

# Chapter 1

## Introduction & Background

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## 1.1 General introduction and background

South Africa is a country rich in resources, however many of these are not yet used to its full capacity. In South Africa there are about 25 000 plant species, a diversity that is found in very few countries. This accounts for 10 % of the world's plant species. About 70% of these species are also endemic to South Africa (Torszel, 1997).

Poverty and the lack of knowledge support the spread and mortality rates of several diseases in our country. Most of these diseases are curable, but the treatment method and cost account for the increasing infection and mortality rates. Many people in South Africa still rely on traditional medicine, because it is their only access to health care. 80% of people living in developing countries are almost completely dependent on traditional medicine for their primary health care needs, and higher plants are known to be the main source of drug therapy in traditional medicine (Torszel, 1997). The development of affordable medicines providing solutions to the diseases in our country is therefore critical.

The need for development of new medicines are being realised all over the world, as pathogens become drug resistant. Medicines effective against these drug-resistant organisms are frequently in demand all over the world. This study is one of only a few that investigates South African plant treasures. One of our indigenous plants might prove to be effective in inhibiting the growth of the most feared virus ever – the Human Immunodeficiency Virus (HIV) that eventually leads to Acquired Immune Deficiency Syndrome (AIDS). The path of drug discovery to find useful and effective

treatments for patients is still a long and steep one, which might prevent many of these plant extracts to be commercialised as a treatment against HIV.

More than a decade has passed since the emergence of HIV and yet the impact of HIV/AIDS still remains a major threat and continues to have a devastating effect on the population. Nowhere has the impact been more devastating than in sub-Saharan Africa where more than 11 million people have died of it. The World Health Organisation (WHO) and the joint United Nations Programme on HIV/AIDS (UNAIDS, 2005) estimated that 30 million people have been living with HIV/AIDS in Africa by 2000 (UNAIDS, 2005) and there was no decrease in this number by 2003. The Population Reference Bureau estimated that by 2002 nearly 5 million of the population in South Africa was already infected with HIV. This is the highest number of HIV infections in any country in Africa (Population Reference Bureau, 2002). The Department of Health estimates that, on average, 1500 people are infected daily with the virus (HIV/AIDS case studies in South Africa, 2002). HIV and three HIV-related diseases namely tuberculosis, influenza and pneumonia are the major causes of death in South Africa. More than a million South Africans are dying each year of HIV/AIDS. According to UNAIDS (2004) there have been 2.3 million deaths by the end of 2004 due to HIV/AIDS (Statistics South Africa, 2002). Statistics South Africa reported that 9% of all deaths can be contributed to HIV, but the Medical Research Council (MRC) believes that the deaths due to HIV are closer to 25% (UNAIDS 2005). The percentage of deaths for each of the HIV related diseases is given in Figure 1.1.

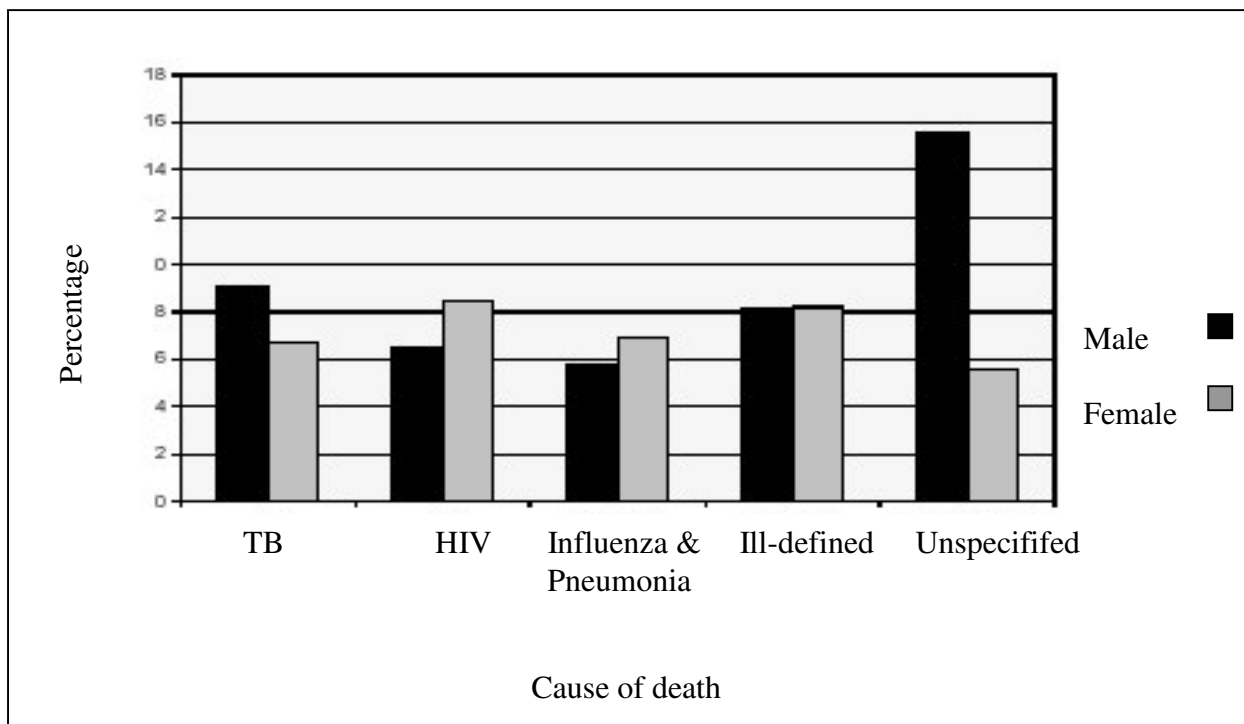


Figure 1.1 Causes of death in South Africa, by sex from 1997 to 2001 (Statistics South Africa, 2002).

## 1.2 Background on traditional medicine

“Nature distributed medicine everywhere”. – Pliny the Elder, circa A.D. 77

While the invention of synthetic chemistry in the 1930s reduced our reliance on the natural world as our sole source of medicines, an electrifying renaissance is well under way as we search the far corners of our planet for healing compounds. Within the course of the past decade, this quest has gone from being a marginal exercise to a mainstream concern all over the world. Mother Nature has been devising extraordinary compounds for more than 3.5 billion years, and new technologies increasingly facilitate our ability to discover them. New technologies therefore

enhance, rather than diminish nature's value as a source of healing compounds (Plotkin, 2000).

Plants have fed the world and cured its ills since time immemorial. A vast knowledge of medicinal and poisonous plants have therefore accumulated. Most of this knowledge still only exists as verbal tradition and only a fraction is yet available to science. Less than 10% of plants have been subjected to investigations of secondary metabolites and their effects. In order to evaluate the prospects of medicinal plant research we must know something about the results already obtained. History is important since it gives us the key to the present and should help in planning the future (Hedberg, 1987).

According to The World Health Organisation, up to 80% of Africans – or more than a half billion people visit traditional healers for some or all of their medical care. In Africa and in many developing countries, medical services are limited or unobtainable for the majority of the population (Iwu & Wooten, 2002). In South Africa today the same phenomenon is found. Many people still uses a wide variety of plants in their daily lives as food, water, shelter, fuel, medicine and for other important necessities in life. Modern health care and educational changes shifted populations from rural to urban areas, and these changes all contribute to the erosion of the indigenous knowledge of our country (Van Wyk & Gericke, 2000).

It is difficult to determine when scientific research started on plants used in traditional medicine. It would probably be safe to date it back to the late 18<sup>th</sup> century when investigations were carried out on the effect of *Digitalis* (Whithering, 1785).

Up to early 1800 drugs were used raw or as simple extracts. A new epoch was initiated when morphine was isolated for the first time. From there the interest seemed to have gradually diverged from research on medicinal plants to research on plant medicines. The research became increasingly orientated towards the chemical aspects and manufacturing of pure compounds like strychnine, caffeine and quinine (Hedberg, 1987).

The improving possibilities to synthesise desirable products meant that searching in the plant kingdom for remedies diminished. The discovery of the oncolytic properties in the alkaloids of *Catharanthus roseus* probably turned the wheel to research of plant products again. This discovery has in 30 years resulted in hundreds of scientific papers and stimulated the search for other anti-tumour agents of plant origin (Hedberg, 1987). The case of *Catharanthus roseus* is by no means unique, as many of our modern medicines are based or modelled on compounds occurring in plants which have been used for hundreds or thousands of years in traditional medicine (Hedberg, 1987).

More Americans are visiting “traditional healers” than physicians. The ineffectivity of Western medicines in treating certain disorders leads people to investigate alternatives. The future of Western healing is probably not in alternative medicines, but complementary medicines, which brings together the best of different healing traditions (Plotkin, 2000).

To ensure that these medicines will be available for research and further discovery, more controlling measures need to be implemented. Environmental destruction and degradation remain the major threats to the use of traditional medicines as complementary medicine to Western medicines. Overpopulation, deforestation, pollution and wildlife trade threaten endangered species, and even our own species as well (Plotkin, 2000).

### **1.3 Objectives and hypothesis**

Due to the alarming statistics of HIV/AIDS over the world and the rich plant species diversity of South Africa, a project was launched between the Department of Botany at the University of Pretoria and the University of Cordoba in Spain to investigate the anti-HIV properties of some indigenous plants in South Africa.

It was important to isolate and identify the active components of the most promising extract, which is however a very lengthy and costly method. It was therefore also important to determine the effectiveness and toxicity of the crude and semi-purified extracts which have a much lower cost of preparation.

The hypothesis of the thesis was that plant extracts from selected indigenous South African plants would be effective against HIV. These plants were selected on their antibacterial and antiviral activity determined in previous studies. If the extracts inhibited HIV, it would be necessary to isolate and identify the pure compounds from the plant extracts for further studies, and to determine the possibility of developing these into commercial products.

The thesis comprises of ten chapters. Chapter one provides a general background on the study. It also includes a literature review on the genus *Elaeodendron* and the species *Elaeodendron croceum*. Chapter two discusses the HIV/AIDS disease, the effects it has on humankind and its pathogenesis. Chapter three describes the preparation of the plant extracts, the steps followed to isolate the active ingredient, and also the structure elucidation. In Chapters four and five the different assays performed to determine the efficacy and toxicity of the crude extract, the semi-purified extract and the pure compound is discussed. The importance of the use of different assays and toxicity tests is highlighted in these chapters. Chapter six gives an overview of the isolated compound digitoxigenin-glucoside and the cardiac glycosides. It describes the uses and characteristics of this group of compounds. Chapter seven is a general discussion and conclusion of all the work covered in the thesis and Chapter eight contains the summary of the study. The individuals, groups and companies that contributed to the study are acknowledged in chapter nine, and the last chapter, Chapter ten has all the references used during the course of the study.

#### **1.4 Plant selection**

All the selected plants had previously been shown to have good antibacterial and antiviral properties in other studies at the Department of Botany at the University of Pretoria. It is however not a 100% effective method, as viruses act very differently from bacteria and fungi, and the results from the anti-HIV assays could give negative results. Even the mode of action between viruses differs completely, and it is therefore important to prepare as many solvent extracts as possible to increase the efficacy of plant screening. Four different anti-HIV assays were used to further



increase the possibility of finding compounds that inhibited the virus using different mechanisms.

Twelve species were extracted according to a specific extraction procedure that will be discussed in Chapter 3. The only extract from the 12 species that showed inhibition of the virus, was the crude ethanolic extract of *Elaeodendron croceum*. As promising results were obtained from *E. croceum* the rest of the study focussed on the isolation and identification of the active compounds from this plant extract.

### **1.5 The genus *Elaeodendron* Jacq.**

*Elaeodendron* is one of the largest genera in the Celastraceae family, consisting mostly of trees and shrubs. The African species are more frequently found in southern Africa and the eastern lowland parts of the continent (Archer & Van Wyk, 1998).

This genus comprises of evergreen or rarely deciduous shrubs to trees. The bark occasionally contains layers of yellow pigment, with the lenticels usually prominent. The leaves are opposite to subopposite, or occasionally alternate. The petals are cream to greenish with erect stamens. The flowers also contain a disc with the ovary immersed and adnate to the disc. The fruits are drupaceous, spheroid, white to yellow with a smooth surface. The seeds are brownish and flattened with fleshy cotyledons (Archer & Van Wyk, 1998).

**1.6 *Elaeodendron croceum* (Thunb.) DC.**

This is a medium to tall evergreen tree with a greyish bark containing layers of powdery yellow pigment. The lenticels are very prominent on the bark, and the leaves are opposite, elliptic, dark green above and a paler green below. The inflorescences are inconspicuous containing flowers with whitish green petals, a disc and an inconspicuous style and stigma. The fruits are drupaceous, ellipsoid and cream in colour or pale brown with fleshy cotyledons (Archer & Van Wyk, 1998). The typical characteristics are shown in an artist's representation in Figure 1.2 and photographs in Figures 1.3 and 1.4.

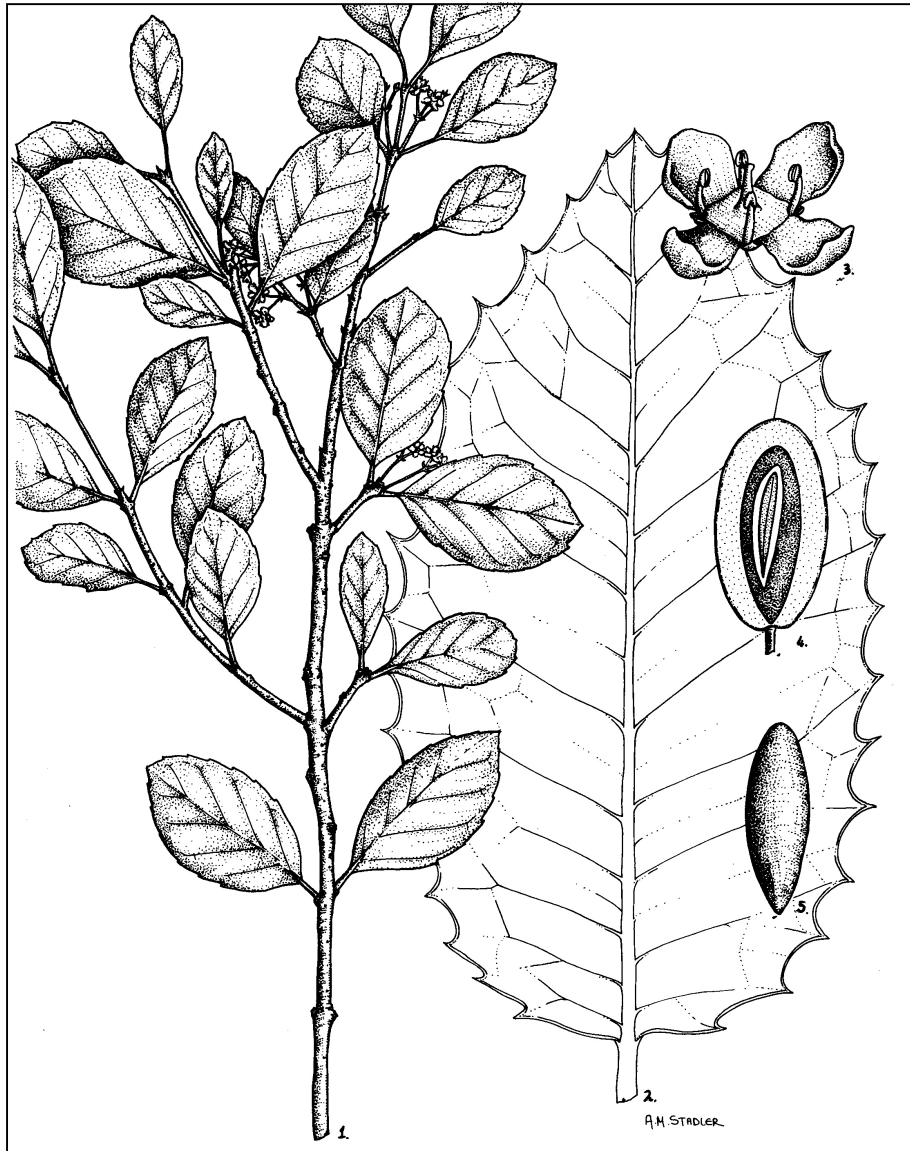


Figure 1.2 An artist's representation of the aerial parts of *E. croceum*. 1 = part of branch, 2 = leaf, 3 = flower, 4 = fruit and 5 = seed (Archer & Van Wyk, 1998).



Figure 1.3 Photograph showing the shiny leathery leaves of *E. croceum*.



Figure 1.4 Photograph showing the olive-shaped fruit.

This tree occurs on the margins of coastal and montane forests from near Ladismith in the Western Cape to Northern KwaZulu-Natal in the east. It is also found in isolated spots along the Mpumalanga and Eastern Zimbabwean escarpment (Figure 1.5). It is most abundant in the southern Cape forests where it was once popular for its bright yellow, durable wood.

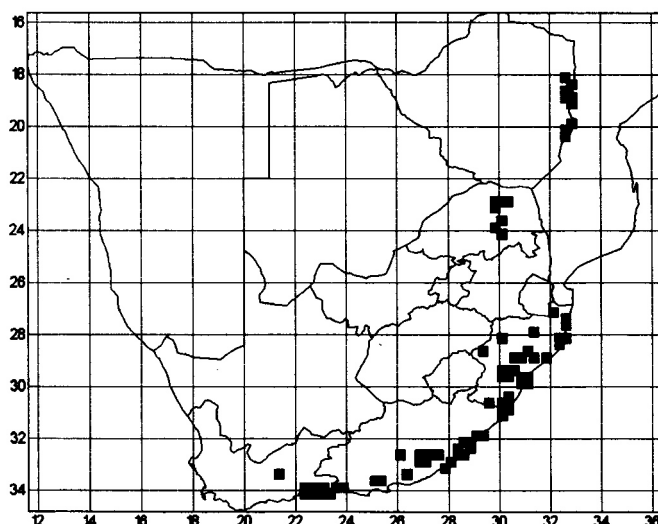


Figure 1.5 Distribution of *E. croceum* (Archer & Van Wyk, 1998).

Thunberg already described the uses of the fine and durable wood in 1794. The wood was used for making all kinds of furniture, building material, wagons as well as buttercasks. The wood is also excellent for firewood and produces long-lasting coals (Van Wyk *et al.*, 2000). Most parts of the plants are poisonous and valued for medicinal and magical properties (Watt & Breyer-Brandwijk, 1962).

Several vernacular names, including ‘Saffron’, ‘Common Saffron’, ‘Saffron wood’ and ‘geelhout’ (yellow wood) were first recorded by Thunberg (1794). Other names include umbomvane (Xhosa/Zulu), ikhukhuzi (Xhosa/Zulu), ummakhankatha (Xhosa) and izinama (Zulu) (Von Breytenbach *et al.*, 2002). The names commonly refer to the yellow pigment found in the bark, which lead to confusion with *Podocarpus* spp. (Archer & Van Wyk, 1998).

### 1.7 Compounds previously isolated from *Elaeodendron croceum*

The only compounds that have been isolated from *E. croceum* were identified and described by Drewes and Mashimbye (1993). The compounds isolated are mostly terpenoid and flavonoid structures which are shown in Figures 1.6-1.8.

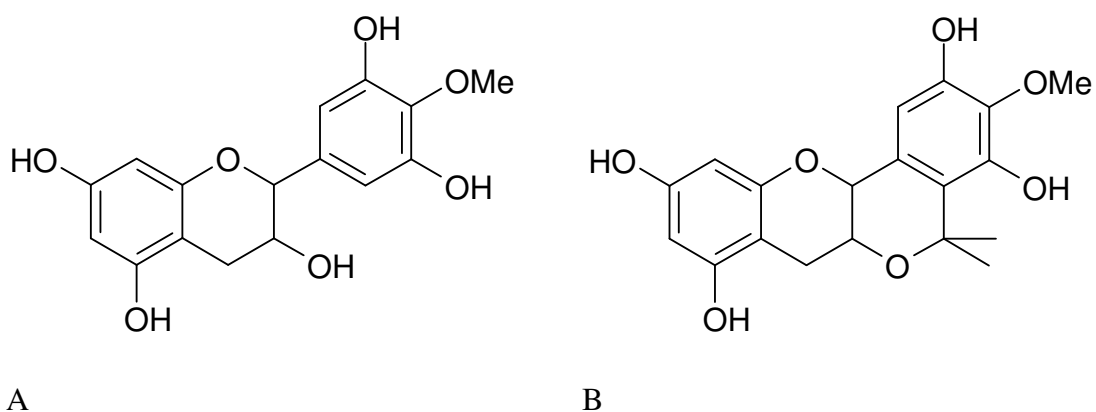
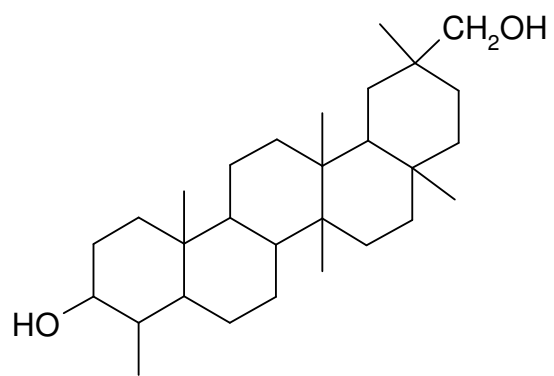
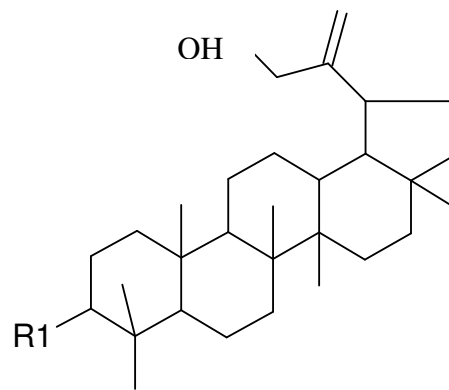


Figure 1.6 A=(-)-4'-O-methyl epigallocatechin & B=(+) 6R, 13R-11, 11-dimethyl-1, 3, 8, 10-tetra-hydroxy-9-methoxy-peltogynan.



A



B

R1= O      30-hydroxylup-20(29)-en-3-one

R1=  $\beta$ -OH      30-hydroxylupeol

Figure 1.7 A= Canophyllol & B=30-hydroxylup-20(29)-en-3-one and 30-hydroxylupeol.

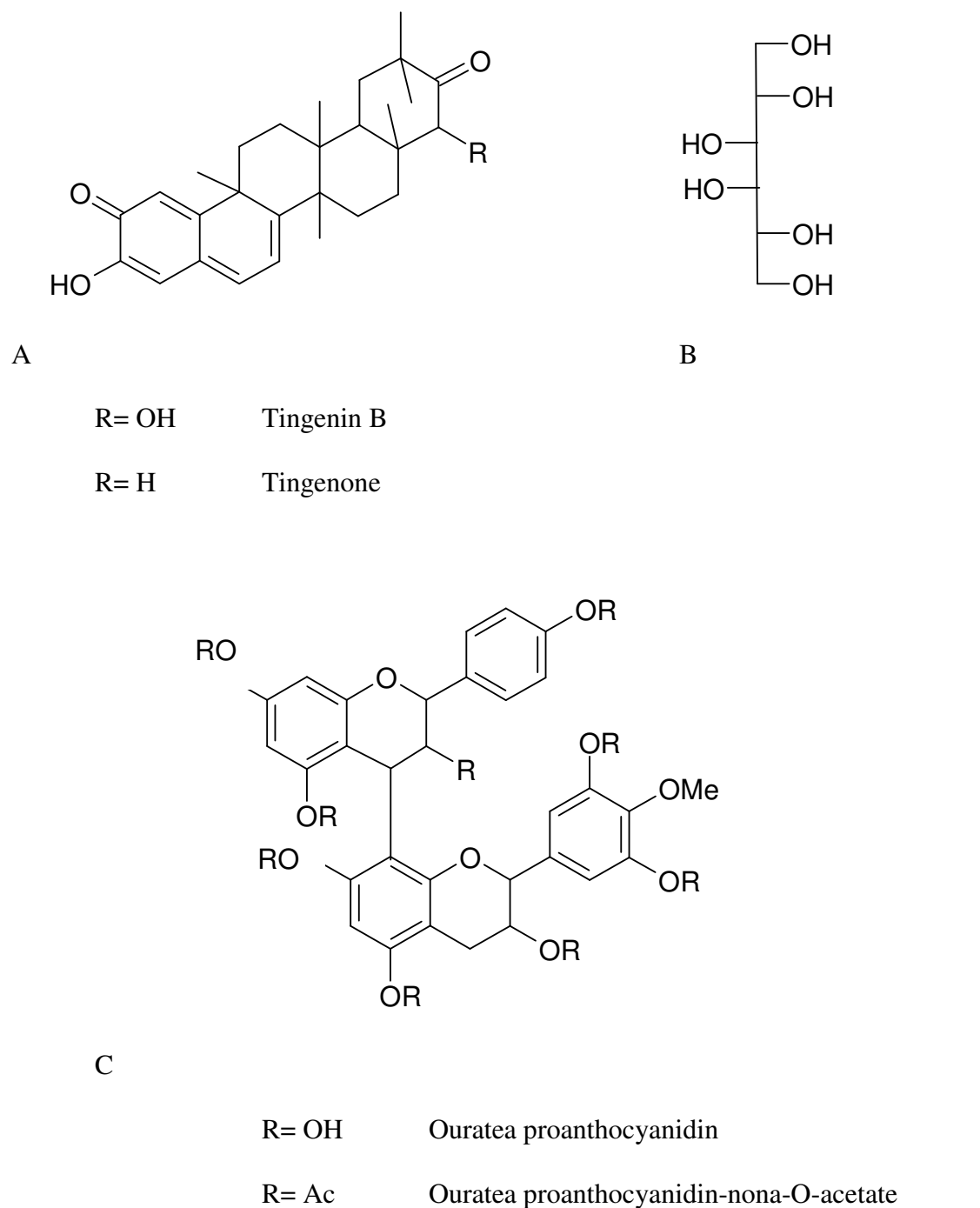


Figure 1.8 A=Tingenin B and Tingenone, B=Galacticol & C=Ouratea proanthocyanidin and Ouratea proanthocyanidin-nona-O-acetate.



Anti-tumour activity has been discovered for only one compound namely tingenone. The other compounds are not very well-known compounds, and more research needs to be conducted to identify the potential of them (Drewes & Mashimbye, 1993).

### **1.8 Compounds previously isolated from other *Elaeodendron* spp.**

Several authors have isolated and identified compounds from this genus, and the compounds isolated cover a broad spectrum of different structures. A number of these compounds exhibit medicinal value, and is already used as traditional medicine to treat several diseases. Previous phytochemical investigations of some *Elaeodendron* species have revealed the presence of cardiac steroids from the bark of *E. glaucum* (Anjaneyulu & Naranyana, 1980), tetranortriterpenoid cardiac glycosides (Shimada *et al.*, 1985), triterpenoid quinine methides (Fernando & Gunatilaka, 1989 & Fernando *et al.*, 1988) and flavonoids from the root bark of *E. balae* (Weeratunga & Kumar, 1985 & Weeratunga *et al.*, 1984). Other uses include antifeedant chemicals from *E. buchananii* (Tsujino *et al.*, 1995 and Tsanuo *et al.*, 1993) and a steroidal compound with moderate cytotoxicity against leukaemic cells from the root bark of *E. buchananii* (Kubo & Fukuhara, 1990).

Some of the uses and structures of the compounds that have been isolated from *E. buchananii*, *E. glaucum*, *E. transvaalensis* and *E. balae* are described and given below.

### 1.8.1 *Elaeodendron buchananii*

This tropical tree grows in east Africa and is poisonous to animal stock and human beings. Ingestion of its leaves, fruits and bark is said to cause sudden death. The compounds related to these deaths are shown in Figure 1.9. Chewing of the plant has been said to cure diarrhoea and dried powdered roots can be used in treatment of wounds and the primary symptoms of syphilis (Tsujino *et al.*, 1995). The compounds isolated from this plant often exhibit anti-feedant activity against the nut grass worm (Tsujino *et al.*, 1995) and a lepidopteran (Tsanuo *et al.*, 1993).

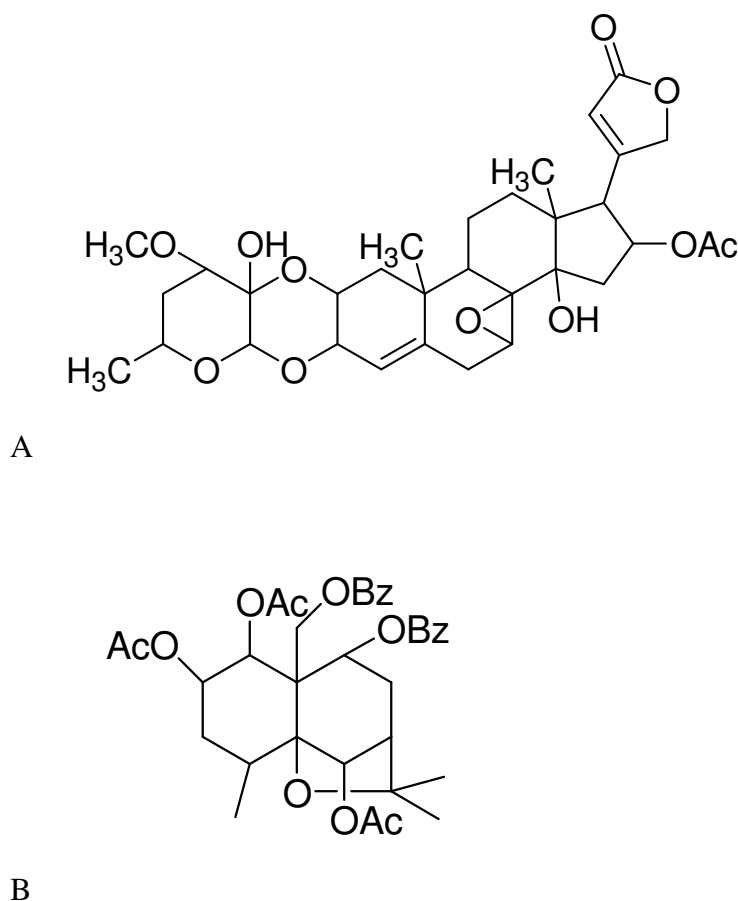
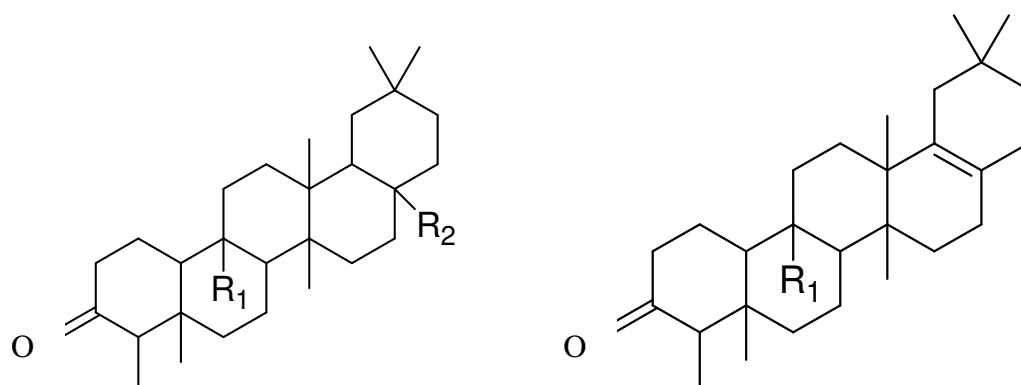


Figure 1.9 A=A glycoside of 2 $\alpha$ ,3 $\beta$ -14-trihydroxy-16 $\alpha$ -acetoxy-14 $\beta$ -carda-4,20(22)-dienolide-7 $\beta$ ,8 $\beta$ -epoxide (buchaninoside) & B=Mutangin (OBz=Benzoyl, Ac=Acetate).

### 1.8.2 *Elaeodendron glaucum*

A number of compounds and several derivatives have been isolated from this species. The isolated compounds which include several cardiac glycosides (Shimada *et al.*, 1985) form part of the compounds isolated from *E. glaucum*. The compounds are shown in Figures 1.10 & 1.11.



A

B

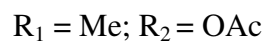
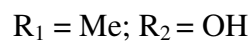
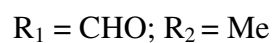
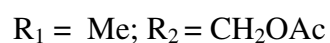
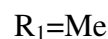
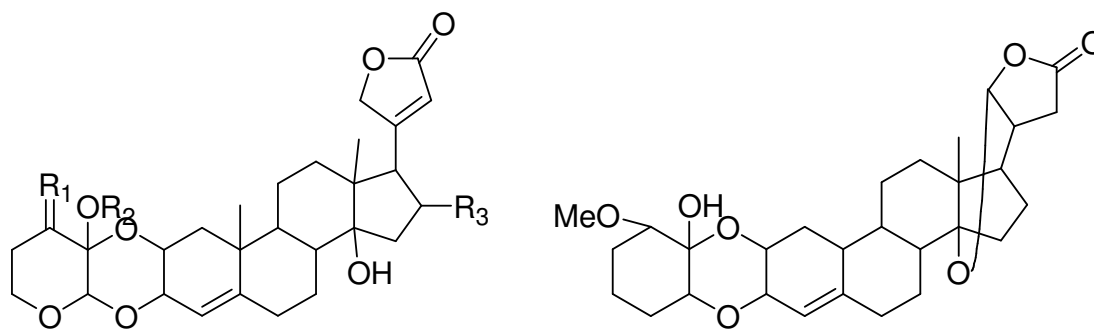


Figure 1.10 A & B=Compounds isolated from *E. glaucum* (Anyaneyulu & Narayana, 1980).



A

$R_1 = \text{H, OMe}; R_2 = \text{H}; R_3 = \text{H}$

$R_1 = \text{H, OMe}; R_2 = \text{Ac}; R_3 = \text{H}$

$R_1 = \text{OMe, H}; R_2 = \text{H}; R_3 = \text{H}$

$R_1 = \text{OMe, H}; R_2 = \text{Ac}; R_3 = \text{H}$

$R_1 = \text{H, OMe}; R_2 = \text{H}; R_3 = \text{OAc}$

$R_1 = \text{OMe, H}; R_2 = \text{H}; R_3 = \text{OAc}$

$R_1 = \text{OMe, H}; R_2 = \text{H}; R_3 = \text{OH}$

B

Figure 1.11 A=Structures of elaeodendroside and related compounds & B=Isocardenolide (Shimada *et al.*, 1985).

### 1.8.3 *Elaeodendron transvaalensis*

*E. transvaalensis* is a well-known plant and used to treat several conditions. Venda and Zulu people drink large quantities of the bark infusion as a general stomach conditioner. It is also used to prepare an enema to relief stomachaches and fevers. Four compounds have been isolated from the root bark of this species and the two basic structures are given in Figure 1.12 (Drewes *et al.*, 1991).

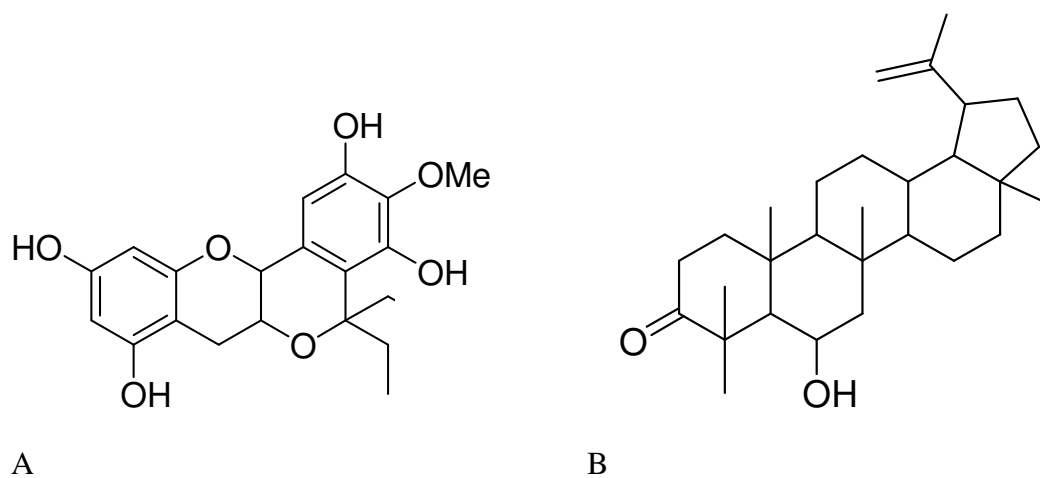


Figure 1.12 A=(+)-11,11dimethyl-1,3,8,10-tetrahydroxy-9-methoxypeltogynan & B= 6-β-hydroxy-lup-20(30)-en-3-one.

### 1.8.4 *Elaeodendron balae*

Several authors isolated compounds and derivatives from *E. balae*, which are shown in Figure 1.13. Very little medicinal data is available on any of these compounds (Tezuka *et al.*, 1993, Fernando & Gunatilaka, 1989, Fernando *et al.*, 1988 and Weeratunga & Kumar, 1985).

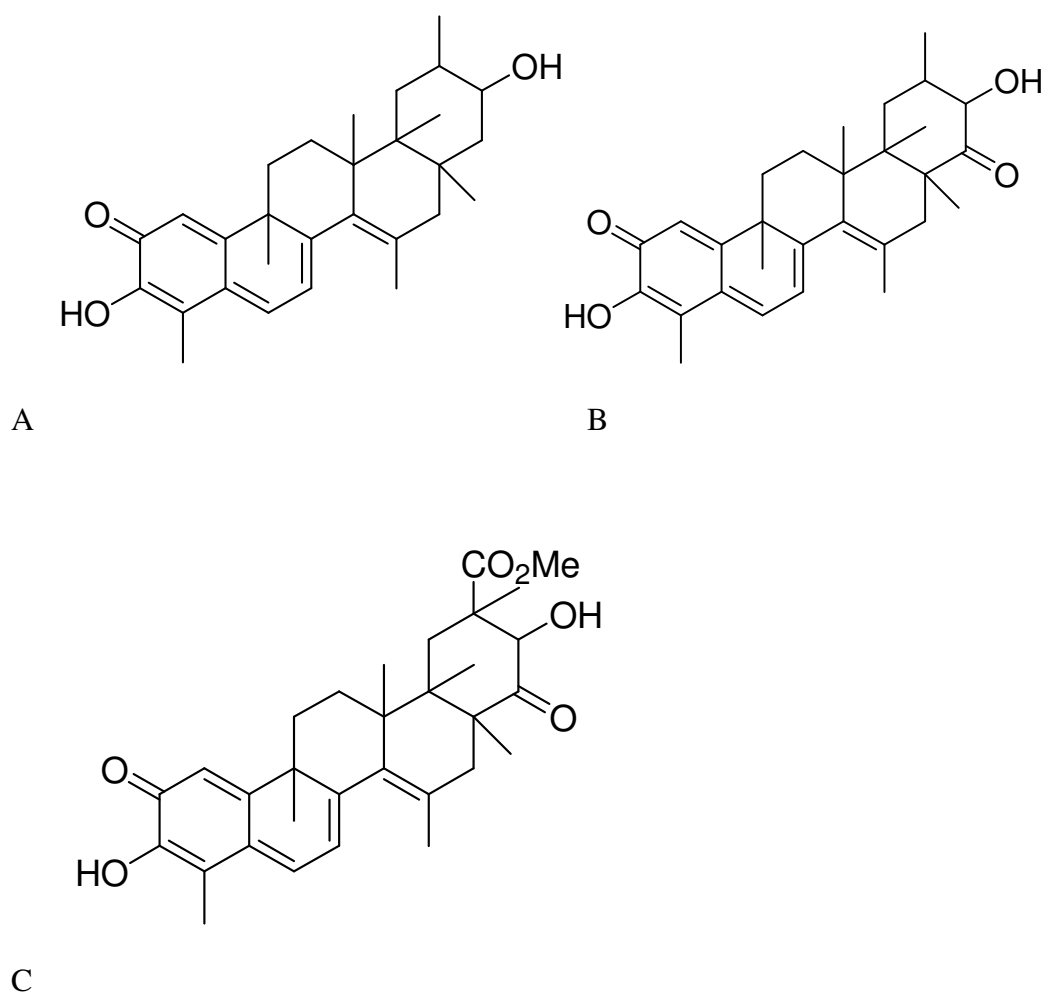


Figure 1.13 A=Balaenol , B=Balaenonol & C=Netzahualcoyone.

## 1.9 Discussion

HIV/AIDS is a threat to people all over the world. The fact that the disease is still spreading daily, underlines the fact that Africa, and especially sub-Saharan Africa will still feel the effect on several levels such as the economy and social impact for many years to come. The urgent need for a safe and cheap treatment against this virus is critical and needs urgent attention (Smith *et al.*, 2001).

Plants are used to treat many people in developing countries, and since 80% of the world's population resides in developing countries, about 64% of the total population utilizes plants as drugs (Torssel *et al.*, 1997). Our country contains such a wealth of plant species (10% of the world's species), and we are therefore in a good position to discover and develop new medicines.

The *Elaeodendron* plant family have been the focal point of many research investigations determining their ethnobotanical uses and several are being used as treatments already. None of these species have been indicated to treat any viral infections, and therefore also not HIV. The extract of *E. croceum* is not used as a treatment, because of its toxicity.