CHAPTER 1: Introduction

1.1 Background

Plants have not only provided mankind with food, clothing, flavours and fragrances, but have also served humanity to treat different ailments. According to the World Health Organization (WHO), about three-quarters of the world population rely on plants for the treatment of many illnesses. Plants are always surrounded by an enormous number of potential enemies such as bacteria, viruses, fungi etc. By nature plants cannot avoid these enemies simply by moving away, they protect themselves through chemical defence systems (Van Wyk & Gericke, 2000). Therefore it is logical to expect biological active compounds to be produced by plants as a chemical defence measure against their enemies. The search for biological active agents from plants is part of a wider resurgence of scientific interest to produce new chemotherapeutics. Plants synthesize very complex molecules with specific stereochemistry and can show biological activity with novel modes of action (Houghton, 1996). Many useful drugs have been developed from medicinal plants used in traditional medicine in the treatment of a variety of illnesses. According to Gilani & Atta-ur-Rahman (2005), “The use of plants, plant extracts or plant-derived pure chemicals to treat diseases is a therapeutic modality, which has stood the test of time”. Most of the clinical drugs that are currently in use were derived from plants and developed because of their use in traditional medicine. Asprin (antipyretic), atropine, digoxin, morphine (pain killer), quinine, respine (hypertension) and tubocurarine are a few examples of

drugs, which were discovered through the study of ethnobotany (Gilani & Atta-ur-Rahman, 2005).

1.2. HIV/AIDS

1.2.1 HIV life cycle

Acquired immunodeficiency syndrome (AIDS), is a global health problem affecting more than 42 million people worldwide (Figure 1.1 & 1.2). Human immunodeficiency virus (HIV) is the pathogen that causes AIDS, a complex array of disorders resulting from the deterioration of the immune system (Silva *et al.*, 2004). The infected individuals become susceptible to opportunistic pathogens such as common microbes and often suffer from tuberculosis (TB), pneumonia and rare forms of cancer. HIV uses the macrophages and T cells as sites for reproduction and production of multiple copies of viral genetic material ready to infect new viral hosts. During each round of infection more cells of the immune system are damaged or killed. The host cells produce antibodies and helper T-cells in order to fight the virus, but eventually the virus prevails and opportunistic diseases associated with AIDS appear (Gurib-Fakim, 2006).

HIV-1 infection (Figure 1.3) begins with a virus binding to a susceptible cell (step 1). Following binding, membrane fusion facilitated in part by the CKR-5 protein, results in the introduction of the HIV-1 (step 2). Reverse transcription of the viral RNA (step 3) and the integration of resulting DNA copy into the host-cell chromosomes ensues (step 4). Once integrated in the chromosome,

the transcriptal activity of the HIV-1 provirus is regulated in part by the virally encoded tat protein (step 5). Following synthesis of a full- length viral RNA, viral mRNAs can be produced. The HIV-1 rev protein controls this process and makes mRNA transcripts available in the cytoplasm for the translation of viral

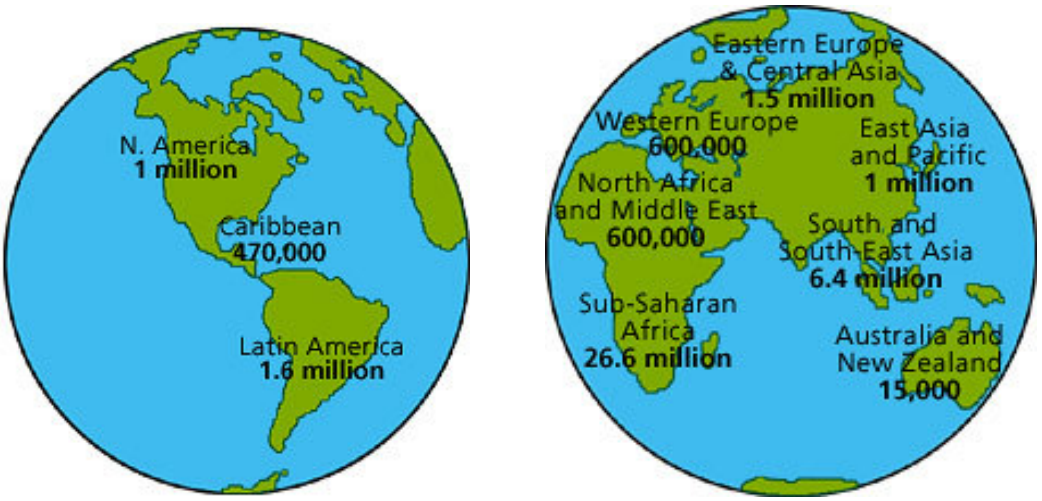

Figure 1.1 Worldwide HIV infections in 2005 (www.scripps.edu)

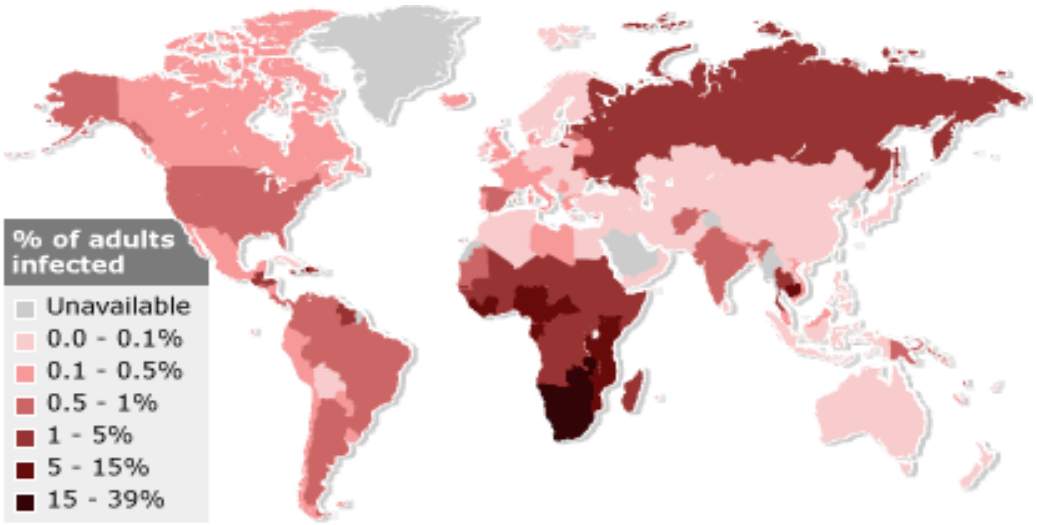

Figure 1.2 Worldwide HIV prevalence rates in 2005 (www.unaids.com)

proteins (step 6). Synthesis of viral structure proteins proceeds (step 7), HIV-1 particles assemble (step 8), and acquire viral envelope proteins as they bud through the host-cell membrane. The viral polyproteins are cleaved by viral protease during or shortly after budding, generating mature infectious virions (step 9) (Perry, 1997).

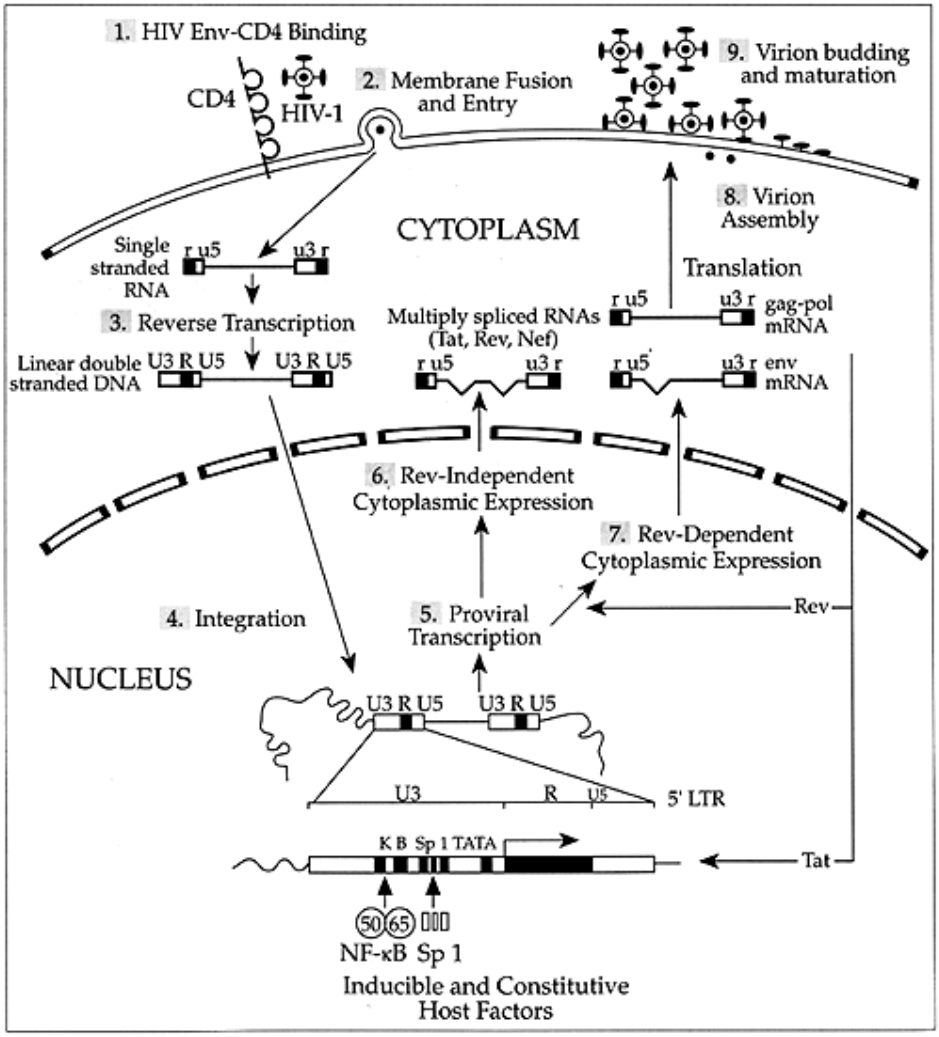

Figure 1.3 The replication cycle of HIV-1 (Perry, 1997).

1.2.2 HIV treatment and research on natural products

The antiretroviral agents which are currently available for the treatment of HIV infection targets enzymes essential to the life cycle of the virus. HIV reverse transcriptase (RT) is crucial for viral replication, HIV protease facilitates maturation and infectivity of virion particles and HIV intergrase mediates HIV intergration into the host genetic material (Ng *et al*., 1997). More than twenty drugs have already been licensed for HIV treatment, including formulations of both individual and combined antiretroviral agents. Specific inhibitors of several stages of the viral cycle, including viral attachment and entry are subject to preclinical investigation or have already entered clinical trials (Notka *et al*., 2003). Efforts to find other anti-HIV agents have been mainly focused on the development of drugs that targets viral proteins, which are essential for viral replication. The current antiviral therapy presents important limitations such as side effects and appearance of mutant viruses that are resistant and makes drugs which are currently in use to be insufficient to maintain a safe theraupeutic arsenal against HIV (Bedoya *et al*., 2006; Ma *et al*., 2002).

There is a global need or demand for broader, safer and cheaper drugs for the treatment of HIV infection. One of the approaches is to find anti-HIV agents from medicinal plants. New anti-HIV compounds from natural sources are often reported, some are essentially unproven and others with distinct promise based on *in vitro* research. Natural products have been a consistently successful source in drug discovery and may offer more opportunities to find anti-HIV drugs or lead compounds (Wang *et al*., 2006).

A number of medicinal plants have been screened and resulted in the isolation of some active compounds with inhibitory activity on HIV. One of the most promising anti-HIV compounds, calanolide was isolated from a Malaysian tree belonging to the Garcinia family. Calanolide, a coumarin, is now being tested in human trials (Gurib-Fakim, 2006). Other coumarins with anti-HIV activity have been reported by Bedoya *et al*. (2005) and Uchiumi *et al*. (2003). Active compounds like benzo[c]phenanthridine decarine and others isolated from *Zanthoxylum ailanthoides* (Figure 1.4) also showed anti-HIV activity in acutely infected H9 cells as reported by Cheng *et al*. (2005).

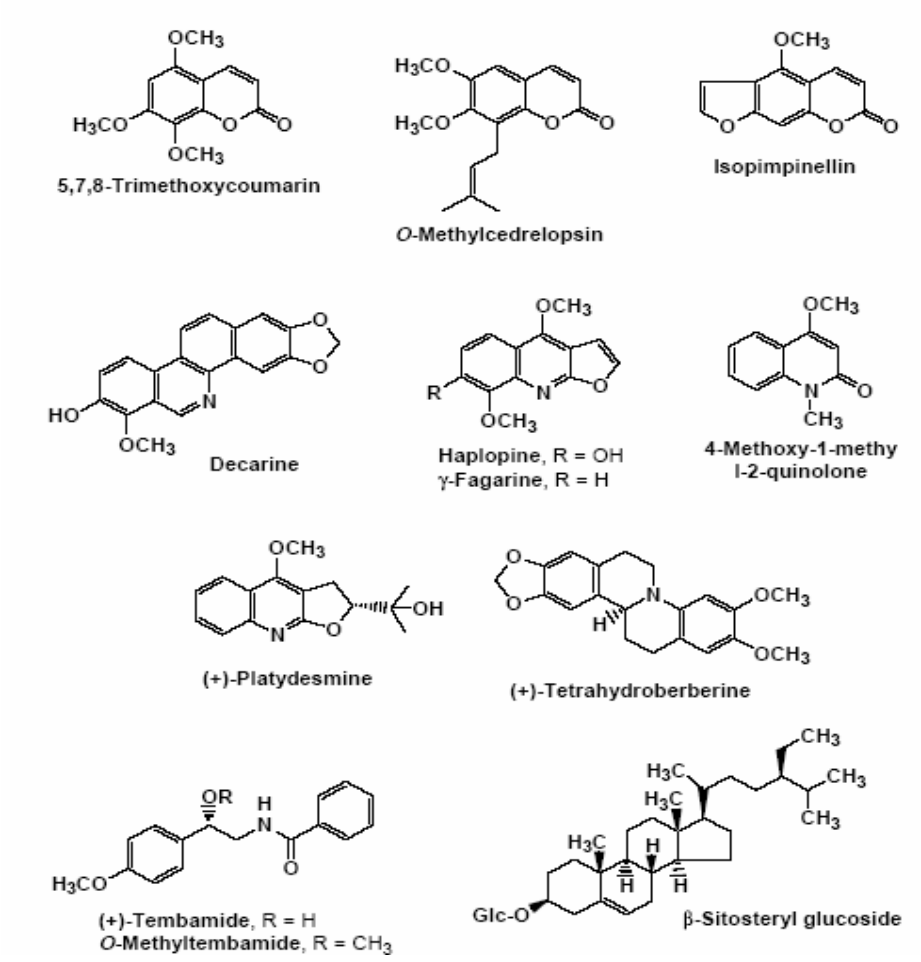

Figure 1.4 Anti-HIV constituents obtained from root bark of *Zanthoxylum ailanthoides* (Cheng *et al*., 2005).

A new kind of potential AIDS treatment by using long terminal repeat (LTR) inhibitors has now emerged since there are various factors that increase HIV activity by activating the LTR. Viral activation can be caused by the enhanced expression of the TAT gene that produces the protein, NF-κB which is normally present in the body in low concentrations. Each of these proteins binds to the LTR and activates HIV (Figure 1.5). The search for LTR inhibitors also led to the investigation of medicinal plants. Reports have shown that anti-NF-κB and anti-Tat agents from medicinal plants could be possible therapeutic agents against HIV infection. These agents seem to have the capacity of inhibiting HIV activation from latency, mainly through interference of NF-κB and Tat functions (Bedoya *et al.*, 2005 and Akesson *et al.*, 2003).

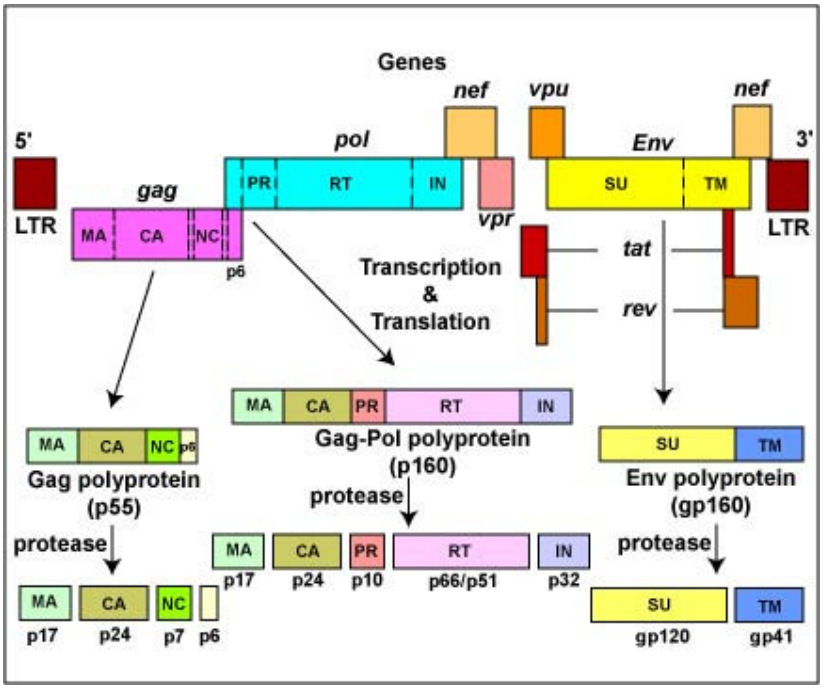

Figure 1.5 The RNA genome of HIV-1 (www.aids.harvard.edu)

1.2.3 Nuclear factor kappa B (NF-κB) and viral Tat transactivator

NF-κB constitutes a family of transcription factors that is important for cellular functions such as cell cycle progression and proliferation (Akesson *et al.*, 2003). It plays an important role in the regulation of a multitude of genes (Figure 1.6) involved in cell survival and it has also been implicated in inflammatory diseases such as rheumatoid arthritis, multiple sclerosis, asthma and atherosclerosis.

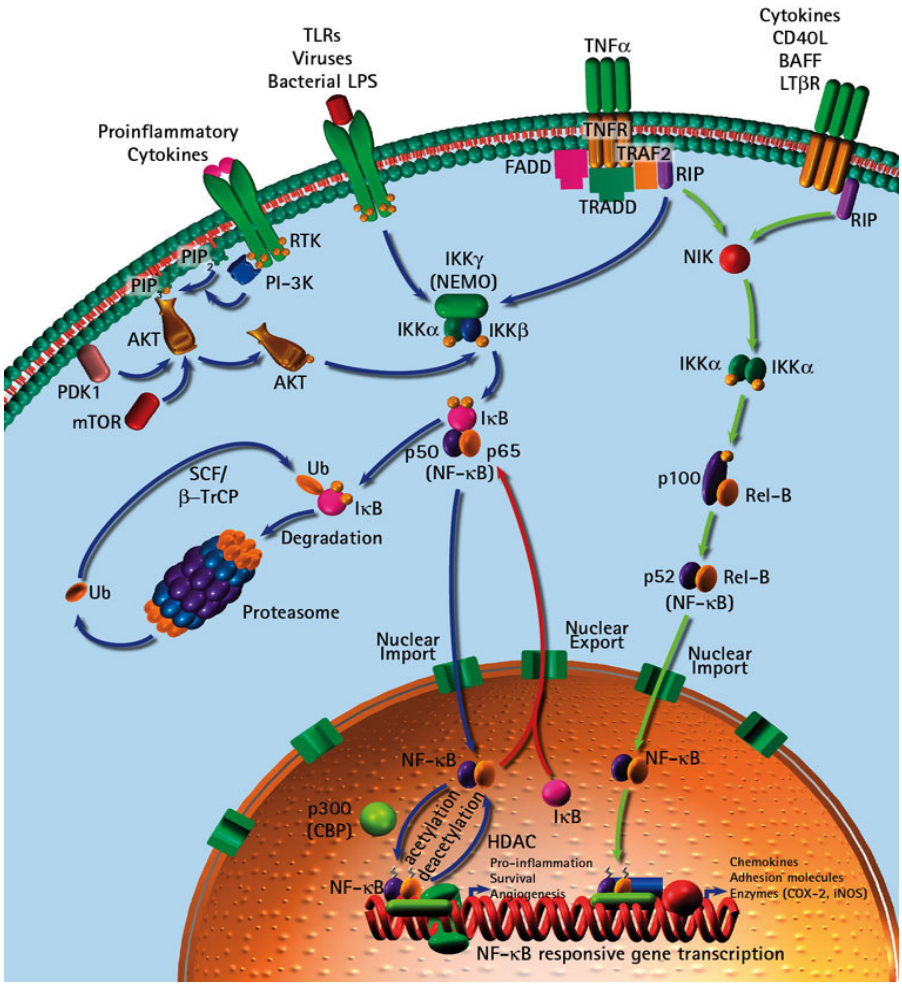

Figure 1.6 Nuclear factor kappa B (NF-κB) pathway (www.emdbioscience.com)

When cells are not stimulated, NF-κB is retained in the cytoplasm in a complex with the inhibitor of κB (IκB) proteins. NF-κB can be activated by stimuli including pro-inflammatory cytokines TNF-∝ which promotes phosphorylation of the IκB proteins followed by degradation of IκB and subsequent translocation of NF-κB into the nucleus (Lindgren *et al*., 2001). The Tat protein of HIV-1 is a small polypeptide of 101 amino acid essential for the transcription of viral genes and for viral replication. Tat activates HIV-1 transcription by promoting the assembly of transcriptionally active complexes at the LTR through interaction with TAR (Figure 1.7).

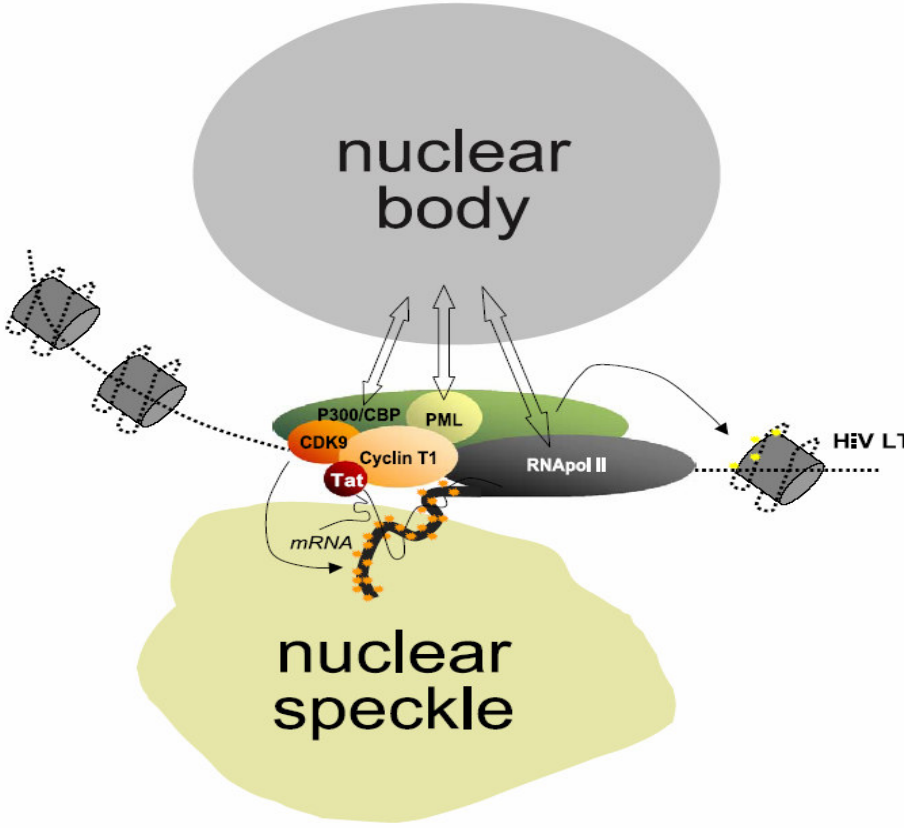

Figure 1.7 A model for regulation of Tat mediated transcriptional activation of the chromatinized HIV LTR promoter (www.clarku.edu/faculty/shuo/homepage/research).

Upon transcriptional activation, multiple spliced, short transcripts arise, encoding for viral accessory proteins including Tat and Rev. These accessory proteins enhance viral transcription and promote the expression and export of the late, unspliced RNAs from the nucleus (Marcello *et al*., 2004).

1.3 Aims and objectives of the study

HIV continues to pose an unprecedented public health problem and current treatment options have not been satisfactory. The quest for effective curative or preventive therapies continues with plants increasingly seen as an alternative source for discovery of novel anti-HIV molecules. Several studies have demonstrated the inhibitory properties of variety of crude plant extracts, as well as isolated compounds against different stages of HIV life cycle. Compounds such as papavarine, glycyrrhizin and trichosanthin were seen to have promise and have been evaluated in AIDS patients (Bessong & Obi, 2006).

South African plant extracts such as *Lobostomon trigonus* (Boraginaceae), and the gallotannin isolated from *Peltophorum africanum* (Fabaceae) strongly inhibited HIV reverse transcriptase functions (Bessong *et al*., 2005). People often claim that their medicinal plant remedies could improve an AIDS patient's quality of life. However, the efficacy of these remedies or plants has mostly not been proved (Woradulayapinij *et al*., 2005).

The main objectives of the study were to determine the:

- anti-HIV activity of plant extracts and isolated compounds against glycohydrolase, reverse transcriptase, NF-κB and Tat proteins.
- cytotoxicity of isolated compounds.

1.4 Plant selection

The plant selection for this study was based on interviews with three traditional healers and a literature review of traditional medicinal plant usage in South Africa. Four of the selected plants (*Clerodendrum glabrum*, *Polianthes tuberosa*, *Rotheca myricoides* and *Senna occidentalis*) were collected after the interview response given by the late Mr Anthony, who was a traditional healer in Soshanguve and often received HIV/AIDS patients. He worked closely with medical doctors and his patients showed an increase in CD4 counts after treatment with the abovementioned plants.

Anredera cordifolia, *Elaeodendron transvaalense*, *Rauvolfia caffra* and *Zanthoxylum davyi* are used by Vhavenda people (Limpopo Province) in the treatment of sexually transmitted diseases (STDs) and two traditional healers (Mr T. Ramudingane and Mrs V. Nemutandani) interviewed claimed that these plants can also help AIDS patients. *Senna pertesiana* and *Terminalia sericea* also used traditionally by Vhavenda people to treat STDs and recent

reports have shown these plants to possess some compounds with anti-HIV activity (Eldeen, 2006; Mabogo, 1990).

It was not possible to select plants used historically for treatment of AIDS, since it does not seem to have been known as a disease until the early 1980s. However, this study investigated *in vitro* anti-HIV properties of some ethnobotanically selected plants used in the treatment of sexually transmitted diseases. It is commonly recognized that bioactive compounds are more likely to be discovered from screening guided by traditional medicine than from random screening (Lee & Houghton, 2005).

1.5 Scope of the thesis

The activity of crude extracts against glycohydrolase and reverse transcriptase enzymes is described in Chapter 2. NF-κB, Hela-Tat and cytotoxicity assays on plant extracts are described in Chapter 3. Chapter 4 deals with the isolation and identification of pure compounds from *E. transvaalense* extract. Chapter 5 describes the anti-HIV activity of isolated compounds and Chapter 6 describes their cytotoxicity. Chapter 7 comprises of the general discussion and conclusions.

1.6 Hypothesis

The extracts and/or isolated compounds from medicinal plants used to treat STD's will be active against HIV.

1.7 References

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