15- 22.

MOSHI, M.J. & MBWAMBO, Z.H. 2005. Some pharmacological properties of extracts of Terminalia sericea roots. Journal of Ethnopharmacology 97: 43-47.

MUREGI, F.W., ISHIH, A., MIYASE, T., SUZUKI, T., KINO, H., AMANO, T., MKOJI, G.M. & TERADA, M. 2007. Antimalarial activity of methanolic extracts from plants used in Kenyan ethnomedicine and their interactions with chloroquine (CQ) against a CQ-tolerant roent parasite, in mice. Journal of Ethnopharmacology 111: 190-195.

NOTKA, F., MEIER, G. & WAGNER, R., 2004. Concerted inhibitory activities of *Phyllanthus amarus* on HIV replication *in vitro* and *ex vivo*. Antiviral Research 64: 93-102.

PALGRAVE, K.C. 1977. Trees of Southern Africa. Struik Publishers. Cape Town. South Africa.

ROCHE, 2005. Reverse Transcriptase Assay colorimetric Manual (Cat. No. 11 468 120 910)

STEENKAMP, V., MATHIVHA, E., GOUWS, M.C. & VAN RENSBURG, C.E.J., 2004. Studies on antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa. Journal of Ethnopharmacology 95: 353-357.

TARUS, P.K., COOMBES, P.H., CROUCH, N.R. & MULHOLAND, D.A. 2006. Benzo [*c*] phenanthridine alkaloids from stem bark of the forest Knobwood, *Zanthoxylum davyi* (Rutaceae). South African Journa of Botany 72: 555- 558.

TONA, L., CIMANGA, R.K., MESIA, K., MUSUAMBA, C.T., DE BRUYNE, T., APERS, S., HERNANS, N., VAN MIERT, S., PIETERS, L., TOTTE, J. & VLIETINCK, A.J. 2004. In Vitro antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. Journal of Ethnopharmacology 93: 27-32.

TORNOS, M.P, SAENZ, M.T, CARCIA, M.D. & FERNANDEZ, M.A. 1999. Antinocieptive effects of the tubercles of *Anredera leptostachys*. Journal of Ethnopharmacology 68: 229-234.

UCHIUMI, F., HATANO, T., ITO, H., YOSHIDA, T. & TANUMA, S. 2003. Transcriptional suppression of the HIV 1 promoter by natural compounds. Antiviral Research 58: 89-98.

WORADULAYAPINIJ, W., SOONTHORNCHAREONNON, N. & WIWAT, C. 2005. *In vitro* HIV type 1 reverse transcriptase inhibitory activities of Thai medicinal plants and *Canna indica* L. rhizomes. Journal of Ethnopharmacology 101: 84-89.