

**SCREENING OF
TRADITIONAL MEDICINAL PLANTS
FROM ZIMBABWE
FOR PHYTOCHEMISTRY, ANTIOXIDANT,
ANTIMICROBIAL, ANTIVIRAL AND
TOXICOLOGICAL ACTIVITIES**

By

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**Thesis submitted in partial fulfillment of the requirements for the degree of
Master of Philosophy**



**School of Pharmacy
College of Health Sciences
University of Zimbabwe
2009**

ABSTRACT

Fourteen indigenous medicinal plants used by traditional medical practitioners in treating sexually transmitted diseases including HIV/AIDS and opportunistic infections were selected after an ethno-botanical pilot survey of five districts from Zimbabwe. The plant materials were collected and extracted separately with methanol. The 28 extracts were lyophilized and screened for phytochemical groups, and biological: antioxidant, antiviral, antibacterial, antifungal and toxicological activities. The phytochemical screening was carried out using Thin Layer Chromatography and UV detection, followed by standard confirmatory tests. The results indicated that seven (25.9%) extracts were positive for alkaloids, ten (35.7%) for anthraquinones, thirteen (46.4%) for coumarins, seventeen (60.7%) for flavonoids, twenty-three (82.1%) for saponins and twenty-five (89.3%) for tannins. Flavonoids, saponins and tannins were the most frequent phytochemical groups found. All extracts contained at least three of the chemical groups. In order to determine the antioxidant activity, the plants were screened for Radical Scavenging Activity using DPPH (2,2-diphenyl-picrylhydrazyl) with β -carotene as reference and their Total Phenolic Contents were measured by the Folin-Ciocalteu reagent using gallic acid as reference. Eight extracts exhibited antioxidant activity with percentages higher than 90% (*Rhus chirindensis* leaves & roots-both 96.9%; *Khaya anthotheca* bark-96.1%) and the lowest result was 27.4% for *Dichrostachys cinerea* roots. Their TPCs ranged from 0.596mg/mg GAE for *Khaya anthotheca* bark to 0.105mg/mg GAE for *Dichrostachys cinerea* roots. The phenolic compounds in the extracts correlate with their antiradical activity ($r^2=0.57$), confirming that the phenolics are likely to cause the radical scavenging activity. The antiviral activity was examined using End Point Titration Technique (EPTT) and Neutralisation Test (NT) after calculating the cytotoxicity of the plant extracts on VERO cells. The HSV-2 virus titre was calculated using the Reed and Muench method ($TCID_{50} = 10^{-8.5}$ per 0.1ml). The reduction factor (RF) was calculated and it was considered a promising antiviral result if the RF was $\geq 10^3$. Out of 26 extracts, 13 (50%) showed considerable antiviral activity against the HSV-2 virus. The best results were obtained from the extracts of *Dichrostachys cinerea* leaves (RF 10^4), *Kigelia africana* fruit (RF 10^4) and *Hypoxis rooperi* tuber (RF 10^3) with concentrations ranging from 10.41 μ g/ml (*Dichrostachys cinerea* leaves) to 125.0 μ g/ml (*Flacourtia indica* roots). The reference acyclovir was active at 1.50 μ g/ml. Their cytotoxicity could also be beneficiary in developing new anti-tumour drugs. The antibacterial and antifungal activities of the plant extracts (10mg/ml) were investigated by the agar well assay. The chosen microorganisms were *Staphylococcus aureus*, *Streptococcus group A*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger*. The best results were *Terminalia sericea* roots, *Warburgia salutaris* roots, *Gymnosporia senegalensis* roots and *Kigelia africana* bark which were active against all micro-organisms. *T. sericea* roots inhibited the growth of *S. aureus* with inhibition zone of 7.88 \pm 0.48mm where the reference amoxicillin (10 μ g) gave a zone of 9.00 \pm 0.41mm and against *P. aeruginosa*, gave a larger zone of inhibition, 10.00 \pm 0.82mm, than the reference gentamicin (10 μ g), 7.00 \pm 0.40mm. *W. salutaris* roots were active against both fungal strains with inhibition zones of 10.00 \pm 0.82mm for *C. albicans* and 8.25 \pm 0.50mm for *A. niger* which were even bigger than the zones of the reference amphotericin B (10 μ g) 6.35 \pm 0.50mm and 6.75 \pm 0.58mm respectively. The toxicity tests were conducted using the Brine Shrimp (*Artemia salina*) Lethality Test (BSLT). Five of the extracts showed significant toxicity levels of $LC_{50} < 300\mu$ g/ml. The lowest readings of LC_{50} , *Terminalia sericea* leaves (66.7ppm) and *Kigelia africana* fruit (117.4ppm) were even lower than the positive control, *Nerium oleander* leaves (141.7ppm) which is a plant with well-established anti-tumour activity. These results confirm the ethno-botanical claims by traditional medical practitioners treating viral, bacterial and fungal infections caused by HIV/AIDS, cancer and cardiovascular diseases with traditional medicinal plants due to the rich phytochemistry, their high levels of antioxidant activity as well as bioactivity of the plants. They should be preserved and harvested with caution not only because of their medicinal value but also the role they play in the rich African heritage.

ACKNOWLEDGEMENTS

I would like to give my most sincere gratitude to my supervisor who is also the Director of the School of Pharmacy, Professor L S Chagonda for supporting me throughout the project. I am also grateful to my co-supervisors, Prof R S Moyo in Medical Microbiology at the UZ and Prof A H Mericli, Chairman of Pharmacognosy at the University of Istanbul for their guidance and for availing their laboratory facilities.

I want to extend my heartfelt gratitude to all the Chairpersons, the lecturers and the technicians of the following UZ Departments: Pharmacy, Veterinary Sciences, Medical Microbiology, Biochemistry, Food Science and Agriculture, of the Pharmacognosy Department at the University of Istanbul and to the Director of Medicines Control Authority of Zimbabwe. I would also like to thank The National Botanical Gardens for identifying all the plants in this study. Special thanks go to Mrs D. Moyo at the Virology clinic, Faculty of Veterinary Sciences for her tireless support throughout the project. I also would like to acknowledge Dr. F Chinyanganya who helped me to get started in Zimbabwe.

I owe a great deal to my workmate Tafadzwa Munodawafa for her astonishing determination and motivation. I thank my lovely sister Dicle Turkoglu who has sent me literature papers all the way from her own university in USA.

I am indebted to my dear husband Gordon Viol for his financial and moral support, patience and love. Our sons, Kaan Konrad and Destan Gerhard, are the joys of my life.

Most importantly, I dedicate this work to my dear mother and dear father who have done nothing but their best and given their all to make me come this far...

This thesis is the result of a project supported largely by the Government of Zimbabwe, Ministry of Environment and Tourism, and by the University of Zimbabwe Research Board. This work was done at the School of Pharmacy, University of Zimbabwe between 2006 and 2008.

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CHAPTER I

1.0 INTRODUCTION

1.1 Traditional Medicine

The use of medicinal plants is accepted as the most common form of traditional medicine. Among the entire flora, it is estimated that the 35000 to 70000 species have been used for medicinal purposes. Some 5000 of these have been studied in biomedical research (NGO Natural Products info, 2000).

In 1964, the Organisation of African Unity (OAU) set up the Scientific and Technical Research Commission (OAU/STRC) which organised, in Dakar in 1968, the Inter-African Symposium on the Development of African medicinal plants. The Symposium decided that the efficacy of herbs used by traditional health practitioners (THPs) should be tested. The areas given priority in the screening of medicinal plants to provide proofs for claims of efficacy were anticancer, antimalarial, anti-helminthic, antimicrobial, antihypertensive, cardiac activity, anti-sickling and antiviral. The OAU/STRC has thus funded 17 research centres all over Africa in order to stimulate research in this virgin area of proof of efficacy of medicinal plants in the Region (21st session of AACHRD, 2002). These initiatives have greatly enhanced the development of medicinal plant research, the drawing up of an African Pharmacopoeia, the conduct of phytochemical and biological screening of medicinal plants, ethno botanical surveys and the development of some phytomedicines.

Since more than 80% of the African population use traditional medicines for their primary health care needs, the 19th session of the African Advisory Committee for Health Research and Development (AACHRD) in 2000, recommended that the Regional Office should revitalise research on traditional medicine, particularly for common problems such as HIV/AIDS, tuberculosis, malaria and childhood illnesses. Then, in 2001, the Organisation of

African Unity (OAU) Heads of State declared at the Summit Meeting in Abuja that research on traditional medicine should be made priority. Later in the same year the OAU Summit held in Lusaka, declared the period 2001-2010 as the decade for African Traditional Medicine.

In Zimbabwe, a significant proportion of the population consults traditional medical practitioners because of the widely held belief that good health, disease, success or misfortune are not chance occurrences but are caused by the action of individuals or ancestral spirits (GEF project summary, 2001). Furthermore, the treatment of certain ailments through traditional medicine is not attributed to herbs alone, but to a combination of herbs and religious rites where religion is defined as “...the outward sign of man’s appeasement of forces that he does not understand” (Oliver-Bever, 1986).

The special powers of traditional healers, *n’angas* (**Fig 1-2**), are either given by the spirit of a departed relative (mudzimu) or of someone unrelated who had the talent of healing and divining (shavi) (Gelfand *et al*, 1985). Therefore, during the pre-colonial era traditional medical practitioners enjoyed tremendous power since they were regarded as ministers of religion who were spiritually endowed and had the gift of healing and divining (Chavunduka, 1997). However, under colonial rule, governments and Christian missionaries attempted to suppress traditional medicine by labelling it a propagator of witchcraft while the present government is encouraging co-operation between traditional and modern medical practitioners (Chavunduka, 1997).

Fig 1: N'anga Mangemba with his spiritual tools



Fig 2: Three generations of female n'angas in Chipinge, Zamchiya ward



Government of Zimbabwe fully recognizes the important role played by traditional medicine in the delivery of primary health care and its potential contribution to modern medicine. This recognition manifests itself in the Traditional Medical Practitioners Act (Chapter 27:14) which was promulgated in 1981. This Act created a Traditional Medical Practitioners Council and paved the way for the largest organization of traditional healers, the *Zimbabwe National Traditional Healers Association (ZINATHA)*. There are over 55,000 traditional healers registered with ZINATHA and many more who do not belong to any association.

Despite the considerable progress made in conventional medicines and the establishment of several health institutions, a growing number of people are turning to alternative medicine to address their health needs because of the increasingly inadequate healthcare system plus the current prices of conventional medicine and the high costs of hospitalization. Therefore the interest in drugs of plant origin is increasing. The general public is starting to recognize the effectiveness of alternative medicine's approach to health, which blends body and mind, science and experience, and traditional and cross-cultural avenues of diagnosis and treatment (Andoh, 1991)

In Zimbabwe, the co-operation between traditional and modern medical practitioners has been encouraged through activities such as the setting up of clinics/pharmacies that specialize in traditional medicine with one of the clinics housing both traditional and modern doctors. Such an arrangement offers patients the choice of either consulting a traditional healer and or a modern doctor. The decision to consult which one depends on the nature of the illness. For example, common illnesses such as short-term stomach and headaches are referred to the modern doctor whilst those with abnormal aetiology such as persistent stomach and headaches go to the traditional healers (Chavunduka, 1997).

Furthermore, traditional medical practitioners in Zimbabwe are involved in the search for the AIDS cure and are allowed to conduct clinical trials on AIDS patients (The Herald, 2008). Along with Zimbabwe, *Benin, Burkina Faso, DRC, Ghana, Côte d'Ivoire, Kenya, Mali, Nigeria, South Africa, Tanzania, Togo* and *Uganda* are countries in Africa that are conducting research on evaluation of herbal preparations for the management of HIV/AIDS with institutions such as the University of Zimbabwe. Preliminary results show that some herbal preparations reduce viral load. In addition, improvements have been noted in the quality of life and clinical conditions of patients treated with the locally produced medicines. Blood tests to monitor the level of immunity (CD4 and CD8 counts) of patients, all of whom are being treated exclusively with traditional medicines, have shown a marked increase in blood cell counts. In *Burkina Faso* and *Zimbabwe* where, apart from baseline CD4/CD8 and viral load values measured at the inception of the study and re-assessed every three months, liver and kidney function tests are being undertaken, using specific protocols. In some countries such as *Burkina Faso*, a weight gain of up to 20 kilograms has been noted in some patients within four months of treatment. (21st session of AACHRD, 2002).

However, the expanded use of herbal medicines has led to concerns relating to the assurance of safety, quality and rational use as well as the danger of over-exploitation. Endemic medicinal plants are threatened from the unsustainable use and habitat destruction. Whilst most of the over 500 plant species used for medicinal purposes in Zimbabwe are still available, some are endangered and many more are vulnerable. There is also need to address the issue of protecting the indigenous knowledge and intellectual property rights.

1.2 Drugs of Plant Origin

Natural product research has been the single most successful strategy for discovering new pharmaceuticals and has contributed dramatically to extending human life and improving clinical practice. Whatever their natural protective functions, natural products are a rich

source of biologically active compounds that have arisen as the result of natural selection, over perhaps 300 million years. The challenge to the medicinal chemist is to exploit this unique chemical diversity. Among the estimated 500,000 plant species, however, only a small percentage has been investigated for phytochemistry. Over 90% of bacterial, fungal, and plant species are still waiting to be investigated (Coombes, 1992).

The history of herbal medicine has become a pre-history for many compounds that are now commonplace in modern pharmacology. Morphine is an example of a secondary metabolite which is present in the tissues of *Papaver somniferum* and being commonly used as opioid analgesic. To chemically produce morphine outside the plant, 14 steps are required from available amino acids, including at least one step that is highly substrate specific (Gerardy, 1993). The presence of morphine must therefore confer a selectional advantage on the plant. The anti-febrile properties of Cinchona bark evolved into the discovery and the use of the major biologically active constituent thereof, quinine. The Ipecac root was the basis for the extraction of the emetic with the major biologically active constituent of emetine which is used clinically as an anti-amebic agent. Even the modern vaso-active agent, ephedrine, was derived from the Chinese plant, Ma Huang (*Ephedra vulgaris*), known since about 3100B.C.

If we look at the recent history, of the 520 new pharmaceuticals approved between 1983 and 1994, 39% were derived from natural products, the proportion of antibacterials and anticancer agents of which was over 60% (Cragg et al, 1997). Between 1990 and 2000, a total of 41 drugs derived from natural products were launched on the market by major pharmaceutical companies, listed on **Table 1**, including *azithromycin*, *orlistat*, *paclitaxel*, *sirolimus (rapamycin)*, *Synercid*, *tacrolimus*, and *topotecan*. In 2000, one-half of the top-selling pharmaceuticals were derived from natural products, having combined sales of more than US \$40 billion. These included the biggest selling anticancer drug *paclitaxel*, the “statin” family of hypolipidemics, and the immunosuppressant *cyclosporin*. During 2001, the market

has seen the launch of *caspofungin* from Merck and *galantamine* from Johnson & Johnson, with *rosuvastatin*, *telithromycin*, *daptomycin*, and *ecteinascidin-743* due to follow in 2002 (Buss et al, 2003).

Table 1: Drugs Derived from Natural Products (1990–2000)

Name	Originator	Indication/Use
Acarbose	Bayer	Diabetes
Artemisinin	Kunming & Guilin	Malaria
Azithromycin	Pliva	Antibiotic
Carbenin	Sankyo	Antibiotic
Cefetamet pivoxil	Takeda	Antibiotic
Cefozopran	Takeda	Antibiotic
Cefpimizole	Ajinomoto	Antibiotic
Cefsulodin	Takeda	Antibiotic
Clarithromycin	Taisho	Antibiotic
Colforsin daropate	Nippon Kayaku	Asthma
Docetaxel	Aventis	Cancer
Dronabinol	Solvay	Alzheimer's disease
Galantamine	Intelligen	Alzheimer's disease, arthritis
Gusperimus	Nippon Kayaku	Arthritis
Irinotecan	Yakult Honsha	Cancer
Ivermectin	Merck & Co	Parasiticide
Lentinan	Ajinomoto	Cancer
LW-50020	Sankyo	Immunomodulation
Masoprocol	Access	Cancer
Mepartricin	SPA	Benign prostatic hyperplasia
Miglitol	Bayer	Diabetes
Mizoribine	Asahi Chemical	Arthritis
Mycophenolate mofetil	Hoffman-LaRoche	Arthritis
Orlistat	Hoffman-LaRoche	Obesity
Paclitaxel	Bristol-Myers Squibb	Cancer
Pentostatin	Warner-Lambert	Leukemia
Podophyllotoxin	Nycomed Pharma	Human papillomavirus
Policosanol	Dalmer	Hyperlipidaemia
Everolimus	Novartis	Immunomodulation
Sirolimus	American Home Products	Immunomodulation
Sizofilan	Taito	Cancer, hepatitis-B virus
Subreum	OM Pharma	Arthritis
Synercid	Novartis	Antibiotic
Tacrolimus	Fujisawa	Immunomodulation
Teicoplanin	Aventis	Antibiotic
Tirilazad mesylate	Pharmacia & Upjohn	Subarachnoid haemorrhage
Topotecan	GlaxoSmithKline	Diabetes
Ukrain	Nowicky Pharma	Cancer, HIV/AIDS
Vinorelbine	Pierre Fabre	Cancer
Voglibose	Takeda	Diabetes, obesity
Z-100	Zeria	Immunomodulation

Pokeweed antiviral protein (PAP) with molecular weight 29-kDa is a plant-derived protein isolated from leaves of *Phytolacca americana*, is a promising nonspermicidal broad-spectrum antiviral microbicide (D'Cruz et al. 2004). The molecular mechanism of the PAP was investigated by directly measuring the amount of adenine released from the viral RNA species using quantitative high-performance liquid chromatography. It was found that PAP29 is another single-chain RIP purified from *Phytolacca americana*.

Colombian medicinal plant extracts of the *Euphorbia* genus were screened for antiviral activity and 11 % showed antiherpetic activity (Betancur-Galvis et al. 2002). Isolated from the *Euphorbia jolkini* plant, the chemical constituent called *Putranjivain A* was proven to inhibit *HSV* type 2 and is now used as antiviral agent (Hua-Yew et al. 2004).

However, when we target HIV/AIDS, it is not only the antiviral effect we should be looking for since the late stage of the condition leaves individuals prone to opportunistic infections, tumours and degeneration of tissues. The most important and common of those infections are sexually transmitted diseases (STDs), tuberculosis, other upper respiratory tract infections, chronic diarrhoea, toxoplasmosis, candidiasis of oesophagus, trachea, bronchi or lungs, cervical cancer and Kaposi's sarcoma (type of skin cancer). Therefore, in the search of an ideal herbal medicine against AIDS, it is necessary to determine *antiviral, antibacterial, antifungal* and *antioxidant activity* of the substance as well as its *phytochemistry* to reveal important knowledge in terms of its action. Another important point of the search should be the *toxicity* of the drug and to establish a safe dose.

In order to achieve all these parameters, there is a series of pharmacological screening that is carried out in this project.

1.3 Phytochemistry

Phytochemistry is concerned with the enormous variety of organic substances that are accumulated by plants and deals with the chemical structures of these substances their biosynthesis, turnover and metabolism, their natural distribution and their biological function (Harborne, 1998).

The classifications of the chemical constituents of the plants are numerous. In biology, the classification can be based on *biosynthetic origin* such as terpenoids, phenylpropanoids and polyketides, on *biological activity* such as antibodies, hormones or on *material source* such as plants, microorganisms. In chemistry, the classification can be based on *structural skeleton* such as terpenoids, flavonoids, alkaloids and steroids, on *functional groups* such as alkanes, ketones, acids or on *physiochemical properties* such as volatile oils, organic acids (Chitsamanga, 2001).

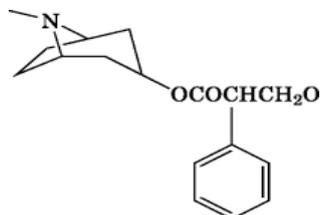
Only through the extraction of bioactive compounds from medicinal plants, demonstration of their physiological activity will be plausible and it also will facilitate pharmacology studies leading to synthesis of more potent drugs with reduced toxicity. The major chemical substances of interest in this survey have been the *alkaloids, flavonoids, saponins, coumarins, anthraquinones* and *tannins*.

1.3.1 Alkaloids

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. The name derives from the word alkaline and was used to describe any nitrogen-containing base. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid-base extraction. Many alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications and recreational drugs. Examples are atropine, the local anesthetic and stimulant

cocaine, the stimulant caffeine, nicotine, the analgesic morphine, or the antimalarial drug quinine. Some alkaloids have a bitter taste.

Fig 3: Chemical structure of the alkaloid *Atropine*



Alkaloids are usually classified by their common molecular precursors, based on the metabolic pathway used to construct the molecule. When not much was known about the biosynthesis of alkaloids, they were grouped under the names of known compounds, even some non-nitrogenous ones (since those molecules' structures appear in the finished product; the opium alkaloids are sometimes called "phenanthrenes", for example), or by the plants or animals they were isolated from. When more is learned about a certain alkaloid, the grouping is changed to reflect the new knowledge, usually taking the name of a biologically-important amine that stands out in the synthesis process.

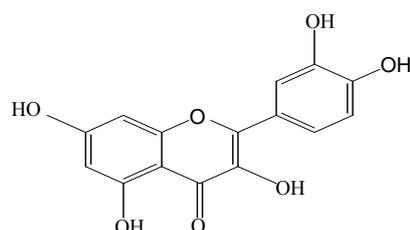
- *Pyridine group*: piperine, coniine, trigonelline, arecaidine, guvacine, pilocarpine, cytisine, nicotine, sparteine, pelletierine.
- *Pyrrolidine group*: hydrine, cuscohygrine, nicotine
- *Tropane group*: atropine, cocaine, ecgonine, scopolamine, catuabine
- *Quinoline group*: quinine, quinidine, dihydroquinine, dihydroquinidine, strychnine, brucine, veratrine, cevadine
- *Isoquinoline group*: The opium alkaloids (morphine, codeine, thebaine, Isopapadimethoxy-aniline, papaverine, narcotine, sanguinarine, narceine, hydrastine, berberine), emetine, berbamine, oxyacanthine
- *Phenethylamine group*: mescaline, ephedrine, dopamine, amphetamine

- *Indole group*:
 - Tryptamines: DMT, N-methyltryptamine, psilocybin, serotonin
 - Ergolines: the ergot alkaloids (ergine, ergotamine, lysergic acid, LSD etc.)
 - Beta-carbolines: harmine, harmaline, yohimbine, reserpine
 - Rauwolfia alkaloids: Reserpine
- *Purine group*:
 - Xanthines: caffeine, theobromine, theophylline
- *Terpenoid group*:
 - Aconite alkaloids: aconitine
 - Steroids: solanine, samandaris (quaternary ammonium compounds): muscarine, choline, neurine
 - *Vinca alkaloids*: vinblastine, vincristine. They are antineoplastic and binds free tubulin dimers thereby disrupting balance between microtubule polymerization and depolymerization resulting in arrest of cells in metaphase.
 - *Miscellaneous*: capsaicin, cynarin, phytolaccine, phytolaccotoxin

1.3.2 Flavonoids

Flavonoids are a group of polyphenolic phytochemicals that include flavones, isoflavones, (iso)flavanones, flavonols, catechins, anthocyanidins and chalcones. Over 4,000 flavonoids have been identified and they occur in relatively high concentrations in fruits, vegetables, nuts and grains, beverages (tea, coffee, beer, wine and fruit drinks) and in various herbs and spices (Sanderson et al, 2004).

Fig 4: Chemical structure of the flavonoid *Quercetin*



The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health. Flavonoids are known to have widely diverse beneficial biological effects, such as anti-inflammatory (Middleton, 1998), antioxidant (Pietta, 2000), antiviral (Jassim and Naji, 2003), and anticancer effects (Adlercreutz, 2002; Frei and Higdon, 2003; Rietveld and Wiseman, 2003). They also modulate the function of sex hormones and their receptors. Certain flavonoids, such as the isoflavone genistein, are estrogenic (Wang et al., 1996; Zand et al., 2000), whereas others, such as chrysin, can interfere with steroid synthesis and metabolism.

The antiviral activities of bioflavonoids extracted from medicinal plants have been evaluated (Beladi et al. 1977; Tsuchiya et al. 1985). The black tea flavonoid, theaflavin is a well-known antioxidant with free radical-scavenging activity and it was able to neutralize *bovine rotavirus* and *bovine corona virus* infections (Clark et al. 1998).

The flavonoid *chryso splenol C* is one of a group of compounds known to be a potent and specific inhibitor of picornaviruses and rhinoviruses, the most frequent causative agents of the common cold (Semple et al. 1999). The *Dianella longifolia* and *Pterocaulon sphacelatum*, were found to contain flavonoid chryso splenol C and anthraquinone chrysophanic acid, respectively, which inhibit the replication of *poliovirus* types 2 and 3 (Picornaviridae) in vitro (Semple et al. 1999, 2001). Recently, new flavonol glycoside the iridoid glycosides and three phenylpropanoid glycosides, named luteoside A, luteoside B and luteoside C were isolated from *Barleria prionitis* and from the roots of the medicinal plant *Markhamia lutea*, respectively, and shown to have potent in vitro activity against RSV (Chen et al. 1998; Kernan et al. 1998). In another study, five groups of biflavonoids (amentoflavone, agathisflavone, robustaflavone, rhusflavanone and succedane flavanone) were isolated from medicinal plants of *Rhus succedanea* and *Garcinia multiflora*, and exhibited various antiviral effects against a number of viruses including respiratory viruses (*influenza A*, *influenza B*,

parainfluenza type 3, RSV, adenovirus type 5 and measles) and herpes viruses (*HSV-1, HSV-2, HCMV* and *varicella zoster virus, VZV*) (Lin et al. 1999). Amentoflavone and robustaflavone, demonstrated significant activity against anti-HSV-1 and anti-HSV-2 with only moderate anti-HSV-2 from rhusflavanone. A significant anti-influenza A and B activity was achieved by amentoflavone, robustaflavone and agathisflavone. By comparison, rhusflavanone and succedaneoflavanone were found to produce a selective anti-influenza type B only. The inhibitory activities against *measles* and *VZV* were demonstrated with rhusflavanone and succedaneoflavanone, respectively. In general, none of groups of biflavonoids exhibited anti-HCMV (Lin et al. 1999).

Baicalein (BA), a flavonoid compound purified from the medicinal plant *Scutellaria baicalensis Georgi*, has been shown to possess anti-inflammatory and anti-HIV-1 activities. BA may interfere with the interaction of *HIV-1* envelope proteins with chemokine co-receptors and block *HIV-1* entry of target CD4 cells and BA could be used as a basis for developing novel anti-HIV-1 agent (Li et al. 2000).

Morin is another type of flavonoid group extracted from *Maclura cochinchinensis* that exhibited a powerful anti-HSV-2 activity in contrast with a synthesized morin pentaacetate that was inactive (Bunyaphatsara et al. 2000). This would suggest that free hydroxyl groups are required for anti-HSV-activity, as demonstrated previously for the antiviral activity of other flavonoids (Hudson 1990; Bunyaphatsara et al. 2000). Such studies clearly indicate that antiviral activity varies with the compound and the virus.

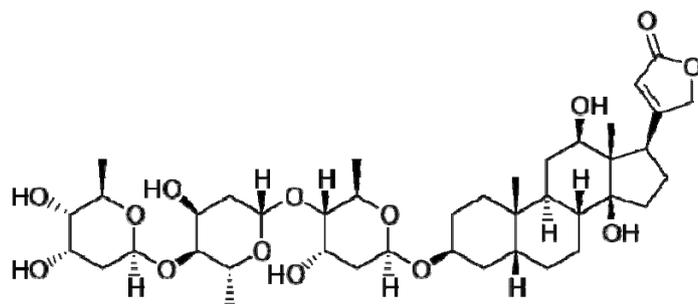
One stage of viral replication that may be inhibited by flavonoids is viral DNA synthesis. Most of the potent anti-HIV flavonoids such as baicalein, quercetin and myricetin have shown inhibitory activity not only against the virus-associated RT but also against cellular DNA or RNA polymerase (Ono and Nakane 1990). The fact that the RT plays a very important role in controlling the replication of *HIV* makes it one of the most attractive targets in the

development of anti-AIDS drugs. The inhibition of DNA and RNA polymerase by these flavonoids was extensively analysed to elucidate the inhibition mechanism(s) by Ono and Nakane (1990). Once again the degree of inhibition also varied depending on the flavonoid.

1.3.3 Saponins

Saponins are glucosides with foaming characteristics. Saponins consist of a polycyclic aglycones attached to one or more sugar side chains. The aglycone part, which is also called sapogenin, is either steroid (C27) or a triterpene (C30).

Fig 5: Chemical structure of the steroid saponin *Digoxin*



The foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part. Saponins have a bitter taste. Some saponins are toxic and are known as sapotoxin (<http://www.phytochemicals.info/phytochemicals/saponins.php>).

Saponins have been found to have significant bioactivities like anti-inflammatory (Wang et al, 2008; Recio et al, 1995), anti-tumour (Jung et al, 2004), antispasmodic (Trute, 1996), antileishmanicidal (Majester et al, 1991), and anti-proliferative activity (Denby 1994). Although a number of saponins, as well as their prosapogenins or sapogenins, could be developed as anti-cancer agents due to their cytotoxicity and anti-inflammatory activity, benefit could also be expected to follow inducible nitric oxide inhibition. Excessive production of NO is associated with various diseases, including arthritis, diabetes, stroke, septic shock, autoimmune diseases, chronic inflammatory diseases, and atherosclerosis (Bredt, 1994).

Dioscin, was extracted from the root of *Polygonatum zanlanscianense Pamp.* It exerted significant inhibitory effects on the growth of the human leukaemia cell HL-60, inducing differentiation and apoptosis (Wang et al, 2001).

1.3.4 Coumarins

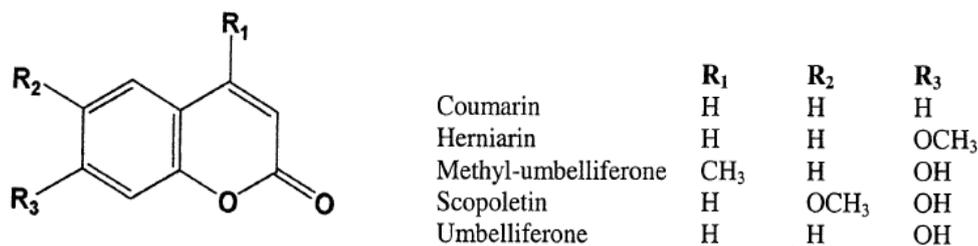
Coumarins owe their class name to 'coumarou', the vernacular name of the tonka bean (*Dipteryx odorata* Willd., Fabaceae), from which coumarin itself was isolated in 1820 (Bruneton, 1999).

Coumarins belong to a group compounds known as the benzopyrones, all of which consist of a benzene ring joined to a pyrone. Coumarin and the other members of the coumarin family are benzo- α -pyrones, while the other main members of the benzopyrone group – the flavonoids – contain the γ -pyrone group (Keating and O'Kennedy, 1997; Murray et al, 1982). Coumarins may also be found in nature in combination with sugars, as glycosides. The coumarins can be roughly categorised as follows (Ojala, 2001):

- *simple* – these are the hydroxylated, alkoxyated and alkylated derivatives of the parent compound, coumarin, along with their glycosides
- *furanocoumarins* – these compounds consist of a five-member furan ring attached to the coumarin nucleus, divided to linear and angular types with substitutes at one or both of the remaining benzenoid positions
- *pyranocoumarins* – members of this group are analogous to the furanocoumarins, but contain a six-member ring
- coumarins substituted in the pyrone ring.

Like other phenylpropanoids, coumarins arise from the metabolism of phenylalanine via a cinnamic acid, *p*-coumaric acid (Bruneton, 1999; Matern *et al.*, 1999).

Fig 6: Chemical Structures of Coumarins



The coumarins exist in larger quantities in the plants of certain families such as *Leguminosae* (bean family), *Rutaceae* (citrus family) and *Umbelliferae* (a.k.a. Apiaceae) (parsley-fennel family). They are also available in fungi and bacteria (Munay, 1982).

They have been reported to have many biological activities without evidence of toxicity, including inhibition of lipidic peroxidation and neutrophil-dependent anion superoxide generation, anti-inflammatory and immunosuppressor actions (Luccini et al, 2008). In addition, coumarin and two of its mono-hydroxylated derivatives (4-hydroxycoumarin and 7-hydroxycoumarin) inhibit prostaglandin biosynthesis (Lee, 1981). It has clinical medical value as the precursor for several anticoagulants, notably warfarin, and is used as a gain medium in some dye lasers.

1.3.5 Anthraquinones

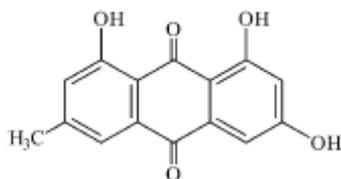
Anthraquinone-containing extracts from different plant sources have been widely used since ancient times due to their laxative and cathartic properties (Thomson, 1986). Anthraquinones are present in the roots, bark or leaves of numerous plants such as senna, cascara, aloe, frangula and rhubarb.

Besides their laxative properties, this class of compounds have shown a wide variety of pharmacological activities such as anti-inflammatory, wound healing, analgesic, antipyretic, anti-tumour (Alves et al, 2004), antifungal (Chrysai-Tokousbalides et al, 2003; Agarwal et al, 2000), antiviral (Semple et al, 2001) and in vivo inhibitory effects towards P388 leukemia

in mice (Lu, 1989) . They were reported containing the photoprotease activities. They are also used in industry as textile dyes, food colourants (Nemeikaite-Ceniene, 2002) and bugs repellents.

Emodin (1,3,8-trihydroxy-6-methylanthraquinone) (Fig. 4) is the active principle of herbal medicines deriving from genus *Rheum* and *Polygonum* (*Polygonaceae*), *Rhamnus* (*Rhamnaceae*) and *Senna* (*Cassieae*). This anthraquinone has been reported to exhibit anti-inflammatory properties by reduction of cytokine production in human T-lymphocytes and endothelial cells (Kuo, 2001). Emodin has also demonstrated antiproliferative effects in several cancer cell lines by promoting apoptosis via caspase-dependent pathways (Srinivas, 2003). Emodin has been recently found to inhibit to proteinkinase CK2, feature which is suspected to be related to its anticarcinogenic and antiviral activities (Sarno et al, 2002) and later was found to be a virucidal agent by Alves et al in 2004.

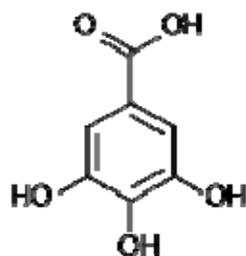
Fig 7: Chemical structure of the anthraquinone *Emodin*



1.3.6 Tannins

Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of red wine, strong tea, or an unripened fruit (McGee, 2004). The term tannin refers to the use of tannins in tanning animal hides into leather; however, the term is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules. Tannins have molecular weights ranging from 500 to over 3,000 (Hemingway, 1989).

Fig 8: A hydrolysable tannin; *Gallic acid*



Tannins have shown potential antiviral (Quideau et al, 2004; Lin et al, 2004; Cheng, 2002), antibacterial (Funatogawa et al, 2004; Akiyama et al, 2001) and antiparasitic effects (Kolodziej, 2005). In the past few years tannins have also been studied for their potential effects against cancer through different mechanisms (Susumu et al, 2005; Ling Ling et al, 2000).

Tannins, including gallo and ellagic acid (epigallitannins), are inhibitors of *HIV* replication. 1,3,4-tri-O-galloylquinic acid, 3,5-di-O-galloyl-shikimic acid, 3,4,5-tri-O-galloylshikimic acid, punicalin and punicalagin inhibited *HIV* replication in infected H9 lymphocytes with little cytotoxicity. Two compounds, punicalin and punicacortein C, inhibited purified *HIV* reverse transcriptase (Nonaka et al, 1990)

The Table 2 shows a summary of the different activities each of the chemical groups is responsible for.

Table 2: The Chemical groups, Activities and associated Ethno-pharmacology

Chemical Group	Activity	Ethno-pharmacology
Alkaloids	Antibacterial Antifungal Antiviral Analgesic effects	- Venereal diseases, HIV - GIT infections. - Skin inf., wounds, Candida, eczema - Colds, coughs, chest pains, TB, - Pneumonia

Flavonoids	Antibacterial, Antifungal Antiviral, Antinephrotoxic Anti-inflammatory Antihepatotoxic	Same as above plus - Cancer, HSV-1,2 - Allergies, eczema Abdominal pains - Thrombosis
Saponins	Anti-inflammatory Anti-tumour Antibacterial Anti fungal	- Venereal diseases, HIV, - TB, Pneumonia, Cancer - Colds, coughs, chest pains , - Hormonal disorders - GIT inf. , Skin inf., wounds, - Candida, thrush, inflammation
Coumarins	Anti-inflammatory Anti fungal, Antioxidant Anti-tumour	- Eczema, HIV, Venereal diseases - Chest pains, Bronchitis, Asthma, - Cancer, Inflammation
Anthraquinones	Laxative, purgative Antibacterial, Antiviral Anti fungal	- Tapeworm, Ringworm, Bilharzias, - Dysentery - Constipation , Diarrhoea
Tannins	Astringent Antibacterial, Anti fungal, Antiviral Antioxidant, Anti-inflammatory	- Diarrhoea, - Inflammations, Wounds, - Cancer, - HIV

1.4 Oxidative Stress and Antioxidant Activity

The oxygen molecule is changed into reactive oxygen species (ROS) such as O_2^- , H_2O_2 and OH through endogenous sources like normal aerobic respiration, reduction to H_2O in living tissues and exogenous sources like environmental pollutants, UV and X-rays causing oxidative stress (Yildirim et al, 2001). Oxidative stress has been linked to inducing cancer, cardiovascular diseases, neurodegenerative diseases such as Alzheimer's and Parkinson's, inflammation and ageing (Dasgupta, 2004). This harmful action can, however, be blocked by *Antioxidant substances* which in small quantities are able to prevent or greatly retard the oxidation of easily oxidisable materials such as lipids, proteins, DNA and carbohydrates and protect cells against the damaging effects of reactive oxygen species (Becker, 2004).

The traditional medicinal plants chosen for this study were good candidates for having antioxidant activity because of their current use in HIV/AIDS, cancer, cardiovascular diseases, opportunistic infections and rheuma. Phenolic compounds (flavonoids, coumarins, tannins and anthraquinones) in plants have been found to play an important role in Antioxidant activity. Flavonoids may help provide protection against these diseases by contributing, along with antioxidant vitamins and enzymes, to the total defence system of the human body. Epidemiological studies have shown that flavonoid intake is inversely related to mortality from coronary heart disease and to the incidence of heart attacks (Miranda, 2000).

A dietary antioxidant that has received attention with regard to antioxidant effect is the polyphenolic compound, *hesperidin* (hesperetin 7-rhamnoglucoside) (**Fig 9**) and its aglycone *hesperetin* (3,5,7-trihydroxy-4_-methoxy flavanone) (**Fig 10**). Both flavonoids present extensively in the plant kingdom especially in many citrus fruits such as grapefruits and oranges, which are commonly used in traditional medicines (Garg et al, 2001; Tosun, 2003). It has been reported that hesperetin shows a wide spectrum of pharmacological effects such as anti-inflammatory, anticarcinogenic, antihypertensive and anti-atherogenic effects

(Galati et al, 1996). Hesperetin has been reported to inhibit low-density lipoprotein oxidation in vitro (Shin et al, 1999). It has also been reported that *hesperetin* inhibited HMG-CoA reductase and lowers the plasma cholesterol level in rats (Bok et al, 1999). The role of *hesperetin* and the structurally related *naringenin*, a citrus flavanone, in the prevention and treatment of atherogenic disease has recently received considerable attention, with particular interest in the use of these flavanones as anticancer and anti-atherogenic compounds (Sanderson et al, 2004; Wilcox et al, 2001).

Fig 9: *Hesperidin*; hesperetin 7-rhamnoglucoside

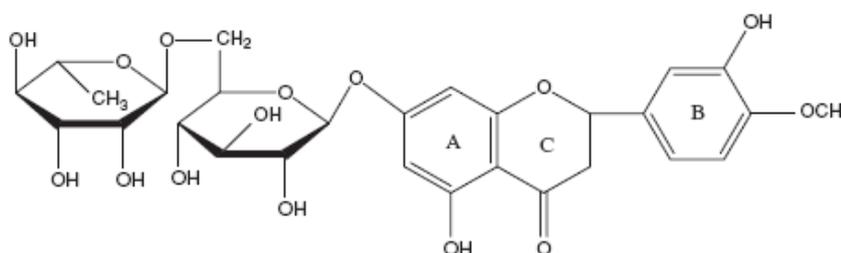
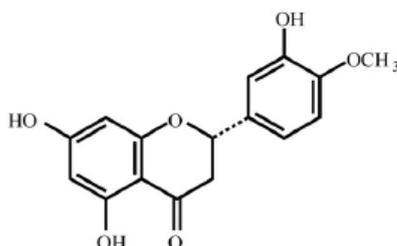


Fig 10: *Hesperetin*; 3,5,7-trihydroxy-4-methoxy flavanone



1.5 Virology and Antiviral Activity

After Koch and his colleagues found out that anthrax, tuberculosis and diphtheria were caused by bacteria, it was assumed that all infectious diseases would be caused by similar organisms. However, for some important diseases, no bacterial cause could be established such as rabies. Infectious material could still pass through bacteria-free filters. These filter-passing agents were originally called filterable viruses which, by the dropping of ‘filterable’ with time, became what are now known to be a distinctive group of microorganisms different in structure and method of replication; **viruses** (Christie, 1981).

All forms of life, animal, plant and even bacterial, are susceptible to infection by viruses. Viruses have no metabolic system of their own. They are intracellular parasites, only growing in other living cells whose energy and protein producing systems they redirect for the purpose of manufacturing new viral components which means the death of the host cell. Three main properties distinguish viruses from their host cells: size, nucleic acid content and metabolic capacities.

Their sizes vary such as poliovirus is 28 nm in diameter whereas poxvirus is 250 nm in diameter but mostly they are beyond the limit of resolution of the light microscope and have to be visualized by the electron microscope.

Viruses contain only a single type of nucleic acid; either DNA or RNA. There are seven families of DNA viruses that are pathogenic for humans. These pathogens come from the *Adenoviridae*, *Hepadnaviridae*, *Herpesviridae*, *Polyomaviridae*, *Papillomaviridae*, *Parvoviridae*, and *Poxviridae* families. Herpesviruses, hepadnaviruses, and papillomaviruses are well established as human health problems and as targets for antiviral chemotherapy.

They are composed of genetic material surrounded by a coat of protein, which is called the capsid. Therefore heat is the most reliable method of virus disinfection. Most human pathogenic viruses are inactivated following exposure of 60°C for 30 minutes. Viruses are stable at low temperatures and are stored at -40°C to -70°C. Ultraviolet light inactivates viruses by damaging their nucleic acid and has been used to prepare viral vaccines.

Many animal virus particles, in addition to their capsid, are surrounded by a lipoprotein envelope, which has generally been derived from the cytoplasmic membrane of their last host cell. Viruses that contain lipid are inactivated by organic solvents such as chloroform and ether, therefore many of the chemical agent used against bacteria have minimal virucidal activity.

They use the reproductive machinery of cells they invade causing ailments as benign as a common wart, as irritating as a cold, or as deadly as what is known as the bloody African fever. The viruses that cause Lassa fever and Ebola fever and the retrovirus that causes acquired immunodeficiency syndrome (AIDS) are examples of what researchers call hot agents viruses that spread easily, kill sometimes swiftly, and for which there is no cure or vaccine.

1.5.1 Human Immunodeficiency Virus (HIV)/ AIDS

AIDS is a collection of symptoms and infections resulting from the specific damage to the immune system caused by the *Human Immunodeficiency Virus (HIV)* (Marx, 1982). Although treatments for AIDS and *HIV* exist to slow the virus's progression, there is no known cure. *HIV* is transmitted through direct contact of a mucous membrane or the bloodstream with a bodily fluid containing *HIV*, such as blood, semen, vaginal fluid, preseminal fluid, and breast milk (San Francisco AIDS Foundation, 2006; Divisions of HIV/AIDS Prevention, 2003). This transmission can come in the form of anal, vaginal or oral sex, blood transfusion, contaminated hypodermic needles, exchange between mother and baby during pregnancy, childbirth, or breastfeeding, or other exposure to one of the above fluids.

Most researchers believe that *HIV* originated in sub-Saharan Africa during the twentieth century (Gao, 1999); it is now a pandemic, with an estimated 38.6 million people now living with the disease worldwide (UNAIDS, 2006). As of January 2006, the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) have estimated that AIDS has killed more than 25 million people since it was first recognized on June 5, 1981, making it one of the most destructive epidemics in recorded history. In 2005 alone, AIDS claimed an estimated 2.4–3.3 million lives, of which more than 570,000 were children (UNAIDS, 2006). More than three quarters of all AIDS deaths globally in 2007 occurred in sub-Saharan Africa ((UNAIDS, 2008).

Zimbabwe is one of the worst affected countries in the world. Latest *HIV* prevalence estimates obtained from antenatal clinic surveillance match those reported in the most recent population-based *HIV* survey, which estimated national adult (15–49 years) *HIV* prevalence at 18% in 2005–2006. An estimated 1,820,000 people are living with the virus. 1,540,000 adults (15–49) are infected and 56.5% of these are women (UNAIDS 2007 epidemic update, 2008). Available information indicates that women are more likely to be *HIV* infected than men; statistically 11% of young women (15–24 years) and 4% of young men are infected with *HIV*. Infections levels in pregnant women vary considerably, ranging from 11% in Mashonaland Central to more than 20% in Matabeleland South and Mashonaland West (Central Statistical Office Zimbabwe & Macro International, 2007). It is estimated that 50% of all bed occupancies in hospitals throughout the country are a result of the *HIV/AIDS* pandemic. (Zimbabwe AIDS Network, 2006).

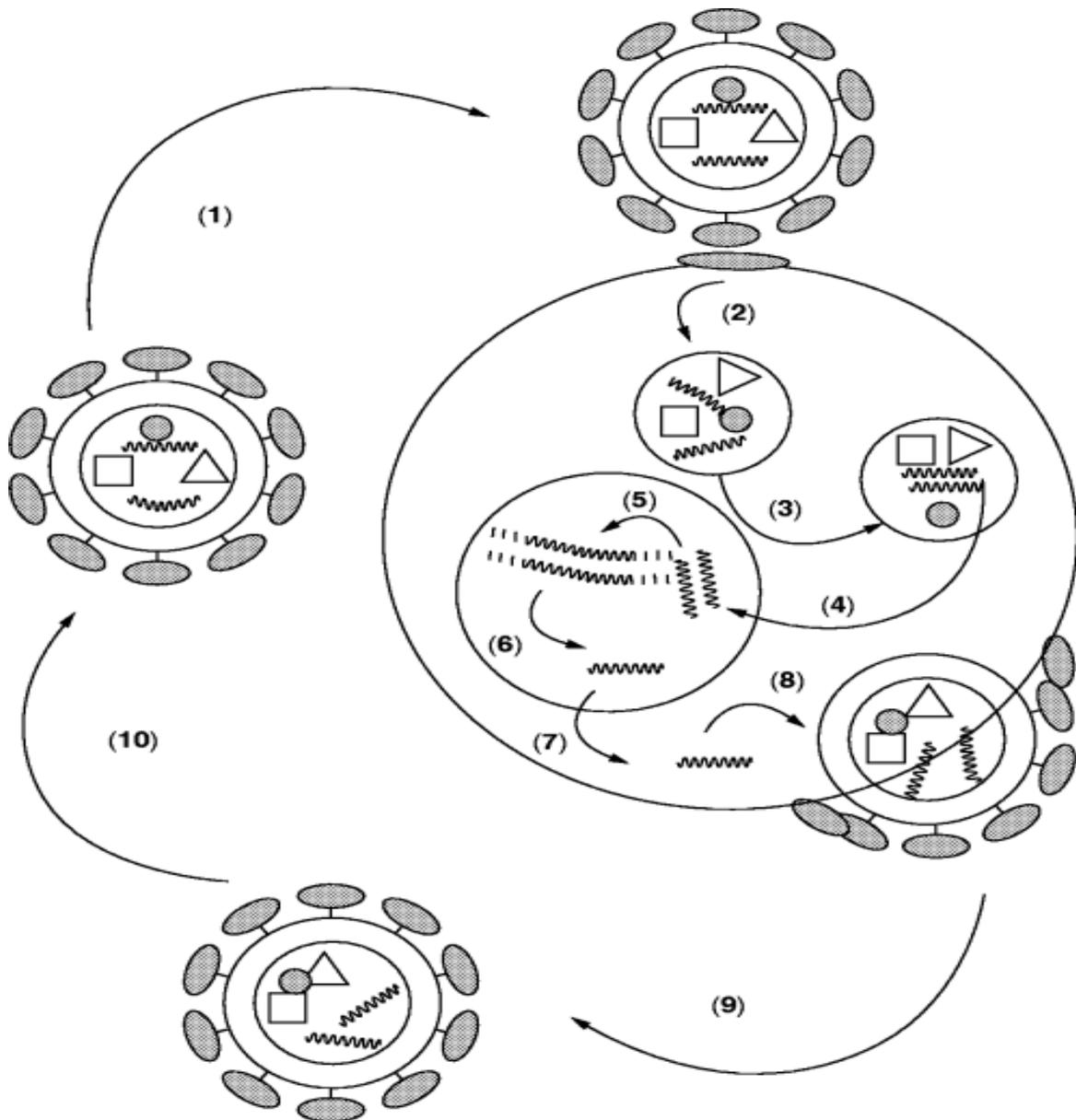
HIV/AIDS stigma is severe and extends beyond the disease itself to providers and even volunteers involved with the care of people living with *HIV*.

1.5.1.1 *HIV* life cycle

The overall process of *HIV* life cycle and replication starts with the virus binding to the host cell through specific surface receptor interactions. After the binding, the virus fuses itself with host cell cytoplasm through a very complex process, which involves a second set of surface protein interactions. After the virus fusion and its entry to the host cell cytoplasm, it makes use of the host cell machinery to express the genetic material necessary to produce its functional proteins. The last stage of the virus life cycle is the stage when the virus assembles itself inside the cell into new virus particles, followed by budding it out then its maturation, to become infective again. Knowledge of *HIV* life cycle is essential to understand the rationale of design of various anti-*HIV* therapeutic agents. As shown in **Fig 11**, the virus life cycle replication process can be described in 10 consecutive steps.

Fig 11: Schematic representation for *HIV* life cycle

(1) Binding of the virus to the T-cell through the gp120 and CD4 receptors. (2) Fusion through viral gp41 and loss of its envelope, the uncoating. (3) Viral DNA formation by reverse transcriptase followed by RNase. (4) Viral DNA entry to the host cell nucleus through its nuclear pores. (5) Viral DNA integration into host cell DNA by integrase. (6) Splicing of viral RNA by host RNA polymerase to produce viral mRNA. (7) Migration of viral RNA to the cytoplasm as mRNA to encode the synthesis of viral proteins. (8) Assembly of the virion containing the viral proteins as a single chain. (9) Viral budding through the host cell membrane with proteins as single chain. (10) Breakdown of the polyprotein precursor by the protease to give structural proteins and enzymes.



*Diagram taken from Mehanna AS, 2003.

1.5.1.2 *HIV* Drugs in Clinical Use

Antiretroviral treatment reduces both the mortality and the morbidity of *HIV* infection, but routine access to antiretroviral medication is not available in all countries (Palella, F. J, 1998). Therefore, World Health Organization has embarked on an ambitious plan to have 3 million people taking antiretroviral therapy by 2005. The large-scale production of generic antiretroviral drugs will allow increased access for impoverished patients. In response to the crisis, the South African National Department of Health has recently accredited 27 facilities, whose mandate to provide AIDS care includes the provision of ‘interventions that delayed the progression of the disease, including nutritional and micronutrient supplementations, and providing complementary and traditional medicines (Mills et al, 2005).

In the fight with viral diseases, an ideal drug would be the one that interferes with viral replication without affecting normal cellular process. Unfortunately only some of the antivirals can do that and many of the drugs have proved toxic to human at therapeutic levels. That’s why antivirals haven’t developed as rapidly as antimicrobials or antiprotozoals (Sethi, 1995).

All currently available drugs for *HIV* therapy belong to one of three classes of inhibitors: the nucleoside reverse transcriptase inhibitors (NRTIs), the nonnucleoside reverse transcriptase inhibitors (NNRTIs), and the protease inhibitors (PIs). These drugs have gained a definite place in the treatment of *HIV*-1 infections because they interfere with crucial events in the *HIV* replication cycle. NRTIs, which target the substrate binding site, include six drugs: zidovudine, didanosine, zalcitabine, stavudine, lamivudine, and abacavir. NNRTIs, which target nonsubstrate binding sites, include three drugs: nevirapine, delavirdine, and efavirenz. Protease inhibitors bind to the active site and act as either enzyme inhibitors or dimer-stabilizing factors; these include five drugs: indinavir, ritonavir, saquinavir, neflinavir, and

amprenavir. Table 3 lists for each compound the generic name, brand name, the pharmaceutical firm that manufactures it, and its mechanistic classification.

Table 3: HIV Approved Drugs for the Treatment of AIDS

Generic Name	Brand Name	Firm	Class
Zidovudine (AZT)	Retrovir	Glaxo Wellcome	NRTI
Didanosine (ddI)	Videx	Bristol-Myers Squibb	NRTI
Zalcitabine (ddC)	Hivid	Hoffman-La Roche	NRTI
Stavudine (d4T)	Zerit	Bristol-Myers Squibb	NRTI
Lamivudine (3TC)	Epivir	Glaxo Wellcome	NRTI
Abacavir (ABC)	Ziagen	Glaxo Wellcome	NRTI
Neveirapine	Viramune	Boehringer Ingelheim	NNRTI
Delavirdine	Rescriptor	Pharmacia	NNRTI
Efavirenz	Sustiva	Hoffman-La Roche	NNRTI
Idinavir	Crixivan	Mercke	PI
Ritonavir	Norvir	Abbott	PI
Saquinavir	Invirase	Hoffman-La Roche	PI
Nelfinavir	Viracept	Agouron Pharma	PI
Amprenavir	Agenerase	Glaxo Wellcome	PI

1.5.1.3 Traditional medicine against *HIV/AIDS*

The number of studies on plants and herbs for antiviral activity is small compared to the numerous clinical researches and screenings done for the antibacterial and antifungal activity (Kambizi, 2001; Hamburger, 1991). However, the studies are giving promising results for potential new antivirals for future.

In Africa, *Hypoxis hemerocallidea* (African potato), *Lessertia frutescens* (Sutherlandia), *Artemisia afra* and *Warburgia* species are used effectively for the treatment of people living with *HIV/AIDS* (Rabe et al, 2000; Hostettman, 2000; Mills et al, 2005).

There is also the extreme caution that should be taken in introducing herbal drugs into the routine care of *HIV* patients in any setting including the developing world, and underscore the need for appropriately designed pharmacokinetic studies to unveil the true drug interaction

potential of herbal drugs with antiretroviral agents. Failure to do this may result in bidirectional drug interactions, which may put patients at risk of treatment failure, viral resistance or drug toxicity. This was proven so in a study where the effect of two herbs in common medical use for HIV in Africa, *Hypoxis hemerocallidea* (African potato) and *Lessertia frutescens* (Sutherlandia), have been analysed for their potential to cause drug interactions with common antiretroviral agent metabolising mechanisms *in vitro* (Mills et al, 2005). The findings suggest that the co-administration of these drugs with antiretroviral agents may result in the early inhibition of drug metabolism and transport followed by the induction of decreased drug exposure with more prolonged therapy.

1.5.2 Herpes simplex virus type 2 (HSV-2)

The fact that the transmission rate of *HIV* increases twofold to sixfold with the presence of a sexually transmitted disease (Orroth et al, 2000) shows how important it is to deal with these secondary diseases in human. In a recent study in Zimbabwe, has shown that Genital Herpes caused by *HSV-2*, was found to be the most common sexually transmitted disease among Zimbabwean rural women (Kjetland, 2005). Therefore, ***Herpes Simplex Virus type 2*** was chosen for this study.

1.5.2.1 General information

Herpes is one of the oldest causes of infections to man. It is recorded that the Romans, in an attempt to eliminate this disease, banned kissing (Steiner et al, 1984). The virus itself was discovered in 1912 and finally isolated from genital tract in 1946 (Yen, 1965). Genital herpes was not officially recognized as a disease, however, until 1966. Since then, reported cases for this disease have increased almost 10 times.

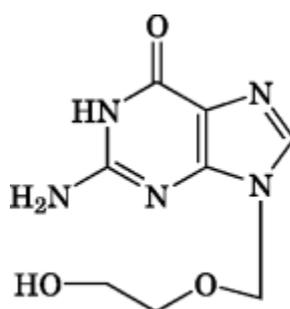
Humans serve as the only host to this DNA-containing virus. The classic presentation of primary HSV-2 is herpes genitalis, an infection characterized by extensive, bilaterally distributed, blister type lesions in the genital area accompanied by fever, lymphadenopathy

and dysuria. The most serious consequence of genital HSV-2 is neonatal herpes. This infection usually results from exposure of the baby to virus being excreted by the mother at time of the vaginal delivery. The neonate may present with infection localized to skin, eyes, mucosa or the central nervous system. The mortality rate for untreated infants who develop disseminated infection exceeds 70% (Arvin, 1995).

1.5.2.2 HSV-2 Drugs in clinical use

Today, in the treatment of herpes virus infections, antiviral drugs like *5-iodo-2-deoxyviridine*, *cytarabine*, *vidarabine*, and *fluorothymidine* are used (Fahad and Stepher, 1996). The mechanism of action of these drugs is basically dependent on their abilities to inhibit the virus-specific enzyme, thymidine kinase, and the DNA polymerase (Dagna and Stuart, 1995). Because of their cytotoxic effects, however, these drugs are not widely used. A relatively less cytotoxic drug, *acyclovir*, is the most preferred and potent drug employed in the treatment of herpes virus infections (Middleton, 2003). In recent years, however, acyclovir and other drugs have been reported to be inefficient in treating genital herpes infections. *HSV-2* has also been reported to acquire resistance to these drugs (Dagna and Stuart, 1995; Wagstaff et al., 1994; Darby and Larder, 1992). For all these reasons, the search for new antiviral drugs active against *HSV* is on the increase. The main goal of such investigations has been the provision of effective treatment with the lowest toxicity (Duran et al, 2003).

Fig 12: The chemical structure of antiviral agent Acyclovir



1.5.3 Antiviral Susceptibility Testing

Cell culture (tissue culture) has its origins in the 19th century when people began to examine in some detail the tissues and the organs of the body in glass vessels. The major purpose was to study the cells themselves, how they grow, what they require for growth and how and when they will stop growing. The term *in vitro* literally means ‘in glass’, although today most of cell culture is performed in or on plastic (Gey et al, 1952).

When cells are isolated from a tissue, grown *in vitro* and before subculture, they are regarded as a primary culture. Transferring cells from primary culture and dispersing them with trypsin and fresh batch of medium will give secondary cell cultures or subcultures. A limited number of subcultures can be performed, up to a maximum of about 50, before the cells degenerate (Freshney et al, 1992).

Human cell lines present dangers, as they may contain pathogenic organisms, which can be shed into the medium. Infectious agents, when released into the medium from cell lines will cause aerosols that can infect via contact with mucous membranes or abrasions. Non-human cell lines present a lesser danger, as it is unlikely that contaminating cells would escape host immunologic defences.

To avoid any kind of contamination, all procedures except cell counting were carried out aseptically. For aseptic conditions, tissue culture hoods were used. There are two principles considered in hood design;

1. Protecting tissue culture from the operator
2. Protecting the operator from the tissue culture.

In this project, Class II hoods were used which offer protection to both the operator and the cell culture. Filtered air is drawn in through the top of the hood, down over the tissue culture, through the bottom of the working area and down through the grill in front of the working area. In this way the cell culture is protected in a stream of sterile air and the operator is

protected from the contamination by the inflow of air into the base of the work area (Hsiung, 1989).

1.6 Bacteriology, Mycology and Anti-infective Activity

Bacteria are single-celled microorganisms that lack a nuclear membrane, are metabolically active and divide by binary fission. Medically they are a major cause of disease. Superficially, bacteria appear to be relatively simple forms of life; in fact, they are sophisticated and highly adaptable. Many bacteria multiply at rapid rates, and different species can utilize an enormous variety of hydrocarbon substrates, including phenol, rubber, and petroleum. These organisms exist widely in both parasitic and free-living forms. Because they are ubiquitous and have a remarkable capacity to adapt to changing environments by selection of spontaneous mutants, the importance of bacteria in every field of medicine cannot be overstated. In developing countries, a variety of bacterial infections often exert a devastating effect on the health of the inhabitants. Malnutrition, parasitic infections, and poor sanitation are a few of the factors contributing to the increased susceptibility of these individuals to bacterial pathogens.

The World Health Organization has estimated that each year, 3 million people die of tuberculosis, 0.5 million die of pertussis, and 25,000 die of typhoid. Diarrhoeal diseases, many of which are bacterial, killing 5 million people annually are the second leading cause of death in the world after cardiovascular diseases.

Fungi are eukaryotic microorganisms. Fungi can occur as yeasts, molds, or as a combination of both forms. Some fungi are capable of causing superficial, cutaneous, subcutaneous, systemic or allergic diseases. Of the approximately 70,000 recognized species of fungi, about 300 are known to cause human infections. In addition, some bacteria and fungi have economic importance as plant and animal pathogens. Fungal diseases of healthy humans tend to be relatively benign, but the few life-threatening fungal diseases are extremely important. Fungal diseases are an increasing problem due to the use of antibacterial and

immunosuppressive agents. Individuals with an altered bacterial flora or compromised defence mechanisms (e.g., AIDS patients) are more likely than healthy people to develop opportunistic fungal infections such as candidiasis. Consequently, opportunistic fungal pathogens are increasingly important in medical microbiology.

1.6.1 Traditional Medicine as Anti-infective Treatment

Medicinal plants are both potential antimicrobial crude drugs as well sources for natural compounds that act as new anti-infection agents. In the past few decades, the search for new anti-infection agents has occupied many research groups in the field of ethnopharmacology. In a recent study, the number of articles published on the antimicrobial activity of medicinal plants in PubMed were reviewed and for the period between 1966 and 1994, the number of articles found was 115; however, in the following decade between 1995 and 2004, this number more than doubled to 307 (Rios, 2005). In this study, a wide range of criteria was found concerning antimicrobial screening. It was reported that many focus on determining the antimicrobial activity of plant extracts found in folk medicine, essential oils or isolated compounds such as alkaloids, flavonoids, sesquiterpene lactones, diterpenes, triterpenes or naphthoquinones, among others (Akinyemi et al, 2005; Karou et al, 2005; Chagonda et al, 2000). Some of these compounds were isolated or obtained by bio-guided isolation after previously detecting antimicrobial activity on the part of the plant. A second block of studies were reported to focus on the natural flora of a specific region or country; the third relevant group of papers was made up of specific studies of the activity of a plant or principle against a concrete pathological micro-organism. As a conclusion, it was suggested that some general considerations must be established for the study of the antimicrobial activity of plant extracts, essential oils and the compounds isolated from them, especially the definition of common parameters, such as plant material, techniques employed, growth medium and micro-organisms tested.

1.6.2 Micro-organisms chosen for the study

The micro-organisms which were chosen for the study are all clinically important human pathogens and all are known to cause serious infections in especially immune suppressed individuals and these infections are called opportunistic infections. . The microorganisms were all supplied by the Medicines Control Authority of Zimbabwe (MCAZ) and Medical Microbiology, College of Health Sciences, University of Zimbabwe.

Two strains of gram-positive, two strains of gram-negative bacteria and two kinds of fungi were chosen for this study;

Staphylococcus aureus: (NCTC 10788) Gram-positive, non-motile, non-sporing coccus, aerobic or anaerobic; causes superficial skin lesions (boils, styes), localized abscesses in other sites, deep-seated infections, such as osteomyelitis and endocarditis, more serious skin infections (furunculosis), hospital acquired (nosocomial) infection of surgical wounds, food poisoning by releasing enterotoxins into food, toxic shock syndrome by release of superantigens into the blood stream and urinary tract infections, especially in girls (Easmon, 1983).

Streptococcus group A: (NCTC 5775) Gram-positive, nonmotile, nonsporeforming, catalase-negative cocci, anaerobes; have a hyaluronic acid capsule.; causes pharyngitis, scarlet fever (rash), impetigo, cellulitis, or erysipelas, myositis and streptococcal toxic shock syndrome (Bisno,1991)

Escherichia coli: (NCTC 10418) Gram-negative rod, motile, facultatively aerobic, enteric pathogen; causes gastroenteritis, urinary tract infections, nosocomial (hospital-acquired) infections (Foxman, 1995)

Pseudomonas aeruginosa: (NCTC 6750) Gram-negative rod, motile, aerobic; causes opportunistic infections in patients hospitalized with HIV/AIDS, cancer, cystic fibrosis, and burns, endocarditis, septicaemia, pneumonia, and infections of the urinary tract, central nervous system, wounds, eyes, ears, skin, and musculoskeletal system; high resistance to antimicrobial agents (Poole, 1994).

Candida albicans : (NCPF 3179) unicellular, yeast-like, eukaryotic fungus that can undergo rapid transformation from the yeast to the hyphal phase in vivo, which partly contributes to its success in invading host tissue; causes oral and genital candidiasis, gastrointestinal infections.

Aspergillus niger: (NCPF 2275) opportunistic mould, break into body through break in epidermis or by the way of lungs; causes, sinus, ear, nail, cornea infections, cellulites, endocarditis and peritonitis.

1.6.3 Infections on Body Parts and the Associated Micro-organisms

Mouth: *Staphylococcus aureus*, *Streptococcus*, *Escherichia coli*, *Candida albicans*

Throat: *Staphylococcus aureus*, *Candida albicans*

Nose: *Staphylococcus aureus*, *Candida albicans*

Lung: *Staphylococcus aureus*, *Streptococcus*, *Pseudomonas aeruginosa*, *Escherichia coli*

Gastrointestinal Tract: *Staphylococcus aureus*, *Streptococcus*, *Escherichia coli*

Stomach: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*

Genital: *Staphylococcus aureus*, *Streptococcus*, *Escherichia coli*, *Candida albicans*

Urinary tract infections: *Staphylococcus aureus*, *Streptococcus*, *Escherichia coli*,
Pseudomonas aeruginosa *Candida albicans*

Vagina: *Staphylococcus aureus*, *Streptococcus*, *Escherichia coli*, *Pseudomonas*
aeruginosa, *Candida albicans*

Skin, Wounds and Burns: *Staphylococcus aureus*, *Candida albicans*, *Pseudomonas*
aeruginosa

Eye: *Staphylococcus aureus*, *Streptococcus*, *Pseudomonas aeruginosa*, *Candida albicans*

Ear: *Staphylococcus aureus*, *Pseudomonas aeruginosa*

Blood Infections: *Pseudomonas aeruginosa*, *Escherichia coli*

1.7 Toxicology and Bioactivity

Plant poisons are highly active substances that may cause acute effects when ingested in high concentrations and chronic effects when accumulated (Pfänder, 1984). In many cases of poisoning resulting from consumption of endogenous toxicants such as those in medicinal plants, hospital admissions with serious clinical presentations have been reported (Tagwireyi, 2002).

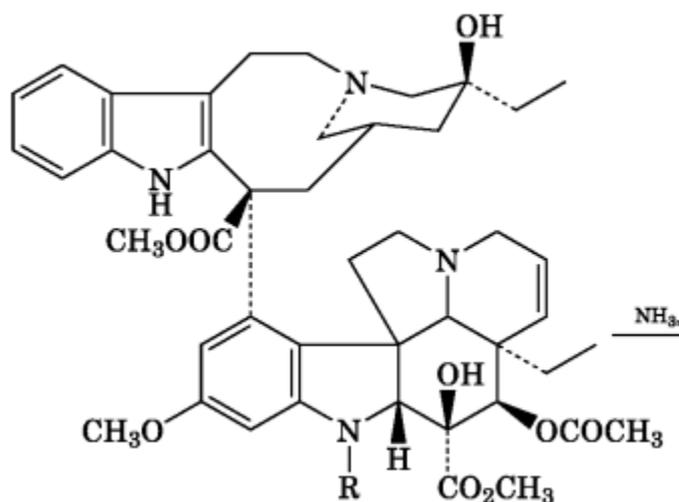
Poisoning or toxic principles as relates to vegetables generally fall into various phytochemical groups, which include alkaloids, oxalates, phytotoxins (toxalbumins), resins, essential oils, amino acids, furanocoumarins, polyacetylenes, protein, peptides, coumarins, flavonoids and glycosides (Concon, 1988). The phytochemical investigations of the traditional medicinal plants those were chosen for this study have shown us that groups like alkaloids, coumarins, flavonoids, glycosides and essential oils are present in our medicinal plants.

Safety in usage of the traditional medicinal plants and their potential bioactivity can be measured by a simple assay called Brine Shrimp Lethality Test. Brine Shrimp (*Artemia salina*) have been previously utilized in various bioassay systems such as analysis of pesticide residues, mycotoxins, stream of pollutants, toxicity of oil dispersants etc. In terms of Traditional medicine, it is simply a search for safety of use and /or bioactive natural products which could be future sources of anti-tumour and cytotoxic agents.

It is also a common knowledge that products used in the anticancer chemotherapy are generally toxic and non-selective/restrictive to cancer cells. Local herbalists have been treating various cancers- and cancer-related conditions for ages (Sofowora, 1984) and many plants have been reported as useful in the management of such conditions. Plants like *Catharanthus roseus* have provided many anticancer drugs such as taxanes, vincristine and vinblastine (**Fig 13**) and still serve as a veritable source of new products through the use of standard bioassay methods (Buss et al, 2003).

Fig 13: The chemical structures of antitumor agents

Vincristine(R=CHO) and Vinblastine(R=CH₃)



Thirty-five extracts from sixteen plants native to the north of Argentina and south Bolivia were submitted to BSLT bioassays in order to evaluate toxicity against *Artemia salina*. The most toxic extracts were the chloroform extracts from *V. tweediana* (LD₅₀=1 ppm), *M. calvescens* (LD₅₀=5 ppm), *D. salicifolia* (LD₅₀=46 ppm) and *S. santelisis* (LD₅₀=49 ppm) and the MeOH extracts from *S. santelisis* (LD₅₀=1 ppm) and *G. scorzonerifolia* (LD₅₀=76 ppm) (Bardon et al, 2007).

Terminalia sericea Burch. Ex. DC (Combretaceae) extracts from Tanzania were toxic to brine shrimps giving LC₅₀ (95% confidence intervals) values ranging from 5.4 to 17.4µg/ml, while that of cyclophosphamide, a standard anticancer drug, was 16.3µg/ml (Moshi, 2005).

Extracts of 17 plant species used for ethnoveterinary purposes in South Africa in rural areas, were tested for toxic effects against brine shrimp larvae. With the lowest LC₅₀ of 0.55mg/ml, the extracts tested in this study do not possess toxic effects (McGaw et al, 2007).

1.8 Aim

To screen the activity of some traditional medicinal plants from selected Zimbabwean districts for possible sources of antimicrobial, antiviral drugs and pharmaco-actives.

1.9 Objectives

1. To study medicinal plants in selected districts of Zimbabwe those are commonly used by traditional medical practitioners and could be threatened with extinction.
2. To obtain crude plant extracts.
3. To run Phytochemical screening.
4. To determine the Antioxidant activity and Total Phenolic Contents of the plant samples and evaluate these results in connection with phytochemistry.

5. To screen for Antiviral activity.
6. To screen for Antimicrobial (antibacterial and antifungal) activity.
7. To screen for Biological activity and Toxicity and comment on potential use of certain plants as anti-tumour agents.
8. To prepare plant monographs with the information gathered from literature search and the results achieved from the study.
9. To evaluate Traditional Healers' claims on indigenous medicinal plants according to the established *in vitro* results.

CHAPTER II

1. MATERIALS AND METHODS

2.1 Chemicals, Reagents and Equipment

Solvents;

Ammonia 25% AR (Batch 503542) Skylabs; *Ethanol* AR (Batch 3875) Associated Chemical Enterprises, RSA ; *Methanol* Spectrophotometric grade (Batch no 68F-0898) Sigma; *Methanol* univAR (Batch 16229) Saarchem Pvt Ltd, RSA; *Toluene* CP (Cat No 15, 500-4) Aldrich; *Ethyl acetate*, AR (Batch no 20774) Aldrich; *Formic acid* (Batch 107F-0658) Sigma; *Gl. Acetic acid* AR (Batch 20040824P); *Diethyl amine* AR (Batch 43410) Microlabs; *Dimethylsulphoxide* AR (1.02952.2500) Merck; *Benzene* (Batch 6317/584) Associated Chemical Enterprises,RSA; *Hydrochloric acid* AR (Batch 4504) Associated Chemical Enterprises, RSA; *Chloroform* AR (Batch 1010060) Saarchem Pvt Ltd, RSA; *Petroleum ether* univAR (Batch 15060) Saarchem Pvt Ltd, RSA; *Acetonitrile* (Lot 96F 3484) Sigma, USA.

Chemicals;

Potassium hydroxide, (Batch 19216) Saarchem; *Sodium hydroxide*, (Batch 1410507), Skylabs; *Potassium Iodide* AR (Batch 69153) Skylabs; *Potassium Chloride* AR (Batch 1029052) Saarchem; *Sodium Chloride* AR (Batch 1028306) Saarchem; *Ferric chloride* (Lot 37F-3478) Sigma, USA; *Bismuth nitrate* (No B-9383) Lot 47F- 0698, Sigma; *Ninhydrin crystalline* (No N-4876) Lot 97F-0081, Sigma; *Fast Blue Salt*, (No 1133) Michrome, Sigma; *p-Coumaric acid* (C-9008) Lot 48H-3430, Sigma; *Caffeic acid* (C-0625) Lot 38H0639 Sigma; *Atropine*, University of Zimbabwe,School of Pharmacy; *Digitonin* (No D-5628) Lot 34F-0141 Sigma; *Vanillin* (S4551918) Merck, Germany; *Dinitrobenzene* (S05456) Merck.

Media material;

Sabouraud Dextrose Agar (Batch B001468) Biotec Laboratories; *Nutrient Broth* (Batch 1066724) Art No C24 Biolab Diagnostics, Merck; *Fetal Calf Serum* Highveld Lab, Johannesburg, RSA; *Sea Salt* (LA 060670917) Baleine Germany.

Bioactive material;

Brine Shrimp (Artemia salina) eggs Aqua Africa, Grahamstown, RSA; *VERO cell line*, Highveld Lab, Johannesburg, RSA; *Herpes Simplex Virus type-2* Highveld Lab, Johannesburg, RSA.

Equipment;

TLC Plates (Batch 126 F-0130) T-6770, Polyester silica gel, 250µm layer thickness, 2-25µm mean particle size, 20x20cm, Sigma; *Whatman filter paper* No 1 125mm (Cat No 1001125) Schleicher and Schuel; *Microtitre plates*, 96-well flat-bottomed, with lid, sterile, Nunc, Denmark; *Cell culturing flasks*, 20ml, canted neck, sterile, Nunc, Denmark; *Membrane filters* (Batch no R3PN58187), 0.22µm sterile filter unit, Millipore MCE membrane, Millex GS.

Grinding Mill, Thomas-Wiley Laboratory Mill Model 4; *Rotary Evaporator*, Heidolph Laborota 4000, Germany; *UV Visible Spectrophotometer*, Shimadzu UV-1601, Chart no 200-91527, Japan.

2.2 Plant Material

2.2.1. Plant Selection Criteria

This thesis was done in connection with the *Ministry of Environment and Tourism & School of Pharmacy Project*. The objective of the project was to promote the conservation, sustainable use and cultivation of endangered medicinal plants in Zimbabwe, by demonstrating effective models at the local level, and developing a legal framework for the

conservation, sustainable use, and equitable sharing of benefits from medicinal plants. The baseline course of action will see increasing use of indigenous medicinal plants by local people and traditional healers as an effective complement to modern medicines.

The thesis was focused on adding value to commonly-used and threatened traditional medicinal plants being used to treat common ailments in Zimbabwe. 23 reports were compiled by Safire and the Ministry of Environment and Tourism on basis of communications with the traditional healers, *n'angas*, from various wards in five districts namely Chipinge, Chimanimani, Matobo, Bulilima and Mangwe. After these were reviewed, the plants with antibacterial, antifungal, antiviral, anthelmintic and antioxidant activities were noted and the literature available on background knowledge of these plants was collected and filed as a part of value addition process. 30 plants of greater interest were chosen from these reports and were compiled into '*plant monographs*' consisting of names of plants (vernacular, Latin, synonyms), description, cultivation, ethno botany, tested pharmacological activities, known chemical constituents, toxicology and marketing status. The anthology of the plants chosen for the study with the added information obtained from the project can be read in the "conclusion" part on page 133.

Table 4: Plants chosen for the study and their Ethnobotany

No	Botanical Name & Family	Vernacular Name	Ethnobotany	Part Used	Part Collected
1	<i>Cassia abbreviata Oliv.</i> <i>Caesalpinioideae</i> Family	Long-pod cassia (Eng) Muremberembe (Sh) Isihaqa (Nd)	Gonorrhoea	Roots	Leaves Bark Roots
			Abdominal pain & Diarrhoea		
			Menorrhagia		
			Backache		
2	<i>Dichrostachys cinerea (D. glomerata)</i> <i>Mimosaceae</i> Family	Mupangara, Musekera Mumhangara (Sh) Ugagu (Nd) Chilitsenge (Tonga)	Venereal diseases, Impotence	Leaves Roots	Leaves Roots
			Syphilis		
			Eye diseases		
			Pneumonia		
			Wounds, injuries		

No	Botanical Name & Family	Vernacular Name	Ethnobotany	Part Used	Part Collected
3	<i>Elaeodendron matabelicum</i> (<i>Cassine matabelica</i>) <i>Celastraceae</i> Family	Condiment saffron (Eng) Murunganyama, Murungamunyu (Sh) Umgugudu (Nd)	Venereal diseases, Syphilis	Roots	Roots
			Abdominal pain & Diarrhoea		
			Chest complaints		
			Abscesses, carbuncles		
			Purgative, Dysentery remedy		
4	<i>Elephantorrhiza goetzei</i> <i>Leguminosae</i> Family	Long-pod cassia (Eng) Muzezepasi (Sh) Intolwane (Nd)	Venereal diseases, Syphilis	Roots	Roots
			Anthelmintic		
			Abdominal pain & Diarrhoea		
			To increase blood in the body		
			Depressed fontanel		
5	<i>Flacourtia indica</i> <i>Flacourtiaceae</i> Family	Governor's plum (Eng) Mundudwe (Sh) Umthunduluka (Nd)	Venereal diseases	Roots	Leaves Roots
			Cough, chest pains		
			Pneumonia		
			Bilharzias		
			Diarrhoea		
6	<i>Gymnosporia senegalensis</i> (<i>Maytenus senegalensis</i>) <i>Celastraceae</i> Family	Chivhunabadza, musosawafa (Sh) Isihlangu (Nd) Ibalalatune (Tonga)	Chickenpox, Measles,	Leaves Twigs Roots	Leaves Twigs Roots
			Varicella		
			Mumps		
			Cough, pneumonia		
			Fever, malaria		
7	<i>Hypoxis hemerocallidea</i> (<i>H. rooperi</i>) <i>Hypoxidaceae</i> Family	Yellow star (Eng) African potato (Eng) Hodo (Sh) Igudu (Nd)	Antiviral (Anti-HIV 1)	Tuber	Tuber
			Urinary infections		
			Heart weakness		
			Internal tumours		
			Nervous disorders		
8	<i>Khaya anthotheca</i> (<i>K. nyasica</i>) <i>Meliaceae</i> Family	Red mahogany (Eng) Muwawa (Sh)	Venereal diseases	Bark	Bark Roots
			Abdominal pains		
			Pneumonia		
			Anthelmintic		
			Colds		
			Antiemetic		
9	<i>Kigelia africana</i> (<i>K. pinnata</i>) <i>Bignoniaceae</i> Family	Sausage tree (Eng) Mubvee (Sh) Umvebe (Nd)	Venereal diseases, Syphilis	Fruit Bark Roots	Fruit Bark Roots
			Skin cancer remedy		
			Antipsoric, antieczama		
			Purgative, Dysentery remedy		
			Swelling of genitalia		
			Haemorrhoids		
			Wounds, abscesses		
			Tapeworm remedy		
10	<i>Rhus chirindensis</i> <i>Anacardiaceae</i> Family	Mubikasadza (Sh)	Measles	Leaves Roots	Leaves Roots
			Cough		
			Chest pains		
			Syphilis		
11	<i>Sclerocarya birrea</i> <i>subsp. caffra</i> <i>Celastraceae</i> Family	Marula (Eng) Mupfura, Mutsomo (Sh) Unganu (Nd)	Cough, pneumonia	Bark	Bark
			Heart pains		
			Diarrhoea, Bilharziasis		
			Malaria		
			Antiemetic		

No	Botanical Name & Family	Vernacular Name	Ethnobotany	Part Used	Part Collected
12	<i>Securidaca longepedunculata</i> <i>Polygalaceae</i> Family	Violet tree (Eng) Mufufu (Sh) Umfufu (Nd)	Venereal diseases,	Roots	Roots
			Syphilis		
			Tuberculosis		
			Pneumonia		
			Epilepsy		
			Pains, fever		
13	<i>Terminalia sericea</i> <i>Combretaceae</i> Family	Silver cluster leaf (Eng) Yellow wood (Eng) Mususu (Sh) Umsusu, Umangwe (Nd)	Gonorrhoea	Roots	Leaves Roots
			Syphilis and other STD		
			Abdominal pain & Diarrhoea		
			Antiemetic		
			Bilharziasis		
			Wounds		
14	<i>Warburgia salutaris</i> <i>Canellaceae</i> Family	Pepper-bark tree (Eng) Muranga (Sh) Isibhaha (Zulu)	Panacea (Remedy for all)	Bark	Leaves Twigs Bark Roots
			Venereal diseases		
			Abdominal pains		
			Headache		
			To cause abortion		
			Aid to divination		
			To increase blood in body		
			Colds and coughs		

2.2.2 Collection

Plants were collected from below mentioned five districts. Districts were chosen according to their climatic zones, altitude, rainfall and soil type. Botanists at the National Herbarium identified the specimens and a sample for each plant was labelled and kept as a reference at the School of Pharmacy University of Zimbabwe.

The plant collection list according to the districts is shown in the **Table 5** on page 44.

2.2.3 Plant Preparation

Leaves, roots and barks were cleaned and cut into small pieces. The specimens were labelled and dried in shade (**Fig 14 & 15**). Once dry, plant material was ground into fine powder using an electric grinder. The powders were placed in black containers, labelled and stored in a dark place.

Table 5: Plants collected according to the districts

DISTRICT	PLANTS COLLECTED
Chimanimani	<i>Kigelia africana</i> , <i>Flacourtia indica</i> , <i>Khaya anthotheca</i>
Chipinge	<i>Warburgia salutaris</i> , <i>Rhus chirindensis</i>
Matobo	<i>Cassia abbreviata</i> , <i>Hypoxis rooperi</i>
Bulilima	<i>Gymnosporia senegalensis</i> , <i>Elephantorrhiza goetzei</i> , <i>Elaedendron matabelicum</i>
Mangwe	<i>Dichrostachys cinerea</i> , <i>Terminalia sericea</i> , <i>Securidaca longepedunculata</i> , <i>Sclerocarya birrea</i>

Fig 14: Plant samples being dried in the research lab



Fig 15: Samples separated, cut into pieces and labelled



2.3 Plant Extraction

According to the literature based on similar ‘screening’ type of work, the most commonly used solvent for extraction was found out to be methanol and these extractions were reported to give better results (Betancur-Galvis L et al, 2002).

The 20-40g of stored plant material was macerated in methanol for 24 hours on a shaker in a water bath at 40°C. This was then vacuum filtered and the filtrate was concentrated under reduced pressure on a rotary evaporator (**Fig 16 & 17**) and the small amounts of wet extracts were then lyophilized by using a vacuum freeze dryer. The dried crystallized extracts were bottled and kept in a refrigerator. The yields of extracts in grams and as percentages of the original dry plant material are in **Table 6** on page 62.



Fig 16: Solvent removed from extract in rotary evaporator



Fig 17: Lyophilized, bottled and labelled plant extract crystals

These crystals were later on dissolved in DMSO to the concentration of 10mg/ml. The solution was sterile filtered through Millipore membrane filters (0.22µm) under aseptic conditions and divided into aliquots in Eppendorf tubes to be kept at -20 C until further use.

2.4 Phytochemical Screening

The major chemical substances of interest in this survey have been the alkaloids, flavonoids, saponins, coumarins, anthraquinones and tannins. Different extraction methods and solvents (methanol, ethanol, ammonia etc.) were used and the extracts were screened for their chemical constituents according to different available literature (Wagner et al., 1984, Harbone 1988, Trease and Evans, 2002 and Mojab et al, 2003).

A major part of the phytochemical screening was done at the Faculty of Pharmacy at the University of Istanbul with the special help of the Department of Pharmacognosy.

There was a wide range of reference compounds especially for flavonoids, alkaloids and anthraquinones which were self obtained from various plant materials and identified through Infrared Spectroscopy.

For each group, all extracts were detected through three different tests, namely Thin Layer Chromatography, UV spectrum readings and Confirmatory tests (**Fig 19-20, p. 64**).

The results for each extract for different groups are presented on **Table 7** on page 63.

2.4.1 Alkaloids

Powdered drug (1g) was dissolved in 5ml of methanol with one ml of 10% ammonia solution. The filtrate (10µl) was applied onto Thin Layer Chromatography plates.

The solvent systems were

- I. Toluene: Ethylacetate (EtOAc): Diethylamine (7:2:1)
- II. Cyclohexane: Chloroform: Diethylamine (7:2:1)
- III. Chloroform: Methanol (MeOH) (8:2)

The extracts were tested against the reference compounds. The reference compounds were

1. Atropine sulphate
2. Berberine
3. Mecambrine

4. Thebaine
5. Narcotine
6. Isocodeine

Once the plates were dry, they were sprayed with *Dragendorf reagent* in 5% ethanolic sulphuric acid with the help of an atomizer. To prepare this reagent, 0.85g Bismuth nitrate was dissolved in 40ml of water and 10ml of glacial acetic acid. This was followed by the addition of 8g potassium iodide dissolved in 20ml of water. The presence of orange-brown spots against a pale yellow background was interpreted as preliminary presence of alkaloids. The **Figure 21** on page 65 shows a TLC plate with alkaloid results.

The plates, before chemical treatment, were examined under UV light at both 254nm and 365nm. Under 254nm, quenching of fluorescence was expected. Under 365nm, blue, green or yellow fluorescence could be seen.

As the confirmatory test, the alkali solution of plant extract was put into test tubes with a couple of drops of 10%HCl and Dragendorf reagent. The presence of orange precipitation was proof of alkaloids.

2.4.2 Flavonoids

Powdered drug (1g) was dissolved in 10ml of methanol. The glycosides and the aglycones were searched both on Paper and Thin Layer Chromatography. The filtrate (10µl) was applied onto paper and TLC plates.

The solvent system was the same for both the glycosides and the aglycones on *Paper Chromatography*;

N-Butanol: Acetic acid: Water (4:1:5)

There were different solvent systems for the glycosides and the aglycones on *Thin Layer Chromatography*.

The solvent systems for *aglycones* were

- I. Toluene: Ethylacetate (EtOAc): Formic acid (5:4:1)
- II. Chloroform: Methanol: Water (80:18:2).
- III. Benzene: Ethanol (8:2)
- IV. Chloroform: Acetone: Formic acid (9:2:1)

The extracts were run against following aglycone reference compounds

- | | |
|----------------------------|----------------------|
| 1. Quercetin | 7. Apigenin |
| 2. Quercetin-3-methylether | 8. Luteolin |
| 3. Cirsilineol | 9. 6-methoxyluteolin |
| 4. Isorhamnetin | 10. Hispidulin |
| 5. Hesperetin | 11. Kaempferol |
| 6. Acacetin | |

The solvent systems for *glycosides* were

- I. Ethylacetate: Ethylmethylketone: Formic acid: Water (5:3:1:1)
- II. Ethylacetate: Methanol: Water (75:15:10)
- III. Benzene: Ethanol: Formic acid (9:7:4)

The extracts were run against following glycoside reference compounds

- | | |
|-----------------------------|------------------------------------|
| 1. Rutin | 7. Hyperoside |
| 2. Quercitrin | 8. Apigenin 7-O-glucoside |
| 3. Isoquercitrin | 9. Vicenin-2 (Vitexin-6,8-di-C-gl) |
| 4. Naringenin 7-O-glucoside | 10. Astragalin |
| 5. Luteolin 7-O-glucoside | 11. Vitexin |
| 6. Hesperidin | 12. Isovitexin |

Once the plates were dry, they were sprayed with either Fast Blue Salt where it is observed to give blue spots for flavonoids or FeCl_3 giving green-brown spots. The **Figure 22** on page 65 shows a TLC plate with flavonoids results.

The plates, before chemical treatment, were examined under UV light at both 254nm and 365nm. Under 254nm, quenching of fluorescence was expected. Under 365nm, blue, green or yellow fluorescence could be seen. After chemical treatment, the colours intensify.

As the confirmatory test, the alcoholic plant extract was put into test tubes with conc. HCl and Magnesium turnings. The flavonols would give pink-magenta red precipitation, the flavones would give orange and the flavonones would give purple precipitation

2.4.3 Saponins

Powdered drug (1g) was dissolved in 5ml of 70% ethanol and was heated in the waterbath at 60°C. The filtrate (10 μ l) was applied onto TLC plates.

The solvent system was Chloroform: Methanol: Water (64:50:10).

The extracts were tested against the reference compound, Digitonin as 0.1% solution in methanol.

Once the plates were dry, they were sprayed with Vanillin-sulphuric acid reagent in 5% ethanolic sulphuric acid with the help of an atomizer. The presence of blue-yellow, blue-violet spots was interpreted as preliminary presence of saponins.

The plates, after chemical treatment, were examined under UV light at 365nm. Saponins are expected to give red-violet, blue, green fluorescence.

As the confirmatory test, the plant extract was put into test tubes with 10ml water and shaken for 10seconds. If foam appears that is stable for 15minutes that is a proof of saponins. The **Figure 23** on page 66 shows a rack of test tubes with results of the confirmatory foam test for saponins.

2.4.4 Coumarins

Powdered drug (1g) was dissolved in 5ml of methanol and was heated in the water bath at 60°C for 30 minutes. The filtrate (10µl) was applied onto TLC plates.

The solvent system was Toluene: Ether (1:1).

The extracts were tested against the reference, Coumarinic acid in 1% methanolic solution.

Once the plates were dry, they were sprayed with 5% KOH ethanolic solution with the help of an atomizer. The presence of blue spots was interpreted as preliminary presence of coumarins.

The plates, before chemical treatment, were examined under UV light at both 254nm and 365nm. Under 254nm, quenching of fluorescence was expected. Under 365nm, simple coumarins would give blue, green fluorescence and furanocoumarins would give yellow, brown, blue fluorescence.

As for the confirmatory test, no method was found.

2.4.5 Anthracene derivatives

Powdered drug (1g) was dissolved in 5ml of methanol and was heated in the water bath at 60°C. The filtrate (10µl) was applied onto TLC plates.

The solvent systems were

- I. Ethylacetate (EtOAc): Methanol: Water (100:17:13)
- II. Petroleum ether: Ethylacetate (90:10)
- III. Toluene: Ethylacetate (75:25)

The extracts were tested against the reference compounds

1. Aloe juice in 1:4 methanol solution
2. Emodin
3. Aloin
4. Chrysophanol

Once the plates were dry, they were sprayed with Dragendorff reagent in 5% KOH ethanolic solution with the help of an atomizer. The presence of red spots was a sign of anthraquinones. The yellow spots were interpreted as preliminary presence of anthrones and anthronols.

The plates, before chemical treatment, were examined under UV light at both 254nm and 365nm. Under 254nm, quenching of fluorescence was expected. Under 365nm, all anthraquinones give yellow or red-brown fluorescence. After chemical treatment, under 365nm, the presence of red fluorescence was a sign of anthraquinones. The yellow fluorescence were interpreted as preliminary presence of anthrones and anthronols.

The Borntrager reaction is the confirmatory test for free anthraquinones. The drug was shaken up with 10ml of benzene, filtered, and 5ml of ammonia was added to the filtrate. The mixture was shaken and the presence of red, violet, pink colour in the lower ammoniac layer indicated the presence of anthraquinones. For the combined anthraquinones derivatives, the drug was boiled with 5ml of sulphuric acid, filtered while hot. The filtrate was shaken with 5ml of benzene, the benzene layer separated and 10% NH₃ added. Pink, red, violet color in the ammoniac lower layer indicated the presence of combined anthraquinones derivatives.

2.4.6 Tannins

Powdered drug (1g) was dissolved in 10ml distilled water, filtered and FeCl₃ reagent added to the filtrate. The presence of tannins was shown by the blue-black, blue-green or green precipitate.

The **Figure 24** on page 66 shows a test tube with the positive tannin recognition test result.

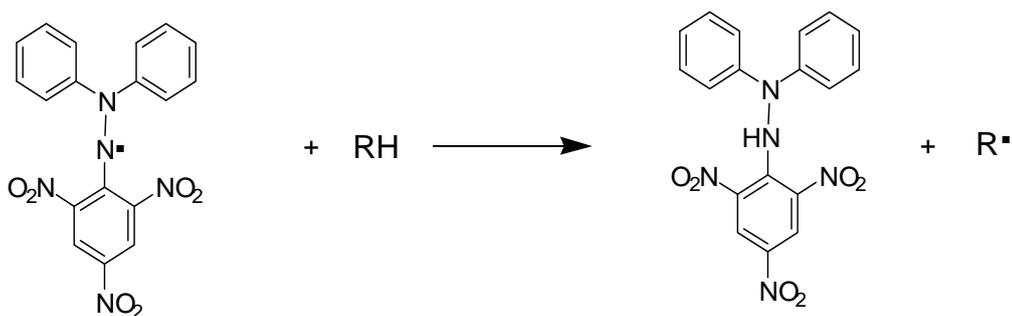
2.5 Antioxidant Activity

The methanolic plant extracts were screened for Antioxidant activity by two assays; the Radical Scavenging Activity and the Determination of Total Phenolic Content.

2.5.1 *Radical Scavenging Activity*

The antiradical activity was measured spectrophotometrically according to the method by Brand-Williams et al, 1995 wherein the bleaching rate of a stable free radical, DPPH (2,2-diphenyl-1—picrylhydrazyl hydrate) is monitored at a characteristic wavelength (λ) in the presence of the methanolic plant extract as the hydrogen-reducing agent.

Fig 15: Reduction of DPPH (2,2-diphenyl-1—picrylhydrazyl hydrate)



0.000625g of DPPH was dissolved in 25 ml of absolute methanol. 1990 μ l of this solution was added to the 10 μ l of plant extract. Before starting the readings, the UV spectrophotometer was set reading a blank with methanol solution only. The changes in the colour from deep-violet to light yellow were measured at 515nm for 20 minutes and the antioxidant activity percentages were calculated according to the equation

$$\% \text{ RSA} = 100 \times \left[1 - \frac{\text{Absorbance of extract } (A_E)}{\text{Absorbance of DPPH } (A_0)} \right]$$

The results of this test are shown on **Table 9** on page 68 and plotted as column graphs on **Figure 25-26** on page 66-67.

2.5.2 Total Phenolic Content Determination

This experiment was carried out according to the method of Velioglu et al, 1998 using Folin Ciocalteu reagent. Extracts were prepared at the concentration of 1mg/ml. 10µl of extract was transferred into test tubes and made up to 1ml with distilled water. 500µl of Folin C reagent (1N) along with 2500µl Na₂CO₃ (5%, w/v) were added, shaken gently and left at room temperature for 40 minutes. A serial dilution of the standard solution of Gallic acid (0.5mg/ml) was prepared to have starting from 0 up to 50µg/ml Gallic acid. The absorbances were read at 725nm using a UV spectrophotometer three fold and the results were expressed as Gallic Acid Equivalents (GAE) in milligrams using plotted standard calibration curve of the Gallic acid. Using the initial amount of plant sample used for extraction, the amount of Total Phenolics was calculated and reported as per mg plant sample. The Total Phenolic Contents of the extracts are shown on **Table 9**, page 68 and drawn as column graphs on **Figure 27**, page 67. Correlation was looked for between these two assays in terms of Antioxidant Activity. The graph for correlation is presented on **Figure 28**, page 69.

2.6 Antiviral Susceptibility Testing

The *VERO Cells* (African Green Monkey Kidney Cells) were purchased from Highveld Ltd, South Africa and were kept in liquid nitrogen tanks at -70°C until used. Before the culturing, the hood should be prepared and cleaned with 70% industrial methylated spirit (IMS). The Media, Phosphate-Buffered Saline Solution (PBS) and the Trypsin were warmed to 37°C in a water bath.

The Media;

RPMI 1640, with L-Glutamine and with NaHCO₃ (2g/l)

Foetal Calf Serum 5%

Amphotericin B, Ampicillin

Tetracycline (Moore GE, 1967)

Phosphate-Buffered Saline Solution (PBS);

NaCl	8.0 g
KH ₂ PO ₄	0.2 g
Na ₂ HPO ₄	1.15 g
KCl	0.2 g
Distilled water	1000 ml (Hsiung, 1982).

Trypsin

1:250 (i.e. trypsin which can digest 250 g substrate for each 1 g trypsin added)

2.6.1 Reviving cell lines from Liquid Nitrogen tanks

The cells were later revived from the liquid nitrogen tanks by thawing quickly at 37°C and washing in 10 ml medium. Afterwards the cells were centrifuged for 10 min at 1000rpm and suspended in 25ml growth medium. They were left in the incubator for 24 hours before they were washed with Phosphate-Buffered Solution (PBS) and fresh growth medium was added.

2.6.2 Subculturing

Once the cells start getting old after 48-72 hours and they are no longer confluent, they need to be passaged onto a new culturing flask with fresh medium. This is called subculturing. The old medium was tipped off from the cell culture flask and the adherent cells were washed with 20ml of Phosphate Buffer Solution (PBS). After this was also poured out to remove all the dead cells, 10 ml of Trypsin was added to rinse the monolayer of cells and all was removed except 2ml, which was then incubated at 30°C for 1 minute to strip the cells attached to the surface of the flask. 5-10 ml of growth medium was added to the flask and mixed up gently to disperse all clumps. At this stage, the counting of the cells was performed (see 2.6.3). Once the cell counting was done, 1×10^5 cells/ml was re-seeded into a new flask with 20 ml of growth medium. The new flask was marked with the name of the cells, the

passage number and the date. The cells were incubated under 5% carbon dioxide at 37°C (Morgan, 1993). The confluent cells can be seen on **Figure 26**, page 72.

2.6.3 Cell counting

The counting of the cells was first done by taking a drop of the cells and mixing it with Trypan Blue to colour the dead cells. Using the Improved Neubauer capillary tube, a couple of drops were taken and put on a slide to count the colourless living cells under the microscope and were counted. When the coverslip is positioned across the central area of the Improved Neubauer, there are two counting chambers with each having five squares (four corners and the centre) enclosing 1 mm². This combined with a 0.1 mm depth between the slide and the coverslip means that the volume of each square is 0.1µl (1mmx1mmx0.1mm). Thus, once the number of cells in a square was counted, the number of cells in 1 ml of the suspension was this value multiplied by 10⁴.

$$\frac{\text{Number of cells counted} \times \text{Dilution factor} \times \text{Volume (10}^4\text{ml)}}{\text{Number of squares counted}}$$

2.6.4 Virus titration

To monitor the antiviral activity, *Herpes Simplex Virus type 2* (HSV-2), purchased from Highveld Ltd, South Africa, was used in this experiments as the virus strain. Virus stocks were kept at -20 °C until use. To titre the virus suspension, confluent monolayer Vero cells were grown in 96-well flat-bottomed microtitre plates and were infected with 100µl of serial tenfold dilution of the virus suspension in quadruplicates to be observed for a period of 7 days. Once the Cytopathic Effect (CPE) was obtained, 50% tissue culture infectious dose (TCID₅₀) was calculated using Reed and Muench method to find the Virus Titer (Reed & Muench, 1938). The appropriate dilution for the antiviral assay was then chosen that contained 100 TCID₅₀ per volume of 0.1ml. The cells with cytopathic effect (CPE) can be seen on **Figure 27**, page 72.

2.6.5 Cytotoxicity of the Plant Extracts

The plant extracts have individual cytotoxicity levels that would cause non-specific cytopathic effect (CPE) on confluent cells and this needed to be known before the antiviral assay to make sure that the CPE observed would entirely be due to the virus suspension and not to the extract. 100µl of the serial two-fold dilution of the plant extracts were introduced to the confluent monolayer Vero cells which were grown in 96-well flat-bottomed microtitre plates in quadruplicates and observed microscopically under 40x magnification for a period of 7 days or evidence of toxicity. This was seen as partial or complete loss of the monolayer or rounding and the shrinkage of the cells. Cytotoxicity levels that caused 50 % CPE in cultured cells were measured according to the Reed and Muench method (Reed & Muench, 1938).

The maximum non-toxic dilution (MNTD) which was the next dilution after the TCID₅₀ of the plant extract was later used in the antiviral screening.

2.6.6 Antiviral Activity Assays

To evaluate the potential antiviral activity of the plant extracts, two assays were carried out. Those were the *End Point Titration Technique (EPTT)* and the *Neutralisation Test (NT)*.

2.6.6.1 End Point Titration Technique (EPTT)

This assay was carried out according to technique described by Cos et al. 2002, Betancur-Galvis et al. 2002, and Vlietinck et al. 1995, with slight modifications. Confluent monolayer VERO cells were grown in 96-well, flat-bottomed microtitre plates. 0.05ml of the maximum non-toxic dilution (MNTD) of the plant extracts in 0.05ml maintenance medium were added in quadruplicates 1h before the viral infection. Cells were infected with the 0.05ml of ten-fold serial dilution of the previously titrated virus suspension and incubated at 37°C. The cells were examined for CPE under the light microscope for 7 days. Controls consisted of noninfected VERO cells and HSV-2 infected untreated cells without any extracts. The antiviral

activity of the plant extract was determined as the reduction factor (RF) of the viral titre. Reduction Factor is the ratio of the virus titre in the absence and in the presence of the extract.

$$\text{Reduction Factor (RF)} = \frac{\text{Virus Titer in absence of extract}}{\text{Virus Titer in presence of extract}}$$

If the RF is $\geq 10^3$, it is a promising antiviral result for the chosen plant extract (Cos et al. 2002, Betancur-Galvis et al. 2002, Vlietinck et al. 1995).

2.6.6.2 Neutralisation Test (NT)

This assay was carried out by adding 0.05ml of the two-fold dilution of the noncytotoxic plant extracts (MNTD) onto the 0.05 ml of the cell suspension in growth medium in quadruplicates. This suspension was infected with 0.05ml of the 100 ID₅₀ of the previously titrated virus suspension. This was sealed tightly, mixed gently and incubated at 37 °C for 7 days and observed for CPE under the light microscope. The endpoint titre is the highest dilution of the plant extract inhibiting 50% of the virus growth expressed as ‘Inhibitory Dose 50’ (ID₅₀).

The calculations were done using the Spearman-Kärber formula (Villegas, 1998)

$$\text{ID}_{50} = x + \frac{1}{2} d - \left(\frac{d \sum r}{n} \right)$$

where

x = the highest dilution at which all cells were uninfected (expressed as the reciprocal)

d = the dilution factor (1)

$\sum r$ = the total number of uninfected cells

n = the number of wells for each dilution

The results for antiviral susceptibility testing are on the **Table 10** on page 71.

2.7 Antimicrobial Susceptibility Testing

2.7.1 Source of microorganisms

The microorganisms used were collected from Medicines Control Authority of Zimbabwe (MCAZ) and these were as Bacteria: *Staphylococcus aureus* NCTC 10788 and *Streptococcus* Group A NCTC 5775, *Escherichia coli* NCTC 10418, *Pseudomonas aeruginosa* NCTC 6750, (National Collection of Type Cultures) and as Fungi: *Candida albicans* NCPF 3179 and *Aspergillus niger* NCPF 2275 (National Collection of Pathogenic Fungi).

2.7.2 Antibacterial Screening

The antibacterial activity of the plant extracts was investigated by the agar well assay, also known as the hole plate diffusion method (Reiner, 1982). Prior to testing, the bacteria from the agar slants were inoculated in sterile Nutrient broth in universal bottles and incubated for 24 hours at 37 C. After incubation, the bottles lacking growth were discarded and new strains obtained.

For the *agar well assay*, 0.1ml of the bacteria suspension was thoroughly mixed with 20ml of autoclave-sterilised Nutrient Agar in sterile Petri dishes. The agar was left to cool and set. The extracts were taken out to thaw in the meantime. Four holes were punched into the agar using a hole borer with diameter of 4mm and the agar was removed from the holes. If the bottom of the Petri dish was exposed, extra amount of agar was squirted in using a micropipette tip in order to avoid leakage of extract from the holes. The plant extracts were aseptically put into the holes at amounts of 25µl for each well. A disk of antimicrobial agents' was put in another Petri dish which was the positive control. The plates were left for an hour to allow diffusion and penetration to the agar. The test substances diffuse into the agar with decreasing concentration towards the periphery. The plates were put into the incubator at 37°C and examined regularly for growth and inhibition.

2.7.3 Antifungal Screening

For antifungal activity, the fungi from the Saboround Dextrose Agar slants were inoculated in sterile Saboround Dextrose Broth in universal bottles and incubated for 72 hours at 37°C. After incubation, the bottles lacking growth were discarded and new strains obtained.

For the *agar well assay*, 0.1 ml of the fungi suspension was thoroughly mixed with 20 ml of autoclave-sterilised Saboround Dextrose Agar in sterile Petri dishes. The rest was the done the same way as in antibacterial testing; the agar was left to cool and set. The extracts were taken out to thaw in the meantime. Four holes were punched into the agar using a hole borer with diameter of 4mm and the agar was removed from the holes. If the bottom of the Petri dish was exposed, extra amount of agar was squirted in using a micropipette tip in order to avoid leakage of extract from the holes. The plant extracts were aseptically put into the holes at amounts of 25µl for each well. A disk of antifungal agents' was put in another Petri dish which was the positive control. The plates were left for an hour to allow diffusion and penetration to the agar. The test substances diffuse into the agar with decreasing concentration towards the periphery. The plates were put into the incubator at 37°C and examined regularly for growth and inhibition.

2.7.4 Sensitivity

The presence of inhibition zones around the wells was interpreted as the indication of antibacterial / antifungal activity. The measurement was taken from the edge of the hole to the end of the inhibition zone for that well. The average zone of inhibition (mm) was calculated for every extract per microorganism with standard deviation.

Another way of expressing the antibacterial / antifungal activity was using the ratio of the inhibition zone of the extract to the zone produced by the control in order to compare and visualize the effectiveness of the extract in a clearer perspective (Vlietinck et al, 1995).

The antibacterial results are expressed on **Table 11-12**, page 73-74 and the antifungal results are on **Table 13-14**, page 75-76. Some of the pictures of the best results for both the antibacterial and antifungal tests can be seen on **Figures 31-36** on page 77.

2.8 Toxicity / Bioactivity Tests

The Bioactivity tests were conducted using the Brine Shrimp (*Artemia salina*) Lethality Test (BSLT) according to McLaughlin *et al*, 1991.

2.8.1 Hatching the Brine Shrimp (Artemia salina) eggs

Artificial seawater was prepared by dissolving sea salt (38.0 g) in distilled water (1 L). The two compartments plastic chamber with several holes on the divider was used for hatching. The eggs were sprinkled into the other compartment which was darkened, while the other compartment was illuminated. After 24 hours of incubation at room temperature (25-28°C) and pH 7.0, nauplii (larvae) were collected by pipette from the illuminated side. In the mean time, the test tubes used were washed and sterilized in an autoclave machine.

2.8.2 Bioassay

Five different concentrations of plant extracts were prepared, using brine in triplicates (1000, 500, 100, 50, 10 µg/ml). Nauplii were drawn through a glass capillary, counted into tens and placed in each vial containing serial dilutions of the extract and brought up to 5 ml of brine solution. Thereafter, recordings were taken at 6, 12 and 24 hours counting the surviving shrimps.

The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. ‘Lethal Concentration₅₀’ (LC₅₀) values, the concentration of the extract to kill 50% of the shrimps, were obtained from the best-fit line plotted concentration versus percentage lethality (Krishnaraju *et al*, 2005).

Nerium oleander leaves were used as a positive control in the bioassay. AnvirzelTM, a patented hot-water extract of *Nerium oleander*, is currently being studied in phase I trials for

its anti-tumour effects. It contains oleandrin and other cardiac glycosides with digoxin-like effects, and the species is toxic with well-described reports of fatal ingestion. The anti-cancer effects of oleander extracts are being investigated largely in *in-vitro* cell line models. Traditional uses have included treatment of swelling, leprosy, eye diseases, and skin disorders. Oleander has been used as an abortifacient, a known instrument of homicide, and gained popularity as an agent used in suicide attempts in Sri Lanka in the 1980s. The "cardiotonic" effects of *oleander* were investigated in the 1930s, but this use was largely abandoned due to significant gastrointestinal toxicity and a perceived narrow therapeutic to toxic window. *Oleander* extracts have been used in China to treat neurologic and psychiatric disorders.

Many trial runs were done as a part of the Laboratory System Suitability tests to find out the best conditions in order to achieve good and acceptable results by making changes in the pH, bowl size, type of light and temperature. The LC₅₀ results of extracts are on **Table 15**, page 78.

2.9 Statistical Analysis

The results were put on the computer program GraphPad Prism 5.0 and using linear regression, best-fit lines were drawn and unknowns were obtained from these graphs. Pearson's two-tailed analysis with 95% confidence intervals was used for finding correlations.

CHAPTER III

3.0 RESULTS

3.1 Plant Extraction

Table 6: Plant extracts' yields in grams and as percentages of original dry material

Plants	Yield in grams	Yield as %
<i>Cassia abbreviata</i> bark	5.12 ± 0.11	12.79 ± 0.27
<i>Cassia abbreviata</i> leaves	4.10 ± 0.17	10.25 ± 0.43
<i>Cassia abbreviata</i> roots	5.87 ± 0.13	16.31 ± 0.37
<i>Dichrostachys cinerea</i> leaves	4.21 ± 0.18	14.53 ± 0.62
<i>Dichrostachys cinerea</i> roots	3.21 ± 0.23	10.71 ± 0.75
<i>Elaeodendron matabelicum</i> roots	2.96 ± 0.12	7.40 ± 0.30
<i>Elephantorrhiza goetzei</i> roots	7.38 ± 0.19	18.46 ± 0.47
<i>Flacourtia indica</i> leaves	2.54 ± 0.19	12.68 ± 0.93
<i>Flacourtia indica</i> roots	3.61 ± 0.23	9.04 ± 0.59
<i>Gymnosporia senegalensis</i> leaves	4.07 ± 0.11	20.37 ± 0.25
<i>Gymnosporia senegalensis</i> roots	1.46 ± 0.13	4.85 ± 0.43
<i>Gymnosporia senegalensis</i> twigs	2.15 ± 0.19	10.77 ± 0.94
<i>Hypoxis rooperi</i> tuber	4.90 ± 0.13	16.34 ± 0.44
<i>Khaya anthotheca</i> bark	3.17 ± 0.09	15.85 ± 0.45
<i>Khaya anthotheca</i> roots	2.87 ± 0.14	14.35 ± 0.62
<i>Kigelia africana</i> bark	1.44 ± 0.21	7.22 ± 1.05
<i>Kigelia africana</i> fruit	3.95 ± 0.25	19.73 ± 1.23
<i>Kigelia africana</i> roots	3.85 ± 0.46	19.27 ± 2.30
<i>Rhus chirindensis</i> leaves	3.38 ± 0.11	8.45 ± 0.28
<i>Rhus chirindensis</i> roots	1.88 ± 0.12	9.38 ± 0.60
<i>Sclerocarya birrea</i> bark	5.99 ± 0.15	14.98 ± 0.37
<i>Securidaca longepedunculata</i> roots	2.22 ± 0.23	11.67 ± 1.21
<i>Terminalia sericea</i> leaves	8.00 ± 0.16	20.00 ± 0.41
<i>Terminalia sericea</i> roots	11.00 ± 0.35	27.51 ± 0.87
<i>Warburgia salutaris</i> bark	1.88 ± 0.12	9.42 ± 0.58
<i>Warburgia salutaris</i> leaves	2.77 ± 0.16	13.87 ± 0.80
<i>Warburgia salutaris</i> roots	2.27 ± 0.26	11.33 ± 1.29
<i>Warburgia salutaris</i> twigs	1.07 ± 0.56	5.35 ± 0.53

3.2 Phytochemical Screening

Table 7: Thin Layer Chromatography, UV and Confirmatory Tests' results of selected plants

PLANT NAMES	ALKALOIDS			FLAVONOIDS			SAPONINS			COUMA		ANTHRAQUI			TANNIN
	UV	TLC	CON	UV	TLC	CON	UV	TLC	CON	UV	TLC	UV	TLC	CON	CON
<i>C. abbreviata</i> bark	-	-	-	++	+++	++	++	++	++	++	++	+++	++	+++	+++
<i>C. abbreviata</i> leaves	+++	++	++	++	++	++	-	-	-	++	+++	+++	+++	+++	-
<i>C. abbreviata</i> roots	+++	++	++	+++	++	++	++	+++	++	++	++	+++	+++	+++	-
<i>D. cinerea</i> leaves	++	+++	++	+++	++	+++	++	+	++	-	-	++	++	++	+++
<i>D. cinerea</i> roots	-	-	-	-	-	-	+	+	++	-	-	-	-	-	+++
<i>E. matabelicum</i> root	-	-	-	-	-	-	++	+++	++	-	-	-	-	-	+++
<i>E. goetzei</i> roots	-	-	-	++	++	+++	++	++	++	+++	++	-	-	-	++
<i>F. indica</i> leaves	-	-	-	-	-	-	++	++	-	+	+	++	++	++	+++
<i>F. indica</i> roots	-	-	-	-	-	-	-	-	-	-	-	++	++	++	++
<i>G. senegalensis</i> leaf	-	-	-	++	++	++	++	++	++	-	-	++	++	++	++
<i>G. senegalensis</i> root	-	-	-	-	-	-	++	++	++	-	-	-	-	-	++
<i>G. senegalensis</i> twig	-	-	-	-	+	++	+	+	+	-	-	-	-	-	++
<i>H. rooperi</i> tuber	-	-	-	-	-	-	+++	+++	++	-	-	++	+	++	++
<i>K. anthotheca</i> bark	-	-	-	++	+	++	+	+	+	+	++	++	++	+++	-
<i>K. anthotheca</i> roots	-	-	-	++	++	+++	+	+	+	++	+	++	+	++	++
<i>K. africana</i> bark	-	-	-	++	++	++	++	+++	++	++	+	+	+	++	+++
<i>K. africana</i> fruit	++	+	+	++	++	-	++	++	++	++	+	+	+	++	+++
<i>K. africana</i> roots	-	-	-	+++	+	++	++	++	+	++	+	+	+	++	+
<i>R. chirindensis</i> leaf	-	-	-	+	++	+++	+	+	-	-	-	+	++	++	++
<i>R. chirindensis</i> root	-	-	-	++	+	+++	+	+	+	++	+	-	-	-	+++
<i>S. birrea</i> bark	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+++
<i>S. longeped.</i> root	+++	+	++	++	++	++	++	++	+++	-	-	++	++	+	+++
<i>T. sericea</i> leaves	-	-	-	+	++	+++	++	+	++	-	-	++	++	+	+++
<i>T. sericea</i> roots	-	-	-	++	+++	++	+++	+++	+++	-	-	-	-	-	+++
<i>W. salutaris</i> bark	++	++	+++	+	+	++	+	+	+	+++	++	-	-	-	+++
<i>W. salutaris</i> leaves	-	-	-	-	-	-	++	+	+	-	-	++	+	++	+++
<i>W. salutaris</i> roots	+++	+	++	++	+	+	+	+	+	+++	++	-	-	-	+++
<i>W. salutaris</i> twigs	+++	+	++	+	+	+	+	+	-	+++	+	-	-	-	+++

KEY

Couma	Coumarin
Anthraqui	Anthraquinones
UV	Ultraviolet Light
TLC	Thin Layer Chromatography
Con	Confirmatory Test

- +** For TLC and UV it means feint small zones, trace quantities detected
For flavonoids, tannins, and anthraquinones confirmatory tests it means very feint coloration, trace quantities detected
For alkaloids it means a cloudy precipitate on confirmatory tests, trace quantities detected.
For saponins it means a froth length of 0.5-1.0cm on confirmatory tests.
- ++** For TLC and UV it means medium coloured and fluorescing zones.
For flavonoids, tannins, and anthraquinones confirmatory tests it means a medium colour of precipitation or layer.
For alkaloids it means a heavy precipitate on confirmatory tests.
For saponins it means a froth length of 2-5cm on confirmatory tests.
- +++** For TLC and UV it means dark and well defined zones.
For flavonoids, tannins and anthraquinones confirmatory tests it means a very dark colour precipitation or layer. For alkaloids it means a heavy precipitate with flocculation on confirmatory tests. For saponins it means a froth length of > 5cm on confirmatory tests.
- Absent (no tested compounds).

Fig 19: Plant extracts and reference compounds



Fig 20: Student, Iklım Viol, doing Paper Chromatography



Table 8: Phytochemical Tests: Compounds indicated/found through PC and TLC when tested against reference compounds as a part of the study at the University of Istanbul, Faculty of Pharmacy.

Plant Extracts	Alkaloids	Glycosides	Aglycones	Anthraquinones
<i>Cassia abbreviata</i> root	positive results but no matching ref; Cassine?	Isovitexin	2-3 positive spots but no matching reference	Chrysophanol Emodin Aloe-emodin?
		Hyperoside		
<i>Dichrostachys cinerea</i> leaf	Berberin	Quercitrin	Hesperetin	positive results but no matching reference
		Isoquercitrin		
<i>Elaedendron matabelicum</i> root	n/a	n/a	n/a	n/a
<i>Elephantorrhiza goetzei</i> root	n/a	Astragalin	n/a	n/a
		Hesperidin		
		Elephantorrhizol?		
<i>Gymnosporia senegalensis</i> leaf	n/a	Hesperidin	n/a	positive results but no matching reference
<i>Gym. senegalensis</i> root	n/a	n/a	n/a	n/a
<i>Securidaca longepedunculata</i> root	positive results but no matching reference	Apigenin 7-O-glucoside	n/a	positive results but no matching reference; Aloin?
		Luteolin 7-O-glucoside		
		Naringenin 7-O-glucoside		
<i>Terminalia sericea</i> root	n/a	Vicenin-2	n/a	n/a
		Vitexin		
		Isoquercitrin		

Fig 21: TLC plate for alkaloids

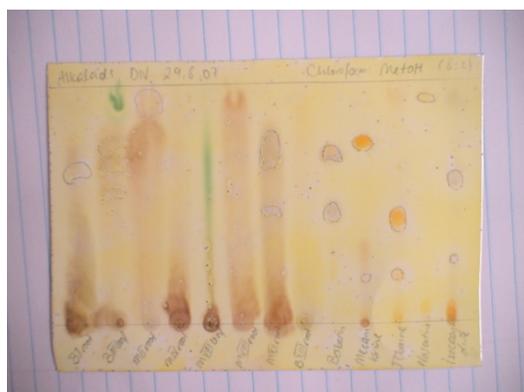


Fig 22: TLC plate for glycoside flavonoids

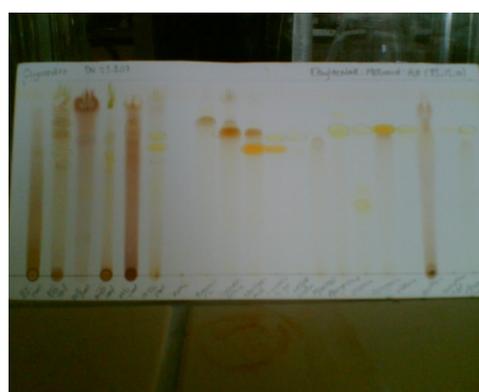


Fig 23: Saponins, confirmatory foam test



Fig 24: Tannins, recognition test (blue-black precipitation)



3.3 Antioxidant Activity

Fig 25: Antioxidant Activity of plant extracts expressed as Radical Scavenging Activity (RSA) in percentages (%) at wavelength (λ) of 515nm.

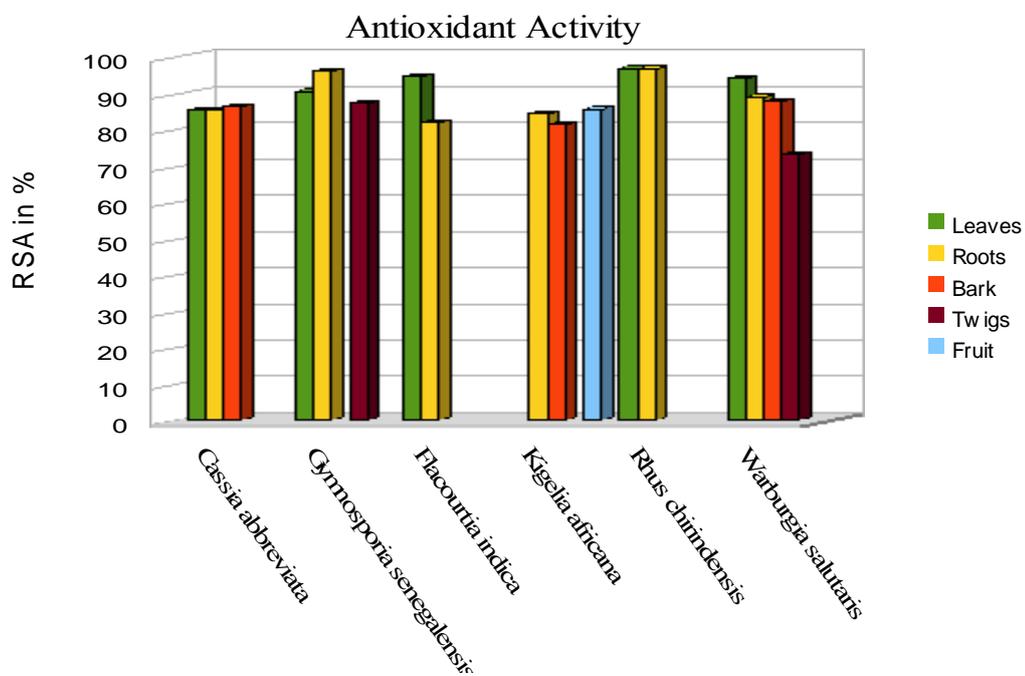


Fig 26: Antioxidant Activity of plant extracts expressed as Radical Scavenging Activity (RSA) in percentages (%) at wavelength (λ) of 515nm.

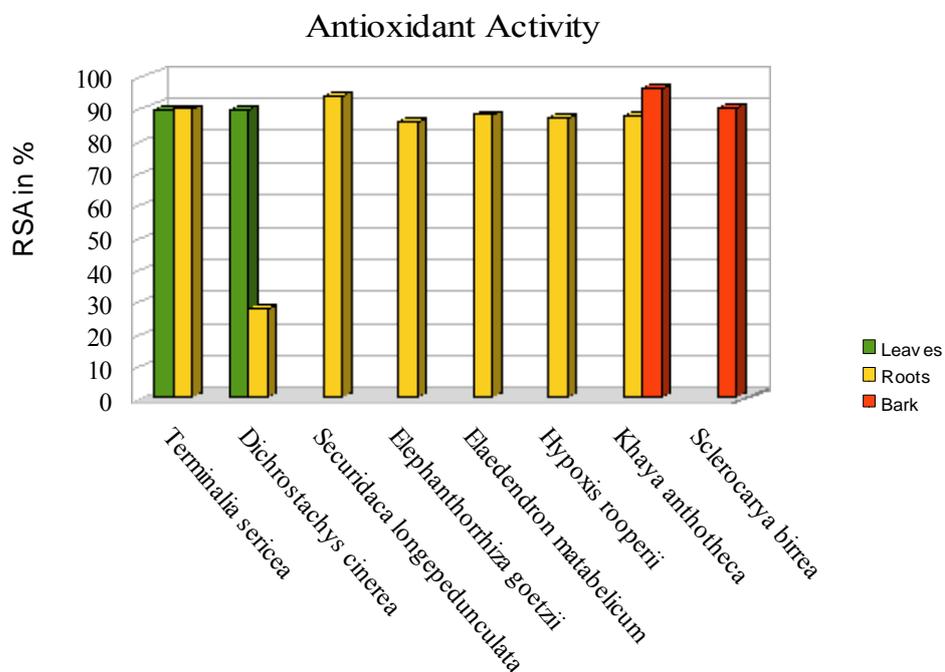


Fig 27: Total Phenolic Contents of plant extracts as mg Gallic Acid Equivalents (GAE) per mg plant material

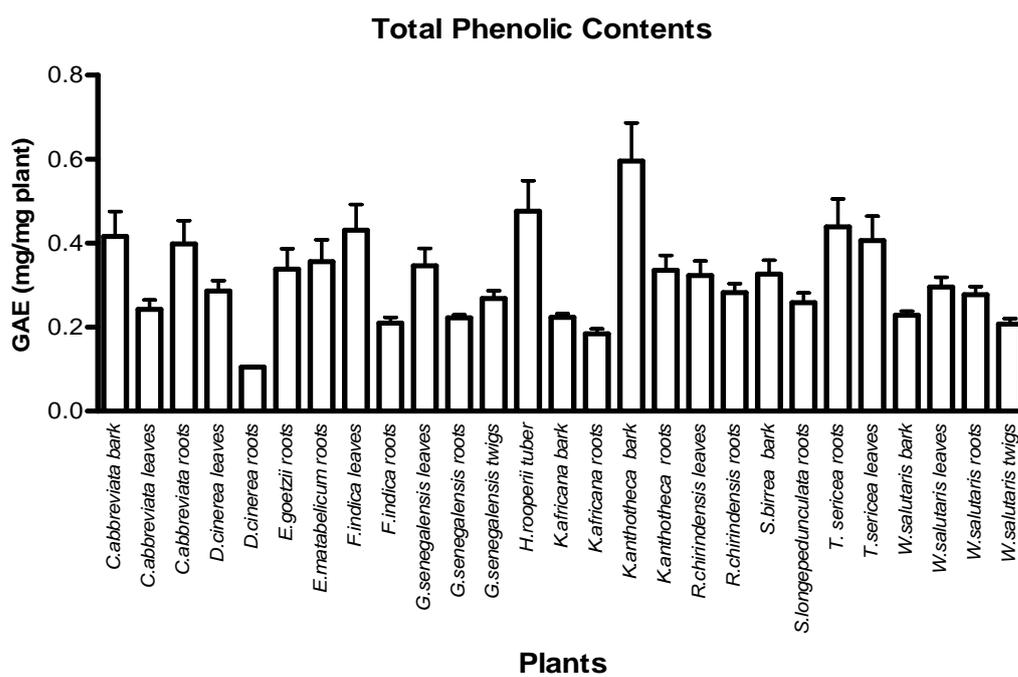


Table 9: Antioxidant Activity expressed as Radical Scavenging Activity (RSA) and Total Phenolic Contents of plant extracts

Plant Parts	RSA %	TPC mg GAE/mg plant sample
<i>Cassia abbreviata</i> bark	86.36 ± 0.04	0.416 ± 0.103
<i>Cassia abbreviata</i> leaves	85.49 ± 0.31	0.243 ± 0.039
<i>Cassia abbreviata</i> roots	85.39 ± 0.04	0.398 ± 0.097
<i>Dichrostachys cinerea</i> leaves	88.97 ± 0.46	0.286 ± 0.043
<i>Dichrostachys cinerea</i> roots	27.39 ± 1.24	0.105 ± 0.003
<i>Elaedendron matabelicum</i> roots	87.64 ± 0.02	0.357 ± 0.090
<i>Elephantorrhiza goetzei</i> roots	85.69 ± 0.03	0.339 ± 0.084
<i>Flacourtia indica</i> leaves	94.87 ± 0.76	0.431 ± 0.106
<i>Flacourtia indica</i> roots	82.01 ± 0.19	0.210 ± 0.025
<i>Gymnosporia senegalensis</i> leaves	90.55 ± 0.67	0.346 ± 0.072
<i>Gymnosporia senegalensis</i> roots	96.05 ± 0.18	0.222 ± 0.014
<i>Gymnosporia senegalensis</i> twigs	87.28 ± 0.10	0.268 ± 0.033
<i>Hypoxis rooperi</i> tuber	86.62 ± 0.26	0.476 ± 0.127
<i>Khaya anthotheca</i> bark	96.05 ± 0.05	0.596 ± 0.157
<i>Khaya anthotheca</i> roots	87.43 ± 0.03	0.336 ± 0.060
<i>Kigelia africana</i> bark	81.49 ± 0.19	0.224 ± 0.015
<i>Kigelia africana</i> fruit	85.64 ± 0.13	0.327 ± 0.061
<i>Kigelia africana</i> roots	84.57 ± 0.03	0.184 ± 0.020
<i>Rhus chirindensis</i> leaves	96.91 ± 0.33	0.323 ± 0.060
<i>Rhus chirindensis</i> roots	96.90 ± 0.49	0.282 ± 0.037
<i>Sclerocarya birrea</i> bark	89.63 ± 0.05	0.326 ± 0.058
<i>Securidaca longepedunculata</i> roots	93.43 ± 0.64	0.258 ± 0.040
<i>Terminalia sericea</i> leaves	89.27 ± 0.13	0.439 ± 0.115
<i>Terminalia sericea</i> roots	89.38 ± 0.02	0.406 ± 0.100
<i>Warburgia salutaris</i> bark	73.28 ± 1.09	0.208 ± 0.022
<i>Warburgia salutaris</i> leaves	87.74 ± 0.03	0.228 ± 0.018
<i>Warburgia salutaris</i> roots	94.08 ± 0.87	0.296 ± 0.040
<i>Warburgia salutaris</i> twigs	89.07 ± 0.02	0.278 ± 0.033
<i>β-carotene, reference</i>	98.84 ± 0.65	-

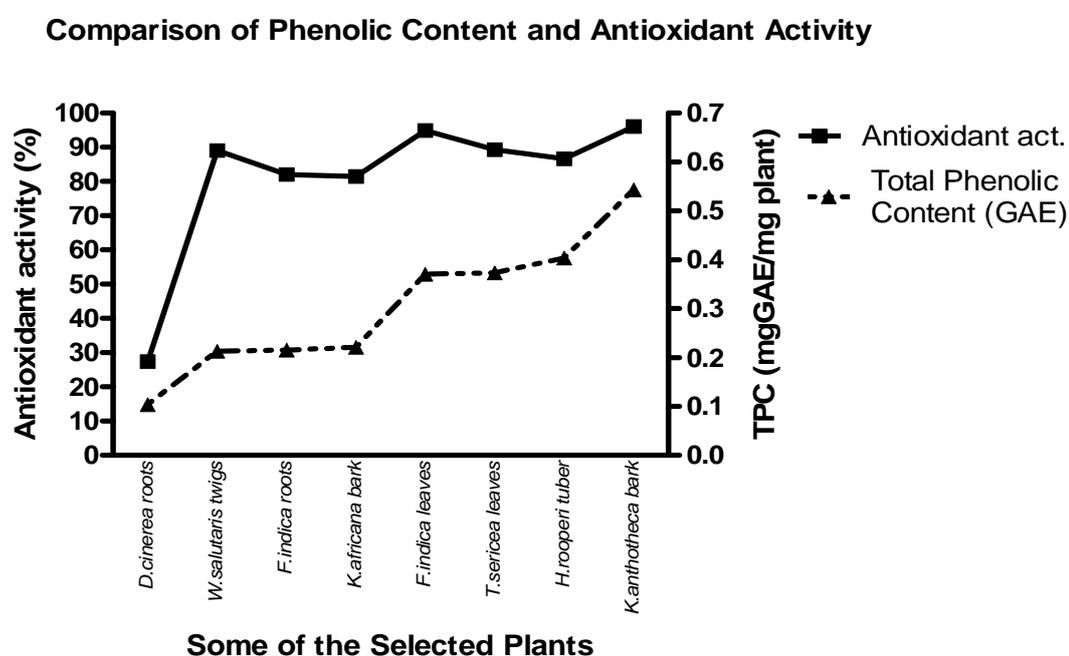
Key:

RSA (%) = Radical Scavenging Activity of Extract expressed as percentage inhibition of the free radical, DPPH.

TPC = Total Phenolic Content expressed as Gallic Acid Equivalent (GAE) in milligrams per mg plant material

The content of phenolic compounds (mg/mg) in methanolic extracts, determined from the calibration curve of the standard Gallic acid ($r^2=0.98$), are summarized in **Tab 9**, page 68 and **Fig 27**, page 67. The highest amount was found in *Khaya anthotheca bark* extract (0.596) which also had the highest antioxidant activity and the lowest was the *Dichrostachys cinerea root* extract (0.105) which had the lowest antioxidant activity.

Fig 28: Comparison of Phenolic Contents and the Percentage Inhibitions of selected extracts



It was observed that the contents of the phenolic compounds in the extracts correlate with their antiradical activity (Pearson's two-tailed, 95% confidence interval, correlation coefficient $R^2=0.57$), confirming that the phenolics are likely to cause the radical scavenging activity, as it can be seen on **Fig 28** above.

3.4 Antiviral Screening

3.4.1 Cytotoxicity of plant extracts

The maximal non-toxic dose (MNTD) was chosen for plant extracts after cytotoxicity tests. The toxicity was seen as partial or complete loss of the monolayer and rounding and shrinkage of the cells. The results for this test are summarised in **Table 10**, page 71.

3.4.2 Herpes Simplex Virus type-2 Titre

The *HSV-2* virus titre was calculated using the Reed and Muench method as previously explained in methodology. It was found to be $TCID_{50} = 10^{-8.5}$ per 0.1ml virus suspension. Therefore, the concentration of the virus that needs to be used in the assay, the 100 $TCID_{50}$, would be $10^{-6.5}$.

3.4.3 Antiviral Assays

3.4.3.1 EPTT Assay

The results of the *End Point Titration Technique (EPTT)* are expressed as the Reduction Factor (RF) and are presented in **Table 10**, page 71. Reduction Factor is the ratio of the virus titre in the absence and in the presence of the extract. A RF value of $10^3 - 10^4$ indicates a pronounced antiviral activity, suitable as selection criterion for further investigation.

3.4.3.2 NT Assay

In *Neutralisation Test (NT)*, the main purpose was to measure the maximal non-toxic dose of the plant extract that inhibits the 50% of the virus, HSV-2 (ID_{50}). The results are summarised in **Table 10** on page 71.

Table 10: Antiviral Screening and Cytotoxicity results of some Zimbabwean Traditional Medicinal Plants

Plant Extracts	Cytotoxicity µg/ml	NT ^a ID ₅₀ in µg/ml	EPTT ^b Reduction Factor
<i>Cassia abbreviata</i> bark	39.06	No activity	1
<i>Cassia abbreviata</i> leaves	156.25	20.83	10 ²
<i>Cassia abbreviata</i> roots	156.25	10.41	10 ³
<i>Dichrostachys cinerea</i> leaves	78.13	10.41	10 ⁴
<i>Dichrostachys cinerea</i> roots	312.50	83.33	10 ²
<i>Elaedendron matabelicum</i> roots	78.13	No activity	1
<i>Elephantorrhiza goetzei</i> roots	156.25	83.33	10 ²
<i>Flacourtia indica</i> leaves	78.13	83.33	10 ²
<i>Flacourtia indica</i> roots	156.25	125.00	10 ³
<i>Gymnosporia senegalensis</i> leaves	39.06	10.41	10 ³
<i>Gymnosporia senegalensis</i> roots	78.13	20.83	10 ³
<i>Gymnosporia senegalensis</i> twigs	19.53	15.63	10 ³
<i>Hypoxis rooperi</i> tuber	156.25	10.41	10 ³
<i>Khaya anthotheca</i> bark	39.06	31.25	10 ²
<i>Kigelia africana</i> bark	39.06	31.25	10 ³
<i>Kigelia africana</i> fruit	39.06	31.25	10 ⁴
<i>Kigelia africana</i> roots	78.13	No activity	1
<i>Rhus chirindensis</i> leaves	312.50	No activity	1
<i>Rhus chirindensis</i> roots	78.13	20.83	10 ²
<i>Sclerocarya birrea</i> bark	39.06	20.83	10 ³
<i>Securidaca longepedunculata</i> roots	78.13	20.83	10 ³
<i>Terminalia sericea</i> leaves	39.06	31.25	10 ²
<i>Terminalia sericea</i> roots	39.06	20.83	10 ³
<i>Warburgia salutaris</i> bark	19.53	No activity	1
<i>Warburgia salutaris</i> leaves	78.13	No activity	1
<i>Warburgia salutaris</i> roots	39.06	31.25	10 ³
<i>Acyclovir</i> , reference antiviral	-	1.50	-

^a = Neutralisation Test; The maximal non-toxic dilution of the plant extract to inhibit 50% of the virus HSV-2 growth, expressed in µg/ml

^b = End Point Titration Technique; The antiviral activity is expressed as the RF (reduction factor) of the viral titre i.e. the ratio of the viral titre of the virus control to the virus titre in the presence of the maximal non-toxic dose of the plant extract.

Fig 29: Control, VERO confluent cells



Fig 30: Cytopathic effect, CPE, 50-60%



3.5 Antimicrobial Activity

The zones of inhibition of the extracts and the reference materials were measured in millimetres. Zones of inhibitions for Antibacterial Activity are given on **Table 11**, page 73 and for Antifungal Activity on **Table 13**, page 75.

The ratio of the zone of inhibition of the plant extract to the zone of inhibition of the most effective antibacterial reference is calculated and these results are summarized on **Table 12**, page 74 for the Antibacterial and on **Table 14**, page 76 for the Antifungal activity.

Table 11: Average Zones of Inhibition (mm) of the Plant Extracts (10mg/ml) and the References (10µg/disk) against Gram (+) and Gram (-) Bacteria Strains

Plant Extracts (10mg/ml) and References (10µg/disk)	Antibacterial Activity			
	Zone of Inhibition (mm)			
	<i>Staphylococcus aureus</i>	<i>Streptococcus grp A</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
<i>Cassia abbreviata</i> bark	3.00±0.41	4.50±0.58	-	-
<i>Cassia abbreviata</i> leaves	-	2.00±0.00	-	-
<i>Cassia abbreviata</i> roots	1.50±0.41	2.13±0.63	-	3.00±0.41
<i>Dichrostachys cinerea</i> leaves	2.13±0.25	2.50±0.58	-	4.75±0.65
<i>Dichrostachys cinerea</i> roots	-	-	-	4.00±0.82
<i>Elaedendron matabelicum</i> root	5.00±0.41	4.63±0.48	2.50±0.58	5.50±0.58
<i>Elephantorrhiza goetzei</i> roots	4.00±0.0	4.50±0.58	-	-
<i>Flacourtia indica</i> leaves	-	-	-	-
<i>Flacourtia indica</i> roots	-	3.00±0.41	-	1.00±0.00
<i>Gymnosp. senegalensis</i> leaves	2.50±0.41	3.00±0.71	4.50±0.58	5.00±0.82
<i>Gymnosp. senegalensis</i> roots	5.13±0.63	2.00±0.00	4.00±0.41	1.00±0.00
<i>Gymnosp. senegalensis</i> twigs	4.00±0.41	2.00±0.00	2.00±0.00	7.00±0.41
<i>Hypoxis rooperi</i> tuber	-	-	-	-
<i>Khaya anthotheca</i> bark	4.00±0.0	2.00±0.00	-	-
<i>Kigelia africana</i> bark	3.00±0.41	5.00±0.41	1.00±0.00	3.75±0.50
<i>Kigelia africana</i> fruit	2.23±0.50	4.00±0.41	-	4.50±0.58
<i>Kigelia africana</i> roots	-	2.00±0.41	-	4.00±0.82
<i>Rhus chirindensis</i> leaves	2.23±0.50	1.00±0.41	-	3.00±0.82
<i>Rhus chirindensis</i> roots	4.88±0.25	3.50±0.58	-	-
<i>Sclerocarya birrea</i> bark	4.50±0.41	5.50±0.58	3.00±0.82	4.00±0.82
<i>Sec. longepedunculata</i> roots	1.50±0.41	3.50±0.58	-	-
<i>Terminalia sericea</i> leaves	1.13±0.25	3.50±0.58	-	-
<i>Terminalia sericea</i> roots	7.88±0.48	8.50±0.58	2.00±0.41	10.00±0.82
<i>Warburgia salutaris</i> bark	5.00±0.82	3.00±0.41	-	-
<i>Warburgia salutaris</i> leaves	2.00±0.0	-	-	-
<i>Warburgia salutaris</i> roots	5.50±0.41	9.50±0.58	3.50±0.58	2.00±0.41
<i>Amoxicillin trihydrate- Ref</i>	9.00±0.41	10.50±0.58	4.75±0.96	0.88±0.63
<i>Chloramphenicol- Reference</i>	9.00±0.82	10.00±0.82	4.00±0.41	1.38±0.58
<i>Co-trimoxazole - Reference</i>	8.00±0.41	12.00±0.82	2.50±0.58	1.38±0.58
<i>Gentamicin- Reference</i>	8.00±0.82	9.25±0.65	12.00±0.82	7.00±0.40

Table 12: Antibacterial Activity of the Plant Extracts in terms of the most active Reference Antibacterial for that specific strain; represented as a ratio

Plant Extracts (10mg/ml) & References (10µg/disk)	Antibacterial Activity ¹			
	<i>Staphylococcus aureus</i>	<i>Streptococcus group A</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
<i>Cassia abbreviata</i> bark	0.33	0.38	0	0
<i>Cassia abbreviata</i> leaves	0	0.17	0	0
<i>Cassia abbreviata</i> roots	0.17	0.17	0	0.43
<i>Dichrostach cinerea</i> leaves	0.22	0.21	0	0.71
<i>Dichrostachys cinerea</i> roots	0	0	0	0.57
<i>Elaeodend matabelicum</i> root	0.56	0.38	0.21	0.79
<i>Elephantorrhhi goetzei</i> roots	0.44	0.38	0	0
<i>Flacourtia indica</i> leaves	0	0	0	0
<i>Flacourtia indica</i> roots	0	0.25	0	0.14
<i>Gymnosp senegalensis</i> leaf	0.28	0.25	0.38	0.71
<i>Gymnosp senegalensis</i> root	0.56	0.17	0.33	0.14
<i>Gymnosp senegalensis</i> twig	0.44	0.17	0.17	1.00
<i>Hypoxis rooperi</i> tuber	0	0	0	0
<i>Khaya anthotheca</i> bark	0.44	0.17	0	0
<i>Kigelia africana</i> bark	0.33	0.42	0.08	0.57
<i>Kigelia africana</i> fruit	0.22	0.33	0	0.64
<i>Kigelia africana</i> roots	0	0.17	0	0.57
<i>Rhus chirindensis</i> leaves	0.22	0.08	0	0.43
<i>Rhus chirindensis</i> roots	0.56	0.29	0	0
<i>Sclerocarya birrea</i> bark	0.50	0.46	0.25	0.57
<i>Sec. longepedunculata</i> root	0.17	0.29	0	0.29
<i>Terminalia sericea</i> leaves	0.11	0.29	0	0
<i>Terminalia sericea</i> roots	0.83	0.71	0.17	1.43
<i>Warburgia salutaris</i> bark	0.56	0.25	0	0
<i>Warburgia salutaris</i> leaves	0.22	0	0	0
<i>Warburgia salutaris</i> roots	0.61	0.79	0.29	0.29
<i>Amoxicillin trihydrate-Ref</i>	1	0.88	0.40	0.13
<i>Chloramphenicol-Ref</i>	1	0.83	0.33	0.20
<i>Co-trimoxazole-Reference</i>	0.89	1	0.21	0.20
<i>Gentamicin-Reference</i>	0.89	0.75	1	1

¹ Hole plate diffusion method; the antibacterial activity is expressed as the ratio of the inhibition zone of the extract (10mg/ml) to the inhibition zone of the most active reference antibacterial for that specific strain (10µg/disk)

Table 13: Average Zones of Inhibition (mm) of Plant Extracts (10mg/ml) and References (10µg/disk) against Fungi Strains

Plant Extracts (10mg/ml) & References (10µg/disk)	Antifungal Activity	
	Zone of Inhibition (mm)	
	<i>Candida albicans</i>	<i>Aspergillus niger</i>
<i>Cassia abbreviata</i> bark	-	3.00±0.41
<i>Cassia abbreviata</i> leaves	-	2.00±0.41
<i>Cassia abbreviata</i> roots	-	3.50±0.41
<i>Dichrostachys cinerea</i> leaves	-	2.13±0.25
<i>Dichrostachys cinerea</i> roots	1.63±0.25	2.00±0.41
<i>Elaeodendron matabelicum</i> roots	-	-
<i>Elephantorrhiza goetzei</i> roots	-	-
<i>Flacourtia indica</i> leaves	-	1.88±0.25
<i>Flacourtia indica</i> roots	-	2.00±0.0
<i>Gymnosporia senegalensis</i> leaves	-	2.00±0.41
<i>Gymnosporia senegalensis</i> roots	2.13±0.25	2.13±0.25
<i>Gymnosporia senegalensis</i> twigs	-	-
<i>Hypoxis rooperi</i> tuber	1.75±0.65	3.13±0.25
<i>Khaya anthotheca</i> bark	-	-
<i>Khaya anthotheca</i> roots	n/a	n/a
<i>Kigelia africana</i> bark	2.00±0.82	3.00±0.41
<i>Kigelia africana</i> fruit	-	2.00±0.0
<i>Kigelia africana</i> roots	-	-
<i>Rhus chirindensis</i> leaves	-	1.00±0.41
<i>Rhus chirindensis</i> roots	-	-
<i>Sclerocarya birrea</i> bark	-	2.00±0.0
<i>Securidaca longepedunculata</i> roots	2.00±0.41	4.25±0.50
<i>Terminalia sericea</i> leaves	-	1.88±0.25
<i>Terminalia sericea</i> roots	2.00±0.41	3.25±0.50
<i>Warburgia salutaris</i> twigs	n/a	n/a
<i>Warburgia salutaris</i> bark	4.25±0.65	5.25±0.50
<i>Warburgia salutaris</i> leaves	2.00±0.82	3.25±0.50
<i>Warburgia salutaris</i> roots	10.00±0.82	8.25±0.50
Miconazole - Reference	10.00±0.41	10.00±0.81
Amphotericin B - Reference	6.35±0.50	6.75±0.58

Table 14: Antifungal Activity of the Plant Extracts in terms of the most active Reference Antifungal for that specific strain; represented as a ratio

Plant Extracts (10mg/ml) & References (10µg/disk)	Antifungal	
	<i>Candida albicans</i>	<i>Aspergillus niger</i>
<i>Cassia abbreviata</i> bark	0	0.3
<i>Cassia abbreviata</i> leaves	0	0.2
<i>Cassia abbreviata</i> roots	0	0.35
<i>Dichrostachys cinerea</i> leaves	0	0.2
<i>Dichrostachys cinerea</i> roots	0.15	0.2
<i>Elaedendron matabelicum</i> roots	0	0
<i>Elephantorrhiza goetzei</i> roots	0	0
<i>Flacourtia indica</i> leaves	0	0.2
<i>Flacourtia indica</i> roots	0	0.2
<i>Gymnosporia senegalensis</i> leaves	0	0.2
<i>Gymnosporia senegalensis</i> roots	0.2	0.2
<i>Gymnosporia senegalensis</i> twigs	0	0
<i>Hypoxis rooperi</i> tuber	0.15	0.3
<i>Khaya anthotheca</i> bark	0	0
<i>Kigelia africana</i> bark	0.2	0.3
<i>Kigelia africana</i> fruit	0	0.2
<i>Kigelia africana</i> roots	0	0
<i>Rhus chirindensis</i> leaves	0	0
<i>Rhus chirindensis</i> roots	0	0
<i>Sclerocarya birrea</i> bark	0	0.2
<i>Securidaca longepedunculata</i> root	0.2	0.4
<i>Terminalia sericea</i> leaves	0	0.2
<i>Terminalia sericea</i> roots	0.2	0.3
<i>Warburgia salutaris</i> bark	0.4	0.5
<i>Warburgia salutaris</i> leaves	0.2	0.3
<i>Warburgia salutaris</i> roots	1	0.8
Miconazole-Reference	1	1
Amphotericin B - Reference	0.64	0.68

¹ Hole plate diffusion method; the antifungal activity is expressed as the ratio of the inhibition zone of the extract (10mg/ml) to the inhibition zone of the most active reference antifungal for that specific strain (10µg/disk).

Fig 31: *Terminalia sericea* roots vs. *Strep* group A



Fig 32: *Elephantorrhiza goetzei* roots vs. *Strep* group A



Fig 33: *Gymnosporia senegalensis* roots vs. *Staphylococcus aureus*



Fig 34: *Terminalia sericea* roots vs. *Pseudomonas aeruginosa*



Fig 35: *Warburgia salutaris* roots vs. *Candida albicans*



Fig 36: *Warburgia salutaris* roots vs. *Aspergillus niger*



3.6 Toxicity / Bioactivity Tests

Table 15: Brine Shrimp Lethality Test (BSLT) results (LC₅₀ µg/ml) for the plant extracts

PLANT EXTRACTS	BSLT LC ₅₀ µg/ml*
<i>Cassia abbreviata</i> bark	454.93 ± 18.60
<i>Cassia abbreviata</i> leaves	445.72 ± 22.15
<i>Cassia abbreviata</i> roots	1319.37 ± 356.63
<i>Dichrostachys cinerea</i> leaves	539.39 ± 78.24
<i>Dichrostachys cinerea</i> roots	4304.59 ± 685.69
<i>Elaeodendron matabelicum</i> roots	1012.31 ± 217.69
<i>Elephantorrhiza goetzei</i> roots	356.55 ± 24.55
<i>Flacourtia indica</i> leaves	281.81 ± 26.13
<i>Flacourtia indica</i> roots	467.31 ± 39.01
<i>Gymnosporia senegalensis</i> leaves	789.37 ± 104.06
<i>Gymnosporia senegalensis</i> roots	2185.61 ± 872.25
<i>Gymnosporia senegalensis</i> twigs	754.70 ± 182.57
<i>Hypoxis rooperi</i> tuber	735.34 ± 89.39
<i>Khaya anthotheca</i> bark	482.19 ± 43.49
<i>Kigelia africana</i> bark	262.20 ± 25.07
<i>Kigelia africana</i> fruit	117.41 ± 30.27
<i>Kigelia africana</i> roots	501.35 ± 34.88
<i>Rhus chirindensis</i> leaves	1023.26 ± 161.69
<i>Rhus chirindensis</i> roots	316.60 ± 30.07
<i>Sclerocarya birrea</i> bark	1112.37 ± 210.04
<i>Securidaca longepedunculata</i> roots	351.89 ± 35.79
<i>Terminalia sericea</i> leaves	66.66 ± 49.31
<i>Terminalia sericea</i> roots	295.33 ± 37.19
<i>Warburgia salutaris</i> bark	359.66 ± 14.33
<i>Warburgia salutaris</i> leaves	351.41 ± 29.58
<i>Warburgia salutaris</i> roots	426.10 ± 55.55
<i>Nerium oleander</i> (+ control)	141.67 ± 68.15

*LC₅₀: Lethal Concentration to kill 50% of the *Artemia salina* shrimps

3.7 Compilation of Overall Results

Table 16: Manicaland Plants – Results of Biological and Antimicrobial Screening Tests

No	Plant, Family and Vernacular name	Plant Part Meth Ext.	Responses by Microbial Strains to Plant Extracts and Biological Activity Results									
			<i>Sa</i> Gm ^{+ve}	<i>S GpA</i> Gm ^{+ve}	<i>Ec</i> Gm ^{-ve}	<i>Pa</i> Gm ^{-ve}	<i>Ca</i> Fungi	<i>An</i> Fungi	<i>HSV-2</i> A/V	A/Cn (Pot.)	A/Ox	<i>BS</i> Tox.
1	<i>Flacourtia indica</i> , <i>Flacourtiaceae</i> Mundidwe, Mubwide, Munhunguru(Sh)	leaf	–	–	–	–	–	w	M	M	VS	T
2	<i>Flacourtia indica</i> , <i>Flacourtiaceae</i> Mundidwe, Mubwide, Munhunguru(Sh)	root	–	w	–	vw	–	w	S	S	M	MT
3	<i>Khaya anthotheca</i> (<i>nyasica</i>), <i>Meliaceae</i> Muwawa (Sh) Mahogany	bark	m	w	–	–	–	–	M	M	VS	MT
4	<i>Khaya anthotheca</i> (<i>nyasica</i>), <i>Meliaceae</i> Muwawa (Sh) Mahogany	root	NT	NT	NT	NT	NT	NT	NT	NT	M	NT
5	<i>Kigelia africana</i> (<i>pinnata</i>) <i>Bignoniaceae</i> Mubvee (Sh), Umvebe (Nd)	bark	w	w	w	m	w	w	S	S	M	T
6	<i>Kigelia africana</i> (<i>pinnata</i>) <i>Bignoniaceae</i> Mubvee (Sh), Umvebe (Nd)	fruit	w	w	–	m	–	w	VS	VS	M	VT
7	<i>Kigelia africana</i> (<i>pinnata</i>) <i>Bignoniaceae</i> Mubvee (Sh), Umvebe (Nd)	root	–	w	–	m	–	–	–	–	M	MT
8	<i>Rhus chiridensis</i> , <i>Fabaceae</i> Mubikasadza (Sh)	leaf	w	w	–	w	–	–	–	–	VS	Sf
9	<i>Rhus chiridensis</i> , <i>Fabaceae</i> Mubikasadza (Sh)	root	m	w	–	-	–	–	M	M	VS	T
10	<i>Warburgia salutaris</i> <i>Canellaceae</i> Muranga(Sh), Isibhaha (Z)	bark	s	w	–	–	m	s	–	–	S	T
11	<i>Warburgia salutaris</i> <i>Canellaceae</i> Muranga(Sh), Isibhaha (Z)	leaf	w	–	–	–	w	w	–	–	VS	T
12	<i>Warburgia salutaris</i> <i>Canellaceae</i> Muranga(Sh), Isibhaha (Z)	root	m	s	m	w	vs	vs	S	S	S	MT
13	<i>Warburgia salutaris</i> <i>Canellaceae</i> Muranga(Sh)	twigs	NT	NT	NT	NT	NT	NT	NT	NT	M	NT

No	Plant, Family and Vernacular name	Plant Part	Responses by Microbial Strains to Plant Extracts and Biological Activity Results											
			Meth Extr.	Sa Gm ^{+ve}	S GpA Gm ^{+ve}	Ec Gm ^{-ve}	Pa Gm ^{-ve}	Ca Fungi	An Fungi	HSV-2 A/V	A/Cn (Pot.)	A/Ox	BS Tox.	
	Anti/Mic Controls Amoxicillin Gm ^{+ve} (10 µg/disk) Chloramphenicol Gm ^{+ve} (10µg/disk) Gentamicin Gm ^{-ve} (10µg/disk) Amphotericin B (10µg/disk) Miconazole (10µg/disk)			s	s	m								
	Anti-oxidant β-carotene												VS	
	Tox. Control <i>Nerium oleander</i> (oleandrin)													T

Key for Table 16 and Table 17:

Meth Extr: Methanol Extract

Bacteria/Fungal Strains:

Sa=*Staphylococcus aureus*, S/Gp A= *Streptococcus Group A*, Ec=*Escherichia coli*, Pa=*Pseudomonas aeruginosa*,

Ca=*Candida albicans*, An=*Aspergillus niger*

Bacterial and fungal responses to extracts:

w=weak, m=medium, s=sensitive, vs=very sensitive, - resistant,

A/V= Anti-viral :

W=weak, M=medium, S=strong, VS=very strong

A/C Pot=Anti cancer potential:

W=weak, M=medium, S=strong, VS=very strong

BS Tox= Brine shrimp Toxicity:

T=toxic, VT=very toxic, MT=moderate toxic, Sf=safe, VSf=very safe

A/Ox=Antioxidant:

VS= very strong, S=strong, M=moderate, W=weak

Table 17: Matabeleland Plants - Results of Biological and Antimicrobial Screening Tests

No	Plant name, Family and Vernacular name	Plant Part	Responses by Microbial Strains to Plant Extracts and Biological Activity Results										
			Meth Extr.	Sa Gm ^{+ve}	S GpA Gm ^{+ve}	Ec Gm ^{-ve}	Pa Gm ^{-ve}	Ca Fungi	An Fungi	HSV-2 A/V	A/Cn (Pot.)	A/Ox	BS Tox.
1	<i>Cassia abbreviata</i> , <i>Caesalpinioideae</i> Muremberembe (Sh), Isihaqa (Nd)	leaf		-	w	-	-	-	w	M	M	M	MT
2	<i>Cassia abbreviata</i> , <i>Caesalpinioideae</i> Muremberembe (Sh), Isihaqa (Nd)	bark		w	m	-	-	-	w	-	-	M	MT
3	<i>Cassia abbreviata</i> , <i>Caesalpinioideae</i> Muremberembe (Sh), Isihaqa (Nd)	root		w	w	-	m	-	m	S	S	M	Sf
4	<i>Dichrostachys cinerea</i> (<i>D. glomerata</i>), <i>Mimosaceae</i> Mupangara, (Sh) Ugagu (Nd)	leaf		w	w	-	s	-	w	VS	VS	S	MT

No	Plant name, Family and Vernacular name	Plant Part	Responses by Microbial Strains to Plant Extracts and Biological Activity Results										
			Meth Extr.	<i>Sa</i> Gm ^{+ve}	<i>S GpA</i> Gm ^{+ve}	<i>Ec</i> Gm ^{-ve}	<i>Pa</i> Gm ^{-ve}	<i>Ca</i> Fungi	<i>An</i> Fungi	<i>HSV-2</i> A/V	A/Cn (Pot.)	A/Ox	<i>BS</i> Tox.
5	<i>Dichrostachys cinerea</i> (<i>D. glomerata</i>), Mimosaceae Mupangara, Musekera, Mumhangara (Sh) Ugagu (Nd)	root		-	-	-	m	w	w	M	M	VW	VSf
6	<i>Elaeodendron matabelica</i> , Celastraceae Murunganyama, Murungamunyu (Sh) Umugugudu (Nd)	root	m	m	w	s	-	-	-	M	S	Sf	
7	<i>Elephantorrhiza goetzei</i> , Leguminosae Muzezepasi (Sh), Intolwane (Nd)	root	m	m	-	-	-	-	M	M	M	T	
8	<i>Gymnosporia (Maytenus) senegalensis</i> , Celastraceae Chivhunabadza, musosawafa (Sh), Isihlangu (Nd), Ibalalatune (T)	leaf	w	w	m	s	-	w	S	VS	VS	MT	
9	<i>Gymnosporia (Maytenus) senegalensis</i> , Celastraceae Chivhunabadza, musosawafa (Sh), Isihlangu (Nd), Ibalalatune (T)	twig	m	w	w	vs	-	-	S	S	M	MT	
10	<i>Gymnosporia (Maytenus) senegalensis</i> , Celastraceae Chivhunabadza, musosawafa (Sh), Isihlangu (Nd), Ibalalatune	root	m	w	m	w	w	w	S	S	VS	Sf	
11	<i>Hypoxis hemerocallidea (rooperi)</i> , Hypoxidaceae : Hodo (Sh), Igudu (Nd)	tuber	-	-	-	-	w	w	S	S	M	MT	
12	<i>Sclerocarya birrea subsp. caffra</i> Anacardiaceae Mupfura, Mutsomo (Nd) Umanganu (Nd)	bark	m	m	m	m	-	w	S	S	S	Sf	
13	<i>Securidaca longepedunculata</i> , Polygalaceae Mufufu (Sh), Umfufu (Nd)	root	w	w	-	w	w	m	S	S	VS	T	
14	<i>Terminalia sericea</i> , Combretaceae Mususu (Sh), Umsusu, Umangwe (Nd)	leaf	w	w	-	-	-	w	M	M	S	VT	
15	<i>Terminalia sericea</i> , Combretaceae Mususu (Sh), Umsusu, Umangwe (Nd)	root	s	s	w	vs	w	w	S	S	S	T	

Table 18: Traditional Medicinal Plants: Prioritised Summary of Results on Phytochemical Groups and their Biological and Anti-infective Activities

No	Plant and Vernacular names	Plant Part Investigated	No Phytochemical Groups Confirmed / Investigated				Microbial Strains : Sensitivity to Plant Extract				Biological Activities		
			PCG	PPG	Alk	Sap	Gm ^{+ve} Bact.	Gm ^{-ve} Bact.	Fungi	HSV-2 Virus A/V	A/Can Pot.	Biol/Act. A/Ox	Tox . BS
1	<i>Kigelia africana (pinnata)</i> Bignoniaceae Mubvee (Sh), Umvebe (Nd)	bark	5/6	4/4	-	++	w	m	w	S	S	M	T
2	<i>Kigelia africana (pinnata)</i> Bignoniaceae Mubvee (Sh), Umvebe (Nd)	Fruit	5/6	3/4	+	++	w	m	w	VS	VS	M	VT
3	<i>Cassia abbreviata</i> , Caesalpinioideae Muremberembe (Sh), Isihaqa (Nd)	bark	5/6	4/4	-	++	m	-	w	-	-	M	MT
4	<i>Cassia abbreviata</i> , Caesalpinioideae Muremberembe (Sh), Isihaqa (Nd)	root	5/6	3/4	++	++	w	m	m	S	S	M	Sf
5	<i>Dichrostachys cinerea (D.glomerata)</i> , Mimosaceae Mupangara (Sh), Ugagu (Nd)	leaf	5/6	3/4	++	++	w	s	w	S	S	S	MT
6	<i>Securidaca longepedunculata</i> Polygalaceae, Mufufu (Sh) Umfufu (Nd)	root	5/6	3/4	++	+++	w	w	m	S	S	VS	T
7	<i>Warburgia salutaris</i> , Canellaceae Muranga(Sh), Isibhaha (Z)	root	5/6	3/4	++	+	s	m	vs	S	S	M	MT
8	<i>Warburgia salutaris</i> , Canellaceae Muranga(Sh), Isibhaha (Z)	bark	5/6	3/4	+++	+	m	-	m	-	-	M	T
9	<i>Warburgia salutaris</i> , Canellaceae Muranga(Sh), Isibhaha (Z)	twig	4/6	3/4	++	-	s	m	vs	S	S	M	MT
10	<i>Cassia abbreviata</i> , Caesalpinioideae Muremberembe (Sh), Isihaqa (Nd)	leaf	4/6	3/4	++	-	w	-	w	M	M	M	MT

No	Plant and Vernacular names	Plant Part Investigated	No Phytochemical Groups Confirmed / Investigated				Microbial Strains : Sensitivity to Plant Extract				Biological Activities		
			PCG	PPG	Alk	Sap	Gm ^{+ve} Bact.	Gm ^{-ve} Bact	Fungi	HSV-2 Virus A/V	A/Can Pot.	Biol/Act. A/Ox	Tox . BS
11	<i>Elephantorrhiza goetzei</i> , Leguminosae Muzezepasi (Sh), Intolwane (Nd)	root	4/6	3/4	-	++	m	-	-	M	M	M	T
12	<i>Gymnosporia (Maytenus) senegalensis</i> , Celastraceae Chivhunabadza, musosawafa (Sh), Isihlangu (Nd)	leaf	4/6	3/4	-	++	w	s	w	S	S	S	MT
13	<i>Khaya anthotheca</i> (nyasica), Meliaceae Muwawa (Sh)	bark	4/6	3/4	-	+	m	-	-	M	M	S	MT
14	<i>Rhus chirindensis</i> , Fabaceae Mubikasadza (Sh)	root	4/6	3/4	-	+	m	-	-	M	M	S	T
15	<i>Terminalia sericea</i> , Combretaceae Mususu (Sh), Umangwe (Nd)	leaf	4/6	3/4	-	++	w	-	w	M	M	S	VT
16	<i>Terminalia sericea</i> , Combretaceae Mususu (Sh), Umangwe (Nd)	root	3/6	2/4	-	+++	s	vs	w	S	S	S	T

Key:

PCG=Phytochemical groups , PPG= Polyphenolic groups, Alk=Alkaloids, Sap=Saponins
Gm^{+ve} Bact.= Gram –positive bacteria, Gm^{-ve} Bact.= Gram-negative bacteria, HSV-2=Herpes simplex virus Type2
Bacterial and fungal responses to extracts: w=weak, m=medium, s=sensitive, vs=very sensitive, -= resistant,
A/V= Anti-viral: W=weak, M=medium, S=strong, VS=very strong, NT=not tested
A/C Pot=Anti-cancer potential : W=weak, M=medium, S=strong, VS=very strong, NT=not tested
BS Tox= Brine shrimp Toxicity: T=toxic, VT=very toxic, MT=moderate toxic, Sf=safe, VSf=very safe
A/Ox=Antioxidant: S=strong, M=moderate, W=weak

CHAPTER IV

4.0 DISCUSSION

Many of the plant extracts have given good results for most of the assays. Some of these names were pronounced as best results at each assay. Therefore, it would be wise to first discuss the findings according to each assay and later examine each plant individually.

4.1 Phytochemistry Assay

The continuous interest in laboratory screening of medicinal plants is not only to determine the scientific rationale for their usage, but also to discover new active principles.

Alkaloids have been well investigated for many pharmacological properties including antiprotozoal, cytotoxic, anti-inflammatory properties but there are also few reports about their antimicrobial properties. In a study in 2005, Karou et al have revealed the presence of two major alkaloids, cryptolepine and quindoline, through the GC/MS analysis of the *Sida acuta* extract which displayed good antimicrobial activity against several test microorganisms. In this study, Alkaloids were found present in extracts of *Warburgia salutaris* (twigs, bark, roots), *Kigelia africana* (fruit), *Securidaca longepedunculata* root, *Dichrostachys cinerea* leaf and *Cassia abbreviata* (leaves, roots) giving definite positive results for all three tests. These results confirm the literature findings for *Kigelia africana* fruit which is known to contain naphthoquinones (Weiss et al, 2000). For *Securidaca longepedunculata*, the positive results should mean the existence of ergot alkaloids that were mentioned in Lannang et al, 2006. According to these results, it is possible to conclude the presence of alkaloid (-)**cassine** in *Cassia abbreviata* which was isolated from *Cassia excelsa* and *Cassia racemosa* (Kim, 2007; Moo-Puc et al,2007). The positive results for *Warburgia salutaris* extract could be due to the sesquiterpenoids known to exist in the plant (Rabe,

2000). It would be a new finding for *Dichrostachys cinerea* which gave similar spots to **berberine** reference but to say for sure, it would need spectroscopical identification.

17 of 28 (60.7%) showed positive results for the test of Flavonoids. Especially nine of the extracts gave undoubtedly good confirmatory test results; the root extracts of *Kigelia africana*, *Securidaca longepedunculata*, *Elephantorrhiza goetzei*, *Cassia abbreviata*, *Khaya anthotheca*, *Rhus chirindensis* and the leaf extracts of *Rhus chirindensis*, *Terminalia sericea* and *Dichrostachys cinerea*. These results coincide with the known information from the literature that *Kigelia africana* contains flavonoids quercetin and luteolin (Azuine et al, 1997); that the roots of *Elephantorrhiza goetzei* contain 3,3',4',5,6,7,8-heptahydroxyflavan named *elephantorrhizol* (Moyo et al, 1999) and stilbene glycoside, 5-methoxy-(E)-resveratrol-3-O-rutinoside (Wanjala, 2001); that roots of *Cassia abbreviata* might contain the 2,4-trans-7,4'-dihydroxy-4-methoxyflavan that has been isolated from this tree's leaves and twigs (Dehmlow et al.,1998) and (2R,3S)-guibourtinidol which was identified in the heartwood of tree (Nel et al., 1999); and that *Dichrostachys cinerea* contains mesquitol (Jagadeeshwar, 2003). Biflavonoids isolated from *Rhus succedanea* (Lin et al, 1999) might also be present in *Rhus chirindensis* and must be further tested for identification.

In addition to the literature, according to the tests held at University of Istanbul against their glycoside references, there is enough evidence to believe that *Securidaca longepedunculata* roots may contain apigenin 7-O-glucoside, luteolin 7-O-glycoside (**Figures 47 & 48**, page 122) and maybe naringenin 7-O-glucoside; that *Cassia abbreviata* roots may contain isovitexin and hyperoside; that *Dichrostachys cinerea* leaves might contain quercitrin and isoquercitrin; that *Elephantorrhiza goetzei* roots might contain astragalin and hesperidin; that *Gymnosporia senegalensis* leaves may contain hesperidin and that *Terminalia sericea* roots might contain vicenin-2, vitexin and isoquercitrin. These would all be new findings which need to be further evaluated through spectroscopy.

Maybe not the best results but all bark extracts have given positive results for flavonoids. This result of the vast majority of the extracts containing flavonoids is supported by the fact that flavones and their close relations are the widely distributed in nature and are more common in the higher plants and in young tissues. These results also shine light on their high antioxidant activities since antioxidant activity depends on phenolic compounds.

Saponins were found in 23 of 28 (82.1%) screened plant extracts which makes it the second abundant phytochemical group after tannins in this research. The best results were the root extracts of *Securidaca longepedunculata*, *Terminalia sericea*, *Elephantorrhiza goetzei*, *Elaeodendron matabelicum*, *Hypoxis rooperi* and *Gymnosporia senegalensis*. It was known from literature that *Securidaca longepedunculata* contains trypanocidal securida-saponin (Atawodi, 2002), that *Terminalia sericea* contains a lysoytic saponin, sericoside (Mochizuki, 2006) and that *Hypoxis rooperi* tuber contains a steroidal saponin (Mahomed, 2003). No reports were found on the remaining three plants suggesting presence of saponins, therefore the positive results could be new findings which need further looking into. Mainly the negative results were leaf extracts which shows that leaves mostly do not contain saponins.

For Coumarins only two methods were used since no confirmatory method is available in literature. 13 of 28(46.4%) extracts contained coumarins and another one with a question mark. The best results were the different extracts of *Warburgia salutaris*, *Cassia abbreviata*, *Kigelia africana*, *Khaya anthotheca* and the root extract of *Elephantorrhiza goetzei*. The only plant between these plants with known coumarins was the *Kigelia africana* (Weiss et al, 2000). The other four plants would need further investigations. The interesting trend in coumarins was if one extract of the plant had it, mostly did the other extract of the plant.

For Anthraquinones, 10 of 28(35.7%) extracts have given very convincing results for all three tests. In testing anthraquinones, *Cassia abbreviata* was almost like a reference material which had both free and combined anthraquinones. The main anthraquinones of this species

are absin, chaksine and cassic acid, which is also known as Rhein, a crystalline antibiotic compound. An antiprotozoal anthraquinone, chrysophanol (**Fig 37**, page 99) was isolated from the leaves of *Cassia racemosa* (Moo-Puc et al, 2007). Running *Cassia abbreviata* root extract against the reference **chrysophanol**, there is enough evidence to believe that this anthraquinone is present in this plant which would be a new finding. On TLC, this extract gave also very similar spots to hydrolysable part of **emodin** (**Fig 7**, page 17). Along with *Cassia*, the extracts of *Flacourtia indica* and *Khaya anthotheca* were among the best results.

Tannins were the most abundant phytochemical group that was screened. 25 of 28(89.3%) extracts did contain tannins. The best results were the extracts of *Warburgia salutaris*, *Flacourtia indica*, *Terminalia sericea*, *Rhus chirindensis*, and *Dichrostachys cinerea*. Through literature, it is proven that *Elephantorrhiza goetzei* root has anthelmintic tannins, gallic acid, catechin and gallocatechin (Molgaard, 2001;Wanjala, 2001); that *Gymnosporia senegalensis* root has (-)-4'-methylepigallocatechin (Drewes, 1993), (-)-epigallocatechin, epicatechin, epigallocatechin, procyanidin (Nonaka, 1983; Nonaka, 1981; Porter, 1982; Hashimoto 1989), phloro-glucinol 1-O- β -D-glucopyranoside and (-)-4'-methylepigallocatechin 5-O- β -glucopyranoside, (+)-4'-methylgalocatechin 3'-O- β -glucopyranoside and (-)-epicatechin (-)-4'-methylepigallocatechin (Ghazi et al, 1999)(**Fig 44**, page 109) and that *Sclerocarya birrea* pulp had high quantity of flavones and condensed tannins, 202 μ g catechin/g and 6.0% condensed tannins (Ndhlala et al,2007).The good results of *Terminalia sericea* extracts suggest that the tannins found might be the ellagitannins, punicalin and 2-O-galloylpunicalin, which were isolated from *Terminalia triflora* leaves (Martino et al, 2004). The three extracts which gave negative results were the bark extract of *Khaya anthotheca*, the leaf and root extracts of *Cassia abbreviata*.

Since tannins fall under phenols, the results are justified as phenols constitute the largest group of plant secondary metabolites and are widespread in nature (Trease and Evans, 2002).

Of the twenty-four extracts containing tannins only six extracts contained condensed tannins. The observed high antioxidant activity by plant extracts can definitely be explained by the high and frequent presence of tannins inside.

4.2 Antioxidant Assay

The Radical Scavenging Activity test results are shown in **Fig 25 & 26**, page 66-67 and eight extracts exhibited Antioxidant Activity with percentages higher than 90%. The highest were *Rhus chirindensis leaves* $96.91 \pm 0.33\%$ & *roots* $96.90 \pm 0.49\%$, *Khaya anthotheca bark* $96.05 \pm 0.05\%$, *Gymnosporia senegalensis roots* $96.05 \pm 0.18\%$, *Flacourtia indica leaves* $94.87 \pm 0.76\%$ and *Warburgia salutaris leaves* $94.08 \pm 0.87\%$. These results can be considered as a full absorption inhibition of DPPH since the final solution after the reaction could actually never be as colourless as the methanol solution therefore never reaches 100% (Miliakuskas et al, 2004). The results of all extracts are presented in **Table 9** on page 68.

The more rapidly the absorbance decreases, the more potent is the antioxidant activity of the extract, in terms of hydrogen atom donating capacity. All extracts were very quick acting in terms of inhibition of DPPH. 12 of 28 (42.9%) extracts already reached the stable stage with a very low absorbance in less than two minutes. Except another 4 which got to that stage in about twenty minutes, all the others took less than ten minutes. This is a very short acting time considering antioxidant vegetable extracts which come to a stable absorbance in 60-120min (Ndhlala et al, 2007).

The content of phenolic compounds (mg/mg) in methanolic extracts, determined from the calibration curve of the standard Gallic acid ($r^2=0.98$), are summarized in **Tab 9**, page 68 and **Fig 27**, page 67. The highest amount was found in *Khaya anthotheca bark* extract (0.596 ± 0.157 mg GAE/mg) which also had the highest antioxidant activity and the lowest was the *Dichrostachys cinerea root* extract (0.105 ± 0.003 mg GAE/mg) which also had the lowest antioxidant activity. It was observed that the contents of the phenolic compounds in the

extracts correlate with their antiradical activity (Pearson's two-tailed, 95% confidence interval, correlation coefficient $R^2=0.57$), confirming that the phenolics are likely to cause radical scavenging activity, **Fig 28**, page 69.

The results correlate well with the phytochemical results where the highest inhibition percentage plants are the same ones that were found to have flavonoids, coumarins, anthraquinones and tannins, in general phenolic compounds. *Khaya anthotheca bark* has given positive results for three of these four groups. The high quantity of total phenolic compounds and therefore the high antioxidant activity is a new finding for this plant and would justify its use against infections in traditional medicine. The high antioxidant activity and the total phenolic content ($0.323\pm 0.060\text{mgGAE/mg}$) of *Rhus chirindensis* extracts matches the antioxidant activity and phenolic composition of Turkish sumac, *Rhus coriaria* extracts which exhibited high antioxidant activity with total phenolic content in ethyl acetate soluble fraction as 540.65mgGAE/g extract (Kosar et al, 2007). *Gymnosporia senegalensis roots* have given good results for tannins and are known to contain gallic catechins (Ghazi et al, 1999) but haven't given positive results for any of the other phenolic groups. This result would prove that tannins have high antioxidant activity. *Flacourtia indica leaves* were found to contain anthraquinones and tannins through phytochemical screening. This correlates with their high total phenolic content with $0.431\pm 0.106\text{mgGAE/mg}$ plant material. In a Zimbabwean antioxidant activity study with the fruit of *Flacourtia indica*, the pulp contained the least total phenolics, flavonoids and condensed tannins $334\mu\text{gGAE/g}$, $41\mu\text{gcatechin/g}$ and 1.4%, respectively (Ndhlala et al, 2006). *Warburgia salutaris leaves* didn't have very high content of total phenolics, $0.296\pm 0.040\text{mgGAE/mg}$ plant, but had very high antioxidant activity.

All the other tested extracts had considerable high antioxidant activity, more than 80%, all except *Warburgia salutaris twigs* ($73.28\pm 1.09\%$) and *Dichrostachys cinerea roots* ($27.39\pm 1.24\%$). This proves how generations of traditional medicine practice chooses the

right extract from wrong since these two extracts are not the plant parts which are currently being used traditionally.

However, the overall high antioxidant activities of the tested plant extracts confirm their use against oxidative stress which has been linked to inducing cancer, cardiovascular diseases, neurodegenerative diseases such as Alzheimer's and Parkinson's, inflammation, aging and opportunistic infections in HIV/AIDS.

4.3 Antiviral Assay

A total of 26 extracts, belonging to 14 different plant species out of 11 families were investigated for their antiviral properties. The results of the antiviral screening are expressed as the Reduction Factor (RF) at the maximal non-toxic dose (MNTD) of the plant extract. The methanol extracts showed mostly good antiviral activity in the End Point Titration Technique, $RF \geq 10^3$, which is considered as a promising antiviral result (Cos et al, 2002; Vlietinck et al, 1995). Out of 26 extracts, 13 (50%) showed considerable antiviral activity against the *HSV-2* virus. The best results were obtained from the extracts of *Dichrostachys cinerea leaves* RF 10^4 and *Kigelia africana fruit* RF 10^4 followed by *Hypoxis rooperi tuber*, *Cassia abbreviata roots*, *Securidaca longepedunculata roots*, *Sclerocarya birrea bark*, *Terminalia sericea roots*, *Warburgia salutaris roots*, *Kigelia africana bark*, *Flacourtia indica roots* and all the extracts of *Gymnosporia senegalensis* with RF 10^3 .

Dichrostachys cinerea was the surprising plant of the antiviral activity. There is no known use of this plant against viral diseases. The leaves are commonly used against bacterial infections and wounds in ethnobotany and have been proven to have antibacterial activity against gram (+) and gram (-) bacteria (Eisa et al, 2000) and free-radical scavenging property (Jagadeeshwar, 2003), but were not tested for antiviral activity. This finding is novel and the leaves could be further investigated for potential activity against HIV and possible

formulation studies. Since the toxicity of the plant parts was also not very strong, this plant could be recommended for use against genital herpes infections as in traditional medicine.

Although extracts of *Kigelia africana* have been assessed for antimicrobial, anti-malarial and cytotoxic activity (Binutu et al, 1996; Akunyili, 1991; Houghton, 1994), the antiviral activity of this plant had not been investigated. The promising results of the fruit and bark extracts attained through this study should be further investigated for better understanding of the role of its phytochemistry and the possible anti-HIV activity.

It was the root extract of *Terminalia sericea* with promising results in this study but in another study, the methanol extract of the leaves of *Terminalia sericea* was found to strongly inhibit the polymerase and the ribonuclease H activities of the *Human Immunodeficiency Virus type1* (HIV-1) (Bessong et al, 2004). In another study with a different species of the same family, the bioassay- guided fractionation of the aqueous extract of *Terminalia triflora* leaves afforded *punicalin* and *2-O-galloylpunicalin* that showed inhibitory activity on HIV-1 reverse transcriptase in a dose-dependent manner (Martino et al. 2004). Looking at these results, further tests should be done to evaluate the anti-HIV activity of Zimbabwean *Terminalia sericea* and to isolate its active principles.

The antibacterial and antifungal activities of *Warburgia salutaris* are well known and it was a big pleasure to see the promising antiviral activity of the roots (Rabe et al 2000, 1997). This also confirms the consumption of this plant against viral diseases. However, the most commonly used part of this tree is the bark and the test results haven't proven this correct.

The roots of *Cassia abbreviata* have given positive results for all the chemical groups tested except the tannins. Especially they were like a reference material for anthraquinones. Considering that different kinds of *anthraquinones* from extracts of *Cassia angustifolia* were found to be quite active against HSV-1 (Sydiskis et al. 1991), it could be assumed that the

anthraquinones present in *Cassia abbreviata* are responsible for this anti-HSV-2 activity. Isolation, identification and further antiviral screening would be necessary for final statement.

All the extracts of *Gymnosporia (Maytenus) senegalensis* have given promising results against *HSV-2*. This complies with the traditional use of the twigs and leaves against viral disease like chickenpox, measles, mumps and rubella. The methanol extracts of *Gymnosporia (Maytenus) senegalensis* (stem-bark) showed considerable inhibitory effects against HIV-1 (Ghazi, 1999).

In terms of treating AIDS/HIV, *Hypoxis hemerocallidea tuber* is one of the mainly used plants in Southern African traditional medicine (Thomson, 2001). In terms of cancer, corms used to treat bladder disorders and testicular tumours (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997). In a recent study to reveal the cytotoxicity of South African plants, the extracts of *H. hemerocallidea* stimulated DU-145 and MCF-12A cell growth and inhibited the growth of the MCF-7 cells (Steenkamp, 2006). This plant has also been reported to display anti-inflammatory activity (Ojewole, 2002), an activity related to cancer. With the results of this study, RF 10^3 and $10.41\mu\text{g/ml}$, combined with the previous findings, there is enough evidence to confirm the use of *Hypoxis* in the treatment of cancer and immunodeficiency related diseases.

It was disappointing not to see a promising antiviral activity from *Rhus chirindensis*, RF 10^2 , since another species from Chinese traditional medicine, ***Rhus chinensis***, showed that the petroleum ether extract of the stems was effective against HIV-1 and *Rhus chinensis* would be a useful medicinal plant for the chemotherapy of HIV-1 infection (Wang et al, 2006). Also, ***Rhus javanica***, a medicinal herb, has been shown to exhibit oral therapeutic anti-herpes simplex virus activity in mice with its two major anti-HSV compounds, *moronic acid* and *betulonic acid* (Kurokawa et al, 1999). There should be further evaluations done with extracts

of different solvents or of different plant parts especially because of the use of leaves against measles in traditional medicine.

The extracts were active against the virus at concentrations ranging from 10.41 to 125.0µg/ml. None of the extracts have shown concentrations as low as acyclovir, the reference anti-HSV2 but there is a big potential if the chemical compounds were isolated and purified. Especially *Dichrostachys cinerea* leaves, *Hypoxis hemerocallidea* tuber, *Cassia abbreviata* roots and *Gymnosporia senegalensis* leaves have shown promising antiviral activity with very low concentrations, all at 10.41µg/ml. However their cytotoxicity levels parallel their activities.

4.4 Antimicrobial Assay

African medicinal plants have been screened for their *in vitro* antibacterial activities and many described antibacterial activities have been focussed on phenolic compounds, terpenoids or essential oils (Bassole et al., 2003; Viljoen et al., 2003). The plants have been found to exert good *in vitro* antimicrobial activities and some active principles have been isolated. Examples are muzigadial isolated from *Warburgia salutaris* (Bertol. f) Chiov. (Canellaceae) (Rabe and Van Staden, 2000) and vernodalin from *Vernonia colorata* (Willd) Drake (Asteraceae) (Reid et al., 2001).

The *antibacterial* and *antifungal activity* of the plant extracts (10 mg/ml) were investigated by the agar well assay, also known as the hole plate diffusion method. 26 extracts were tested against 6 micro-organisms; two strains of gram-positive (*Staphylococcus aureus*, *Streptococcus group A*), two strains of gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and two kinds of fungi (*Candida albicans*, *Aspergillus niger*).

The most active plant parts against *Staphylococcus aureus*, a gram (+) bacteria, were in the order of *Terminalia sericea* roots, *Warburgia salutaris* roots, *Rhus chirindensis* roots, *Gymnosporia senegalensis* roots, *Elaeodendron matabelicum* roots and *Sclerocarya birrea*

bark. The most active parts of the plants against this bacterium were found to be the **roots** followed by bark.

Especially *Terminalia sericea* roots and *Warburgia salutaris* roots have inhibited the growth of this bacterium almost as much as the reference amoxicillin. *Terminalia sericea* roots inhibited the growth of *Staphylococcus aureus* with inhibition zone of 7.88 ± 0.48 mm where the reference amoxicillin (10 μ g/disk) gave a zone of 9.00 ± 0.41 mm. This result correlated with the results obtained from other studies in literature where the methanol and water extracts of *Terminalia sericea* were more active compared to the other tested extracts against *Streptococcus pyogenes* and *Staphylococcus aureus* (Steenkamp et al, 2004). On antimicrobial screening of the crude extracts of the selected *Combretum* and *Terminalia* species, the methanol extract of the roots of *Terminalia sericea* showed marked inhibition against Gram-positive bacteria and was also good inhibitor of *Enterobacter aerogenes*. All four of the extracts of the roots of *T. sericea* tested, (methanol, ethanol, acetone and hot water) had good antimicrobial activity (Fyhrquist et al, 2002). In a different study, intermediate and polar extracts of the roots exhibited high antibacterial activity against *Staphylococcus aureus*, *E. coli*, *Bacillus anthracis* and *P. aeruginosa*. *Cassia abbreviata* leaves, *Flacourtia indica* leaves and roots, *Hypoxis rooperi* tuber, *Kigelia africana* roots and *Dichrostachys cinerea* roots have shown no activity against this bacterium.

The most active plant part against *Streptococcus group A*, another gram (+) bacteria, were in the following order; *Warburgia salutaris* roots, *Terminalia sericea* roots, *Sclerocarya birrea* bark, *Kigelia africana* bark, *Cassia abbreviata* bark, *Elaeodendron matabelicum* roots and *Elephantorrhiza goetzei* roots. All the **bark** extracts have shown comparably good activity against this bacterium.

The highest inhibition of growth of this microorganism was achieved by the extract of *Warburgia salutaris* roots, 9.50 ± 0.58 mm which was almost as much as the reference

antibacterial amoxicillin, 10.50 ± 0.58 mm. Crude extracts from 21 South African medicinal plants were screened for in vitro antibacterial activity and the highest activity was found in the methanol extracts from *Warburgia salutaris* among with three other plants (Rabe et al, 1997) The leaves and bark contain numerous *drimane sesquiterpenoids*, including *warburganal*, *polygodial*, *muzigadial*, *mukaadial*, *ugandensidial* and *salutarisolide* (Mashimbye et al, 1999) (**Fig 52**, page 127). First two of these compounds showed to be potently anti-candidal, and also have broad-spectrum antimicrobial activity (Rabe et al, 2000). *Flacourtia indica* leaves, *Hypoxis rooperi* tuber and *Dichrostachys cinerea* roots have also shown no activity against this bacterium along with *Warburgia salutaris* leaves, proving that these plant parts are not to be used against infections caused by these bacteria such as upper respiratory and skin infections.

The most active against both Gram (-) bacteria were *all parts of Gymnosporia senegalensis*, *Terminalia sericea* roots, *Kigelia africana* bark, *Elaeodendron matabelicum* roots and *Sclerocarya birrea* bark.

The greatest inhibition against any bacteria was achieved with the extract of *Terminalia sericea* roots against *Pseudomonas aeruginosa*. The extract has given even larger zones of inhibition, 10.00 ± 0.82 mm, than the reference gentamicin ($10 \mu\text{g}/\text{disk}$), 7.00 ± 0.40 mm. One of the extracts with the highest activity was the bark extract of *Sclerocarya birrea*. This result is similar to the study where the bark and leaves were extracted with acetone and MIC values were determined using a microplate serial dilution technique with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* as test organisms. All extracts were active with MIC values from 0.15 to 3 mg/ml (Eloff, 2001). *Kigelia africana* has been well investigated and the antimicrobial activity of the tree has been long established. For example, the ethanol extract of the bark has been reported to be antibacterial and antifungal (Akunyili, 1991). The extracts were considerably less active against *E. coli*,

especially *Cassia abbreviata*, *Hypoxis*, *Flacourtia indica*, *Elephantorrhiza goetzei* and *Khaya anthotheca* have shown no activity against these bacteria. *Rhus chirindensis* roots had also no activity against gram negative bacteria although it was one of the most active extracts against gram positive bacteria.

Well-pronounced, potent antibacterial activity of the roots of *Elaeodendron matabelicum* is a new finding which hasn't been reported in literature. This extract has shown antibacterial activity against both gram (+) and gram (-) bacteria with large zones of inhibition; 5.00 ± 0.41 mm, 4.63 ± 0.48 mm, 2.50 ± 0.58 mm, 5.50 ± 0.58 mm respectively.

For antifungal activity, the same plants have given the best results for both fungi. *Warburgia salutaris* parts, especially roots, were active against both fungi strains with inhibition zones of 10.00 ± 0.82 mm for *C. albicans* and 8.25 ± 0.50 mm for *A. niger* which were even bigger than the zones of the reference amphotericin B ($10\mu\text{g}$) 6.35 ± 0.50 mm and 6.75 ± 0.58 mm respectively. *Securidaca longepedunculata* roots, *Terminalia sericea* roots, *Kigelia africana* bark and *Cassia abbreviata* all parts were also active against fungi strains. The most surprising plant was *Hypoxis rooperi*, which had previously shown no activity against bacteria but was definitely active against fungi. Reports on the inhibition of *C. albicans* growth by methanolic root extracts of *Terminalia sericea* (Steenkamp et al, 2007; Fyhrquist et al., 2002 and Moshi and Mbwambo, 2005) support the present findings.

Out of 26 extracts, 4 were active against all microorganisms; *Terminalia sericea* roots, *Warburgia salutaris* roots, *Gymnosporia senegalensis* roots and *Kigelia africana* bark.

4.5 Toxicology / Bioactivity Assay

The brine shrimp lethality assay is considered a useful tool for preliminary assessment of toxicity. In addition, the method is rapid, simple, reproducible and economical. A wide variety of biologically active chemical compounds, in particular cytotoxic agents, are toxic to

brine shrimp (*Artemia salina*); the death of this organism when exposed to varying concentrations of these compounds forms the basis of a toxicity test. Bioactive compounds are nearly always toxic in high concentrations and, as toxicology can be described as pharmacology at higher doses, this premise has been applied to the screening of medicinal plant extracts in the brine shrimp toxicity test.

It has been worked with 14 plants and 26 extracts. Different literature papers have taken different levels of LC_{50} ($\mu\text{g/ml}$) as toxic/bioactive. According to D'eciga-Campos et al, 2007 and McLaughlin et al, 1998 publications in which $LC_{50} < 1000\text{ppm}$ is considered toxic, 20 of 26 extracts (77%) showed bioactivity. 5 of these were showing significant toxicity with levels of $LC_{50} < 300\mu\text{g/ml}$. The lowest readings of LC_{50} , *Terminalia sericea leaves* (66.7ppm) and *Kigelia africana fruit* (117.4ppm) were even lower than the positive control, *Nerium oleander* (141.7ppm) which is a plant with well-established anti-tumour activity. The low readings of *Kigelia africana fruit* and *bark* (117.4ppm; 262.2ppm) explain the use of these medicinal extracts in the treatment of skin cancer for their cytotoxic properties (Houghton, 1994). *Securidaca longepedunculata's* toxicity is well known by the traditional medicine since it is a common suicide mean among African women by the insertion of the roots into the vagina (Gelfand et al, 1985). *Flacourtia indica leaves* and *Rhus chirindensis roots'* toxic levels oppose to the non-toxic levels of the parts used in the traditional medicine, roots and leaves respectively, exhibited once again that the practice of traditional medicine for centuries determines the right from wrong. *Terminalia sericea roots*, *Warburgia salutaris bark and leaves* and *Elephantorrhiza goetzei roots* were the other extracts which need further investigation concerning their toxic LC_{50} levels. The other six extracts exhibited levels in the safe zone which helps us to explain their use in traditional medicine. Especially *Dichrostachys cinerea roots* gave the highest reading of $LC_{50} = 4304.6\text{ppm}$. The degree of lethality was found directly proportional to the concentration of the extract.

4.6. Plants

4.6.1. *Cassia abbreviata* Oliv.

The bark, the leaves and the roots were taken separately for the research. All parts gave good yields in the extraction process; $5.12 \pm 0.11\text{g}$ ($12.79 \pm 0.27\%$), $4.10 \pm 0.17\text{g}$ ($10.25 \pm 0.43\%$), and $5.87 \pm 0.13\text{g}$ ($16.31 \pm 0.37\%$) respectively.

In screening Phytochemistry, the Alkaloids were present in extracts of *Cassia abbreviata* leaves and roots. It is inspected that the Rf values in TLC didn't match any of the used references. Knowing from literature the presence of alkaloid (-)cassine (**Fig 38**, page 99) was isolated from *Cassia excelsa* and *Cassia racemosa* (Kim, 2007; Moo-Puc et al,2007), it would be highly possible to find the same alkaloid in *Cassia abbreviata* if it was run against that reference. For Flavonoids, all *Cassia* extracts gave undoubtedly good confirmatory test results; from literature it is known that 2,4-trans-7,4'-dihydroxy-4-methoxyflavan has been isolated from leaves and twigs and the first flavan-3-ol with 4',7-dihydroxy phenolic substitution pattern, the novel (2R,3S)-guibourtinidol, was identified in the heartwood (Nel et al., 1999; Dehmlow et al.,1998). Running the extracts against glycoside and aglycone references, there is enough evidence to believe that *Cassia abbreviata* roots may contain **isovitexin** and **hyperoside**. For Saponins, the roots and bark extracts have definite positive results for all three tests. For Coumarins all three parts gave positive results. In testing Anthraquinones, *Cassia abbreviata* was almost like a reference material which had both free and combined anthrocyanenes. It is known that the main chemical constituents of this species are the anthraquinones such as *absin*, *chaksine* and *cassic acid*, which is also known as *Rhein*, a crystalline antibiotic compound. Although from a different plant from the same species, an antiprotozoal anthraquinone, **chrysophanol** (**Fig 37**, page 99) was isolated from the leaves of *Cassia racemosa* (Moo-Puc et al,2007; Mena-Rejon et al, 2002; Sansores-Peraza et al, 2000). Out of the eight extracts tested against anthraquinone references, *Cassia*

abbreviata roots gave definite positive results and the Rf values and the visible red colour of the spots after spraying suggested that the extract may well contain **chrysophanol** and hydrolysable part of **emodin** (Fig 7, page 17). The leaf and root extracts of *Cassia abbreviata* gave negative results for Tannins.

Fig 37: *Chrysophanol*, anthraquinone

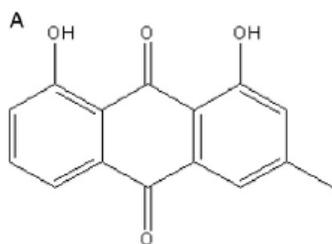
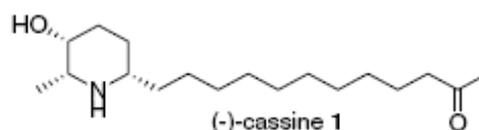


Fig 38: *Cassine*, alkaloid



According to the Antioxidant assay, all three extracts of this plant have given good radical scavenging activity; bark $86.36 \pm 0.04\%$, leaves $85.49 \pm 0.31\%$ and roots $85.39 \pm 0.04\%$, with high content of total phenolic compounds, bark ($0.416 \pm 0.103\text{mgGAE}$) and roots ($0.398 \pm 0.097\text{mgGAE}$) being relatively higher than the leaves ($0.243 \pm 0.039\text{mgGAE}$). The observed high antioxidant activity by plant extracts can definitely be explained by the high and frequent presence of phenolic compounds present in the plant parts such as flavonoids, anthraquinones and small amounts of tannins.

In the Antiviral assay, the root extract of *Cassia abbreviata* has given the best results among the three extracts of this plant. In EPTT, the Reduction Factor of the infectivity of the virus, HSV-2, was 10^3 for this particular extract which is a good antiviral result according to Vlietinck et al, 1995. In NT, the amount of extract necessary to neutralize the viral suspension was as low as $10.41\mu\text{g/ml}$. The cytotoxicity levels were relatively low compared to the other extracts, with $156.25\mu\text{g/ml}$. All three results compiled, the root extract of *Cassia abbreviata* makes a good choice as an antiviral remedy. This could be due to the abundance of phenolic

groups present in the extract which would serve the well established theory (Khan et al, 2005). The antiviral activity of different anthraquinones from extracts of *Cassia angustifolia* was proven against HSV-1 (Sydiskis et al. 1991). Although the leaves haven't achieved the same success in EPTT with RF value of 10^2 , in SNT the extract neutralized the viral suspension at a fairly low concentration of $20.83\mu\text{g/ml}$ and low cytotoxicity levels of $156.25\mu\text{g/ml}$. On the other hand, the bark hasn't shown any antiviral activity.

In the Antimicrobial assay, out of three extracts, the bark and the root extracts have shown moderate antibacterial activity against gram(+) bacteria, *Staphylococcus aureus* and *Streptococcus group A* but, except the moderate activity of the roots against *Pseudomonas aeruginosa*, the extracts were not active against gram(-) bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*. None of the extracts have shown antifungal activity against *Candida albicans* but all have shown moderate to good antifungal activity against *Aspergillus niger*. In a screening of plants used against STDs in Zimbabwe, the methanol extracts of *Cassia abbreviata* showed significant inhibition against Gram-positive and Gram-negative bacteria, while acetone extract inhibited most of the species (Kambizi, 2001). The same antimicrobial results were achieved in another study with methanol, aqueous and DCM extracts (Thomik, 2000) (-)cassine was isolated from *Cassia excelsa*, has antimicrobial activity against *Staphylococcus aureus*, prosopinine shows analgesic, anesthetic, and antibiotic activities and ()-spectaline offers cytotoxic activity.

In the acute Toxicity test done with brine shrimp eggs according to the BSLT methods, all parts, especially the root extract with LC_{50} values of $1319.37 \pm 356.63\mu\text{g/ml}$, were found to safe to use. The bark had LC_{50} values of $454.93 \pm 18.60\mu\text{g/ml}$ and the leaves had LC_{50} values of $445.72 \pm 22.15\mu\text{g/ml}$.

4.6.2. *Dichrostachys cinerea* (Forssk.) Chiov

The leaves and the roots were taken separately for the research. Both parts gave good yields in the extraction process, $4.21 \pm 0.18\text{g}$ ($14.53 \pm 0.62\%$), and $3.21 \pm 0.23\text{g}$ ($10.71 \pm 0.75\%$) respectively.

In screening Phytochemistry, the Alkaloids were present in the leaf extract of *Dichrostachys cinerea* leaf giving definite positive results for all tests. After running chromatographic tests with reference compounds, it is highly possible that *Dichrostachys cinerea* leaves have **berberine** alkaloid. For Flavonoids, out of the eight extracts tested against glycoside and aglycone references, there is enough evidence to believe that *Dichrostachys cinerea* leaves may contain **quercitrin**, **isoquercitrin** (**Fig 39**, page 101) and **hesperetin** (**Fig 10**, page 21). A new isomer of mesquitol (2,3-trans-3',4',7,8-tetrahydroxyflavan-3-ol) (**Fig 40**, page 101) was isolated from *Dichrostachys cinerea* in excellent yields. It has shown free-radical scavenging property and alpha-glucosidase inhibitory activities but, it could not display xanthine oxidase inhibitory property (Jagadeeshwar, 2003). For Saponins, both root and leaf extracts have definite positive results for all three tests. For Coumarins both parts gave negative results. In testing Anthraquinones, there were definite positive results for the leaf extract but the spots in TLC didn't match the references tested. For Tannins, two of the best results were both extracts of *Dichrostachys cinerea*.

Fig 39: Quercitrin, R= Rhamnoside

Isoquercitrin, R= Glucoside

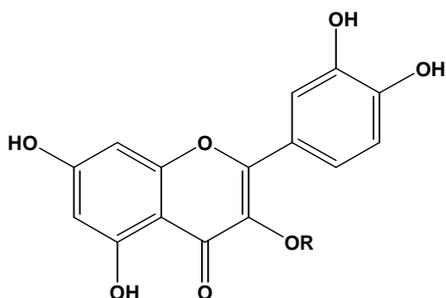
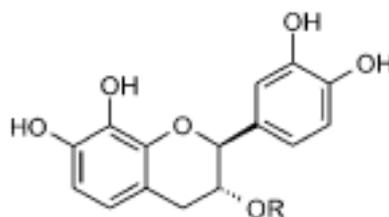


Fig 40: Mesquitol,

2,3-trans-3',4',7,8-tetrahydroxyflavan-3-ol



According to the Antioxidant assay, although the leaf extract of this plant has given very good radical scavenging activity, $88.97 \pm 0.46\%$, with considerable content of total phenolic compounds, $0.286 \pm 0.043\text{mgGAE/mg plant}$, the root extract has given the lowest readings in both DPPH test with only $27.39 \pm 1.24\%$ and TPC test with $0.105 \pm 0.003\text{mgGAE/mg plant}$. This was expected since hardly any phenolic compounds were detected in the phytochemistry screening of this extract. Therefore, the observed low antioxidant activity can be explained by the low content of phenolic compounds in this particular extract and proves once again the point that the presence of phenolic compounds present in the plant parts affects directly their antioxidant activity.

One of the two best results achieved on the Antiviral assay was with the *Dichrostachys cinerea* leaves. Achieving 10^4 RF on the EPTT test and neutralizing the viral suspension with concentration as low as $10.41\mu\text{g/ml}$ in NT, it was proven to be a potent antiviral extract. The cytotoxicity levels, $78.13\mu\text{g/ml}$, were acceptable with caution. The roots however didn't show the same potent activity as the leaves but were still active in NT at the concentration of $88.33\mu\text{g/ml}$ and had the lowest cytotoxicity levels with $312.50\mu\text{g/ml}$. In an antiviral study done on *Caesalpinia pulcherrima*, an herb used in traditional Chinese medicine, and the related quercetin, exhibited potent anti-HSV and -ADV activities. Among the flavonoids tested, only quercetin possessed significant activity against *human herpesviruses* and *adenoviruses*. According to a previous report, rutin (quercetin-3-rutinoside) did not express antiviral activity whereas **quercitrin** (quercetin-3-rhamnoside) possessed similar activity to quercetin. Therefore, the antiviral activity among the flavonoid glycosides containing the quercetin moiety might be correlated with the species of sugar group at the 3 position (Chiang et al, 2003). Through Thin Layer Chromatography runs against references, it was concluded that *Dichrostachys cinerea* leaves may contain **quercitrin**, **isoquercitrin** and **hesperitin**. This would explain their high antiviral and antioxidant activity.

In the Antimicrobial assay, the leaves have shown moderate antibacterial activity against gram (+) bacteria, *Staphylococcus aureus* and *Streptococcus group A* but the roots have shown no activity against the same bacteria. Both extracts were not active against gram (-) bacteria, *Escherichia coli* and both have shown good activity against *Pseudomonas aeruginosa*. The leaf extract was one of the few extracts that have shown antifungal activity against *Candida albicans* but both extracts were highly active against *Aspergillus niger*.

In the acute Toxicity test done with brine shrimp eggs according to the BSLT methods, the leaves were found to be moderately safe with $539.39 \pm 78.24 \mu\text{g/ml}$ and the roots were very safe to use with LC_{50} value of $4304.59 \pm 685.69 \mu\text{g/ml}$.

4.6.3. *Elaeodendron matabelicum* Loes.

The roots were extracted and tested. The yield was $2.96 \pm 0.12\text{g}$ and that was $7.40 \pm 0.30\%$ of the extracted dry material.

In screening Phytochemistry, the methanol extract only gave positive results for Saponins and Tannins. There is not much information in literature available about this plant.

According to the Antioxidant assay, the root extract of this plant has given good radical scavenging activity, $87.64 \pm 0.02\%$, with high content of total phenolic compounds, $0.357 \pm 0.090\text{mgGAE/mg plant}$. The observed high antioxidant activity by this plant extract must be due to its tannin content.

No Antiviral activity was found with the root extracts of this plant. The cytotoxicity levels, $78.13 \mu\text{g/ml}$, were acceptable with caution.

In the Antimicrobial assay, the root extract of *Elaeodendron matabelicum* was one of the best extracts which have shown good antibacterial activity against both the gram(+) and gram(-) bacteria especially one of the most active against *Staphylococcus aureus*, $5.00 \pm 0.41\text{mm}$, and *Pseudomonas aeruginosa*, $5.50 \pm 0.58\text{mm}$. The extract was one of the eight

extracts that have shown any inhibition against *Escherichia coli* with inhibition zone of 2.50 ± 0.58 mm when the reference gentamicin inhibited the growth of bacteria for 12.00 ± 0.82 mm. However, for antifungal activity, this extract has shown no activity against the fungi which were chosen for this study, *Candida albicans* and *Aspergillus niger*.

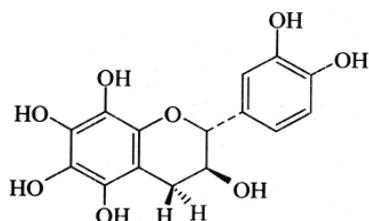
In the acute Toxicity test done with brine shrimp eggs according to the BSLT methods, the root extract was found to be safe to use with LC_{50} value of 1012.31 ± 217.69 μ g/ml.

4.6.4. *Elephantorrhiza goetzei* Harms

The roots were extracted and tested. Roots gave good yield in the extraction process, 7.38 ± 0.19 g which was $18.46 \pm 0.47\%$.

In screening Phytochemistry, the Alkaloids' tests gave positive results for the root extract of *Elephantorrhiza goetzei*. After running chromatographic tests with reference compounds, it is highly possible that *Elephantorrhiza goetzei* roots have **berberine** alkaloid which would be a new finding since there is no mention of it in literature. Out of the eight extracts tested against glycoside and aglycone references, there is enough evidence to believe that *Elephantorrhiza goetzei* roots may contain **astragalin** and **hesperidin** (**Figure 9**, page 21). It is already known that Flavonoids are present, one of them is 3,3',4',5,6,7,8-heptahydroxyflavan, namely **elephantorrhizol** (**Fig 41**, page 104), was isolated from the roots of *Elephantorrhiza goetzei* (Moyo et al, 1999). The stilbene glycoside, 5-methoxy-(E)-resveratrol-3-O-rutinoside was isolated from the root bark of *Elephantorrhiza goetzei*, along with known compounds, namely gallic acid, (E)-resveratrol, catechin, gallic acid and oleanene triterpenoids (Wanjala, 2001).

Fig 41: Elephantorrhizol, flavan



Saponins were found in 22 of 27(81%) screened plant extracts which makes it the second abundant phytochemical group after tannins in this research. One of the best results was achieved with the root extract of *Elephantorrhiza goetzei*. Also for Coumarins and Tannins, the root extract of *Elephantorrhiza goetzei* gave some of the best results. No Anthraquinones were found in the methanol extract. The other chemical constituents known about this plant are stilbene glycosides and triterpenoids (Wanjala, 2001).

According to the Antioxidant assay, the root extract of this plant has given good radical scavenging activity, $85.69 \pm 0.03\%$, with high content of total phenolic compounds, $0.339 \pm 0.084\text{mg GAE}$. The observed high antioxidant activity by this plant extract can definitely be explained by the high and frequent presence of phenolic compounds present in the plant parts such as flavonoids, coumarins and tannins.

Although packed with chemical compounds, in EPTT test, the Antiviral activity achieved with the methanol root extract of this plant was not significant enough with the Reduction Factor of only 10^2 . However, in NT, the viral suspension was neutralized with the concentration of $83.33\mu\text{g/ml}$. The cytotoxicity levels were low compared to the other extracts, with $156.25\mu\text{g/ml}$. This extract could be used against viral diseases as a relatively potent antiviral.

In the Antimicrobial assay, the root extract of *Elephantorrhiza goetzei* has shown one of the best results against gram(+) bacteria, *Staphylococcus aureus*, $4.00 \pm 0.0\text{mm}$ and *Streptococcus group A*, $4.50 \pm 0.58\text{mm}$ but the extract was not active against gram(-) bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*. For antifungal activity, this extract has shown no activity against the fungi which were chosen for this study, *Candida albicans* and *Aspergillus niger*.

In the acute Toxicity test done with brine shrimp eggs according to the BSLT methods, the root extract has given low readings, $356.55 \pm 24.55\mu\text{g/ml}$, which suggests caution is necessary using this part of this plant.

4.6.5. *Flacourtia indica* (Burm.f.) Merr.

The leaves and the roots were taken separately for the research. Both parts gave good yields in the extraction process, $2.54 \pm 0.19\text{g}$ and $3.61 \pm 0.23\text{g}$ respectively which were respectively $12.68 \pm 0.93\%$ and $9.04 \pm 0.59\%$ of the plant material.

In screening Phytochemistry, the only definite groups found in both of the extracts were Anthraquinones and Tannins. Through literature, it is known that this plant contains a fatty acid mixture consisting of stearic acid, margaric acid, palmitic acid, linolic and linolenic acids. Also new compounds have been identified such as a glucoside ester, named **flacourside**, 4-oxo-2-cyclopentenylmethyl 6-O-(E)-p-coumaroyl-b-D-glucopyranoside (**Fig 42**, page 106) that has been isolated together with known methyl 6-O-(E)-p-coumaroyl glucopyranoside (**Fig 43**, page 107) and 6-O-(E)-p-coumaroyl glucopyranose from the n-butanol extract of fruit juice of the *Flacourtia indica* (Amarasinghe et al, 2007) . Another known compound in this plant is sitosterin-(6-O-acyl)- β -D-glucopyranoside (Dehmlow EV, 2000).

Fig 42: Flacourside,

4-oxo-2-cyclopentenylmethyl 6-O-(E)-p-coumaroyl-b-D-glucopyranoside

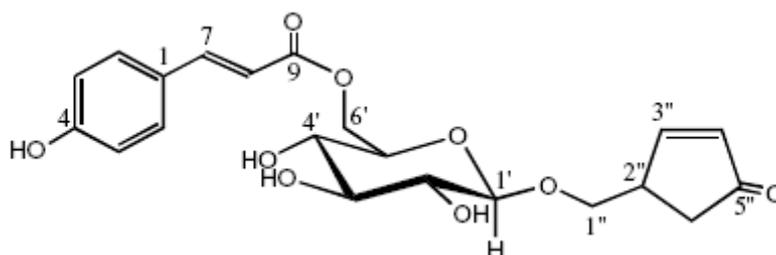
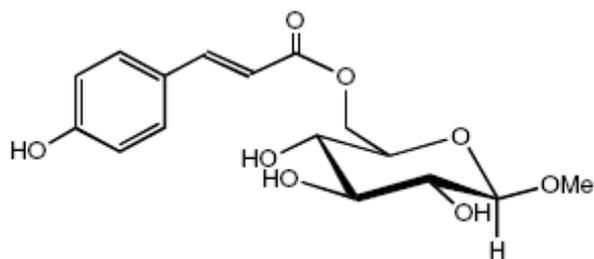


Fig 43: methyl 6-O-(E)-p-coumaroyl glucopyranoside



According to the Antioxidant assay, the leaf extract of this plant has given very good radical scavenging activity, $94.87 \pm 0.76\%$, with very high content of total phenolic compounds, $0.431 \pm 0.106 \text{mgGAE}$. The observed high antioxidant activity by this plant extract must be due to its anthraquinone and tannin content. Although not as good, the roots have also given good radical scavenging activity, $82.01 \pm 0.19\%$, with considerable amount of total phenolic compounds, $0.210 \pm 0.025 \text{mgGAE}$.

Of the two extracts tested for Antiviral activity, the roots have given better results at the EPTT with the RF value of 10^3 whereas the leaves have only reduced the infectivity of the virus at 10^2 level. However, in NT, the leaf extract neutralised the viral suspension at lower concentrations, $83.33 \mu\text{g/ml}$ versus $125.0 \mu\text{g/ml}$. Both extracts had relatively safe levels of cytotoxicity with concentrations of extracts of leaves at $78.13 \mu\text{g/ml}$ and the roots at $156.25 \mu\text{g/ml}$. Considering all these results, we can conclude that both leaf and root extracts of *Flacourtia indica* could be used against viral diseases with moderate safety.

In the Antimicrobial assay, the leaves have shown no activity against any of the gram (+) or gram (-) bacteria. The roots were moderately active against *Streptococcus group A* and slightly active against *Pseudomonas aeruginosa* but not active against the other two strains of bacteria. For antifungal activity, both extracts were not active against *Candida albicans* but moderately active against *Aspergillus niger*.

In the acute Toxicity test done with brine shrimp eggs according to the BSLT methods, the leaf extract was found to be toxic, giving readings such as $281.81 \pm 26.13\mu\text{g/ml}$. The root extract was found to be moderately safe to use, $467.31 \pm 39.01\mu\text{g/ml}$. This result exhibited once again that the practice of traditional medicine for centuries determines the right from wrong because the part of *Flacourtia indica* used in traditional medicine is the roots and not the leaves.

4.6.6. *Gymnosporia (Maytenus) senegalensis (Lam.) Loes*

The twigs, the leaves and the roots were taken separately for the research. Twigs and leaves gave good yields in the extraction process, $2.15 \pm 0.19\text{g}$ and $4.07 \pm 0.11\text{g}$ respectively whereas the roots gave a yield which was only $1.46 \pm 0.13\text{g}$, $4.85 \pm 0.43\%$ of plant material.

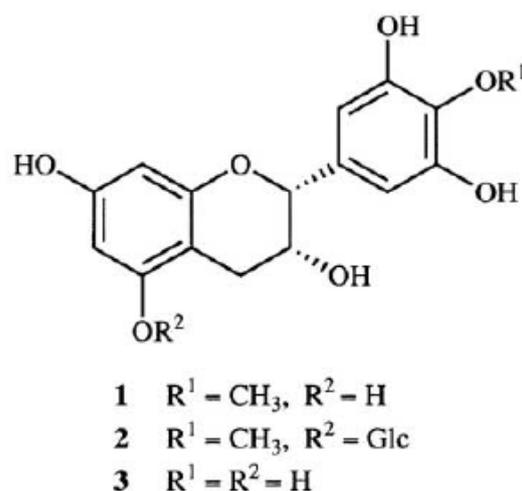
In screening Phytochemistry, although reported by Pistelli et al. in 1998 to be present, the Alkaloids and Coumarins were not found in any extract of *Gymnosporia senegalensis*. For Flavonoids, out of the eight extracts tested against various glycoside and aglycone references, there is enough evidence to believe that *Gymnosporia senegalensis* leaves may contain **hesperidin** (Fig 9, page 21). For Saponins, some of the best results were the root and leaf extracts of *Gymnosporia senegalensis*. Only the leaves gave positive results for the Anthraquinone tests. However the Rf values of the spots didn't match any of the references available to the project. All three extracts gave good positive results for Tannins. This was expected since the presence of different types of catechins was known from literature. The known compounds are (-)-4'-methylepigallocatechin (Drewes, 1993), (-)-epigallocatechin, epicatechin, epigallocatechin, procyanidin (Nonaka, 1983; Nonaka, 1981; Porter, 1982; Hashimoto 1989), phloro-glucinol 1-O-b-D-gluco-pyranoside and (-)-4'-methylepigallocatechin-5-O- β -gluco-pyranoside, (+)-4'-methylgallo-catechin-3'-O- β -gluco-pyranoside and (-)-epicatechin(-)-4'-methylepigallocatechin (Ghazi et al, 1999)(Fig 44, page 109).

Fig 44: Catechins

1= (-)-4'-methylepigallocatechin

2=(-)-4'-methylepigallocatechin-5-O- β -
glucopyranoside

3=(-)-epigallocatechin



According to the Antioxidant assay, all three extracts of this plant have given well to very good radical scavenging activity. Especially the root extract, with $96.05 \pm 0.18\%$, was the third best result in all of the extracts. This extract also had high content of total phenolic compounds, $0.222 \pm 0.014\text{mg GAE}$. The other two extracts, leaves $90.55 \pm 0.67\%$ and twigs $87.28 \pm 0.10\%$, had also considerable amounts of total phenolic compounds, $0.346 \pm 0.072\text{mgGAE}$ and $0.268 \pm 0.033\text{mgGAE}$ respectively. The observed high antioxidant activity by plant extracts can definitely be explained by the high and frequent presence of phenolic compounds present in the plant parts such as flavonoids, anthraquinones and tannins.

All three extracts of *Gymnosporia senegalensis* gave very good results in Antiviral testing. In EPTT, the Reduction Factor of the infectivity of the virus was 10^3 for all extracts for this particular plant which is a good antiviral result according to Vlietinck et al, 1995. In NT, the amount of extract necessary to neutralize the viral suspension was as low as $10.41\mu\text{g/ml}$ for the leaves, $15.63\mu\text{g/ml}$ for the twigs and $20.83\mu\text{g/ml}$ for the roots. Chiang et al. (2002) examined the antiviral (on HSV-1, -2 and adenoviruses-3, -8, 11) activity of aqueous extracts and pure compounds of *Plantago major*. It was concluded that the pure compounds isolated from *P. major* exhibiting anti-HSV activities are mainly derived from phenolic compounds, especially **caffeic acid**. Again, the antiviral and antioxidant activity of some fractions and of a series of flavonoids and proanthocyanidins obtained from *Crataegus sinaica* (Rosaceae) was

evaluated by Shahat et al. (2002). In general, more pronounced anti-HSV activities were seen with **epicatechin-containing dimmers**. Therefore it wouldn't be wrong to assume the good antiviral activity of *Gymnosporia senegalensis* could be due to the catechins present in its phytochemistry. However, the cytotoxicity levels were relatively high compared to the other extracts, with toxicity already at concentration of 19.53µg/ml for twigs, 39.06µg/ml for the leaves and 78.13µg/ml for roots. All three results complied, all extracts of *Gymnosporia senegalensis* make good and potent choices as an antiviral remedies which need to be used with caution.

In the Antimicrobial assay, the root extract of *Gymnosporia senegalensis* was one of the four extracts in all those tested which gave positive results and have shown activity against all six micro-organisms chosen for this project. Other than that, the other two extracts of this plant have also shown good to high antibacterial activity against the entire gram (+) and gram (-) bacteria, especially the leaves and the twigs were some of the best results among all results. For antifungal activity, the root extract was the only extract of *Gymnosporia senegalensis* that was active against *Candida albicans* and one of the rare extracts which were active against this fungi strain at all. The leaves and the roots were also moderately active against *Aspergillus niger*. The twigs were not active against any of the fungi strains.

Gymnosporia (Maytenus) senegalensis was screened for its anti-malarial activity against *Plasmodium falciparum* in vitro along with others. It was found to be one of the four most active plants (Gessler, 1994). Liquid-liquid partitioning of the methanol extracts indicated that fractions of *M. senegalensis* in dichloromethane and ethyl acetate had the highest antileishmanial activity (El Tahir, 1998).

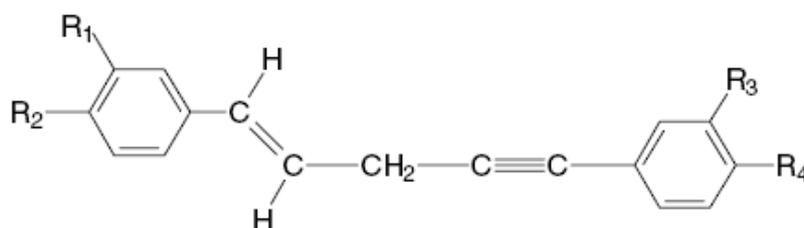
In the acute Toxicity test done with brine shrimp eggs according to the BSLT methods, all parts, especially the root extract with LC₅₀ value of 2185.61 ± 872. 25µg/ml, were found to be safe to use.

4.6.7. *Hypoxis rooperi* (hemerocallidea)

The tuber part of *Hypoxis* was extracted in methanol and the yield was good with $4.90 \pm 0.13\text{g}$ and $16.34 \pm 0.44\%$.

In screening Phytochemistry, the Alkaloids, Flavonoids and Coumarins were not found to be present in the extract although a major constituent of the corms of *Hypoxis hemerocallidea* as well other *Hypoxis* species is the pentenyne glycoside, hypoxoside (up to 4.5%), which on hydrolysis gives an aglycone with the trivial name of rooperol which is cytotoxic and inhibits the growth of cancer cells (**Fig 45**, page 111). Rooperol is also a lipoxygenase inhibitor and effective against mutagenesis in Ames test (Laporta et al, 2007). For Saponins *Hypoxis rooperi* gave one of the best results. It is known from literature that it contains sapogenin. There is a possibility of the presence of Anthraquinones in the extract. For Tannins, the extract gave positive result. In addition, from literature, the corms are reported to contain β -sitosterol, sterolins (sterol glycosides, up to 9mg/100g), monoterpene glycosides and organic acids.

Fig 45: Chemical structures of the norlignans derived from *Hypoxis rooperi* extract



Compound		R ₁	R ₂	R ₃	R ₄
<u>1</u>	Hypoxoside:	-OH	-O- β -D-glucopyranosyl	-OH	-O- β -D-glucopyranosyl
<u>2</u>	Dehydroxyhypoxoside:	-H	-O- β -D-glucopyranosyl	-OH	-O- β -D-glucopyranosyl
<u>3</u>	Bis-dehydroxyhypoxoside:	-H	-O- β -D-glucopyranosyl	-H	-O- β -D-glucopyranosyl
<u>4</u>	Rooperol:	-OH	-OH	-OH	-OH
<u>5</u>	Dehydroxyrooperol	-H	-OH	-OH	-OH
<u>6</u>	Bis-dehydroxyrooperol	-H	-OH	-H	-OH

According to the Antioxidant assay, the tuber extract of this plant has given good radical scavenging activity, $86.62 \pm 0.26\%$, with the second highest content of total phenolic compounds in all of the extracts tested, $0.476 \pm 0.127\text{mgGAE}$. The observed high antioxidant activity by this plant extract must be due to its anthraquinone and tannin content. This also is an indicator for use in cancer/tumour therapy. Through literature, this use of *Hypoxis* has been well investigated and proven. Lipophilic extracts of *H. rooperi* bulbs are used in the treatment of prostate problems; they have anti-inflammatory activity and relieve symptoms (Hostettman, 2000). Hypoxoside and its glycone rooperol have been shown to possess antimutagenic and cytotoxic properties (Albrecht, 1996). A clinical assessment of the effects of whole plant extracts of *H. hemerocallidea* (randomised, placebo-controlled, double blind study involving 200 adult male patients with mild to moderate BPH) reported a statistically significant decrease in symptoms (Lowe FC *et al*, 1998). Other clinical studies have demonstrated an improvement in symptoms associated with BPH in patients treated with *Hypoxis* extracts (Buck, 1996; Muller-Christiansen, 1993; Dreikorn & Schonhofer, 1995). The efficacy of β -sitosterol in the treatment of BPH (benign prostatic hyperplasia) is well-documented (Pegel, 1984; Berges *et al*, 1995), as is its immunomodulatory (Bouic, 1998) and antimutagenic activity (Merck & Co. Inc., 1989). A general immunomodulatory effect, attributable to phytosterols, is the basis of efficacy.

For Antiviral activity, the methanol extract of the tuber of this plant has given good results. In EPTT, the Reduction Factor of the infectivity of the virus was 10^3 and in NT, the amount of extract necessary to neutralize the viral suspension was as low as $10.41\mu\text{g/ml}$. The cytotoxicity levels were relatively low compared to the other extracts, with $156.25\mu\text{g/ml}$. All three results compiled, the tuber extract of *Hypoxis* makes a good choice as an antiviral remedy.

In the Antimicrobial assay, the tuber extract of *Hypoxis hemerocallidea* was not active against any of the bacteria strains, gram (+) or gram (-), therefore showed no antibacterial activity. However, this extract was one of the most active extracts against the fungi strains chosen for this project, *Candida albicans*, 1.75 ± 0.65 mm, and *Aspergillus niger*, 3.13 ± 0.25 mm. Therefore it is appropriate to say that this extract is a good choice for fungal diseases but not bacterial infections.

In the acute Toxicity test done with brine shrimp eggs according to the BSLT methods, the tuber extract was found to be safe to use with LC_{50} value of 735.34 ± 89.39 µg/ml.

4.6.8. *Khaya anthotheca* (nyasica)

The barks and the roots were extracted in methanol. The yield was good with 3.17 ± 0.09 g and 2.87 ± 0.14 g respectively which was $15.85 \pm 0.45\%$ and $14.35 \pm 0.62\%$ of the dry plant material respectively.

In the Phytochemistry assay, no Alkaloids were found in both parts. For flavonoids, one of the nine extracts that gave undoubtedly good confirmatory test results was the root extracts of *Khaya anthotheca*. There are traces of Saponins and Coumarins in both extracts. In testing Anthraquinones, bark extract of *Khaya anthotheca* definitely gave one of the best results. Tannins were the most abundant phytochemical group that was screened. However, the bark extract of *Khaya anthotheca* was one of the only three extracts which gave negative results. The bark of *Khaya senegalensis* for northern Nigeria has also given negative results for alkaloids, glycosides and resins but has yielded 10.2% tannin. Ferreol et al have isolated nimbosterol which is identical with β -sitosterol and its glycoside nimbosterin which is the β -D-glycoside of the β -sitosterol.

According to the Antioxidant assay, the bark extract of this plant has given very good radical scavenging activity and with $96.05 \pm 0.05\%$, was the third best result in all of the

extracts. This extract also had highest content of total phenolic compounds, $0.596 \pm 0.157\text{mgGAE}$, which proves once again the point that the presence of phenolic compounds present in the plant parts affects directly their antioxidant activity. Although not as good, the roots have also given good radical scavenging activity, $87.43 \pm 0.03\%$, with high amount of total phenolic compounds, $0.336 \pm 0.060\text{mgGAE}$. The observed high antioxidant activity by this plant's extracts must be due to its flavonoids, coumarins, anthraquinones and tannins present in the plant parts.

In the Antiviral assay, the bark extract of the plant hasn't achieved the significant success in EPTT with RF value of only 10^2 , but in NT the extract neutralized the viral suspension at a fairly low concentration of $31.25\mu\text{g/ml}$. There has to be caution in using the extract though because of the cytotoxicity levels at even low concentrations such as $39.06\mu\text{g/ml}$.

In the Antimicrobial assay, the bark extract has shown moderate antibacterial activity against gram(+) bacteria, *Staphylococcus aureus* and *Streptococcus group A* but the extract was not active against any of the gram(-) bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*. The extract has also shown no antifungal activity against *Candida albicans* or *Aspergillus niger*.

In the acute Toxicity test done with brine shrimp eggs according to the BSLT methods, the bark extract was found to be almost toxic, giving readings $482.19 \pm 43.49\mu\text{g/ml}$. This initial test for toxicity could be an indication of its possible use as an anticancer agent. This toxicity could also be as done in a test done in Cameroon; the decoction of the bark has supplied antihelminthic results in-vivo (Nfi, 1999).

4.6.9. *Kigelia africana (pinnata) DC*

The bark, the fruit and the roots were extracted separately for the research. The fruit and the roots gave very good yields in the extraction process, $3.95 \pm 0.25\text{g}$ ($19.73 \pm 1.23\%$) and $3.85 \pm 0.46\text{g}$ ($19.27 \pm 2.30\%$). The bark extract gave less than half the yield with $1.44 \pm 0.21\text{g}$ ($7.22 \pm 1.05\%$).

Screening Phytochemistry, only the fruit extract gave positive results for alkaloids and the bark and root extracts gave negative results for Alkaloids. By reviewing the current literature this was expected because of the isolation of naphthoquinones (Akunyili and Houghton, 1993; Weiss et al, 2000). For Flavonoids, Saponins, Coumarins and Anthraquinones all extracts gave positive results which was also expected since iridoids (Gouda et al, 2003; Akunyili and Houghton, 1993), flavonoids (El-Sayyad, 1981) and coumarins (Govindachari et al, 1971) from *Kigelia africana* have been identified. For Tannins, only the bark extract gave considerable positive result.

According to the Antioxidant assay, all three extracts of this plant have given good radical scavenging activity; bark $81.49 \pm 0.19\%$, fruit $85.64 \pm 0.13\%$, roots $84.57 \pm 0.03\%$, with considerable content of total phenolic compounds. The fruit extract had the highest amount with $0.327 \pm 0.061\text{mgGAE}$, roots, $0.184 \pm 0.020\text{mgGAE}$, being slightly lower than the bark, $0.224 \pm 0.015\text{mgGAE}$. The observed good antioxidant activity by plant extracts can definitely be explained by the high and frequent presence of phenolic compounds present in the plant parts such as flavonoids, coumarins, anthraquinones and small amounts of tannins. It also explains the use of this plant as an anti-tumour agent. Dichloromethane extract of the bark and alcohol extract of the fruit have shown cytotoxicity (Houghton, 1994; Jackson, 1996). In an in vivo study, extracts given intra-peritoneally to mice have been recorded as anti-tumour (Azuine et al, 1997).

In the Antiviral assay, one of the two best results achieved was with the methanol extract of *Kigelia africana fruit*. Achieving 10^4 RF on the EPTT test and neutralizing the viral suspension with concentration as low as $31.25\mu\text{g/ml}$ in SNT, it was proven to be a potent antiviral extract. The cytotoxicity levels, however, were on the high side with toxicity already at concentrations of $39.06\mu\text{g/ml}$. The bark extract was also significantly potent as an antiviral agent with RF 10^3 in EPTT and active at concentration as low as $31.25\mu\text{g/ml}$ in NT. The issue of cytotoxicity is also relevant for this extract with same toxicity levels as the fruit extract. Both parts of *Kigelia africana* would be potent choices against viral infections but need to be used with great caution. The roots however didn't show any antiviral activity as the other extracts of this plant.

In the Antimicrobial assay, the bark extract of *Kigelia africana* was one of the four extracts in all those tested which gave positive results and have shown activity against all six microorganisms chosen for this project. Out of other two extracts, the fruit extract has also shown good antibacterial activity against gram (+) bacteria, *Staphylococcus aureus* and *Streptococcus group A*. The roots were moderately active against only *Streptococcus group A*. Except the slight activity of the bark against *Escherichia coli*, the extracts could not inhibit the growth of *E. coli*. All the extracts have shown good antibacterial activity against one of the gram (-) bacteria, *Pseudomonas aeruginosa*. For antifungal activity, the bark extract was one of the rare extracts which has shown good antifungal activity against both strains. The fruit extract was moderately active against only *Aspergillus niger*. The roots have shown no antifungal activity at all. These results have also been confirmed by other studies such as that the ethanol extract of the bark has been reported to be antibacterial and antifungal (Akunyili, 1991). These results correlate with the ethno-botanical use of this tree against all kinds of infections especially skin infections because of their high antimicrobial activity.

Dichloromethane extract of the rootbark is found out to be antiplasmodial (Weiss, 2000) and antitrypanosomal (Moideen, 1999). Ethanol extract of the fruit is reported as anti-inflammatory (Azuine et al, 1997).

In the acute Toxicity test done with brine shrimp eggs according to the BSLT methods, the fruit and the bark ($262.20 \pm 25.07\mu\text{g/ml}$) were found to be toxic, giving readings less than $300\mu\text{g/ml}$. Especially the fruit extract had a LC_{50} value of $117.41 \pm 30.27\mu\text{g/ml}$ which was even lower than LC_{50} value of the used reference toxic plant, *Nerium oleander* with $141.67 \pm 68.15\mu\text{g/ml}$. This initial test for toxicity could be a proof of its use against skin cancer (Houghton, 1994) and an indication to use parts of this plant as an anticancer agent. The roots, however, were found to be moderately safe to use.

4.6.10. *Rhus chirindensis*

The leaves and the roots were taken separately for the research. Both parts gave yields less than 10% in the extraction process, $3.38 \pm 0.11\text{g}$ ($8.45 \pm 0.28\%$) and $1.88 \pm 0.12\text{g}$ ($9.38 \pm 0.60\%$).

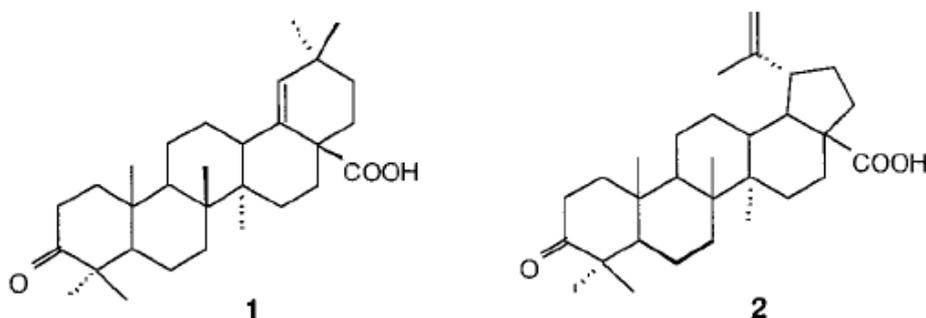
In screening Phytochemistry, for Flavonoids, both extracts of *Rhus chirindensis* gave undoubtedly good test results. There has been biflavonoids isolated from genus *Rhus succedanea* such as amentoflavone, agathisflavone, robustaflavone, hinokiflavone, volkensiflavone, rhusflavanone and succedaneflavanone (Lin et al, 1999). These flavonoids could as well be present in *Rhus chirindensis* if the extracts were tested against these reference flavonoids. In both extracts there were no Saponins and there were no Coumarins in the leaf extract. There was high possibility of the presence of Anthraquinones in the leaves but nothing in the roots. For Tannins both extracts gave good results.

According to the Antioxidant assay, both extracts of this plant have given very good radical scavenging activity and in fact were the top two scorers of this assay with the leaves

giving $96.91 \pm 0.33\%$ inhibition of DPPH and the roots $96.90 \pm 0.49\%$. Both extracts had good content of total phenolic compounds, leaves ($0.323 \pm 0.060\text{mgGAE}$) being slightly higher than the roots ($0.282 \pm 0.037\text{mgGAE}$). The observed high antioxidant activity by plant extracts can definitely be explained by the high and frequent presence of phenolic compounds present in the plant parts such as flavonoids, anthraquinones, coumarins and tannins. Antioxidant activity and phenolic composition of Turkish sumac, *Rhus coriaria* extracts were investigated and found to exhibit antioxidant activity (Kosar et al, 2007).

In the Antiviral assay, although the roots haven't achieved the significant success in EPTT with RF value of only 10^2 , in NT the extract neutralized the viral suspension at a fairly low concentration of $20.83\mu\text{g/ml}$. The cytotoxicity levels, $78.13\mu\text{g/ml}$, were acceptable with caution. On the other hand, there was no activity of the leaf extract of *Rhus chirindensis* on the antiviral assay but had the safest level of cytotoxicity with a concentration of $312.50\mu\text{g/ml}$. These results were slightly disappointing since in recent tests experimented on another genus of species, commonly found in China, *Rhus chinensis* showed that the petroleum ether extract of the stems was effective against *HIV-1* and *Rhus chinensis* would be a useful medicinal plant for the chemotherapy of *HIV-1* infection (Wang et al, 2006). *Rhus javanica*, a medicinal herb, has been shown to exhibit oral therapeutic anti-herpes simplex virus (*HSV*) activity in mice. Two major anti-*HSV* compounds, moronic acid (**Fig 46**, page 119) and betulonic acid, were purified from the herbal extract by extraction with ethyl acetate at pH 10 followed by chromatographic separations and their anti-*HSV* activity in vitro and in vivo were examined (Kurokawa et al, 1999).

Fig 46: Structures of Moronic acid (1) and Betulonic acid (2) isolated from *Rhus javanica*.



In the Antimicrobial assay, both the leaf and, especially, the root extracts have shown moderate to good antibacterial activity against gram(+) bacteria, *Staphylococcus aureus* and *Streptococcus group A* but, except the moderate activity of the leaves against *Pseudomonas aeruginosa*, the extracts were not active against gram(-) bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*. None of the extracts have shown antifungal activity against *Candida albicans* or *Aspergillus niger*.

In the acute Toxicity test done with brine shrimp eggs according to the BSLT methods, the leaf extract was found to be safe to use with the LC_{50} value of $1023.26 \pm 161.69 \mu\text{g/ml}$. However, the root extract has given low readings, $316.60 \pm 30.07 \mu\text{g/ml}$, which suggests caution is necessary using this part of this plant. This initial test for toxicity could be an indication of its possible use as an anticancer agent.

4.6.11. *Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.)

The bark part was extracted for the project. The yield was good with $5.99 \pm 0.15\text{g}$ and therefore $14.98 \pm 0.37\%$ of the dry plant material.

In screening Phytochemistry, the only group that was definitely present in the bark extract was the Tannins although traces of alkaloids were reported in Watt and Breyer-Brandwijk, 1962. Otherwise, all the other tested groups gave trace amounts (saponins) or negative results. In the fruit pulp, flavonoids were also detected along with the tannins (Ndhlala et al, 2007).

According to the Antioxidant assay, the bark extract of this plant has given very good radical scavenging activity, $89.63 \pm 0.05\%$, with high content of total phenolic compounds, $0.326 \pm 0.058\text{mgGAE}$, which would be the tannins that were found present in its phytochemistry. The phenolic acid composition of the peel and pulp of the fruits of Zimbabwe were analysed using traditional colorimetric as well as HPLC methods. *Sclerocarya birrea* pulp had the highest total phenolics, flavanoids and condensed tannins, i.e., 2262 lg GAE/g, 202 lg catechin/g and 6.0% condensed tannins, respectively (Ndhala et al, 2007).

For Antiviral activity, the methanol extract of the bark of this plant has given good results. In EPTT, the Reduction Factor of the infectivity of the virus was 10^3 and in NT, the amount of extract necessary to neutralize the viral suspension was as low as $20.83\mu\text{g/ml}$. Both test results indicate a potential as an antiviral agent. This could be due to the tannins present because in a recent study, the ethanol extract of whole plant of *Youngia japonica* exhibited antiviral activity against RSV cultured in HEp-2 cells. Two partially purified fractions (Fr.10 and Fr.11) from the 95% ethanol extract exhibited significant anti-RSV with 50% inhibitory concentration (IC₅₀) in the range of 3.0–6.0 $\mu\text{g/ml}$. A positive test of the fractions 10 and 11 for FeCl₃ reaction suggested that they might contain tannins or related phenolic compounds which may contribute to anti-RSV activity. (Ooi et al, 2004). However there should be caution towards its cytotoxicity levels which were relatively high compared to the other extracts, with $39.06\mu\text{g/ml}$.

In the Antimicrobial assay, the bark extract of *Sclerocarya birrea* was one of the best extracts which have shown good antibacterial activity against all the gram(+) and gram(-) bacteria especially one of the most active against *Streptococcus group A*, $5.50\pm 0.58\text{mm}$, and *Escherichia coli*, $3.00\pm 0.82\text{mm}$. Similar results have been achieved in a recent study where the bark and leaves were extracted with acetone and MIC values were determined using a

microplate serial dilution technique with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* as test organisms. All extracts were active with MIC values from 0.15 to 3 mg/ml (Eloff, 2001). However, for antifungal activity, this extract has shown no activity against the fungi *Candida albicans* and only moderate activity against *Aspergillus niger*.

The stem bark methanol/methylene chloride extract of *Sclerocarya birrea* exhibited at termination, a significant reduction in blood glucose and increased plasma insulin levels in diabetic rats. The extract also prevented body weight loss in diabetic rats. The effective dose of the plant extract (300 mg/kg) tended to reduce plasma cholesterol, triglyceride and urea levels toward the normal levels (Dimo et al, 2007). The results of an experimental animal study indicate that both the aqueous and methanolic extracts of *S. birrea* stem-bark possess anti-inflammatory activity although they are less potent than ASA as anti-inflammatory agent and thus lend credence to the suggested folkloric use of the plant in the management and/or control of arthritis and other inflammatory conditions in certain communities of South Africa (Ojewole, 2003). Methanolic extract of *Sclerocarya birrea* was screened for molluscicidal activity and found active. (Kela, 1989).

In the acute Toxicity test done with brine shrimp eggs according to the BSLT methods, the bark extract was found to be safe to use with LC₅₀ value of $1112.37 \pm 210.04 \mu\text{g/ml}$.

4.6.12. *Securidaca longepedunculata* Fresen.

The roots were extracted with methanol. The yield was good with $2.22 \pm 0.23\text{g}$ which was $11.67 \pm 1.21\%$ of the dry plant material.

In screening Phytochemistry, the Alkaloids were present in the root extract of *S. longepedunculata*, giving definite positive results for two tests. The presence of ergot and indole alkaloids has already been reported in literature (Costa et al, 1992; Scandola et al,

1994). Out of the eight extracts tested against glycoside and aglycone references, there is enough evidence to believe that *S. longepedunculata* roots may contain **apigenin 7-O-glucoside** and **luteolin 7-O-glucoside** (Figures 47 & 48 as aglycones) which would be new finding.

Fig 47: Apigenin

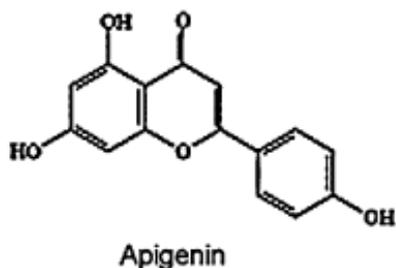
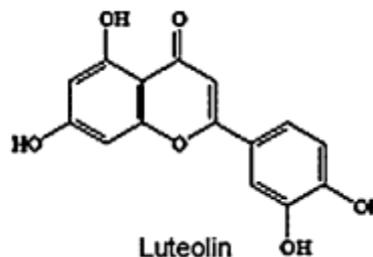


Fig 48: Luteolin



The other main constituents of *S. longepedunculata* are 6-methoxy-salicylic acid (Fig 49, page 122), two known xanthenes and saponins which were found in abundance in our tests as well. Two minor bitter compounds: β -D-(6-sinapoyl)-glucopyranoside and β -D-(3-sinapoyl)-fructofuranosyl- α -D-(6-sinapoyl) glucopyranoside have also been reported in bark extracts. A new hepta-oxygenated xanthone has also been recently isolated, 1,5-dihydroxy-2, 3, 6, 7, 8 pentamethoxy-xanthone (Fig 50, page 122) (Lannang et al, 2006).

Fig 49: 6-methoxy-salicylic acid

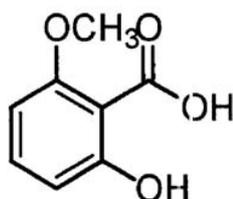
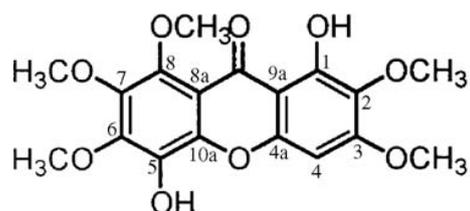


Fig 50: 1,5-dihydroxy-2, 3, 6, 7, 8 pentamethoxy-xanthone



According to the Antioxidant assay, the root extract of this plant has given very good radical scavenging activity, $93.43 \pm 0.64\%$, with considerable amount of total phenolic

compounds, 0.258 ± 0.040 mgGAE. The high antioxidant activity of this plant extract must be due to its flavonoids and tannins. The methanolic extract of the root of *S. longepedunculata* was screened for anti-inflammatory, analgesic and anticonvulsant activities, as well as for effects on phenobarbitone sleeping time. The extract produced an inhibition of the carrageenan-induced rat paw oedema, an inhibition of writhings induced by acetic acid, a complete (100%) protection against leptazol-induced convulsion and a potentiation of the phenobarbitone sleeping time in mice (Olajide et al, 1998). It was investigated again in 2005 to ascertain its analgesic and anti-colic properties using acetic acid-induced abdominal writhing in mice and tri-nitrobenzenesulphonicacid-induced colitis in rats. The results indicate that *S. longepedunculata* possesses analgesic properties, does not possess any anti-colic activity.

For Antiviral activity, the methanol extract of the roots of this plant has given good results. In EPTT, the Reduction Factor of the infectivity of the virus was 10^3 and in NT, the amount of extract necessary to neutralize the viral suspension was as low as $20.83\mu\text{g/ml}$. The cytotoxicity levels were relatively low compared to the other extracts, with $78.13\mu\text{g/ml}$. All three results compiled, the root extract of *S. longepedunculata* makes a good choice as an antiviral remedy.

In the Antimicrobial assay, the root extract of *S. longepedunculata* was one of the best extracts which have shown good antibacterial and antifungal activity against five of the six micro-organisms tested in this project. Only *Escherichia coli* could not be inhibited.

In the acute Toxicity test done with brine shrimp eggs according to the BSLT methods, the root extract was found to be almost toxic, $351.89 \pm 35.79\mu\text{g/ml}$, giving readings close to $300\mu\text{g/ml}$. This was expected since this part of *S. longepedunculata* is a well known suicide mean in traditional medicine amongst women by insertion of the root into the vagina (Gelfand, 1985). In addition, the plant was found to be toxic to rats since it damaged the

gastrointestinal mucosa (Buabeng, 2005). However, this initial test for toxicity could also be an indication of its possible use as an anticancer agent.

4.6.13. *Terminalia sericea* Burch ex. DC

The leaves and the roots were taken separately for the research. Both parts gave very good yields with $8.00 \pm 0.16\text{g}$ ($20.00 \pm 0.41\%$) and $11.00 \pm 0.35\text{g}$ ($27.51 \pm 0.87\%$) respectively.

In screening Phytochemistry, no Alkaloids were found as also reported by Raffauf, 1996. For Flavonoids, both extracts of *Terminalia sericea* gave undoubtedly good test results. Out of the eight extracts tested against glycoside and aglycone references, there is enough evidence to believe that *Terminalia sericea* roots may contain **isoquercitrin**, **vicenin-2** and **vitexin** which would be a new finding (**Figure 51**).

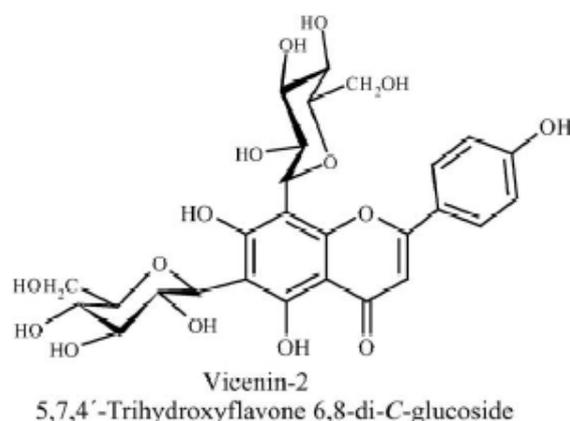


Fig 51: Vicenin-2

In Saponin tests, two of the best results were the extracts of *Terminalia sericea*, which is most probably due to sericoside, a triterpenoidal saponin (Miyako, 2006). The leaves and roots of *Terminalia sericea* have been found to contain sericoside with antioxidant activity (Steenkamp et al., 2004). There were no positive results for Coumarins and for Anthraquinones only the leaves gave some positive results. However, the two of the best results for the Tannins test were the extracts of *Terminalia sericea*.

According to the Antioxidant assay, both extracts of this plant have given very good radical scavenging activity, leaves $89.27 \pm 0.13\%$ and roots $89.38 \pm 0.02\%$, with very high content of total phenolic compounds, $0.439 \pm 0.115\text{mgGAE}$ and $0.406 \pm 0.100\text{mgGAE}$ respectively. The observed good antioxidant activity by plant extracts can definitely be explained by the high and frequent presence of phenolic compounds present in the plant parts such as flavonoids, tannins and small amounts of anthraquinones.

In the Antiviral assay, the root extract of *Terminalia sericea* has given better results than the leaf extract of this plant. In EPTT, the Reduction Factor of the infectivity of the virus was 10^3 for this particular extract which is a good antiviral result according to Vlietinck et al, 1995. In NT, the amount of extract necessary to neutralize the viral suspension was as low as $20.83\mu\text{g/ml}$. Although the leaves haven't achieved the same success in EPTT with RF value of only 10^2 , in NT the extract neutralized the viral suspension at a fairly low concentration of $31.25\mu\text{g/ml}$. However, the cytotoxicity levels for both extracts were relatively high compared to the other extracts, with $39.06\mu\text{g/ml}$. All three results compiled; the extracts of *Terminalia sericea* make good choices as antiviral remedy although need be used with great caution. The methanol extract of the leaves of *Terminalia sericea* was found to strongly inhibit the polymerase and the ribonuclease H activities of the *Human Immunodeficiency Virus type 1* (Bessong et al, 2004).

In the Antimicrobial assay, the root extract of *Terminalia sericea* was one of the four extracts which gave positive results and have shown activity against all six micro-organisms chosen for this project giving the second highest activity results in all those tested. Especially against *Staphylococcus aureus*, $7.88 \pm 0.48\text{mm}$, and *Pseudomonas aeruginosa*, $10.00 \pm 0.82\text{mm}$, the extract has given a zone of inhibition which was equivalent to or even larger than the reference antibacterials tested. The leaves have also shown slight to moderate activity against both of the gram (+) bacteria and the fungi strain *Aspergillus niger*. Similar findings have

been seen in literature such as in an antibacterial and antioxidant activity screening, methanol and water extracts of *Terminalia sericea*, were more active compared to the other extracts against *Streptococcus pyogenes* and *Staphylococcus aureus* (Steenkamp et al, 2004). On antimicrobial screening of the crude extracts of the selected *Combretum* and *Terminalia* species, the methanol extract of the roots of *Terminalia sericea* showed marked inhibition against Gram-positive bacteria and was also good inhibitor of *Enterobacter aerogenes*. All four of the extracts of the roots of *T. sericea* tested, (methanol, ethanol, acetone and hot water) had good antimicrobial activity (Fyhrquist et al, 2002). In a different study, intermediate and polar extracts of the roots exhibited high antibacterial activity against *Staphylococcus aureus*, *E. coli*, *Bacillus anthracis* and *P. aeruginosa*. They also exhibited antifungal activity against *C. albicans* and *Aspergillus niger* (Moshi et al, 2004)..

In the acute Toxicity test done with brine shrimp eggs according to the BSLT methods, both parts were found to be toxic, giving readings less than 300µg/ml. Especially the leaf extract had a result, $66.66 \pm 49.31\mu\text{g/ml}$, which was the lowest result in this assay, much lower than the used reference toxic plant, *Nerium oleander*, $141.67 \pm 68.15\mu\text{g/ml}$. Even though it is a very potent plant in every aspect, it should be used with great caution and in small doses. However, this initial test for toxicity could also be an indication of its possible use as an anticancer agent and tests should be considered.

4.6.14. *Warburgia salutaris* (Bertol.f.) Chiov.

The bark, the leaves, the roots and the twigs were taken separately for the research. All three parts gave good yields in the extraction process; $1.88 \pm 0.12\text{g}$ ($9.42 \pm 0.58\%$), $2.77 \pm 0.16\text{g}$ ($13.87 \pm 0.80\%$), $2.27 \pm 0.26\text{g}$ ($11.33 \pm 1.29\%$) and $1.07 \pm 0.56\text{g}$ ($5.35 \pm 0.53\%$) respectively.

In screening Phytochemistry, the Alkaloids and Coumarins were present in three of the four extracts of *Warburgia salutaris* which were tested for phytochemistry, all except the leaves.

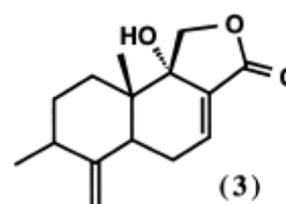
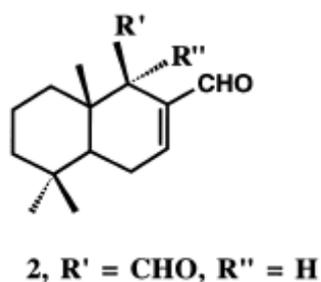
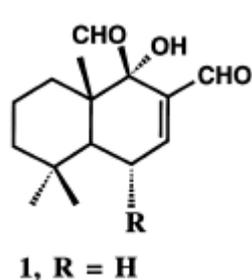
There were trace amounts of Flavonoids in three extracts again, all except the leaves. On the other hand, the leaves were the only extract which seemed to have Anthraquinones. For Saponins, all of the extracts were found to contain trace amounts. All four extracts gave some of the best results for Tannins. From literature, it is known that the leaves and bark contain numerous drimane sesquiterpenoids, including warburganal, polygodial, muzigadial, mukaadial, ugandensidial and salutarisolide (Mashimbye et al, 1999) (**Fig 52**, page 127).

Fig 52: Drimane sesquiterpenoids of *Warburgia salutaris*;

1= Warburganal,

2= Polygodial,

3= Salutarisolide



According to the Antioxidant assay, out of four extracts of this plant tested, except the twigs ($73.28 \pm 1.09\%$), other three extracts have given good radical scavenging activity, all more than 87%. Leaves have also shown very good activity with $94.08 \pm 0.87\%$. All extracts had more or less same levels of total phenolic compounds, leaves and roots being slightly higher than the others, $0.296 \pm 0.040\text{mgGAE}$ and $0.278 \pm 0.033\text{mgGAE}$. The observed high antioxidant activity by plant extracts can definitely be explained by the presence of phenolic compounds present in the plant parts such as flavonoids, coumarins, anthraquinones and tannins.

Out of three extracts of *Warburgia salutaris*, the only extract which gave significant positive Antiviral activity results was the root extract. In EPTT, the Reduction Factor of the infectivity of the virus was 10^3 and in NT, the amount of extract necessary to neutralize the

viral suspension was as low as 31.25µg/ml. However, the cytotoxicity levels for this extract was relatively high compared to the other extracts, with 39.06µg/ml. The methanol extracts of the bark and the leaves haven't demonstrated any antiviral activity and had very high cytotoxicity at concentration levels as low as 19.53µg/ml and 78.13µg/ml respectively. Overall results for *Warburgia salutaris* came as a surprise since the bark of this plant is a well established antiviral remedy against HIV/AIDS and other viral diseases. Either the test could be repeated with extracts of different solvents or it could mean the success of the plant depends on its high antibacterial and antifungal activity.

In the Antimicrobial assay, the root extract of *Warburgia salutaris* was the most successful extract showing high activity against all six micro-organisms chosen for this project. This extract has given the best results against three of the six strains, namely *Streptococcus group A*, *Candida albicans* and *Aspergillus niger*, reaching same zones of inhibition as the used references. The bark extract was also active against both of the gram (+) bacteria and the fungi strains without showing any activity against any of the gram (-) bacteria. *Warburgia salutaris* roots were active against both fungi strains with inhibition zones of 10.00±0.82mm for *C. albicans* and 8.25±0.50mm for *A. niger* which were even bigger than the zones of the reference amphotericin B (10µg) 6.35±0.50mm and 6.75±0.58mm respectively and as much as the zones of the reference antifungal miconazole (10µg) 10.00±0.41mm and 10.00±0.81mm respectively. The least active extract of *Warburgia salutaris* was the leaf extract by being slightly active against *Staphylococcus aureus* and moderately active against both of the fungi strains. These results indicate that any extract of *Warburgia salutaris* could be used against fungal infections and especially the roots could be very useful against bacterial infections caused by gram (+) bacteria. Crude extracts from 21 South African medicinal plants were screened for in vitro antibacterial activity and the highest activity was found in the methanol extracts from *Warburgia salutaris* among with three other plants (Rabe

et al, 1997). Warburganal and polygodial have been proven to be potently anti-candidal, and also have broad-spectrum antimicrobial activity (Rabe et al, 2000).

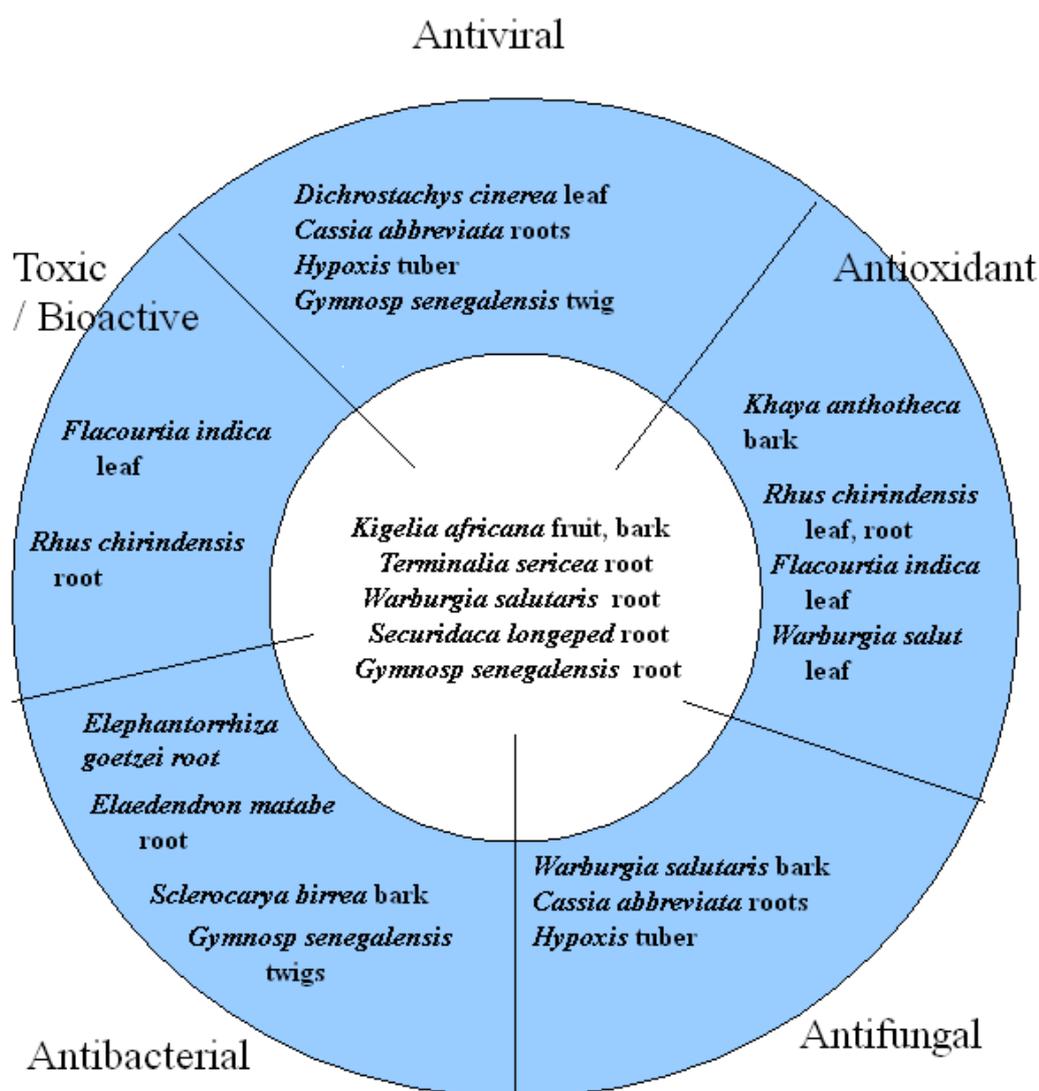
In the acute Toxicity test done with brine shrimp eggs according to the BSLT methods, all parts, especially the leaf and the bark extract, have given low readings, $351.41 \pm 29.58\mu\text{g/ml}$ and $359.66 \pm 14.33\mu\text{g/ml}$ respectively, which suggests caution is necessary when using this plant.

CHAPTER V

5.0 CONCLUSION

Some of 26 extracts have shown good activity in all pharmacological tests. These extracts are *Gymnosporia senegalensis* roots, *Kigelia africana* fruit, *S. longepedunculata* roots, *Terminalia sericea* roots and *Warburgia salutaris* roots. In case of virology and antifungal activity, it is necessary to add *Hypoxis rooperi* tuber and *Cassia abbreviata* roots to this list.

Fig 50: Summary Chart, displaying the best results for each assay. The group of extracts in the middle of the circle were present in all the assays as best results.



The observed high **antioxidant activity** by almost all of the plant extracts can definitely be explained by the high and frequent presence of tannins inside. Since tannins fall under phenols, the results are justified as phenols constitute the largest group of plant secondary metabolites and are widespread in nature (Trease and Evans, 2002). All extracts were very quick acting in terms of changing the colour of DPPH. 11 of 27 extracts already reached the stable stage with a very low absorbance in less than two minutes. Except another 4 which got to that stage in about twenty minutes, all the others took less than ten minutes. This is a very short acting time considering antioxidant vegetable extracts which come to a stable absorbance in 120min. The results correlate well with the phytochemical results where the highest percentage plants are the same ones that were found to have flavonoids, coumarins, anthraquinones and tannins, in general phenolic compounds. Although have not been very potent antiviral or antimicrobials, *Flacourtia indica leaves*, *Khaya anthotheca bark* and *Rhus chirindensis leaves* have shown the best antioxidant activity. It proves the point once again that in fighting HIV/AIDS, it is necessary to treat a group of syndromes and not only the infection aspect. The degeneration of tissues in an immuno-suppressed body may lead to far worse pathological conditions.

When we look at **antiviral activity** in terms of **phytochemistry**, it is easy to see the presence of abundant phenolic groups in the most active extracts. These results of the vast majority of the extracts containing flavonoids is supported by the fact that flavonoids and their close relations are the widely distributed in nature and are more common in the higher plants and in young tissues. This theory has been pronounced in many different literature papers with similar kind of work. A large number of small molecules, like phenolics, polyphenols, terpenes (e.g., mono-, di-, tri-), flavonoids, sugar-containing compounds, were found to be promising anti-herpetic agents. The major conclusion is that natural products from medicinal plant extracts are very important sources of anti-HSV agents (Khan et al, 2005).

The results and conclusions are not different for the **antimicrobial activity**. The same extracts were again the most active against tested strains of bacteria and fungi. In some cases like *Warburgia salutaris*, *Terminalia sericea* and *Gymnosporia senegalensis* extracts, the activity was as high as the antimicrobial reference compounds (**Table 11-14**, page 73-76). The important fact is again that they have alkaloids and flavonoids present.

In the **toxicity assay**, some of the most toxic plant extracts have given the best antimicrobial and antiviral results such as *Kigelia africana*, *Securidaca longepedunculata*, *Terminalia sericea* and *Warburgia salutaris* extracts. Therefore it is not appropriate to say that the use of these extracts should be prohibited since they are toxic. Looking at their in-vitro cell-toxicity, therapeutic levels should be calculated for safer administrations. These plants should be considered for anti-tumour activity.

These results clearly indicate that the plants under investigation are rightfully being used in **traditional medicine** to treat HIV/AIDS, opportunistic infections like viral, bacterial and fungal infections, cancer and cardiovascular diseases due to their rich phytochemistry, especially phenolic compounds and high levels of antioxidant activity as well as bioactivity.

5.1 Anthology of Plants

5.1.1. *Cassia abbreviata* Oliv.

Family

Caesalpinioideae (*Leguminosae*)

Synonyms

Cassia afrofitula Brenan

Cassia beareana Holmes

Cassia kassneri Bak. F.

Common names

Long-pod cassia (Eng.)

Muremberembe, muvheneka (Sh)

Isihaqa (Nd)

Status

Very commonly used.

District

Matobo, Halale ward



Plant Description and Distribution

Cassia abbreviata is a single-stemmed shrub or small tree 2-15 m with a medium round canopy. Bark grey to brown, very rough on older trees. Young branchlets glabrous, pubescent or puberulous. Leaves with petiole and rachis (5-25 cm long) eglandular. Leaflets in 5-12 pairs, petiolulate, ovate-elliptic to oblong-elliptic, sometimes elliptic-lanceolate, 1-7.5 cm long, 0.8-4.5 cm wide, rounded to obtuse or subacute at apex, usually pubescent or puberulous. Flowers fragrant, racemes 0.5-9 cm long. Bracts persistent while flowers are open. Petals yellow, 1.5-3.5 cm long, 0.7-1.8 cm wide. Stamens 10; filaments of 3 each with an S-bend near base and a swelling half-way along their length. Pods cylindrical, 30-90 cm long, 1.5-2.5 cm in diameter, from velvety to glabrous and blackish, transversely but not longitudinally partitioned within. Seeds embedded in pulp, brown-black, 9-12 x 8-9 x 3 mm. Based on petal size, pubescence and geographical distribution three subspecies, namely *abbreviata* Brenan, *beareana* (Holmes) Brenan and *kassneri* (Bak. f.) Brenan are recognized for *C. abbreviata* (Palgrave, 2002; Wild, 1972).

The generic name is from the Greek name 'kassia'.

In Zimbabwe, it is widespread and commonly found throughout the country.

Propagated by seedlings and wildings. Seeds are sown in a sand:compost mixture (1:1) and should be kept warm and moist. It is better to sow seed directly into polythene bags or into the ground.

Ethnopharmacological Uses

In Matobo, the dried bark is boiled for stomach-aches, fever, blood pressure, pneumonia and as laxative and aphrodisiac for men. Generally in Zimbabwe, the infusion or decoction of the root is mainly used for abdominal pains, constipation, diarrhoea, bloat, toothache, pneumonia and gonorrhoea and as aphrodisiac for men (Gelfand et al, 1982; Chavunduka, 1976). The powder of the root is also taken for backache.

In East Africa, it is used for fever, malaria, stomach troubles, uterine complaints, gonorrhoea, syphilis, pneumonia and as purgative (Kokwaro, 1976). In Malawi, it is also used for snakebites and as charm (Williamson, 1975). The Lamba, Lenje, Lilima and Swaka all use the infusion of the root for toothache by holding it in the mouth. The plant is a dysentery

and diarrhoea remedy for the South African Bantu. The Tonga use the decoction of the bark and the root for diarrhoeas.

Biological and Chemical Studies

In a screening of plants used against STDs in Zimbabwe, the methanol extracts of *Cassia abbreviata Oliv* showed significant inhibition against Gram-positive and Gram-negative bacteria (Kambizi, 2001). Antimalarial activity study of Congo plants has revealed that *Cassia occidentalis* and *C. sanguinolenta* have inhibited 60% of the parasite growth (Tona et al, 1999). Methanol extracts of leaves, roots and bark of *Cassia racemosa* Mill. showed good antiprotozoal activity against *Giardia intestinalis* and *Entamoeba histolytica* (Moo-Puc et al, 2007).

The main chemical constituents of this species are the anthraquinones such as absin, chaksine and cassic acid, which is also known as Rhein, a crystalline antibiotic compound. This explains the use against constipation and infections. 2,4-trans-7,4'-dihydroxy-4-methoxyflavan has been isolated from leaves and twigs (Dehmlow et al.,1997). Also, the first flavan-3-ol with 4',7-dihydroxy phenolic substitution pattern, the novel (2R,3S)-guibourtinidol, was identified in the heartwood of *Cassia abbreviata* (Nel et al., 1999). Although from a different plant from the same species, an antimicrobial, antiprotozoal alkaloid, cassine, and antiprotozoal anthraquinone, chrysophanol, were isolated from the leaves of *Cassia racemosa* (Mena-Rejon et al, 2002; Sansores-Peraza et al, 2000). Medicinal doses produce the evacuation of soft stool. The action takes about eight hours. It will be excreted in the urine and the milk, so the baby might be affected as well.

Other Research Findings

According to our phytochemical analysis, the leaves, the bark and the roots of this tree contain alkaloids (cassine?), flavonoids (hyperoside, isovitexin), coumarins, anthraquinones (chrysophanol, aloemodine?), saponins and tannins.

In the Radical Scavenging Activity test (RSA) and Total Phenolic Compounds tests, all extracts of this plant have given very good results for potent antioxidants.

Methanol extracts of bark and the root were active against both of the gram (+) bacteria tested, *Staphylococcus aureus* and *Streptococcus gr A*. Only the root extract was moderately active against gram (-) bacteria, *Pseudomonas aeruginosa*. All extracts showed moderate antifungal activity against *Aspergillus niger*.

The roots and the leaves have shown moderate-good antiviral activity against *Herpes Simplex Virus Type-2*. There was no activity from the bark extract.

Toxicity

The anthracene bodies are irritant purgatives and if uterus were pregnant, it might cause abortion (Watt JM, Breyer-Brandwijk, 1962). According to our in vitro tests for acute toxicity, the plant parts were found to be moderately safe.

Marketing Status

Most of the harvested products are sold at Cecil Rhodes cultural site to the tourists and to the local community in forms of roots, leaves, bark and powder.

5.1.2. *Dichrostachys cinerea* (L.) Wight & Arn.

Family

Mimosaceae (*Leguminosae*)

Synonyms

Dichrostachys glomerata Chiov.
Dichrostachys nutans (Pers.) Benth.
Dichrostachys nyassana Taub
Mimosa cinerea L.
Cailliea dichrostachys Guill. et Perrot.

Common names

Sickle bush (Eng)
Mupangara, Musekera, Mumhangara (Sh)
Ugagu (Nd)
Chilitsenge (Tonga)

Status

Not threatened; Commonly used

District

Bulilima, Matjinge Ward



Brief Description of the plant and Distribution

Dichrostachys cinerea is a semi-deciduous to deciduous tree up to 7 m tall with an open crown. Bark on young branches green and hairy but dark grey-brown and longitudinally fissured on older branches and stems; smooth on spines formed from modified side shoots. Slash cream coloured to light yellow. Strong alternate thorns, up to 8 cm long, almost at right angles, slightly recurved, grow out of the branches and may bear leaves at the base. Twigs grey brown violet, with prominent light lenticels. Leaves bipinnate; rachis 4-8 cm, with 5-15 (max. 19) pairs of pinnae, which each bear (min. 9) 12-22 (max. 41) pairs of leaflets; terminal pair of pinnae shorter, dark green, underside pale. Leaflets about 8 x 2.5 mm wide; leaflets and petioles very tomentose and ciliate. Flowers very characteristic in bicoloured cylindrical, dense, petioled, pendulous spikes (bottlebrush), 6-8 cm long and fragrant. Terminal lower flowers hermaphroditic, with 1 pistil and 10 yellow stamens each. Upper flowers of a hanging spike are sterile, reddish or pale purple, with protruding staminodes. Pods narrow, yellow or brown; generally twisted or spiralled, up to 100 x 15 mm, in dense, stalked, intertwined clusters; indehiscent. About 4 black seeds with a spot at one end per pod. It seems possible that 2 subspecies can be recognized: *D. cinerea* ssp. *africana* and *D. cinerea* ssp. *nyassana*. The latter tends to grow larger and has larger and less hairy leaves and leaflets.

The generic name 'Dichrostachys' means '2-coloured spike', and 'cinerea' refers to the greyish hairs of the typical subspecies, which is confined to India; from the Greek 'konis' and the Latin 'cineres'. In South Africa it is called the 'Kalahari Christmas tree', and because of the attractive 2-coloured hanging flowers some people call it 'tassels for the chief's hat'. But most commonly it is known as the 'sickle bush', because the young pods are curved like sickles.

In Zimbabwe, it is widespread and common at most altitudes in several types of woodland and wooded grassland (Wild, 1972). It is native to Africa but found all over the world.

Artificial propagation by transplanting root suckers or by using root cuttings is likewise the easiest and usually most successful method of propagation. Direct sowing of seed is also possible. The freshly collected ripe seeds take long to germinate (15-20 days), mainly due to a thick seed coat. Scarified seeds give better germination, and a pre-treatment of 25 minutes

in concentrated sulphuric acid gives optimum germination of 3-7 days for freshly collected seeds.

Ethnopharmacological Uses

In Bulilima, it is used in sexually transmitted diseases.

Generally in Zimbabwe, the root is made into an ointment and applied to the fontanel for the depressed fontanel. Infusion of the roots with the roots of *Ormacapum trichocarpum* (*mupotanzau-Sh, ingobamakoshi-Nd*) is taken for abdominal pains. The roots mixed with the roots of *Terminalia sericea* (*mususu-Sh;umsusu, umangwe-Nd*) and the body of the person who has influenza is washed while he is seated naked in a bowl. The infant's body can be washed with the infusion of the leaves to fatten him. The infusion of the roots taken by mouth is used for cough, pneumonia, diarrhoea with blood, backache, swelling of the body and scorpion bites.

The powder of the leaves taken in porridge is used against leprosy. The ointment of the seeds is applied for scabies. In Syphilis, the powder of the fruit can be put on sores.

In Kenya, South Africa and Tanzania, the decoctions of the leaves and roots are used against venereal disease, eye injury, skin rash, and pimple, snake bite, wound and as astringent, detoxifying, antalgic and aphrodisiac. It is a Tonga toothache remedy. The root is used for chest complaints and the twigs for gonorrhoea and syphilis. The sprinkled rootbark on wounds is said to promote healing. The smoke of the leaf and the root are used for pulmonary tuberculosis.

Biological and Chemical Studies

A new isomer of mesquitol (2,3-trans-3',4',7,8-tetrahydroxyflavan-3-ol) was isolated from *Dichrostachys cinerea* in excellent yields. It has shown free-radical scavenging property and alpha-glucosidase inhibitory activities but, it could not display xanthine oxidase inhibitory property (Jagadeeshwar, 2003). The mature pods are said to yield hydrocyanic acid.

Leaves are highly palatable, rich in protein (11-15% crude protein) and mineral content.

Other Research Findings

According to our findings through phytochemical screening, the leaves also have flavonoids (quercitrin, isoquercitrin, hesperetin), anthraquinones, saponins and tannins whereas the roots only have coumarins, saponins and tannins.

Through the Radical Scavenging Activity, they both showed good results to be a fast potent antioxidant.

Methanol extract of the leaves was slightly active against both of the gram (+) bacteria tested, *Staphylococcus aureus* and *Streptococcus gr A*. Both the root and the leaf extracts were moderately active against gram (-) bacteria, *Pseudomonas aeruginosa*. Both extracts showed slight antifungal activity against *Aspergillus niger*.

The root extract has especially shown very good antiviral activity against *Herpes Simplex Virus Type-2*. There was moderate activity from the leaf extract.

Toxicity

According to our in vitro tests for acute toxicity, the plant parts were found to be moderately safe-safe.

Marketing Status

Most of the harvested products are sold in Bulawayo's Makhokhoba Market. Products are usually sold in the form of roots, leaves or powder. The market is from the local community and surrounding urban towns like Lupane and Tsholotsho. In some instances buyers are from neighbouring countries like Botswana and South Africa.

5.1.3. *Elaeodendron matabelicum* Loes.

Family

Celastraceae

Synonym

Cassine matabelica (Loes.) Steedman

Common names

Condiment saffron (Eng.)

Murungamunyu (Sh)

Umugugudu (Nd)

Status

Endemic; Threatened

District

Matobo, Halale ward



Plant Description and Distribution

It is often a shrub or a small tree 4m high, occurring on low-medium altitudes, wooded grassland, frequently on termite mounds. In Zimbabwe it is common in medium altitudes, especially Matabeleland. The bark is grey; is smooth when young and gets fissured later. Leaves are leathery, shiny, greyish green on both sides. Flowers are small, yellowish green. Fruit is an ellipsoid drupe about 15mm long. (Palgrave, 2002; Wild, 1972).

Ethnopharmacological Uses

In Matobo, the bark of the tree is usually used for female related illnesses such as sexually transmitted diseases and as sexual stimulant for women. The roots are also used for body fitness, blood purification and as charm. Generally in Zimbabwe, the Ndebele use the infusion of the root orally for venereal diseases, abdominal pains, chest pains and menorrhagia. The infusion of the bark is used orally for diarrhoea with blood and topically to reduce the size of the vagina. The Shona drink the infusion and the decoction of the root and the bark as aphrodisiac. The roots are source of yellow dye. Also in Zimbabwe, it is used for trial by ordeal. If the accused becomes rapidly unconscious, without vomiting, he is guilty. The administration is frequently followed by vomiting and purging, in which event the accused recovers. In Tanzania the Nyamwezi apply a paste of the root to abscesses and carbuncles.

Biological and Chemical Studies

This specific genus of this species is not well researched. However, according to literature the species that belong to this family contain glycosides.

Other Research Findings

Through the phytochemical analysis, we found out that the roots contain saponins and tannins. Through the Total Phenolic Contents assay and Radical Scavenging Activity test, the root showed high total phenolic content levels and high percentage of RSA which proves the extract to be a potent antioxidant and therefore could be effective for all the claimed uses.

Methanol extract of the root was moderately-highly active against all of the bacteria strains tested. However it has shown no antifungal activity against *Candida albicans* and *Aspergillus niger*.

The root extract has shown no antiviral activity against *Herpes Simplex Virus Type-2*.

Toxicity

The leaf of *Cassine capensis* is toxic to rabbit, 2.5 g/kg of the fresh material being the minimum lethal dose. The symptoms, which commence soon after the administration, are marked dyspnoea, general paralysis, and acceleration and weakness of the heart. Death is due to the respiratory paralysis, post mortem the heart is found in systole and there is hyperemia of the lung and the liver with multiple petechiae in the lung parenchyma. According to our in vitro tests for acute toxicity, the plant roots were found to be safe.

Marketing Status

Most of the harvested products are sold at Cecil Rhodes cultural site to the tourists and to the local community in forms of roots, bark and powder.

5.1.4. *Elephantorrhiza goetzei* Harms subsp. *Goetzei*

Family

Leguminosae

Synonyms

No data

Common names

Narrow-pod elephant foot (Eng.)

Muzezepasi (Sh)

Intolwane (Nd)

Status

Endemic; Threatened.

District

Mangwe, Macingwana ward



Plant Description and Distribution

This is an indigenous species for Zimbabwe, growing in northern and western regions. It is a shrub or a small tree to 5m, frequently growing on rocky outcrops. It has a grey-brown smooth bark. The leaves are bipinnate, with many pairs of narrow leaflets. The flowers are cream-yellow in axillary false spikes and appear in October-November. The pod is dark brown, swollen above the seeds.

Elephantorrhiza species are known for their large underground rhizomes. That is what gives the name 'Elephant-root' to the genus (Palgrave, 2002; Wild, 1972).

Ethnopharmacological Uses

In Mangwe, the roots are used to strengthen the knees and the back and as laxative and *ingumbane* (*ingubhane?*). Generally, the infusion of the roots taken orally is used as a remedy in Zimbabwe for abdominal pains, diarrhoea, gonorrhoea, painful uterus, fever, depressed fontanels, bilharzias, backache, and heart pains and to increase the blood in body. The red colour of the roots signals the presence of tannin which is often present in the plants used for sore throat, diarrhoea and dysentery. Indeed, the root and the bark are used for tanning as well as a diarrhoea and dysentery remedy (Hutchings, 1989). This species seem to play a major role in stopping or slowing down diarrhoea recurrence, but usually the patient's need for hydration requires an intervention of modern medicine.

Since this species is endemic to Zimbabwe, for the uses outside Zimbabwe, we can only discuss other species belonging to Elephantorrhiza genus. *Elephantorrhiza elephantina*'s

roots are used as dysentery and diarrhoea remedy by Zulu and Xhosa. The infusion of the root is also for heart conditions and the relief of haemorrhoids. But this plant has long been known as a poison in South Africa, the root contains tannic acid and the leaves also contain the active principle which is poisonous to stock. In Zimbabwe it has also been reported as poisonous. *Elephantorrhiza burkei* (intolwane ecinyane-Nd) is used by Ndebele people as infusion of the root taken by mouth to increase blood in the body, for constipation and as anti-emetic.

Biological and Chemical Studies

Extracts of 23 plant species used popularly against schistosomiasis in Zimbabwe were screened for their anthelmintic effect. The best results against schistosomes were obtained from *Elephantorrhiza goetzei* which proves it to be effective anthelmintic (Molgaard, 2001).

The chemical constituents known about this plant are flavonoids, stilbene glycosides and tannins.

Other Research Findings

According to our findings through phytochemical screening, the roots also have flavonoids (astragalins, hesperidin), coumarins and saponins.

In the Radical Scavenging Activity test (RSA) and Total Phenolic Compounds tests, the root extract of this plant has given very good results for potent antioxidants.

Methanol extract of the root was moderately active against both of the gram (+) bacteria tested, *Staphylococcus aureus* and *Streptococcus gr A*. The root extract was not active against gram (-) bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* or tested fungi, *Candida albicans* and *Aspergillus niger*.

The root extract has shown moderate antiviral activity against *Herpes Simplex Virus Type-2*.

Toxicity

Elephantorrhiza elephantina has long been known as a poison in South Africa, the root contains tannic acid and the leaves also contain the active principle which is poisonous to stock. In Zimbabwe it has also been reported as poisonous.

According to our in vitro tests for acute toxicity, the plant parts were found to be moderately safe. Until further results are produced, *E. goetzei* should still be used with caution.

Marketing Status

Most of the harvested products are sold in Bulawayo's Makhokhoba Market. Products are usually sold in the form of roots, bark or powder. The market is from the local community and surrounding urban towns like Lupane and Tsholotsho. In some instances buyers are from neighbouring countries like Botswana and South Africa.

5.1.5. *Flacourtia indica* (Burm.f.) Merr

Family

Flacourtiaceae

Synonyms

Flacourtia hirtiuscula Oliv; *Flacourtia latifolia* T.Cooke; *Flacourtia ramontchi* L'Hér.;
Flacourtia sepiaria Roxb.; *Gmelina indica* Burm.f.

Common names

Governors-plum (Eng.)

Mutudza (Sh)

Umthunduluka (Nd)

Status

Threatened

District

Chimanimani, Saurombe ward



Plant Description and Distribution

This is a small tree up to 5m high, occurring naturally in *Brachystegia* and *Combretum* woodland and bush from sea level up to 1600m. Tolerates a variety of well-drained soils. It prefers mostly sandy soils near watercourses and red clay soils (FAO 1983). The bark is pale grey and smooth, may become brown to dark grey and flaking, revealing pale orange patches. Branches are often spiny. Leaves alternate and are glabrous or hairy, red or pink when young, widest about the middle and the stalk is upto 2 cm.. Male and female flowers are on separate plants. Male flowers in axillary racemes 0.5-2 cm long; pedicles slender, may be pubescent, up to 1 cm long, the basal bracts minute and caducous. Sepals (min. 4) 5-6 (max. 7), broadly ovate, apex acute to rounded, pubescent on both sides, 1.5-2.5 mm long and broad. Filaments 2-2.5 mm long; anthers 0.5 mm long. Disk lobulate. Female flowers in short racemes or solitary; pedicles up to 5 mm. Disk lobulate, clasping the base of the ovoid ovary; styles 4-8, central, connate at the base, spreading, up to 1.5 mm long; stigmas truncate. Fruit is a small, red, fleshy, round berry. It turns a dark reddish-black when mature, and contains 4 to 10 brown, flattened, wrinkled seeds (Palgrave, 2002; Palmer and Pitman 1972; Wild, 1972).

The botanical name is of particular historical and geographical interest in South Africa. 'Flacourtia' honors E. de Flacourt (1607-60), a governor of Madagascar, who knew the Cape before van Riebeeck and 'indica' indicates that the east is equally the home of this little tree of the Transvaal bushveld.

In Zimbabwe, it is common except at the highest altitudes.

This tree can be propagated through coppice and from seed, cracking or scarifying the hard seed coat may improve germination.

Ethnopharmacological Uses

In Chimanimani, the roots and the leaves are used against diarrhea, gonorrhea, other sexually transmitted diseases, and sore eyes. In Zimbabwe in general, the Shona take the infusion of the root orally for gonorrhea, diarrhea, painful uterus, chest pains, cough and bilharziasis. When the root is burnt, the ashes taken in porridge is pneumonia and kidney remedy. The Shona and the Lobedu also take infusion of the root for the relief of body pains. The roots cooked with the roots of *Burkea africana* (mukarati-Sh;umnondo-Nd) and the decoction taken by mouth is a Ndebele remedy for *chidyiso*, condition caused by ingestion of food that has been bewitched. The dry leaf is said to be expectorant, tonic, astringent, good for asthma and a gynecological remedy. In Madagascar, the bark, triturated

in oil, is used as an antirheumatic liniment. In India, the infusion of the bark is used as gargle for hoarseness.

Biological and Chemical Studies

This plant contains a fatty acid mixture consisting of stearic acid, margaric acid, palmitic acid, linolic and linolenic acids. A new found compound in this plant is sitosterin-(6-O-acyl)- β -D-glucopyranoside (Dehmlow, 2000).

Other Research Findings

According to our findings through phytochemical screening, the leaves also have coumarins, anthraquinones, saponins and tannins. The roots have flavonoids, anthraquinones and tannins. In the Radical Scavenging Activity test (RSA) and Total Phenolic Compounds tests, extracts of both parts of the plant have given very good results for potent antioxidants.

Methanol extracts of the leaf was not active against any of the bacteria strains tested. The root extract was slightly active against *Streptococcus gr A*. Both extracts showed slight antifungal activity against *Aspergillus niger*.

The root extract has shown good antiviral activity against *Herpes Simplex Virus Type-2*. There was moderate activity from the leaf extract.

Toxicity

According to our in vitro tests for acute toxicity, the leaves were found to be potentially toxic and the roots were moderately safe. The plant needs to be used with caution.

Marketing Status

Most of the harvested products are sold in Chipinge wards and at the traditional practitioners' homesteads. Products are sold in the form of root, leaves and/or powder. The market is from the local community and surrounding neighbouring countries like Mozambique.

5.1.6. *Gymnosporia (Maytenus) senegalensis (Lam.) Exell*

Family

Celastraceae

Synonyms

Maytenus senegalensis (Lam.) Loes.

Common names

Red-spike thorn (Eng)
Chivhunabadza, Musosawafa (Sh)
Isihlangu (Nd)
Ibalalatune (Tonga)

Status

Not threatened; Commonly used

District

Mangwe, Macingwana ward



Plant Description and Distribution

It's a multistemmed small tree up to 9m occurring in bushveld. The branches are stiff and spiny. Bluish-green leaves are often in tufts, elliptic, hairless and leathery. Flowers are in

many-flowered axillary heads, cream white, sweetly scented, appear in February-June. Fruits are red capsules about 5mm in diameter, smooth and red (Van Wyk, 1997). In Zimbabwe, it is found at low to higher altitudes, in woodlands and wooded grasslands.

Ethnopharmacological Uses

In Mangwe, the roots are used in *indingindi*, gonorrhoea and chest pains.

Generally in Zimbabwe, other than above it is also used in diarrhoea, wounds and ulcers.

For chest pains the root of the plant is boiled and applied by the Shangana as a poultice first to the opposite side of the chest and then to the painful part. The decoction is made to porridge and given to the patient to eat. In Botswana, infusions of leaves are used for chickenpox, measles, varicella and mumps, which points out for antiviral activity. In other parts of Africa it is also used for diarrhoea fever, malaria earache parasites and as sedative and analgesic. Infusion of the leaf is drunk as snakebite if the snake cannot be caught. Otherwise the root is burnt with the head of the snake and the ashes are put on the bite and the tongue. The Karanga use *Gymnosporia sp.* as a remedy of epilepsy and madness. In Senegal the plant is used as a source of textile fibre and dye for hair and nails. The bark is used as dysentery remedy. In India the powdered bark is made into a paste with mustard oil and applied to the head for the destruction of head lice.

Biological and Chemical Studies

The methanol extracts of *Maytenus senegalensis* (stem-bark) showed considerable inhibitory effects against *HIV-1* (Ghazi, 1999) *Maytenus senegalensis* was screened for its antimalarial activity against *Plasmodium falciparum* in vitro along with others. It was found to be one of the four most active plants (Gessler, 1994).

Liquid-liquid partitioning of the methanol extracts indicated that fractions of *M. senegalensis* in dichloromethane and ethyl acetate had the highest antileishmanial activity (El Tahir, 1998). As chemical compounds, it contains celastrin, furfuraldehyde, dulcitol and tannins.

Other Research Findings

According to our findings through phytochemical screening, the roots and leaves also have anthraquinones, saponins and tannins. The twigs and leaves gave positive results for flavonoids (hesperidin).

Through the Radical Scavenging Activity, all three parts showed good results to be fast potent antioxidants.

Methanol extracts of twigs, leaves and roots were all moderately-highly active against all bacteria strains tested. However, only the roots have shown antifungal activity against both strains of fungi, *Candida albicans* and *Aspergillus niger*.

All extracts have shown good antiviral activity against *Herpes Simplex Virus Type-2*.

Toxicity

According to our in vitro tests for acute toxicity, the plant parts were found to be moderately safe-safe.

Marketing Status

Most of the harvested products are sold in Bulawayo's Makhokhoba Market. Products are usually sold in the form of roots, leaves or powder. The market is from the local community and surrounding urban towns like Lupane and Tsholotsho. In some instances buyers are from neighbouring countries like Botswana and South Africa.

5.1.7. *Hypoxis rooperi* S. Moore

Family

Hypoxidaceae

Synonyms

Hypoxis hemerocallidea

Common names

African potato (Eng)

Hodo (Sh)

Igudu (Nd)

Status

Threatened

District

Matobo, Halale ward



Plant Description and Distribution

Hypoxis hemerocallidea is a well-known genus of the family, easily recognizable by its bright yellow star-shaped flowers and strap-like leaves. The plant overcomes winter conditions in the form of an underground rootstock called the corm or the tuber. Corms are hard, fleshy, mucilaginous and white or yellow-orange within. Sliced corms, when exposed to the atmosphere, turn black with oxidation. The leaves are deciduous, in three distinct groups, strap shaped, up to 30cm long \times 3.2 cm in width, folded from the midrib, distinctly ribbed, glabrous on the upper surface, softly pilose on the margin and lower surface. The flowers which bloom from October to January are yellow, borne on slender villous pedicels with perianth segments ca. 20mm long and 15mm wide, bearing soft hairs on the margin and lower surface; calyx, developing fruit and bracts all villous.

In Zimbabwe it is common at medium to higher altitudes in grasslands (Wild, 1972).

Ethnopharmacological Uses

The rootstocks were used by Zulu traditional healers for centuries in the treatment of urinary infections, heart weakness, internal tumours and nervous disorders. Corms of the latter species are used as an emetic against fearful dreams. The Sotho people use *Hypoxis* as a charm against lightning and storms (Gillmer et al, 1999). The African potato is currently being used in South Africa in primary health care as an immune booster for patients with HIV/AIDS.

Biological and Chemical Studies

A major constituent of the corms of *Hypoxis hemerocallidea* as well other *Hypoxis* species is the pentenyne glycoside hypoxoside (up to 4.5%), which on hydrolysis gives an aglycone with the trivial name of rooperol which is cytotoxic and inhibits the growth of cancer cells. Rooperol is also a lipoxygenase inhibitor and effective against mutagenesis in Ames test. The corms are reported to contain, in addition to hypoxoside, β -sitosterol, sterolins (sterol glycosides, up to 9mg/100g), monoterpene glycosides, sapogenin which is hemolytic and organic acids. The cytokinins zeatin, zeatin riboside and zeatin glucoside have also been isolated from corms of this species. Since Hypoxide acts as a prodrug in humans, clinical trials are underway in South Africa. Lipophilic extracts of *H. rooperi* bulbs are used in the treatment of prostate problems; they have anti-inflammatory activity and relieve symptoms (Hostettman, 2000). Hypoxoside and its glycone rooperol have been shown to possess antimutagenic and cytotoxic properties (Albrecht, 1996). Preliminary tests with hypoxoside indicated low or no toxicity following oral and intraperitoneal administration to mice (LD₅₀

= 0 for 500mg/kg) and intravenous administration to rabbits (>100mg/kg). An *in vitro/in vivo* assessment of antineoplastic activity of Southern African plant species was unable to show cytotoxic activity in cell culture (CA-9KB) of ethanol:water (50:50) fresh leaf extracts, or antitumour activity of similar extracts in mice against Leuk-L1210 and Leuk-P388 (Charlson, 1980). In an *in vitro/in vivo* study of 5-alpha reductase inhibitory activity of a tuber extract, no activity was demonstrated. (Rhodes *et al*, 1993). A clinical assessment of the effects of the whole plant extract (randomised, placebo-controlled, double blind study, 200 adult male patients with mild to moderate BPH) reported a statistically significant decrease in symptoms (Lowe *et al*, 1998). Peak flow rate was increased from 9.9 to 15.2 ml/sec and a decreased post void residual volume observed, compared with placebo (dose: 60.0mg/day; duration of trial: 6 months). An 18-month follow-up to the study showed that patients previously randomised to the placebo group, then later treated with the extract, had an improvement both in symptom scores and flow rates. The follow-up also showed that patients who had received *Hypoxis* extract for the first 6 months of the trial continued to improve during the subsequent 12-month period, irrespective of whether medication was continued or not. Other clinical studies have demonstrated an improvement in symptoms associated with BPH in patients treated with *Hypoxis* extracts (Buck, 1996; Muller-Christiansen, 1993; Dreikorn & Schonhofer, 1995). The efficacy of β -sitosterol in the treatment of BPH (benign prostatic hyperplasia) is well-documented (Pegel 1984; Berges *et al*, 1995), as is its immunomodulatory (Bouic, 1998) and antimutagenic activity (Merck & Co. Inc., 1989). A general immunomodulatory effect, attributable to phytosterols, is the basis of efficacy.

Other Research Findings

According to our findings through phytochemical screening, it also has tannins.

Through the Total Phenolic Contents assay and Radical Scavenging Activity test, the tuber showed high total phenolic content levels and high percentage of RSA which proves the corm to be a potent antioxidant and therefore could be effective for all the claimed uses.

Methanol extract of the tuber was not active against any of the bacteria strains tested. However, the extract has shown moderate antifungal activity against *Candida albicans* and *Aspergillus niger*.

The tuber extract has shown good antiviral activity against *Herpes Simplex Virus Type-2*.

Toxicity

No foetotoxic or teratogenic effects were noted in mice following oral administration of up to 100mg/kg. Because of the sterol content, the use during pregnancy should be undertaken with caution. Taken orally, this species is reputed to cause purging.

According to our *in vitro* tests for acute toxicity, the tuber was found to be moderately safe.

Marketing Status

There is a South African patent remedy, Moducare[®], which claims for the reputed to be derived from *Hypoxis hemerocallidea* but in fact manufactured from pine wood extracts. The latter are good sources of β -sitosterol and its glucoside, both of which are common in nature. As crude drug, most of the harvested products are sold at Cecil Rhodes cultural site to the tourists, patients and to the local community in forms of corms. The corms usually seen in the marketplace are ca. 60mm in diameter but may be up to 25cm in diameter, bearing a ring of stout vertical bristles at the apex and a fringe of numerous secondary roots at the base. They are brown-black externally, bright yellow internally when freshly cut, darkening rapidly on exposure to air. It exudes a sticky resinous yellow juice from the cut surface.

5.1.8. *Khaya anthotheca* (Welw.) C. DC.

#

Family

Meliaceae

Synonyms

Khaya nyasica Stapf. ex. Baker f.

Common names

Red mahogany (Eng)

Mururu, mubarwa, muvava, muwawa (Sh)

Status

Not threatened; commonly used

District

Chimanimani, Hot Springs ward



Plant Description and Distribution

The tree is large to very large; some trees grow from 40m to taller with straight, cylindrical boles clear to 90 feet and diameters of 3 to 6 feet. The Bark is grey to brown, mainly smooth but flaking off in characteristic scales. The branches can hang half way the length of the tree. Leaves are compound, paripinnate, and large, up to 10cm., 2-7 pairs of leaflets; leaflets oblong-elliptic, 17 x 7 cm, surface dark glossy green, paler green below; margin entire; petiolules and petioles. Flowers are white, sweetly scented, up to 10 mm in diameter, and are inconspicuous. They are produced in large, many-flowered, axillary, branched sprays or panicles. Fruit is an ovoid woody capsule, 3-5 cm in diameter, creamy brown, splitting into 4-5 valves; seeds winged. The heartwood is a light pink-brown that darkens when cut to a reddish brown. It has a medium to coarse texture and a straight to interlocked grain, which can yield a striped or roe figure. The wood finishes well and has a lustrous quality. Of the three species generally called African mahogany, *Khaya anthotheca* is reportedly the only one suitable for steam bending, where the wood is placed in a vessel for a few hours and steamed then removed and held in a mould until it has dried out.

The specific epithet is after Nyasaland (now Malawi) where this splendid tree was collected for scientific identification.

It requires full sun and constant warmth & high humidity. Grows best on moist, well-drained, deep alluvial soils. In Zimbabwe, it is frequent at medium altitudes, in riverine fringes and in evergreen forests.

Khaya senegalensis has been successfully planted as bare root and as stumps. Use of containerized seedlings yields better results. Germination of fresh seed can sometimes be nearly 100% and begins in about 3 weeks. Propagation by seedlings or wildings is also possible.

Ethnopharmacological Uses

In Chimanimani and in Zimbabwe in general, the infusion of the bark taken orally is used against venereal diseases, abdominal pains, pneumonia, and colds and as antiemetic. In Mozambique the bark is an ingredient in a decoction taken for the relief of colds. In Gaza, the crushed seed is boiled to extract the oil, which has a bitter taste and is rubbed onto the hair to kill the vermin. Along the Buzi River the tree is used exclusively for the making of dugouts.

Biological and Chemical Studies

In a test done in Cameroon, the decoction of the bark has supplied anthelmintic results in-vivo (Nfi, 1999). The bark of *Khaya senegalensis* for northern Nigeria has given negative results for alkaloids, glycosides, resins but has yielded 10.2% tannin. It has been stated that the bark that is bitter contains the alkaloid calidedrine. Ferreol et al have isolated nimbosterol which is identical with β -sitosterol and its glycoside nimbosterin which is the β -D-glycoside of the β -sitosterol. The tree exudes a pale greenish yellow gum.

Other Research Findings

According to our findings through phytochemical screening, the roots and the bark have flavonoids, coumarins, anthraquinones, and some traces of saponins and tannins.

Through the Total Phenolic Contents assay and the Radical Scavenging Activity, they both showed good results to be fast potent antioxidants.

Methanol extract of the bark was slightly-moderately active against both of the gram (+) bacteria tested, *Staphylococcus aureus* and *Streptococcus gr A*. However, the extract was not active against gram (-) bacteria, *Escherichia coli* or *Pseudomonas aeruginosa*. It also didn't demonstrate any antifungal activity against *Candida albicans* and *Aspergillus niger*.

The bark extract has shown slight antiviral activity against *Herpes Simplex Virus Type-2*.

Toxicity

The root is said to be poisonous and the bark is bitter.

According to our in vitro tests for acute toxicity, the bark was found to be moderately safe.

Marketing Status

Most of the harvested products are sold in Chimanimani Saurombe ward and at the traditional healers' homesteads. Products are usually sold in the form of root, bark and/or powder. The market is from the local community.

5.1.9. *Kigelia africana* DC

Family

Bignoniaceae

Synonyms

Kigelia pinnata; *Bignonia africana* Lam.; *Crescentia pinnata* Jacq.; *Kigelia abyssinica* A. Rich.; *Kigelia acutifolia* Engl. ex Spreng.; *Kigelia aethiopum* (Fenzl) Dandy; *Kigelia aethopica* Decne.; *Kigelia elliottii* Sprague; *Kigelia elliptica* Sprague; *Kigelia impressa* Sprague

Common names

Sausage tree (Eng)

Mubvee (Sh)

Umvebe (Nd)

Status

Very Commonly Used

District

Chimanimani, Saurombe ward



Plant Description and Distribution

Kigelia africana is native to Africa. The genus is now considered to be monospecific although at one time several species were thought to exist (Burkill, 1985). *Kigelia africana* is a large rounded tree up to 25 m in height, with a dense rounded crown. The bark is grey, generally smooth in large specimens, flaking in thin, round patches. The Leaves are opposite, crowded near the ends of branches, compound, with 3-5 pairs of leaflets plus a terminal leaflet. The leaflets are oblong, up to 6 x 10 cm, leathery, roughly hairy on both surfaces, rather yellowish-green above, paler green below, apex broadly tapering to rounded. The base is square, asymmetric in the lateral leaflets, symmetric in the terminal leaflet; margin entire, sometimes obscurely toothed, wavy; the lower leaflets shortly petiolulate, the terminal pair without petiolules; petiole up to 15 cm long. Flowers are striking, dark maroon with heavy yellow veining on the outside, cup shaped, asymmetric, up to 15 cm across the mouth but they have an unpleasant smell. They are arranged in 6- to 12-flowered, lax, pendulous stalks up to 90 cm long. Calyx shortly tubular with 2-5 ribbed lobes; corolla widely cup shaped with 5 broad spreading lobes; stamens 4, slightly protruding beyond the mouth of the corolla tube; ovary 1-chambered. Fruits are very unusual; sausage shaped can grow up to 1 m x 18 cm and are greyish-brown, heavily dotted with lenticels, indehiscent. They are heavy, weighing up to 12 kg, containing a fibrous pulp in which are embedded many seeds.

The common name 'sausage tree' is derived from the cylindrical shape of the fruit. *Kigelia* is the Latinised version of a Mozambique name and 'africana' means simply 'from Africa'.

In Zimbabwe, they can be found all around the country primarily in wet savannah woodland spreading into gallery woodland and along rivers in moist forests. In open woodland and in riverine fringes, it occurs at low altitudes.

Seedlings and wildings are possible material for propagation. The sausage tree is not a prolific seeder. Seeds are released when fruit rots on the ground, and plants regenerate naturally. Seeds are placed in seedling trays filled with pure river sand; they are pressed into the sand until the tip is level with the sand, covered lightly with a thin layer of sand or pure compost and kept moist. Seed usually germinate after 10-25 days. No pre-treatment is needed, but germination rate is poor. *Kigelia africana* is a relatively slow-growing tree; depending on the climate, it reaches good shade proportions in 4-5 years. The growth rate is

at least 1 m/year, but it is slower in colder areas. It is not frost resistant, but if young plants are protected for the 1st 2-3 years from cold winds in colder areas, they will survive.

Ethnopharmacological Uses

In Chimanimani and generally in Zimbabwe, all parts of the tree are used in traditional medicine for a variety of purposes including the treatment of skin cancer, dysentery, venereal diseases, haemorrhoids, tapeworm, as topical applications on wounds, eczema, psoriasis, abscesses and carbuncles. (Oliver-Bever, 1986). Bark and leaves are used for bladder trouble/kidney disease. An enema or drink of the boiled root and stem bark is used for piles. Wounds, sores and cuts are treated with a leaf and bark decoction or bark. The bark and leaf decoctions are antidotes for snakebite. The unripe fruits are said to be poisonous but are taken as a remedy for syphilis and rheumatism, and boiled fruit is massaged into the body for lumbago. In South Africa, the fruits are used as a dressing for ulcers or to increase the flow of milk in lactating women. In northern Nigeria, the fruit is used in some districts as a purgative, and in others to treat dysentery. The leaf alone, or with other ingredients, is useful for diarrhoea and dysentery. The fruits and bark, ground and boiled in water, are taken either orally or as an enema in treating children's stomach ailments. The fruits and roots of *K. africana* are boiled along with the stem and tassels of a plantain for postpartum haemorrhage. Decoctions of the stem bark are used for spleen infection, gonorrhoea and syphilis. A cream made from fruit extract is used to remove sunspots known as 'solar keratosis', particularly on the face and hands.

The Tonga apply the powdered fruit as addressing to ulcers while the Zulu use the tree medicinally. The Luvale rub pieces of the fruit over the baby of a baby to make it fatter but believe that its application to the head results in development of hydrocephalus. They also rub the breasts of a lactating woman to increase the flow of milk. In Malawi, in times of scarcity, the natives roast and eat the seeds. In Central Africa the tree is regarded as holy and fetishes are cut from it. Religious meetings are held in its shade. In Tanzania, the very fibrous ripe or unripe fruit of various *Kigelia* species are baked and put into the beer of the natives to ferment it. If small portion of the fruit is chewed it is said to cause the swelling of the male genitalia.

Biological and Chemical Studies

Scientifically, this plant has been well researched. Ethanol extract of the bark has been reported to be antibacterial and antifungal (Akunyili, 1991). Dichloromethane extract of the bark and alcohol extract of the fruit have shown cytotoxicity (Houghton, 1994; Jackson, 1996). In an in vivo study, extracts given intraperitoneally to mice have been recorded as antitumour (Azuine et al, 1997). Dichloromethane extract of the rootbark is found out to be antiplasmodial (Weiss, 2000) and antitrypanosomal (Moideen, 1999). Ethanol extract of the fruit is reported as anti-inflammatory (Azuine et al, 1997). Although extracts of the plant have been assessed for antimicrobial, antimalarial and cytotoxic activity, the antiviral activity of this plant has not been investigated. Therefore this plant was chosen for the project

Reviewing the current literature revealed the isolation of naphtoquinones (Akunyili and Houghton, 1993; Weiss et al, 2000), iridoids (Gouda et al, 2003; Akunyili and Houghton, 1993), flavonoids (El-Sayyad, 1981) and coumarins (Govindachari et al, 1971).

Other Research Findings

The leaf and fruit have given negative results for flavonols, alkaloids and tannins, and given positive results for sterols. According to our findings through phytochemical screening, the root and bark extracts have alkaloids, anthraquinones, saponins and tannins.

Through the Total Phenolic Contents assay and Radical Scavenging Activity test, the extracts showed high total phenolic content levels and high percentage of RSA which proves them to be potent antioxidants.

Methanol extracts of fruit, bark and the root were slightly-moderately active against both of the gram (+) bacteria tested, *Staphylococcus aureus* and *Streptococcus gr A*. All extracts were moderately active against gram (-) bacteria, *Pseudomonas aeruginosa*. Only the bark showed slight- moderate antifungal activity against both *Candida albicans* and *Aspergillus niger*.

The bark and fruit extracts have shown good-very good antiviral activity against *Herpes Simplex Virus Type-2*. There were no activities from the root extract.

Toxicity

The unripe fruit is said to be very poisonous. The extracts of the fruit have shown cytotoxic effects. The ripe fruit is also not edible, is purgative.

According to our in vitro tests for acute toxicity, the fruit and the bark were found to be potentially toxic and the root to be moderately safe. The plant parts need to be used with caution.

Marketing Status

Zambesia Botanica, a company in Kadoma, obtains whole fruit pulp and mixes it in aqueous cream. This product is called 'Genuine Sausage Tree Cream' and is sold in 100ml bottles in pharmacies in Zimbabwe.

The Essential Plant Extract Company, in Masasa, Harare, produces 'Kigelia africana Cream' that is sold in 25g tubes to various pharmacies and health shops countrywide by Acacia Herbals and Pharmed companies. *The Essential Plant Extract Company* also buys *Kigelia africana* extract, which is produced by Aroma Chemicals Company, and resells at Jems Mobb Immune Enhancement Clinic.

Most of the harvested products are sold in Chimanimani Saurombe ward and at the traditional healers' homesteads in the form of fruit, root, bark and powder. The market is from the local community.

5.1.10. *Rhus chirindensis* Baker f.

Family

Anacardiaceae

Synonyms

Rhus legatii Schonland

Common names

Red Currant (Eng)

Mubikasadza; Mutsodzo (Sh)

Status

Not threatened; Commonly used

District

Chipinge, Zamchiya ward



Plant Description and Distribution

The red currant, *R. chirindensis*, is named after the Chirinda Forest in Zimbabwe, and is the largest of all the southern African *Rhus* species. It occurs naturally in forests, along forest margins, in riverine bush and scrub forest and rocky hillsides. It is a semi-deciduous shrub to small tree, 6-10 m high (although exceptional specimens may reach 20 m). Young and coppicing branches are armed with spines, although the mature tree is spineless. The flowers are small, yellowish green and are borne in clusters at the ends of the branches from August to March. Male and female flowers occur on separate trees. The fruits, which are round, shiny, slightly fleshy, dark reddish brown and 4-7 mm in diameter, are borne from December to March, in heavy clusters which can weigh down the branches. The large leaves, which may grow to 130 mm long, have three leaflets and are dark green, turning red before falling in autumn. The margin of the leaves is entire and usually undulate and ends in a tapering tip. The midrib is pinkish and usually sunken above and prominent below. The leaf stalk may be up to 70 mm long and is also pinkish red. The young leaves are reddish.

Ethnopharmacological Uses

In Chimanimani, the sap of this tree is used in traditional medicine for treating heart complaints. The bark is also used to strengthen the body, to stimulate circulation and in the treatment of rheumatism and mental disorders. Other than these, in Zimbabwe the roots are used for syphilis. They contain tannic acid and are used for tanning.

Biological and Chemical Studies

This genus of the species hasn't been researched yet but the other ones have been found to have biflavonoids such as amentoflavone, agathisflavone, robustaflavone, hinokiflavone, volkensiflavone, rhusflavanone, succedaneoflavanone, all isolated from *Rhus succedanea* and exhibited inhibitory activities against different strains of viruses. The wood of *Rhus cotinus* contains fisetin and the leaf contains tannin and myricetin.

Other Research Findings

According to our findings through phytochemical screening, the roots and leaves have flavonoids, coumarins, anthraquinones, saponins and tannins.

Through the Radical Scavenging Activity, both extracts showed good results to be a fast potent antioxidant.

Methanol extract of the leaves was slightly and the root was moderately active against both of the gram (+) bacteria tested, *Staphylococcus aureus* and *Streptococcus gr A*. Only the leaf extract was moderately active against gram (-) bacteria, *Pseudomonas aeruginosa*. However, the extracts showed no antifungal activity against *Candida albicans* or *Aspergillus niger*.

The root extract has shown slight antiviral activity against *Herpes Simplex Virus Type-2*. There were no activities from the leaf extract.

Toxicity

According to our in vitro tests for acute toxicity, the plant parts were found to be safe.

Marketing Status

Most of the harvested products are sold in Chipinge wards and at the traditional practitioners' homesteads. Products are sold in the form of root, leaves and/or powder. The market is from the local community and surrounding neighbouring countries like Mozambique.

5.1.11. *Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.)

Family

Anacardiaceae (Mango family)

Synonyms

Sclerocarya caffra (A. Rich.) Hochst.

Spondias birrea A. Rich.

Common names

Marula (Eng.)

Mupfura, Mutsomo (Sh)

Umganu (Nd)

Status

Threatened; Commonly used

District

Bulilima, Matjinge ward



Plant Description and Distribution

Sclerocarya birrea ssp. *caffra* is a medium to large tree, usually 9 m tall, but trees up to 18 m have been recorded. It is single stemmed with a dense, spreading crown and deciduous foliage. The bark is grey and usually peels off in flat, round disks, exposing the underlying light yellow tissue. The young twigs are thick and digitaliform with spirally arranged composite leaves at their ends; it has a thick, relatively short taproot reaching depths of 2.4 m, lateral roots branch at the upper 60 cm of soil. *Mycorrhizae* (root fungus) are found on the fine roots. Leaves 18-25 x 8-15 cm, composite, containing 2-23 leaflets, averaging 11; leaflets oblong elliptic with petioles ranging from very short to 20 mm in length. Although male and female flowers occasionally occur on the same tree, it is considered dioecious. Male flowers are borne in groups of 3s on racemes below new leaves, dark red when young, turning pink or white when open. The female flowers are blood red but change colour from purple to white after opening. They occur below the leaves on long peduncles and consist of 4 curling petals, numerous infertile stamens and a long, shiny ovary. Fruit borne in clusters of up to 3 at the end of the twigs and always on the new growth. Fruit is a round or oval drupe, usually wider than it is long, with a diameter of 30-40 mm. The shape and number of nuts per stone determine the final shape of the fruit. Marula fruit has a thick, soft leathery exocarp with tiny, round or oval spots, enclosing a juicy, mucilaginous flesh that adheres tightly to the stone and can be removed only by sucking. The flesh tastes tart, sweet and refreshing, although the fruit has a slight turpentine-like aroma and can give off a very unpleasant smell when decaying. Each fruit contains an exceedingly hard seed, which is covered by fibrous matter. It is usually trilocular, but sometimes only bilocular. Each seed locule contains a single, light nut filling the entire cavity, which is sealed by a round, hard disk that protects the embryo until germination.

The name 'sclerocarya' is derived from two Greek words, 'skleros' and 'karyon', meaning 'hard' and 'nut', respectively, and refers to the hard stone of the fruit. 'Birrea' comes from 'birr', the common name for the tree in Senegal, and *caffra* from 'Kaffaria' Eastern Cape, South Africa (Peters, 1988).

The Marula is a common and widespread fruit-bearing species throughout much of sub-Saharan and South Africa. In Zimbabwe, it occurs naturally in various types of woodland, on sandy soil or occasionally sandy loam.

The *S. birrea* ssp. *caffra* tree is a prolific seed bearer: between 0.2 and 1.5 tons of fruit have been collected from a single tree in a season in the wild. Seeds should be soaked overnight before sowing. Propagation is by seedlings or cuttings; gregarious root suckering. Over 95%

success has been achieved by grafting 5-10 cm of scion material cut from the tips of branches. It is essential that scion material be collected immediately dormancy breaks.

Ethnopharmacological Uses

It is widely used by rural populations in most countries wherever it grows. It has multiple uses, including the fruits. All parts of the fruit of *S. birrea ssp. caffra* are edible. The pulp can be consumed raw or boiled into a thick, black consistency and used for sweetening porridge. They can be fermented to make a beer, the kernels are eaten or the oil extracted, the leaves are browsed by livestock and have medicinal uses, as does the bark. In Bulilima, the bark is soaked in water to be used against constipation, stomach-ache and backache. The nuts are crushed and used for nosebleed. Generally in Zimbabwe, the decoction of the bark treats cough, pneumonia, dysentery, diarrhoea, rheumatism and has a prophylactic effect against malaria. The bark is an excellent remedy for haemorrhoids. Roots and bark are also used as laxatives. A drink made from Marula leaves is used for the treatment of gonorrhoea. The oil of the kernel is said to have proteins and iodine. This oil is rubbed on the skin shirts to preserve them and keep them soft. The gum which exudes from the tree is rich in tannin and is used as ink substitute.

Biological and Chemical Studies

The vitamin C content of the fruit is 54mg/100 g, which is 2-3 times that of the orange. The seeds are high in fat (56-61%), protein (28-31%), citric acid (2.02 %), malic acids and sugar, phosphorus, magnesium, copper, zinc, thiamine and nicotinic acid. Methanolic extract of *Sclerocarya birrea* was screened for molluscicidal activity and found active (Kela, 1989). The leaves are known to contain tannin and a trace of alkaloids. The gum that exudes from the tree is rich in tannin and is sometimes used in making ink by dissolving it in water and mixing in soot. Bark contains 20.5% tannin and some alkaloids. Lipids: The nuts yield oil with a quality and fatty acid composition comparable to olive oil but with stability that is 10 times greater. Non-drying oil that burns like a candle comprises 56% of the seed. The walnut like stone contains up to 6% edible oil (1 t of fruit yields 60 l of oil), which is occasionally sold on the local market.

Research Findings

According to our findings through phytochemical screening, the bark has saponins and tannins. Through the Total Phenolic Contents assay and Radical Scavenging Activity test, the bark showed highest total phenolic content levels and high percentage of RSA which proves it to be a potent antioxidant.

Methanol extract of the bark was highly active to all the bacteria strains tested. However, the extract showed no antifungal activity against *Candida albicans* or *Aspergillus niger*.

The bark extract has shown good antiviral activity against *Herpes Simplex Virus Type-2*.

Toxicity

The leaves are said to be slightly poisonous.

According to our in vitro tests for acute toxicity, the bark was found to be safe.

Marketing Status

There is a network, Southern Africa Marula Oil Producers' Network, which only focuses on Marula oil from the kernel of the Marula tree in efforts to commercialize (IGO, 2000). Most of the harvested products are sold in Bulawayo's Makhokhoba Market. Products are usually sold in the form of roots, bark or powder. The market is from the local community and surrounding urban towns like Lupane and Tsholotsho. In some instances buyers are from neighbouring countries like Botswana and South Africa.

5.1.12. *Securidaca longepedunculata* Fresen.

Family

Polygalaceae

Synonyms

Lophostylis angustifolia Hochst.

Lophostylis oblongifolia Hochst.

Lophostylis pallida Klotzsch

Securidaca longepedunculata var. *parvifolia* Oliv.

Securidaca spinosa Sim

Common names

Violet tree (Eng.)

Mufufu (Sh)

Umfufu (Nd)

Status

Very Threatened.

District

Bulilima, Matjinga ward



Plant Description and Distribution

Securidaca longepedunculata is a semi-deciduous small beautiful tree up to 6m high, with an often flattened or slightly fluted bole. It is spiny and much branched, with an open, rather straggly looking crown. The bark is light grey and smooth. The leaves are alternate or clustered on dwarf, lateral branchlets and are simple, variable in size and shape, broadly oblong to narrowly elliptic. They are 1-5 x 0.5-2 cm with very fine hairs when young but losing these by maturity; apex rounded; base narrowly tapering; margin entire; petiole slender, up to 5 mm long simple and spirally arranged. It has rather small, lilac purple, sweetly scented flowers on long slender stalks produced in beautiful profusion in terminal axillary sprays that are 3-5 cm long. They appear mainly October-November with the very young leaves and are bisexual. The fruits are more or less a round pale brown nut, somewhat heavily veined, occasionally smooth, bearing a single, oblong, rather curved, membranous wing up to 4 cm long; purplish-green when young, becoming pale, straw-coloured when mature (Palgrave, 2002; Wild, 1972). The wood is spongy and soft. When dried, it approaches cork in lightness.

The hatchetlike appearance of the fruit is referred to in the generic name, while the specific name, 'longepedunculata', refers to the long, slender stalks of the flowers.

It occurs in woodland and bush from sea level up to 1600m. This range of altitude makes this widespread in Zimbabwe, mainly in southern parts.

One-year-old seed germinates freely but the plants are slow growing and the seedlings are hard to transplant.

Ethnopharmacological Uses

In Bulilima, the roots are ground into powder and taken for constipation, blood purification, pneumonia, backache, sexually transmitted diseases like gonorrhoea and aphrodisiac for men.

In Zimbabwe and Malawi, the infusion of the root and in South West Africa, the infusion of the bark is used in treating venereal diseases esp. syphilis, epilepsy, convulsions, bile emesis, constipation, palpitations, tuberculosis, pneumonia, tapeworm, hookworm and as aphrodisiac. The root is also a Shona and Ndebele malaria remedy. The infusion is used to

washing the body to drop down the temperature. Root scrapings are applied to 'nyora' as an antidote for snakebites over the wound and on the limbs for rheumatism. They are also used as snake repellents as placed around homestead since they have the characteristic smell of oil of wintergreen. Spiritually, the body washed with the infusion of the roots is said to arouse spirits and drive away bad spirits. In Nigeria, the root is also used for venereal diseases esp. syphilis, abdominal pains, headaches, rheumatic pains and as purgative and diuretic. For cough the very young leaf, cooked with the native salt, is taken while the decoction of the root is used as mouth wash for the relief of gum-boil. For the control of nose bleed, root scrapings are placed in a funnel-shaped leaf and water poured onto them. Four drops of water which passes through the scrapings are inserted into the nostril. The result is an extremely violent headache but the patient is better in an hour. In Tanzania, a cold infusion of the rootbark is an eye remedy. It is also used for treating venereal diseases, headaches and as expectorant. In Congo, the bark is used as an ordeal poison. In Mozambique, it said to be good for driving away bad spirits. A cold infusion of the powdered root is drunk by person is believed to be possessed by an evil spirit and is taken with an infusion of *Heeria abyssinica* as a purifying medicine after ceremonial defilement. The infusion causes vomiting and diarrhoea.

Biological and Chemical Studies

The root contains methyl salicylate; therefore it can be and is commonly used as anti-inflammatory in relieving fever, rheumatism, toothache, convulsions and dysmenorrhoea. Methanol extracts of *Securidaca longepedunculata* were strongly trypanocidal (Atawodi, 2003). An ethanolic extract of the plant was investigated to ascertain its analgesic and anti-colic properties and was found to possess analgesic properties but no anti-colic activity. In addition, the plant was found to be toxic to rats since it damaged the gastrointestinal mucosa (Buabeng, 2005).

Other Research Findings

The plant contains methyl salicylate, securida-saponins, gaultherin and according to our research it is found that the roots also have flavonoids (apigenin 7-O-glucoside, luteolin-7-O-glucoside) and tannins.

According to the Radical Scavenging Activity, it is a very potent antioxidant.

Methanol extract of the root was moderately active against both of the gram (+) bacteria tested, *Staphylococcus aureus* and *Streptococcus gr A*. The extract was also slightly active against gram (-) bacteria, *Pseudomonas aeruginosa*. It showed moderate antifungal activity against *Candida albicans* and *Aspergillus niger*.

The root extract has shown good antiviral activity against *Herpes Simplex Virus Type-2*.

Toxicity

The plant is the most famous of the intravaginal poisons and that it is the accepted means of suicide in African women. Insertion of a preparation of the root into the vagina proves fatal in twelve to twenty-four hours. If discovered in time, promptly and thoroughly wash out the vagina. According to our in vitro tests for acute toxicity, the plant parts were found to be moderately safe but still needs to be used with caution.

Marketing Status

Most of the harvested products are sold in Bulawayo's Makhokhoba Market. Products are usually sold in the form of roots or powder. The market is from the local community and surrounding urban towns like Lupane and Tsholotsho. In some instances buyers are from neighbouring countries like Botswana and South Africa.

5.1.13. *Terminalia sericea* Burch ex. DC

Family

Combretaceae

Synonyms

Terminalia silozensis Gibbs

Common names

Silver cluster leaf, yellow wood (Eng.)

Mususu, mukonono (Sh)

Umsusu, umangwe (Nd).

Status

Vulnerable; Commonly used.

District

Bulilima, Matjinge ward



Plant Description and Distribution

This is a deciduous tree, which grows in open woodland and on sandy soils. *T. sericea* is common as a shrub or bush 6-9 m tall, but individual trees may reach 23 m in height. The bark is dark grey or brownish in colour and deeply fissured vertically. Young stems and branches often parasitized and bear longish round galls often up to 2-3 cm in diameter frequently with leaves. The leaves are silky silvery hairy and clustered at the ends of the branches, narrowly obovate-elliptic, blue-green and 5.5-12 by 1.5-4.5 cm. The flowers are powerfully and rather unpleasantly scented and are small creamy to yellow, found on small spikes up to 7 cm. The fruit is a single winged pod, oval, 2.5-3.5 by 1.5-2.5 cm, which grows in clusters on the tree, starting out with a pinkish colour, changing to dark brown as they dry out. They are sometimes parasitized and become deformed, twisted and hairy. The wood is yellow, hard, close-grained, strong and elastic. It is used for making furniture, in wagon work, hut building and fencing.

The generic name 'Terminalia' comes from Latin word 'terminus' or 'terminalis' (ending), and refers to the habit of the leaves being crowded or borne on the tips of the shoots.

In Zimbabwe, it is widespread and can be found all over the country.

T. sericea is usually propagated by seed. It naturally seeds and regenerates readily as open sites become available.

Ethnopharmacological Uses

In Bulilima, this plant is used against venereal diseases, for wounds, diarrhoea and as charm.

Generally in Zimbabwe, the roots' infusion in water or powder in porridge has also medical applications against various bacterial infections, such as gonorrhoea, syphilis, and diarrhoea and against hypertension and even cancer. This decoction is very bitter. The leaves are used against stomach disorders and as a cough remedy. The powder can also be applied on the wounds for quicker healing. During birth, the vagina is washed with the infusion to dilate the birth canal.

In South Africa, the root is prepared into a treatment for diarrhoea and a fermentation of the root is used in treating pneumonia (Palgrave K.C. 2002). In East Africa, Tanzania and South Africa, it is taken for stomach disorders and bilharzias (Watt Breyer-Brandwijk, 1962).

In Zambia it is used against dysentery, sores, stomach pains and as hydrocele (Storrs, 1979). Another *Terminalia* species that belongs to this family, *Terminalia stenostachya* (mususumukuru-Sh; umangwe wenduna-Nd), is rarely used against epilepsy and antidote for poison in the form of infusion of the roots (Gelfand et al, 1982). A stick of the wood is stuck, with magical significance, in the floor of a shrine in which the homage is paid to ancestral

spirits at planting and harvesting times and when hunting party sets out. The tree is not cut when crops are growing, as this is believed to bring hail.

Biological and Chemical Studies

On an antibacterial and antioxidant activity screening, methanol and water extracts of *Terminalia sericea*, were more active compared to the other extracts against *Streptococcus pyogenes* and *Staphylococcus aureus* (Steenkamp *et al*, 2004). On antimicrobial screening, the methanol extract of the roots of *Terminalia sericea* showed inhibition against gram-positive bacteria (Fyhrquist *et al*, 2002). In a different study, the extracts exhibited antifungal activity against *C. albicans* and *Aspergillus niger* (Moshi *et al*, 2004).

The fractionation of the aqueous extract of *Terminalia* leaves afforded nerifolin, punicalin and 2-O-galloylpunicalin (Martino *et al*. 2004). Nerifolin is a glycoside inhibits fibroblastic outgrowth in aneural explanted heart tissue *in vitro* and inhibits the pulse rate in a dilution of 1:7000 (Das, 1947).

Other Research Findings

According to our phytochemical analysis, the leaves and roots of this tree contain alkaloids, flavonoids (isoquercitrin, vicenin-2, vitexin), anthraquinones, saponins and tannins.

In the Total Phenolic Compounds test and Radical Scavenging Activity test (RSA), all extracts of this plant have given very good results and they have high total phenolic compounds which make the extracts of this tree potent antioxidants.

Methanol extracts of the leaf and the root were highly active against both of the gram (+) bacteria tested, *Staphylococcus aureus* and *Streptococcus gr A*. Both extracts were moderately active against gram (-) bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*. Both extracts especially the root showed moderate antifungal activity against *Candida albicans* and *Aspergillus niger*.

Both the leaf and the root extracts have shown moderate-good antiviral activity against *Herpes Simplex Virus Type-2*.

Toxicity

The plant has been reported to be poisonous and in Zululand the root is believed to be poisonous. It is used by the sorcerers, who dip their fingers in the boiling root and smear the poison on two assegais, which are then thrown in the direction of the person intended to be bewitched. The victim though he be miles away dies of wounds and coughing (Watt & Breyer-Brandwijk, 1962).

According to our *in vitro* tests for acute toxicity, the plant parts were found to be potentially toxic and have to be used with caution.

Marketing Status

Most of the harvested products are sold in Bulawayo's Makhokhoba Market. Products are usually sold in the form of roots, leaves or powder. The market is from the local community and surrounding urban towns like Lupane and Tsholotsho. In some instances buyers are from neighbouring countries like Botswana and South Africa.

5.1.14. *Warburgia salutaris* (Bertol.f.) Chiov.

Family

Canellaceae

Synonyms

Warburgia breyeri Pott.

Warburgia ugandensis Sprague

Chibaca salutaris Bertl. f.

Common names

Pepper-bark tree (Eng.)

Muranga (Shona)

Isibhaha (Zulu)

Status

Critically Threatened

District

Chipinge, Mount Selinda



Plant Description and Distribution

It is a slender tree attaining a height of 5-10m. The bark is rich brown and rough. Leaves are aromatic, ovate-lanceolate, entire, alternate, 4.5-11 x 1-3 cm, glossy dark green above, paler green and dull below; midrib frequently slightly off-centre; apex and base tapering; margin entire; petiole 1-3 mm long. Flowers are at the beginning of the rains and they are bisexual, up to 7 mm in diameter. They are either solitary or in 3-flowered cymes in axils of leaves, green, 3 sepals, 10 petals, 5 inner petals smaller, thinner in texture and yellower than outer 5, filaments fused to form a prominent staminal tube. Fruits are oval berries 4 cm in diameter, skin leathery, glandular, turning dark purple when ripe, containing 2 or more seeds with oily endosperm. The fruits form late in the rainy season and may remain on the tree for a long time (FAO 1986).

This spreading evergreen is widely distributed in lower rainforests, drier highland forest areas, and in secondary bushlands and grasslands (Dale and Greenway 1961) where the altitude should be between 1000m and 2000m.

The genus is named after Dr Otto Warburg (1859-1938), born in Hamburg, a lecturer in botany at the University of Berlin and author of numerous botanical papers. The specific name 'salutaris' means 'healthy' or 'salutary', presumably in reference to its medicinal properties.

In Zimbabwe, it is rarely found in Chipinge districts and Eastern Highlands.

This tree can be propagated by seed (direct sowing and seedlings), cuttings, and wildlings. *W. salutaris* is fairly slow growing.

Ethnopharmacological Uses

The bark of *Warburgia salutaris*, a very popular species in southern Africa, is used to heal mainly cough and cold. In Chipinge, it is used for colds, coughs and chest pains.

Generally in Zimbabwe, decoction of the bark taken orally is used basically as *panacea* which means 'universal remedy', good for diseases such as abdominal pains, venereal diseases, malaria, colds, chest pains, coughs, diarrhoea, muscle pains and general body pains (Rulangaranga 1989, Gelfand *et al*, 1985). The bark of the stem is widely used in southern Africa as an expectorant in common cold, being often smoked for this purpose. The fumes of the powdered bark are inhaled or the powdered material swallowed with the water for chest pains. The plant is one of the fever trees and bark has been used as malaria

remedy by Bantu but has been proven to be ineffective in experimental malaria. The powdered bark can be applied to *nyora* made on the temples for headache. As an aid to divination, the bark is boiled together with *hakata* and is then chewed and spat on *hakata*.

Biological and Chemical Studies

According to the literature search, the bark contains numerous drimane sesquiterpenoids, including warburganal, muzigadial, mukaadial, ugandensidal and polygodial (Rabe *et al*, 2000). Mannitol seems to be the main chemical constituent in this species. It also contains tannin used for diarrhoea disease (van Wyk *et al.*, 1997). These compounds have been shown to be potently antibacterial, antifungal, antiviral, antiparasitic and antitumour (Rabe, *et al*, 1997; Treurnicht, 1997; Jansen, 1991; Clark, 1997).

Other Research Findings

During our phytochemical analysis of this tree, it has been found out that the roots, bark, leaves and the twigs contain alkaloids, coumarins, flavonoids, anthraquinones, saponins and tannins.

They also had remarkably high free radical scavenging activity and total phenolic contents to show that they could be good antioxidants.

Methanol extracts of bark and the root were highly active against both of the gram (+) bacteria tested, *Staphylococcus aureus* and *Streptococcus gr A*. Only the root extract was moderately active against gram (-) bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*. All extracts especially the root showed good-very good antifungal activity against *Candida albicans* and *Aspergillus niger*.

The root extract has shown good antiviral activity against *Herpes Simplex Virus Type-2*. There were no activities from the bark and the leaf extract.

Toxicity

None reported for this species when used in the traditional manner. Skin irritation and contact dermatitis have however been demonstrated for individual drimanes e.g. warburganal and polygodial. In view of reports of possible toxicity, this species should preferably be used only under the supervision of a competent traditional practitioner.

According to our in vitro tests for acute toxicity, the plant parts were found to be moderately safe.

Marketing Status

There is already a highly marketed product line for *Warburgia salutaris* to be used for different kinds of indications (africandrugs.com). The bark also occurs in the marketplace as crude drug, curved or channelled pieces up to 30cm long and 3-15mm in thickness. The drug is smooth grey-brown when young, showing numerous lenticels. It gets rough-scaly when older, with a thick cork layer; grey-brown on the external surface; pale cream-brown to red-brown on the inner surface; breaking with a splintery fracture; odour aromatic; taste bitter and peppery.

5.2 Future Scope

Comparing overall results after all the pharmacological and phytochemical screening, the plant extracts with very promising results could be chosen for further investigations in terms of chemical *compound isolations* using more complicated chromatography (e.g. VLC, chromatotron etc.) and *chemical structure elucidations* using IR and MS.

REFERENCES

- AACHRD, 2002. Enhancing Research In to Traditional Medicine in the African Region. Document prepared for the 21st Session of the African Advisory Committee for Health Research and Development (AACHRD), Port Louis, Mauritius.
- Adlercreutz, H., 2002. Phyto-oestrogens and cancer. *Lancet Oncology* 3: 364–373.
- Agarwal, S.K., Singh, S.S., Verma, S., Kumar, S., 2000. Antifungal activity of anthraquinone derivatives from *Rheum emodi*. *Journal of Ethnopharmacology* 72: 43–6.
- Akinyemi, K.O., Oladapo, O., Okwara, C.E., Ibe, C.C., and Fasure, K., 2005. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. *BMC Complementary and Alternative Medicine* 5: 6.
- Akiyama, H., Kazuyasu, F., Yamasaki, O., Oono, T., Iwatsuki, K., 2001. Antibacterial action of several tannins against *Staphylococcus aureus*. *Journal of antimicrobial chemotherapy* 48: 487-491.
- Akunyili, D.N., Houghton, P.J., 1993. Monoterpenoids and naphthoquinones from *Kigelia pinnata*. *Phytochemistry* 32(4): 1015-1018.
- Akunyili, D.N., Houghton, P.J., Raman, A., 1991. Antimicrobial activities of the stem bark of *Kigelia pinnata*. *Journal of Ethnopharmacology* 35: 173-177.
- Albrecht, C.F., 1996. Hypoxoside as a putative non-toxic, multi-functional prodrug for the treatment of cancer, HIV-AIDS and inflammatory conditions. *Proceedings of the 2nd IOCD International Symposium, Victoria Falls, Zimbabwe, 25-28 February 1996*.
- Alves, D.S., Pérez-Fons, L., Estepa, A., Micol, V., 2004. Membrane-related effects underlying the biological activity of the anthraquinones emodin and barbaloin. *Biochemical Pharmacology* 68 (3): 549-61.
- Amarasinghe, N.R., Jayasinghe, L., Hara, N., Fujimoto, Y., 2007. Flacourside, a new 4-oxo-2-cyclopentenylmethyl glucoside from the fruit juice of *Flacourtia indica*. *Food Chemistry* 102: 95–97.
- Andoh, A.K., 1991. The science and romance of selected herbs used in medicine and religious ceremony. Library of Congress Catalog in Published Data.
- Arvin, A.M., Prober, C.G., 1995. Herpes Simplex Viruses. In Murray, P.R., E.F. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (eds.). *Manual of Clinical Microbiology*. Sixth Ed. ASM Press, Washington, D.C. pp. 876-883.
- Atawodi, S.E., Ameh, D.A., Ibrahim, S., Andrew, J.N., Nzelibe, H.C., Onyike, E., Anigo, K.M., Abu, E.A., James, D.B., Njoku, G.C., Sallau, A.B., 2002. Indigenous knowledge system for treatment of trypanosomiasis in Kaduna state of Nigeria. *Journal of Ethnopharmacology* 79: 279-282.
- Azuine, M.A., Ibrahim, K., Enwerem, N.M., Wambebe, C., Kolodziej, H., 1997. Protective role of *Kigelia africana* fruits against benzo[a]pyrene-induced forestomach tumorigenesis in mice and

- against albumen-induced inflammation in rats. *Pharmaceutical and Pharmacological Letters* 7: 67-70.
- Bardón, A., Borkosky, S., Ybarra, M.I., Montanaro, S., Cartagena, E., 2007. Bioactive plants from Argentina and Bolivia. *Fitoterapia* 78: 227–231.
- Barnard, D.L., Smee, D.F., Huffman, J.H., Meyerson, L.R., Sidwell, R.W., 1993. Antiherpesvirus activity and mode of action of SP-303, a novel plant flavonoid. *Chemotherapy* 39: 203–211.
- Bassole, I.N.H., Ouattara, A.S., Nebie, R., Ouattara, C.A.T., Kabore, Z.I., Traore, A.S., 2003. Chemical composition and antimicrobial activities of the essential oils of *Lippia chevalieri* and *Lippia multiflora* from Burkina Faso. *Phytochemistry* 62: 209-212.
- Becker, E.M., Nissen, R.L., Skibsted, H., 2004. Antioxidant evaluation protocols: Food quality or health effects. *Food Research Technology* 219: 561-571.
- Beladi, I., Pusztai, R., Mucsi, I., Bakay, M., Gabor, M., 1977. Activity of some flavonoids against viruses. *Annals of the New York Academy of Sciences* 284: 358–364.
- Berges, R.R., Windeler, J., Trampisch, H.J., 1995. Randomised, placebo-controlled, double blind clinical trial of β -sitosterol in patients with benign prostatic hyperplasia. *The Lancet* 345 (17): 1529-1532.
- Bessong, P.O., Chiwelu, L.O., Igumbor, E., Andreola, M.L., Litvak, S., 2004. *In vitro* activity of three selected South African medicinal plants against HIV type1 reverse transcriptase. *African Journal of Biotechnology* 3(10): 555-59.
- Betancur-Galvis, L.A., Morales, G.E., Forero, J.E., Roldan, J., 2002. Cytotoxic and antiviral activities of Colombian medicinal plant extracts of the *Euphorbia* genus. *Memorial Institute Oswaldo Cruz* 97(4): 541-546.
- Binutu, O.A., Adesogan, K.E., Okogun, J.I., 1996. Antibacterial and antifungal compounds from *Kigelia pinnata*. *Planta Medica* 62: 352-353.
- Bisno, A.L., 1991. Group A streptococcal infections and acute rheumatic fever. *The New England Journal of Medicine* 325(11): 783-93.
- Bok, S.H., Lee, S.H., Park, Y.B., 1999. Plasma and hepatic cholesterol and hepatic activities of 3-hydroxy-3-methyl-glutaryl-CoA reductase and acyl CoA: cholesterol transferase are lower in rats fed citrus peel extract or a mixture of citrus bioflavonoids. *Journal of Nutrition* 129: 1182–5.
- Bouic, P.J.D., 1998. Sterols and sterolins-the natural , non-toxic immunomodulators and their role in the control of rheumatoid arthritis. *Health Talk*: 48-49.
- Bouic, P.J.D. *et al.*, 1996. Beta-sitosterol and beta-sitosterol glucoside stimulate human peripheral blood lymphocyte proliferation: implications for their use as an immunomodulatory vitamin combination. *International Journal of Immunopharmacology* 18(12): 693-700.
- Bredt, D.S., Snyder, S.H., 1994. Annual Revision Biochemistry, 63: 175—195.
- Bruneton, J., 1999. Pharmacognosy, Phytochemistry, Medicinal Plants. Intercept Ltd., Hampshire, UK. 2nd Edition pp. 263-277.

- Buabeng, K.O., 2005. Analgesic and anti-colitic evaluation of the ethanolic extract of *Securidaca longepedunculata*. *African Journal of Traditional Complementary and Alternative Medicine* 2 (2): 177 – 205.
- Buck, A.C., 1996. Phytotherapy for the prostate. Review article. *British Journal of Urology* 78(3): 325-336.
- Bunyapraphatsara, N., Dechsree, S., Yoosook, C., Herunsalee, A., Panpisutchai, Y., 2000. Anti-herpes simplex virus component isolated from *Maclura cochinchinensis*. *Phytomedicine* 6: 421–424.
- Burkill, H.M., 1985. *The Useful Plants of West Tropical Africa*, Royal Botanical Gardens, Kew, London, 2nd edition, 1: 254-7.
- Buss, A.D., Cox, B., Waigh, R.D., 2003. Natural products as leads for new pharmaceuticals. In Burger's *Medicinal Chemistry and Drug Discovery*, 6th ed.; Volume 1: *Drug Discovery*; Abraham, D. J., Ed.; Wiley: Hoboken, NJ Chapter 20: 847-900.
- Chagonda, L.S., Makanda, C.D., Chalchat, J.C., 2000. Essential oils of cultivated *Heteropyxis natalensis* (Harv.) and cultivated *Heteromorpha trifoliata* (Wendl.) eckl. & zey. from Zimbabwe. *Journal of Essential Oil Res.* 12: 317-321.
- Charlson, A.J., 1980. Antineoplastic constituents of some Southern African plants. *Journal of Ethnopharmacology* 2(4): 323-335.
- Chavunduka, G.L., 1997. *Traditional Medicine in Modern Zimbabwe*. University of Zimbabwe Publications. Harare.
- Chen, J.L., Blanc, P., Stoddart, C.A., Bogan, M., Rozhon, E.J., Parkinson, N., Ye, Z., Cooper, R., 1998. New iridoids from the medicinal plant *Barleria prionitis* with potent activity against respiratory syncytial virus. *Journal of Natural Products* 61: 1295–1297.
- Cheng, H.Y., Lin, C.C., Lin, T.C., 2002. Antiherpes simplex virus type 2 activity of casuarinin from the bark of *Terminalia arjuna* Linn. *Antiviral Research* 55: 447-455.
- Cheng, H.Y., Lin, T.C., Yang, C.M., Wang, K.C., Lin, L.Z., Lin, C.C., 2004. Putranjivain A from *Euphorbia jolkini* inhibits both virus entry and late stage replication of herpes simplex virus type 2 *in vitro*. *Journal of Antimicrobial Chemotherapy* 53(4): 577-583.
- Chiang, L.C., Chiang, W., Liu, M.C., Lin, M.C., 2003. *In vitro* antiviral activities of *Caesalpinia pulcherrima* and its related flavonoids. *Journal of Antimicrobial Chemotherapy* 52: 194–198.
- Chiang, L.C., Chiang, W., Chang, M.Y., Ng, L.T., Lin, C.C., 2002. Antiviral activity of *Plantago major* extracts and related compounds *in vitro*. *Antiviral Research* 55 (1): 53–62.
- Christie, A.B., 1987. *Infectious Diseases: Epidemiology and Clinical Practice* Churchill Livingstone.
- Chrysayi-Tokousbalides, M., Kastanias, M.A., 2003. Cynodontin: a fungal metabolite with antifungal properties. *Journal of Agricultural Food Chemistry* 51: 4920–3.
- Clark, K.J., Grant, P.G., Sarr, A.B., Belakere, J.R., Swaggerty, C.L., Phillips, T.D., Woode, G.N.,

1998. An *in vitro* study of theaflavins extracted from black tea to neutralize bovine rotavirus and bovine coronavirus infections. *Veterinary Microbiology* 63: 147–157.
- Clark, T.E., Appleton, C.C., 1997. Molluscicidal activity of *Apodytes dimidiata* E. Mey. ex Arn (Icacinaceae), *Gardenia thunbergii* L.f. (Rubiaceae) and *Warburgia salutaris* (Bertol. f.) Chiov. (Canellaceae) plants. *Journal of Ethnopharmacology* 56(1): 15-30.
- Concon, J.M., 1988. Food Toxicology: Part A & B. Marcel Dekker Inc. New York.
- Coombes, J.D., 1992. New Drugs from Natural Sources, IBC Technical Services, London. Pp 59–62; 93–100.
- Cos, P., Hermans, N., De Bruyne, T., Apers, S., Sindambiwe, J.B., Vanden Berghe, D.B., Pieters, L., Vlietinck, A.J., 2002. Further evaluation of Rwandan medicinal plant extracts for their antimicrobial and antiviral activities. *Journal of Ethnopharmacology* 79(2): 155-163.
- Costa, C., Bertozzo, A., Allegri, G., 1992. *Journal of Heterocyclic Chemistry* 29: 1641.
- Cox, S.D., Mann, C.M., Markham, J.L., 2001. Interactions between components of the essential oil of *Melaleuca alternifolia*. *Journal of Applied Microbiology* 91: 492–497.
- Cragg, G.M., Newman, D.J., Snader, K.M., 1997. *Journal of Natural Products* 60: 52.
- D'éciga-Campos, M., Rivero-Cruz, I., Arriaga-Alba M, Castañeda-Corral, G., Angeles-L'opez, G.E., Navarrete, A., Matab, R., 2007. Acute toxicity and mutagenic activity of Mexican plants used in traditional medicine. *Journal of Ethnopharmacology* 110: 334–342.
- Dagna, S.L., Stuart, E.S., 1995. Resistance to antivirals. *Antimicrobial Resistance in Pediatrics* 42 (3): 583–599.
- Dale, I.R., Greenway, P.J., 1961. Kenya Trees and Shrubs. *Buchanan's Kenya Estates Ltd.*, in association with Hatchards, London
- Darby, G., Larder, B.A., 1992. The clinical significance of antiviral drug resistance. *Research in Virology* 143: 116–120.
- D'Cruz, O.J., Waurzyniak, B., Uckun, F.M., 2004. Mucosal toxicity studies of a gel formulation of native pokeweed antiviral protein. *Toxicologic Pathology* 32(2): 212-21.
- Dehmelow, E.V., Guntenhoner, M., van Ree, T., 2000. On the isolation of sitosterol-(6-O-acyl)- β -D-glucopyranosides from *Flacourtia indica* and on the unexpected formation of gels by the triacetate. *Zeitschrift fuer Naturforschung* 55b: 333-335.
- Dehmlow, E.V., Van Ree, T., Guntenhoner, M., 1998. 2,4-trans-,7 4'-dihydroxy-4-methoxyflavan from *Cassia abbreviata*. *Phytochemistry* 49 (6): 1805-1806.
- Dimo, T., Rakotonirina, S.V., Tan, P.V., Azay, J., Dongoc, E., Kamtchouing, P., Cros, G., 2007. Effect of *Sclerocarya birrea* (Anacardiaceae) stem bark methylene chloride/methanol extract on streptozotocin-diabetic rats. *Journal of Ethnopharmacology* 110: 434–438.
- Divisions of HIV/AIDS Prevention, 2003. HIV and Its Transmission. Centers for Disease Control & Prevention. Retrieved on 2006-05-23.

- Dreikorn, K., Schonhofer, P.S., 1995. The place of phytotherapy in the treatment of Benign Prostatic Hyperplasia. *Urologe* 34(2): 119-129. (Review article - in German)
- Drewes, S.E., Mashibaye, M.J., 1993. *Phytochemistry* 32: 1041-1044.
- Drewes, S.E., Hall, A.J., Learmonth, R.A., Upfold, U.J., 1984. Isolation of hypoxoside from *Hypoxis rooperi* and synthesis of (*E*)-1,5-bis (3',4'-dimethoxyphenyl) pent-4-en-1-yne. *Phytochemistry* 23(6): 1313-1316.
- Duran, N., Allahverdiyev, A., Ozguven, M., Koltas, S., 2003. Antiviral activity of the volatile oils of *Melissa officinalis* L. against herpes simplex virus type-2. *Phytomedicine* 11: 657-661.
- Easmon, C.S.F., 1983. Medical Microbiology: Immunization against Bacterial Disease. London & New York, Academic Press.
- Eisa, M.M., Almagboulb, A.Z., Omerb, M.E.A., Elegamib, A.A., 2000. Antibacterial activity of *Dichrostachys cinerea*. *Fitoterapia* 71: 324-327.
- El Tahir, A., Ibrahim, A., Satti, G.M.H., Theander, T.G., Kharazmi, A., Khalid, S.A., 1998. The potential antileishmanial activity of some Sudanese medicinal plants *Phytotherapy Research* 12: 576.
- Eloff, J.N., 2001. Antibacterial activity of Marula, *Sclerocarya birrea* (A. rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro) (Anacardiaceae), bark and leaves. *Journal of Ethnopharmacology* 76: 305-308.
- El-Sayyad, S.M., 1982. Flavonoids of the leaves and fruits of *Kigelia pinnata*. *Fitoterapia* 42: 189-91.
- Fahad, A., Stepher, L., 1996. New antiherpes virus agents. Their targets and therapeutic potential. *Potent Drugs* 52 (1): 17-32.
- Fairbairn, J.W., Betts, T.J., Crellin, J.K., 1964. The analysis and standardization of anthraquinone drugs. *Planta Medica* 12: 260-264.
- FAO, 1986. Natural resources and the human environment for food and agriculture in Africa, *FAO Environment and Energy Paper* No. 6: 88.
- Ferreol, G., *et al.*, 1958. *Comptes Rendus* 246: 3082.
- Foxman, B., Geiger, A.M., Palin, K., Gillespie, B., Koopman, J.S., 1995. First-time urinary tract infection and sexual behavior. *Epidemiology* 6: 162-168.
- Frei, B., and Higdon, J.V., 2003. Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. *Journal of Nutrition* 133: 3275-3284.
- Freshney, R.I., 1992. Introduction to Basic Principles in Animal Cell Culture. IRL Press, Oxford, 2nd Edition pp 1-14.
- Frohne, D., Pfander, J., 1984. A Colour Atlas of Poisonous Plants. Wolfe, ISBN 0723408394.
- Funatogawa, K., Hayashi, S., Shimomura, H., Yoshida, T., Hatano, T., Ito, H., Iría, Y., 2004.

- Antibacterial activity of hydrolysable tannins derived from medicinal plants against *Helicobacter pylori*. *The Journal of Microbiology, Immunology and Infection*, 48(4): 251-261.
- Fyhrquist, P., Mwasumbi, L., Haeggstrom, C.A., Vuorela, H., Hiltunen, R., Vuorela, P., 2002. Ethnobotanical and antimicrobial investigation on some species of *Terminalia* and *Combretum* (Combretaceae) growing in Tanzania. *Journal of Ethnopharmacology* 79(2): 169-77.
- Galati, E.M., Trovato, A., Kirjavainen, S., et al., 1996. Biological effects of hesperidin, a citrus flavonoid. (Note III). Antihypertensive and diuretic activity in rat. *Il Farmaco* 51: 219-21.
- Gao, F., Bailes, E., Robertson, D.L., Chen, Y., Rodenburg, C.M., Michael, S.F., Cummins, L.B., Arthur, L.O., Peeters, M., Shaw, G.M., Sharp, P.M., Hahn, B.H., 1999. Origin of HIV-1 in the Chimpanzee Pan troglodytes. *Nature* 397 (6718): 436-441.
- Garg, A., Garg, S., Zaneveld, L.J., Singla, A.K., 2001. Chemistry and pharmacology of the citrus bioflavonoid hesperidin. *Phytotherapy Research* 15: 655-69.
- Gelfand, M., Mavi, S., Drummond, R.B., Ndemera, B., 1985. The Traditional Medicinal Practitioner in Zimbabwe. *Mambo Press*, Harare.
- Gessler, M.C., Nkunya, M.H., Mwasumbi, L.B., Heinrich, M., Tanner, M., 1994. Screening Tanzanian medicinal plants for antimalarial activity. *Acta Tropica* 56(1): 65-77.
- Gey, G.O., 1952. Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. *Cancer Research* 12: 264-265.
- Ghazi, H., Norio, N., Meselhy, R.M., Masao, H., 1999. Phenolics from *Maytenus senegalensis*. *Phytochemistry* 50: 689-694.
- Gillmer, M., Symmonds, R., 1999. Seed Collection and Germination: *Hypoxis hemerocallidea*. *Plantlife* 21: 36-37.
- Gouda, Y.G., Afaf, M.A., Faten, M.D., Khaked, M.M., Ryoji, K., Kazuo, Y., 2003. Iridoids from *Kigelia pinnata* DC. fruits. *Phytochemistry* 63: 887-892.
- Govindachari, T.R., Patankar, S.J., Viswanathan, N., 1971. Isolation and structure of two new dihydroisocoumarins from *Kigelia pinnata*. *Phytochemistry* 10(7): 1603-1606.
- Harborne, J.B., 1998. *Phytochemical Methods: A guide to modern techniques of plant analysis*. Chapman and Hall Ltd, London.
- Hashimoto, F., Nonaka, G.I., Nishioka, I., 1989. *Chemical and Pharmaceutical Bulletin* 37: 77-85.
- Hemingway, R.W., Karchesy, J.J., 1989. Chemistry and significance of condensed tannins. *Plenum Press*, New York. Pp 47-60.
- Hostettmann, K., Marston, A., Ndjoko, K., Wolfender, J.L., 2000. The potential of African plants as a source of drugs. *Current Organic Chemistry* 4: 973-1010.
- Houghton, P.J., Photiou, A., Udin, S., Shah, P., 1994. Activity of extracts of *Kigelia pinnata* against melanoma and carcinoma cell lines. *Planta Medica* 60: 430-433.

- Hsiung, G.D., 1982. *Diagnostic Virology*, Yale University Press, New Haven and London.
- Hudson, J.B., 1990. *Antiviral Compounds from Plants*. Boca Raton, Ann Arbor, Boston: CRC Press.
- Hutchings, A., 1989. Observations on plant usage in Xhosa and Zulu medicine. *Bothalia* 19 (2): 225-235.
- Jackson, S.J., 1996. Cytotoxic activity of *Kigelia pinnata* against melanoma and other neoplastic cell lines. *PhD Thesis*, University of London, UK.
- Jagadeeshwar, R., Tiwari, A., Kumar, U.S., Reddy, S.V., Ali, A.Z., Rao, J.M., 2003. Novel 3-o-acyl mesquitol analogues as free-radical scavengers and enzyme inhibitors: synthesis, biological evaluation and structure–activity relationship. *Bioorganic & Medicinal Chemistry Letters* 13: 2777–2780.
- Jansen, B.M.J., de Groot, A., 1991. The occurrence and biological activity of drimane sesquiterpenoids. *Natural Products Reports* 8: 309-318.
- Jassim, S.A., Naji, M.A., 2003. Novel antiviral agents: a medicinal plant perspective. *Journal of Applied Microbiology* 95: 412–427.
- Jung, H.J., Lee Chong, O., Lee, K.T., Choi, J., Park H.J., 2004. Structure–activity relationship of oleanane disaccharides isolated from *Akebia quinata* versus cytotoxicity against cancer cells and NO inhibition. *Biological and Pharmaceutical Bulletin* Vol.27: 744-747.
- Kambizi, L., Afolayan, A.J., 2001. An ethnobotanical study of plants used for the treatment of STD (njovhera) in Guruve District, Zimbabwe. *Journal of Ethnopharmacology* 77(1): 5-9.
- Karou, D., Savadogo, A., Canini, A., Yameogo, S., Montesano, C., Simporé, J., Colizzi, V., Traore, A.S., 2005. Antibacterial activity of alkaloids from *Sida acuta*. *African Journal of Biotechnology* 4 (12): 1452-1457.
- Keating, G.J., and O’Kennedy, R., 1997. *The chemistry and occurrence of coumarins*. O’Kennedy & Thornes: 23-66.
- Kela, S.L., Ogunsusi, R.A., Ogbogu, V.C., Nwude, N., 1989. Screening of some Nigerian plants for molluscicidal activity. *Revue d’Elevage et de Médecine Vétérinaire des Pays Tropicaux* 42(2): 195-202.
- Kernan, M.R., Amarquaye, A., Chen, J.L., Chan, J., Sesin, D.F., Parkinson, N., Ye, Z., Barrett, M., et al., 1998. Antiviral phenylpropanoid glycosides from the medicinal plant *Markhamia lutea*. *Journal of Natural Products* 61: 564–570.
- Khan, M.T.H., Ather, A., Thompson, K.D., Gambari, R., 2005. Extracts and molecules from medicinal plants against herpes simplex viruses. *Antiviral Research* 67:107-119.
- Kim, G., and Kim, N., 2007. A new synthetic route to (-)-cassine via asymmetric aminohydroxylation. *Tetrahedron Letters* 48: 4481–4483.
- Kjetland, E.F., Gwanzura, L., Ndhlovu, P.D., Mduluza, T., Gomo, E., Mason, P.R., Midzi, N., Friis, H., Gundersen, S.G., 2005. Herpes simplex virus type 2 prevalence of epidemic proportions in rural Zimbabwean women: association with other sexually transmitted infections. *The Archives*

- Kokwaro, J.O., 1976. Medicinal Plants of East Africa. East Africa Literature Bureau. Nairobi.
- Kolodziej, H., Kiderlen, A.F., 2005. Antileishmanial activity and immune modulatory effects of tannins and related compounds on *Leishmania* parasitised RAW 264.7 cells *Phytochemistry* 66: 2056-71.
- Kosar, M., Bozan, B., Temelli, F., Baser, K.H.C., 2007. Antioxidant activity and phenolic composition of sumac (*Rhus coriaria* L.) extracts. *Food Chemistry* 103: 952–959.
- Krishnaraju, A.V., Rao-Tayi, V.N., Sundararaju, D., Vanisree, M., Tsay, H.S., Subbaraju, G.V., 2005. Assessment of bioactivity of Indian medicinal plant using brine shrimp (*Artemia salina*) lethality assay. *International Journal of Applied Science, Engineering and Technology* 3(2): 125-134.
- Kuo, Y.C., Meng, H.C., Tsai, W.J., 2001. Regulation of cell proliferation, inflammatory cytokine production and calcium mobilization in primary human T lymphocytes by emodin from *Polygonum hypoleucum* Ohwi. *Inflammation Research* 50(2): 73–82.
- Kurokawa, M., Basnet, P., Ohsugi, M., Hozumi, T., Kadota, S., Namba, T., Kawana, T., Shiraki, K., 1999. Anti-herpes simplex virus activity of moronic acid purified from *Rhus javanica* *in vitro* and *in vivo*. *The Journal of Pharmacology and Experimental Therapeutics* Vol. 289: 72–78.
- Lannang, A.M., Lontsi, D., Ngounou, F.N., Sondengam, B.L., Nkengfack, A.E., van Heerden, F.R., Assob, J.C.N., 2006. Securidacaxanthone A, a hepta-oxygenated xanthone from *Securidaca longepedunculata*. *Fitoterapia* 77: 199–202.
- Laporta, O., Funes, L., Garzón, M.T., Villalain, J., Micol, V., 2007. Role of membranes on the antibacterial and anti-inflammatory activities of the bioactive compounds from *Hypoxis rooperi* corm extract. *Archives of Biochemistry and Biophysics*, doi: 10.1016/j.abb.2007.08.013
- Lee, R.E., Bykadi, G., Ritschel, W.A., 1981. *Arzneimittel-Forschung/Drug* 31: 640-642.
- Li, B.Q., Fu, T., Dongyan, Y., Mikovits, J.A., Ruscetti, F.W., Wang, J.M., 2000. Flavonoid baicalin inhibits *HIV-1* infection at the level of viral entry. *Biochemical and Biophysical Research Communications* 276: 534–538.
- Lin, L.U., Shu-wen, L., Shi-bo, J., Shu-guang, W., 2004. Tannin inhibits HIV-1 entry by targeting gp41. *Acta Pharmacologica Sinica* 25(2): 213-218.
- Lin, Y.M., Flavin, M.T., Schure, R., Chen, F.C., Sidwell, R., Barnard, D.L., Huffman, J.H., Kern, E.R., 1999. Antiviral activities of biflavonoids. *Planta Medica* 65(2): 120-5.
- Ling-Ling, Y., Chih-Ying, L., Kun-Ying, Y., 2000. Induction of apoptosis by hydrolyzable tannins from *Eugenia jambos* L. on human leukemia cells. *Cancer Letters* 157: 65-75.
- Lowe, F.C., *et al.*, 1998. A review of recent placebo-controlled trials utilising phytotherapeutic agents for the treatment of benign prostatic hyperplasia (BPH). *Prostate* 37(3): 187-193.
- Lu, M., Chen, Q.H., 1989. Biochemical study of Chinese rhubarb: XXIX. Inhibitory effects of anthraquinone on P388 leukemia in mice. *Journal of China Pharmaceutical University* 12:155-7.

- Luchini, A.C., Rodrigues-Orsi, P., Cestari, S.H., Seito, L.N., Witaicenis, A., Pellizzon, C.H., Cláudio Di Stasi, L., 2008. Intestinal anti-inflammatory activity of coumarin and 4-hydroxycoumarin in the trinitrobenzenesulphonic acid model of rat colitis. *Biological and Pharmaceutical Bulletin* Vol.31: 1343-1350.
- Mahomed, I.M., Ojewole, J.A., 2003. Hypoglycemic effect of *Hypoxis hemerocallidea* corm (African potato) aqueous extract in rats. *Methods & Findings in Experimental & Clinical Pharmacology*, 25(8): 617.
- Majester-Savornin, B., Elias, R., Diaz-Lanza, A.M., Balansard, G., Gasquet, M., Delmas, F., 1991. *Planta Medica* 57: 260-262.
- Martino, V., Morales, J., Martinez-Irujo, J.J., Font, M., Monge, A., Coussio, J., 2004. Two ellagitannins from the leaves of *Terminalia triflora* with inhibitory activity on HIV-1 reverse transcriptase. *Phytotherapy Research* 18(8): 667-9.
- Marx, J.L., 1982. New disease baffles medical community. *Science* 217 (4560): 618–621.
- Mashimbye, M.J., Maumela, M.C., Drewes, S.E., 1999. A drimane sesquiterpenoid lactone from *Warburgia salutaris*. *Phytochemistry* 51: 435-438.
- Matern, U., Lürer, P., Kreuzsch, D., 1999. Biosynthesis of coumarins. *Comprehensive Natural Products Chemistry* 1: 623-637.
- McGaw, L.J., Van der Merwe, D., Eloff, J.N., 2007. *In vitro* anthelmintic, antibacterial and cytotoxic effects of extracts from plants used in South African ethnoveterinary medicine. *The Veterinary Journal* 173: 366–372.
- McGee, H., 2004. On Food and Cooking. Simon & Schuster. New York, NY pp. 714.
- McLaughlin, J.L., 1991. Crown gall tumors on potato discs and brine shrimp lethality: Two simple bioassays for higher plant screening and fractionation. *Methods in Plant Biochemistry*. In: Hostettmann K (ed) Assays for Bioactivity, Academic Press, London, 6: 1 – 33.
- Mehanna, A.S., 2003. Rationale Design of Anti-HIV Drugs. in *Burger's medicinal chemistry and drug discovery*. Vol. 5: *Chemotherapeutic Agents*. Ed. Abraham, D.J. John Wiley & Sons. Chapter 11: 457-483.
- Mena-Rejon, G.J., Perez-Rivas, K., Sansorez-Peraza, P., Rios, T., Quijano, L., 2002. Racemochrysonone, a dihydroanthracenone from *Senna racemosa*. *Zeitschrift fuer Naturforschung C* 57: 777–779.
- Merck & Co. Inc. 1989. The Merck Index 11th edition, Rahway, Massachusetts.
- Middleton, E., 1998. Effect of plant flavonoids on immune and inflammatory cell function. *Advances in Experimental Medicine and Biology* 439: 175–182.
- Miliauskas, G., Venskutonis, P.R., van Beek, T.A., 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry* 85: 231-237.
- Mills, E., Foster, B., Heeswijk, R., Phillips, E., Wilson, K., Leonard, D., Kosuge, K., Kanfer, I., 2005. Impact of African herbal medicines on antiretroviral metabolism. *AIDS*. 19(1): 95-97.

- Miranda, C.L., Stevens, J.F., Ivanov, V., McCall, M., Frei, B., Deinzer, M.L., Buhler, D.R., 2000. Antioxidant and prooxidant actions of prenylated and nonprenylated chalcones and flavanones in vitro. *Journal of Agricultural and Food Chemistry* 48(9): 3876-84.
- Mirotnik, J., Mosellie, B.M., 2004. Genital herpes and the mass media. *The Journal of Popular Culture* 20 (3): 1 – 11.
- Mochizuki, M., Hasegawa, N., 2006. Acceleration of lipid degradation by sericoside of *Terminalia sericea* roots in fully differentiated 3T3-L1 cells. *Phytotherapy Research* 20(11): 1020-1.
- Moideen, S.V.K., Houghton, P.J., Rock, P., Croft, S.L., Aboagye-Nyame, F., 1999. Activity of extracts and naphthoquinones from *Kigelia pinnata* against *Trypanosoma brucei brucei* and *Trypanosoma brucei modiesiense*. *Planta Medica* 65: 493-586.
- Mojab, F., Kamalinejad, M., Ghaderi, N., Vahidipour, H.R., 2003. Phytochemical screening of some species of Iranian plants. *Iranian Journal of Pharmaceutical Research*: 77-82.
- Mølgaard, P., Nielsen, S.B., Rasmussen, D.E., Drummond, R.B., Makaza, N., Andreassen, J., 2001. Anthelmintic screening of Zimbabwean plants traditionally used against schistosomiasis. *Journal of Ethnopharmacology* 74(3): 257-64.
- Moo-Puc, R.E., Mena-Rejon, G.J., Quijano, L., Cedillo-Rivera, R., 2007. Antiprotozoal activity of *Senna racemosa*. *Journal of Ethnopharmacology* 112: 415–416.
- Morgan, S.J., Darling, D.C., 1993. Primary Culture in Animal Cell Culture. *BIOS Scientific publishers*, Oxford, UK: 37-50 & 51-65.
- Moshi, M.J., Mbwambo, Z.H., 2005. Some pharmacological properties of *Terminalia sericea* roots. *Journal of Ethnopharmacology* 97(1): 43-7.
- Moyo, F., Gashe, B.A., Majinda, R.R.T., 1999. A new flavan from *Elephantorrhiza goetzei*. *Fitoterapia* 70: 412.
- Muller-Christiansen, K., 1993. Besonderer Stellenwert der Phytopharmaka. *Therapiewoche* 43(26/27): 1490-1496. (German).
- Murray, R.D.H., Méndez, J., Brown, S.A., 1982. The Natural Coumarins - Occurrence, *Chemistry and Biochemistry*, John Wiley & Sons Ltd., Chichester, UK.
- Nakano, M., Kurokawa, M., Hozumi, T., Saito, A., Ida, M., Morohashi, M., Namba, T., Kawana, T., et al., 1999. Suppression of recurrent genital herpes simplex virus type 2 infection by *Rhus javanica* in guinea pigs. *Antiviral Research* 41: 153–154.
- Narayanan, B.A., Geoffroy, O., Willingham, M.C., Reb, G.G., Nixon, D.W., 1999. p53/p21(WAF1/CIP1) expression and its possible role in G1 arrest and apoptosis in ellagic acid treated cancer cells. *Cancer Letters* 136: 215-221.
- Ndhlala, A.R., Kasiyamhuru, A., Mupure, C., Chitindingu, K., Benhura, M.A., Muchuweti, M., 2007. Phenolic composition of *Flacourtia indica*, *Opuntia megacantha* and *Sclerocarya birrea*. *Food Chemistry* 103: 82–87.
- Nel, R., Mthembu, M., Coetzee, J., van Rensburg, H., Malan, E., Ferreira, D., 1999. The novel

- flavan-3-ol, (2R,3S)-guibourtinidol and its diastereomers. *Phytochemistry* 52: 1153-1158.
- Nemeikaite-Ceniene, A., Sergediene, E., Nivinskas, H., Cenas, N., 2002. Cytotoxicity of natural hydroxyanthraquinones: role of oxidative stress. *Zeitschrift fuer Naturforschung* 57: 822–7.
- Nfi, A., Ndi, C., Bayemi, P.H., Njwe, R., Tchoumboue, J., Njakoi, H., Mopoi, N., Njakoi, M., 1999. The anthelmintic efficacy of some indigenous plants in the Northwest province of Cameroon *Revue d Elevage et de Medecine Veterinaire des Pays Tropicaux* 52(2):103-6.
- NGO Regional Natural Products Development Initiative, Annex 3, 2000. CRIAA SA-DC, Namibia.
- Nonaka, G.I., Hsu, F., Nishioka, I., 1981. *Journal of Chemical Society Chemical Communication*, 781-783.
- Nonaka, G.I., Kawahara, O., Nishioka, I., 1983. *Chemical and Pharmaceutical Bulletin*, 31: 3906-3914.
- Nonaka, G., Nishioka, I., Nishizawa, M., Yamagishi, T., Kashiwada, Y., Dutschman, G.E., Bodner, A.J., Kilkuskie, R.E., Cheng, Y.C., Lee, K.H., 1990. Anti-AIDS agents, 2: Inhibitory effects of tannins on HIV reverse transcriptase and HIV replication in H9 lymphocyte cells. *Journal of Natural Products* 53(3): 587-95.
- Ojala, T., 2001. Biological Screening of Plant Coumarins. *Academic Dissertation*, Faculty of Science of the University of Helsinki, Helsinki.
- Ojewole, J.A.O., 2003. Hypoglycemic effect of *Sclerocarya birrea* {(A. Rich.) Hochst.} [Anacardiaceae] stem-bark aqueous extract in rats. *Phytomedicine* 10: 675–681.
- Ojewole, J.A.O., 2007. Analgesic, anti-inflammatory and hypoglycaemic effects of *Rhus chirindensis* (Baker F.) [Anacardiaceae] stem-bark aqueous extract in mice and rats. *Journal of Ethnopharmacology* 113: 338–345.
- Olajide, O.A., Awe, S.O., Makinde, J.M., 1998. Pharmacological screening of the root extract of *Securidaca longepedunculata*. *Fitoterapia* 69 (3): 249-252.
- Oliver-Bever, B., 1986. Medicinal plants in tropical West Africa. *Cambridge University Press*, Cambridge.
- Ooi, L.S.M., Wang, H., Luk, C.W., Ooi, V.E.C., 2004. Anticancer and antiviral activities of *Youngia japonica* (L.) DC (Asteraceae, Compositae). *Journal of Ethnopharmacology* 94: 117–122.
- Orroth, K.K., Gavyole, A., Todd, J., Mosha, F., Ross, D., Mwijarubi, E., Grosskurth, H., Hayes, R.J., 2000. Syndromic treatment of sexually transmitted diseases reduces the proportion of incident HIV infections attributable to these diseases in rural Tanzania. *AIDS* 14(10): 1429-37.
- Palella, F.J. Jr, Delaney, K.M., Moorman, A.C., Loveless M.O., Fuhrer, J., Satten, G.A., Aschman, D.J., Holmberg, S.D., 1998. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *New England Journal of Medicine* 338 (13): 853–860.
- Palgrave, K.C., 2002. Trees of Southern Africa. Struik Publ. 3rd edition.

- Palmer, E., Pitman, P., 1972. Trees for southern Africa covering all known indigenous species in RSA, South West Africa, Botswana, Lesotho and Swaziland Volume 2. A.A. Balkema. Cape Town.
- Pegel, K.H., 1973. Extraction of phytosterol glycosides from *Hypoxis* tubers. S.A.Patent ZA 7201855.
- Pegel, K.H., 1984. B-sitosterol β -D-glucoside (sitosterolin) as the active agent in Harzol[®] and other phytopharmaka. *Extracta Urologica* 1, suppl. 7: 105-111. (In German)
- Peters, C.R., 1988. Notes on the distribution and relative abundance of *Sclerocarya birrea* (A. Rich) Hochst. (Anacardiaceae). *Monograph in systematic Botany of the Missouri Botanical Gardens*, 25: 403-410.
- Pietta, P.G., 2000. Flavonoids as antioxidants. *Journal of Natural Products* 63: 1035–1042.
- Pistelli, L., Venturi, R., Marsili, A., Morello, I., 1998. Alkaloids and Coumarins from *Gymnosporia senegalensis* var. *spinosa* (Celastraceae). *Biochemical Systematics and Ecology* 26: 677-79.
- Poole, K., 1994. Bacterial multidrug resistance – emphasis on efflux mechanisms and *Pseudomonas aeruginosa*. *Journal of Antimicrobial Chemotherapy*. 34: 453–456.
- Porter, L.J., Newman, R.H., Foo, L.Y., Wong, H., Hemingway, R.W., 1982. *Journal of Chemical Society Perkin Transaction I*: 1217-1221.
- Quideau, S., Varadinova, T., Karagiozova, D., Jourdes, M., Pardon, P., Baudry, C., Genova, P., Diakov, T., Petrova, R., 2004. Main structural and stereochemical aspects of the antiherpetic activity of nonhydroxyterphenoyl-containing C-glycosidic ellagitannins. *Chem Biodivers*. 1(2): 247-58.
- Raffauf, R.F., 1996. Plant Alkaloids: A Guide to Their Discovery and Distribution. The Haworth Press, Binghamton, NY, page 48.
- Rabe, T., van Staden, J., 1997. Antibacterial activity of South African plants used for medicinal purposes. *Journal of Ethnopharmacology* 56: 81-87.
- Rabe, T., van Staden, J., 2000. Isolation of an antibacterial sesquiterpenoid from *Warburgia salutaris*. *Journal of Ethnopharmacology* 73: 171-174.
- Recio, M.D.C., Just, M.J., Giner, R.M., Manez, S., Rios, J.L., Hostettmann, K., 1995. Antiinflammatory activity of saikosaponins from *Heteromorpha trifoliata*. *Journal of Natural Products*. 58: 140-144.
- Reed, L.J., Muench, H., 1938. A simple method of estimating fifty percent endpoints. *The American Journal of Hygiene* 27: 493–497.
- Reid, K.A., Jäger, A.K., van Staden, J., 2001. Isolation of the anti-bacterial vernodalin from traditionally used *Vernonia*. *South African Journal of Botany*, 67(1): 71–73.
- Reiner, R., 1982. Detection of antibiotic activity. In Antibiotics an introduction. *Roche Scientific Services*, Switzerland, 1: 21-25.

- Rhodes, L., *et al.*, 1993. Comparison of finasteride (Proscar), a 5-alpha reductase inhibitor, and various commercial plant extracts in *in vitro* and *in vivo* 5-alpha reductase inhibition. *Prostate* 22 (1): 43-51.
- Rietveld, A., Wiseman, S., 2003. Antioxidant effects of tea: evidence from human clinical trials. *Journal of Nutrition* 133: 3285–3292.
- Ríos, J.L., Recio, M.C., 2005. Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology* 22: 100(1-2): 80-4. Review.
- Rulungaranga, Z.K., 1989. Some Important Indigenous Medicinal and Aromatic Plants in the Wild Flora of Tanzania Mainland. *Tropical Forestry Action Plan, Working Paper 24*. Tanzania Ministry of Lands, Natural Resources and Tourism, Dar es Saalam.
- San Francisco AIDS Foundation, 2006-04-14. How HIV is spread. Retrieved on 2006-05-23.
- Sanderson, T.J., Hordijk, J., Denison, M.S., Springsteel, M.F., Nantz, M.H., van den Berg, M., 2004. Induction and inhibition of aromatase (cyp19) activity by natural and synthetic flavonoid compounds in h295r human adrenocortical carcinoma cells. *Toxicological Sciences* 82: 70–79.
- Sansores-Peraza, P., Rosado-Vallado, M., Brito-Loeza, W., Mena-Rejon, G.J., Quijano, L., 2000. Cassine, an antimicrobial alkaloid from *Senna racemosa*. *Fitoterapia* 71: 690–692.
- Sarno, S., Moro, S., Meggio, F., Zagotto, G., Dal Ben, D., Ghisellini, P., *et al.* 2002. Toward the rational design of protein kinase casein kinase-2 inhibitors. *Pharmacological Therapy* 93(2/3): 159–68.
- Scandola, M., Ganes, D.E., Costa, C., Allegri, G., Bertozzo, A., Curcuruto, O., 1994. *Journal of Heterocyclic Chemistry* 31: 219.
- Semple, S.J., Pyke, S.M., Reynolds, G.D., Flower, R.L.P., 2001. *In vitro* antiviral activity of the anthraquinone chrysophanic acid against poliovirus. *Antiviral Research* 49: 169–78.
- Sethi, M. L., 2002. *Foye's Principles of Medicinal Chemistry*, Lippincott Williams and Wilkins: Philadelphia. Chapter 39.
- Shahat, A.A., Cos, P., De Bruyne, T., Apers, S., Hammouda, F.M., Ismail, S.I., Azzam, S., Claeys, M., Goovaerts, E., Pieters, L., Vanden Berghe, D., Vlietinck, A.J., 2002. Antiviral and antioxidant activity of flavonoids and proanthocyanidins from *Crataegus sinaica*. *Planta Medica* 68 (6): 539–541.
- Shin, Y.W., Bok, S.H., Jeong, T.S., *et al.*, 1999. Hypocholesterolemic effect of naringin associated with hepatic cholesterol regulating enzyme changes in rats. *International Journal for Vitamin and Nutrition Research* 69: 341–7.
- Sofowora, E.A., 1984. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd (Ibadan): 97-145.
- Srinivas, G., Anto, R.J., Srinivas, P., Vidhyalakshmi, S., Senan, V.P., Karunagaran, D., 2003. Emodin induces apoptosis of human cervical cancer cells through poly (ADP-ribose) polymerase cleavage and activation of caspase-9. *European Journal Pharmacology* 473(2/3): 117–25.

- Steenkamp, V., Mathivha, E., Gouws, M.C., van Rensburg, C.E., 2004. Studies on antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa. *Journal of Ethnopharmacology* 95(2/3): 353-7.
- Susumu, T., Ryoji, K., Takashi, T., Ying-Jun, Z., Isao, K., Michiaki, K., 2005. .Suppression of tumor cell invasiveness by hydrolyzable tannins (plant polyphenols) via the inhibition of matrix metalloproteinase-2/-9 activity. *Biochemical and Biophysical Research Communications* 330: 1306–1313.
- Sydiskis, R.J., Owen, D.G., Lohr, J.L., Rosler, K.H., Blomster, R.N., 1991. Inactivation of enveloped viruses by anthraquinones extracted from plants. *Antimicrobial Agents and Chemotherapy* 35: 2463-66.
- Tagwireyi, D., Ball, D.E., Nhachi, C.F.B., 2002. Traditional medicine poisoning in Zimbabwe: clinical presentation and management in adults. *Human & experimental toxicology* 21(11):579-86.
- Thomson, R.H., 1986. *Naturally occurring quinones* III. Recent advances. London: Chapman and Hall.
- Tona, L., Ngimbi, N.P., Tsakala, M., Mesia, K., Cimanga, K., Apers, S., Pieters, L., Totte, J., Vlietinck, A.J., 1999. Antimalarial activity of 20 crude extracts from nine African medicinal plants used in Kinshasa, Congo. *Journal of Ethnopharmacology* 68(1-3): 193-203.
- Tosun, L., Ustun, S.N., 2003. An investigation about antioxidant capacity of fruit nectars. *Pakistan Journal of Nutrition*. 2 (3): 167-169.
- Trease, G.E., Evans, W.C., 2002. *Pharmacognosy*. Williams and Wilkins Company, Baltimore 7th Edn.
- Treurnicht, F.T., 1997. An evaluation of the toxic and potential antiviral effects of some plants used by South Africans for medicinal purposes. *MSc thesis*, University of Stellenbosch.
- Trute, A., Gross, J., Mutschler, E., Nahrstedt, A., 1996. *Planta Medica* 63: 125-129.
- Tsuchiya, Y., Shimizu, M., Hiyama, Y., Itoh, K., Hashimoto, Y., Nakayama, M., Horie, T., Morita, N., 1985. Antiviral activity of natural occurring flavonoids *in vitro*. *Chemical and Pharmaceutical Bulletin (Tokyo)* 33: 3881–3886.
- UNAIDS, 2006. Overview of the global AIDS epidemic, *2006 Report on the global AIDS epidemic*. (PDF).
- UNAIDS, 2008. Sub-Saharan Africa AIDS epidemic update Regional Summary. WHO Library Cataloguing-in-Publication Data.
- Van Staden, J., 1981. Constituents of *Hypoxis rooperi*, a valuable medicinal plant in South Africa. *Deutsche Apotheke Zeitung* 33: 460-464.
- van Wyk, B.E., Oudtshoorn, B., Gericke, N., 1997. *Medicinal Plants of South Africa*. Pretoria: Briza Publications.
- Velioglu, V.S., Mazza, G, Cao, L., Oomah, B.D., 1998. Antioxidant activity and total phenolics in

- selected fruits, vegetables and grain products. *Journal of Agriculture and Food Chemistry*, 46; 4113-7.
- Viljoen, A., Van Vuuren, S., Ernst, E., Klepser, M., Demirci, B., Basser, H., Van Wyk, B.E., 2003. *Osmitopsis asteriscoides* (Asteraceae) –the antimicrobial and essential oil composition of a Cape-Dutch remedy. *Journal of Ethnopharmacology* 88: 137-143.
- Villegas, P., 1998. Titration of biological suspensions, In D. E. Swayne (ed.), A laboratory manual for the isolation and identification of avian pathogens. *American Association of Avian Pathologists*, Kennett Square, Pp. 248-254.
- Vlietinck, A.J., van Hoof, L., Totte, J., Lasure, A., van den Berghe, D., Rwangabo, P.C., Mvukiyumwami, J., 1995. Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. *Journal of Ethnopharmacology* 46: 31-47.
- Wagner, H., Bladt, S., Zgainski, E.M., 1984, Plant Drug Analysis. Springer-Verlag Berlin Heidelberg New York Tokyo: 291-304.
- Wagstaff, A.J., Faulds, D., Gona, K.L., 1994. Acyclovir: a reappraisal of its antiviral activity. Pharmacokinetic properties and therapeutic efficacy. *Drugs* 47 (1): 153–205.
- Wang, J.R., Zhou, H., Jiang, Z.H., Wong, Y.F., Liu, L., 2008. *In vivo* anti-inflammatory and analgesic activities of a purified saponin fraction derived from the root of *Ilex pubescens*. *Biological and Pharmaceutical Bulletin* Vol.31: 643-650.
- Wang, R.R., Gu, Q., Yang, L.M., Chen, J.J., Li, S.Y., Zheng, Y.T., 2006. Anti-HIV-1 activities of extracts from the medicinal plant *Rhus chinensis*. *Journal of Ethnopharmacology* 105: 269–273.
- Wang, Z., Zhou, J., Ju, Y., Zhang, Z., Liu, Li, X., 2001. Effects of two saponins extracted from the *Polygonatum zanlanscianense* pamp on the human leukemia (hl-60) cells. *Biological and Pharmaceutical Bulletin* Vol.24: 159-162.
- Wang, T.T., Sathyamoorthy, N., Phang, J.M., 1996. Molecular effects of genistein on oestrogen receptor mediated pathways. *Carcinogenesis* 17: 271–275.
- Wanjala, C.C.W., Majinda, R.R.T., 2001. A new stilbene glycoside from *Elephantorrhiza goetzei*. *Fitoterapia* 72: 649-655.
- Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern Africa. E & S Livingston Limited, Edinburgh, London.
- Weiss, C.R., Moideen, S.V.K, Croft, S.L., Houghton, P.J., 2000. Activity of extracts and isolated naphthoquinones from *Kigelia pinnata* against *Plasmodium falciparum*. *Journal of Natural Products* 63: 1306-1309.
- Wilcox, L.J., Borradaile, N.M., de Dreu, L.E., Huff, M.W., 2001. Secretion of hepatocyte apoB is inhibited by the flavonoids, naringenin and hesperetin, via reduced activity and expression of ACAT2 and MTP. *Journal of Lipid Research* 42: 725–34.
- Wild, H., 1972. The Compositae of the Flora Zambesiaca area. 3. Mutisieae. *Kirkia* 8(2): 173-207.
- Williamson, J., 1975. Useful plants of Malawi. Revised and extended edition. Limbe, Malawi.

Montfort Press.

Yen, S.S., Reagan, J.W., Rosenthal, M.S., 1965. Herpes simplex infection in female genital tract. *Journal of Obstetrics and Gynecology* 25: 479–492.

Zand, R.S., Jenkins, D.J., Diamandis, E.P., 2000. Steroid hormone activity of flavonoids and related compounds. *Breast Cancer Research Treatment* 62: 35–49.