Screening of some Traditional Medicinal Plants from Zimbabwe for Biological and Anti-microbial Activity

By

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ABSTRACT

In Zimbabwe medicinal plants play a critical role in the healthcare delivery system. Ethnobotanical surveys of five districts from the Matebeleland and Manicaland regions of Zimbabwe were done and most commonly used and endangered plant species used in traditional medical practice were selected. From twelve plants 20 extracts were prepared from the various plant parts and extracted by solvent extraction and freeze dried. The extracts were then screened for phytochemical constituents and biological activity using standard techniques.

Phytochemical screening was carried out using TLC (Thin Layer Chromatography) where UV detection was at 254 and 365nm followed by confirmatory tests. Three of the plant extracts contained alkaloids, five had anthraquinone derivatives, five had coumarins, eight had cardiac glycosides and flavonoids, fifteen had saponins and sixteen contained tannins. Saponins and tannins were the most abundant phytochemicals found. These phytochemical groups and isolated chemical compounds may be responsible for the various biological activities observed by traditional healers in ethnopharmacology.

Total phenolic contents were determined by the Folin-Ciocalteu method and the antioxidant activity was evaluated by DPPH (2, 2-diphenyl picrylhydrazyl) using β-carotene as reference. A high total phenolic content was observed for *P. africanum* bark 0.438±0.00424 and lowest for *V. infausta* leaves with 0.0048±0.00255TAE. Some extracts had high antioxidant activity: *P. africanum* leaves, bark, and roots had 97.6±0.354%, 96.3±0.354% and 96.40±0.00% respectively. The lowest antioxidant value was recorded in *V. infausta* roots with 39.7 ± 0.212%. Most plants showed great potential for use as antioxidants.

Brine shrimp lethality tests were used to predict potential cytotoxic activity of the plant extracts. Most of the extracts were non-toxic with LC_{50} values ranging from 1000 to 4000 µg/ml compared to the known toxic plant *Nerium oleander* used as a positive control with LC_{50} value of 142±68.2 µg/ml. *V. infausta* leaves (338±23.4 µg/ml), root (416±28.3 µg/ml) and *P. angolensis* bark (478±29.7 µg/ml) were moderately safe and should be used with caution. Plants showing significant toxicity to *Artemia salina* may have potential use as anti-tumour drugs.

Antimicrobial activity was carried out on selected microorganisms and fungal strains by the agar well diffusion method. Antibacterial activity was assessed against *Staphylococcus aureus, Streptococcus Group A* (Gram positive bacteria) and *Escherichia coli, Pseudomonas aeruginosa* (Gram negative bacteria). The fungi used were *Candida albicans* and *Aspergillus niger*. Activity was measured as a radius in mm to give a zone of inhibition. In general, the gram positive bacteria were more sensitive to the plant extracts compared to the gram negative bacteria. Out of the runs done for all the bacterial strains; 28 plant extracts showed no activity against the strains at the different concentrations used. The root extracts of *X. caffra, P. angolensis* and *P.africanum* were highly active (about 10mm radius) against *E. coli*. *P. africanum* root extract was also highly active against *P. aeruginosa.* *A. stenophylla* leaves were inactive against *S. aureus*, *E. coli* and *P. aeruginosa.* 65% of the extracts did show activity against *Candida albicans* and *Aspergillus niger*. The highest zone of inhibition was shown by *D. anomala* tuber against *C. albicans*; reading of 5.5±0.58mm and *P. africanum* bark 4.0±0.82mm.The lowest values were recorded from *C. anisata* leaves and *L. edulis* leaves against *A. niger*; readings of 1.00±0mm and 1.00±0.58mm respectively.

The results from these studies indicated that most of the commonly used traditional medicinal plants have merit for use in traditional medical practice as they have shown zones of inhibition on various microorganisms tested, meaning they are potential antimicrobial agents and so should be preserved and harvested with care

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CONTENTS

CHAPTER 2

CHAPTER 5

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

CHAPTER 1

1.0 INTRODUCTION

1.1 Background Information

Amongst the most basic of essential human needs is the provision of adequate health facilities. However it is becoming more and more difficult for everyone to afford this basic need. This is aggravated by the increased occurrence and spreading of diseases due to factors like increased population growth and political instability. Man, perhaps including other animals have used herbal remedies and medicines to cure various diseases from time immemorial (Parekh *et al.,* 2005). The interest in drugs of plant origin is ever increasing. Meanwhile diseases of diverse aetiologies account for high morbidity and mortality rates in developing countries.

Traditional medical practice is an immemorial mode of health care in Zimbabwe as in many parts of Africa. It is still the main vehicle of health care delivery today especially in the rural areas of the country where conventional medical facilities are not within the reach of most people. About 85% of the population relies on it for their medical care (Mdluli, 2002). Increase in the patronage of herbal medicines is likely to continue because of the global economic downturn and as a large proportion of the population rely on herbal medicines for their medical care. It is therefore essential that information about the system be preserved through proper documentation and the constituents of the phytomedicines must be analysed (Amusan *et al.,* 2007).

Africa together with Madagascar is reputed for the extraordinary richness in its flora, totaling several thousands of species. Based on careful observation and a judicious choice of plants, it is possible to discover very interesting new natural products (Hostettmann *et al.,* 2001). Hundreds of millions of people in developing countries depend on plants for their traditional medicine. In certain African countries, up to 90% of the population still relies exclusively on plants as a source of medicines (Drummond *et al.,* 1985). Many of these have been documented and an African Pharmacopoeia has been published by the Scientific Technical Research Commission of the Organisation of African Unity, starting with Volume 1 in 1985. It is well known that Western allopathic medicines alone do not hold the exclusive key to the maladies of modern man (Drummond *et al.,* 1985). Long before the southern tip of Africa was recognized by the European settlers as a potential route to the East Indies members of the indigenous communities were identified as having a special competence to provide and maintain health care in the

African populations. The available knowledge on the use of plant preparations in traditional medicines is enormous but if it is not rapidly researched and documented, indications as to the usefulness of these plants will be lost with succeeding generations.

Since plants have been playing as curative and therapeutic agents in preserving human health against diseases since the beginning of man's life on earth, their use is based on the experience of many generations of physicians and traditional systems of medicines from different ethnic societies (Udding *et al.,* 2003).

Traditional systems of medicine have become a topic of interest for the past few decades especially with the spread of diseases such as the HIV/AIDS pandemic and opportunistic infections. In developing countries, traditional practitioners and medicinal plants primarily support the primary health care system. Traditionally, rural African communities have relied upon spiritual and practical skills of the traditional medicinal practitioners because:

1) Traditional medicines are more readily accessible and acceptable to many people in the rural areas.

2) Even if the medical doctors are available, the consultation only occurs with socio-economic and cultural changes, access to formal education and religious influences (Zimbabwe Republic Police Report 1993).

3) Traditional African medicines take a holistic approach: good health, disease, success or misfortune are not seen as chance occurrences but are believed to arise from the actions of individuals and ancestral spirits according to the balance or imbalance between the individual and the social environment (Drummond *et al.,* 1985).

However there are some disadvantages like:

1) Lack of scientific proof for efficacy.

2) Imprecise diagnosis by the TMP.

3) Lack of precise dosage.

4) Low hygiene standards of handling drugs.

5) More emphasis on the psychological causes of diseases

6) The secrecy of some healing methods and the absence of written records about the patients.

Currently there are several initiatives taking place worldwide to support herb use in the maintenance of health and the prevention or treatment of disease. In fact, hundreds of thousands of scientists around the globe are publishing papers on new plant-derived compounds and their biological activity. Herbs hold a great promise for improving health, but growth will not be possible without continuing research and increasing awareness of sustainable trade (Kilham, 2004). Attempts have been made by scientists to justify or rationalize on a scientific basis, many aspects of the practice of the African Traditional medicinal plants.

1.1.1 Use of plants in traditional medicine

Out of the 5000 plant species growing in Zimbabwe, about 10 percent of the plants with medicinal properties are used in traditional medicine and most of them are rapidly disappearing due to extensive harvesting by traditional medical practitioners. Developments related to economic growth, international trade and science and technology have worsened the situation by converting woodland to other forms of land use such as residential plots and agricultural expansions, which is estimated to clear well over 70 000 hectares of woodland annually (Mavi, 1997).

The knowledge of the traditional practitioners is of a great value. To document this precious oral tradition Gelfand *et al*., (1985) interviewed more than 200 traditional healers and noted all the plants they used, the symptoms of the patients and the ways of administration. This survey constitutes a valuable basis for the selection of plants and search for new active compounds. As traditional healers do not generally make a clear distinction between 'medicine' in its medical sense and the broader sense which includes charms of various sorts, it seems important to give an overview of their methods.

Although plants reputed to have medicinal properties in Zimbabwe have been extensively documented, few studies comparatively have been carried out to evaluate the efficacy and thus substantiate the claims scientifically. The School of Pharmacy and Chemistry at the University of Zimbabwe have been actively involved in the research of medicinal plants for more than 2 decades, and indeed studies reveal that quite a number of the investigated plants have medicinal properties (Gundidza, 1993).

1.1.2 Treatments

Medicines are commonly given in the form of powders, decoctions, infusions or ointments. Fine powder to be taken orally is obtained by drying the plant in the sun, crushing it into small pieces and then grinding it into powder in a stamping block. The powder is usually taken in porridge or *sadza* (thickened porridge made out of pulverized grains, generally white maize), sometimes in water or beer. Ointments are prepared by mixing the prepared portions with castor oil. The decoction is prepared by boiling the roots, bark or leaves in water for a short time and the resulting liquid may be used immediately or stored in a bottle for later use. An infusion may be prepared by putting the required portions of the plants in water for a short period or several hours. Inhalations are commonly used for respiratory disorders such as asthma and to drive away bad spirits. The aromatic ingredients are powdered and added to boiling water the vapour of

which is inhaled by the patient. A popular method of treatment is scarification. Incisions are made, and an irritant powder is rubbed into the incisions to increase blood supply to the affected part. This is often performed in case of rheumatism, headache, or abdomen and chest pains (Gelfand *et al*., 1985).

1.1.3 Importance of traditional medicine

When an individual has decided to seek professional help for a medical problem, he has to decide whether the illness should be referred to a modern or a traditional health practitioner. The decision depends largely upon the cost of each type of treatment, accessibility, knowledge of the probable effect of each type of treatment and the definition given to the illness by those involved. It was observed by Chavunduka (1994) that a patient often tries to find a social cause or a meaning to his illness. A western doctor does not produce an answer to this question and merely tries to cure the physical problem. If the patient fails to respond to the treatment, he will often turn to the traditional healer who, on the other hand, identifies himself with the patient's difficulties and social problems and helps find if the ancestral spirits have been offended; this gives the patient more confidence in his remedies. However, there are cases when the patient, dissatisfied with the treatment, turns back to the scientific practitioner (Gelfand *et al*., 1985). Traditional healers have been represented since 1980 by an organization known as the Zimbabwe National Traditional Healers Association (ZINATHA) (Chavunduka, 1994).

LITERATURE REVIEW

1.2 Drugs Derived from Natural Products

Modern drugs that are derived from natural products have to be extensively studied in terms of their chemistry in order to appreciate the chemical constituents.

Phytochemistry is the study of plant chemistry and deals with the chemical structures of the enormous variety of organic substances that are accumulated by plants (Trease and Evans, 2002). The chemical constituents of plants can be classified in a number of ways for instance biologically where the biosynthetic origin or activity is considered and chemically where the structural skeleton, functional groups or physiochemical properties are considered. Inevitably a compound may be mentioned in more than one grouping and often a compromise is made (Hao and Simon, 1997). Several phytochemical surveys have been published, including the random sampling approach, which involved some plant accessions collected from all parts of the world. The major chemical substances of interest in these surveys have been the alkaloids and steroidal sapogenins (saponins), however other diverse groups of naturally occurring phytochemicals such as flavonoids, tannins, unsaturated sterols, triterpenoids and essential oils have also been reported (Mojab *et al.,* 2003).

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. It is well known that plants produce these chemicals to protect themselves but recent research demonstrates that they can protect humans against diseases. Some of the well known phytochemicals are lycopene in tomatoes, isoflavones in soy and flavanoids in fruits. They are not essential nutrientsand are not required by the human body for sustaining life (www.phytochemicals.info/).

There are many phytochemicals and each works differently. These are some possible actions:

Antioxidant - Most phytochemicals have antioxidant activity. They prevent oxidative damage and reduce the risk of developing certain types of cancer. Phytochemicals with antioxidant activity: allyl sulfides (onions, leeks, garlic), carotenoids (fruits, carrots), flavonoids (fruits, vegetables), polyphenols (tea, grapes).

Hormonal action - Isoflavones, found in soy, imitate human estrogens and help to reduce menopausal symptoms and osteoporosis.

Stimulation of enzymes - Indoles, which are found in cabbages, stimulate enzymes that make the estrogen less effective and could reduce the risk for breast cancer. Other phytochemicals, which interfere with enzymes, are protease inhibitors (soy and beans), terpenes (citrus fruits and cherries).

Interference with DNA replication - Saponins found in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells. Capsaicin, found in hot peppers, protects DNA from carcinogens.

Anti-bacterial effect - The phytochemical allicin from garlic has anti-bacterial properties.

Physical action - Some phytochemicals bind physically to cell walls thereby preventing the adhesion of pathogens to human cell walls. Proanthocyanidins are responsible for the anti-adhesion properties of cranberry. Consumption of cranberries will reduce the risk of urinary tract infections and will improve dental health. Phytochemicals are naturally present in many foods but it is expected that through bioengineering new plants will be developed, which will contain higher levels and would make it easier to incorporate enough phytochemicals together with our food (http://www.phytochemicals.info/).

It has been documented that these plant metabolites are responsible for the pharmacological activities they exhibit (Akinyemi *et al.,* 2005).

Table 1: Chemical groups and associated Ethnopharmacology.

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	Antibacterial Antifungal(Bever, 1986).	- Venereal diseases, HIV, TB, Pneumonia - Colds, coughs, chest pains ,Hormonal disorders - GIT inf. , Skin inf., wounds, Candida, thrush, eczema
Coumarins	Antifungal, Antioxidant	- Eczema, HIV, Venereal diseases - Chest pains, Bronchitis, Cancer, Asthma
Anthraquinones	Laxative, purgative Antibacterial, Antifungal	- Tapeworm, Ringworm, Bilharzias, Dysentery - Constipation , Diarrhea
Cardiac glycosides	Antihypertensive Neuroprotective	- Chest pains, Stroke, Heart weakness, - Nerve system disorders
Tannins	Astringent, Anti-inflammatory Antibacterial, Antifungal Antioxidant,	- Diarrhea, - Inflammations, Wounds, - Cancer, HIV

Phytochemical compounds that were screened for in this study include alkaloids, flavonoids, tannins, saponins, cardiac glycosides, coumarins and anthracene derivatives.

1.2.1 Alkaloids

They are one of the chemical groups that are derived from natural products and has had so many drugs that are derived from their synthesis. Alkaloids are basic substances that contain one or more nitrogen atoms, usually in combination or as part of a cyclic system. Over 5 500 alkaloids are known and they comprise the largest single class of secondary metabolites. Many of the earliest isolated pure compounds with biological activity were alkaloids. This was due to the ease of isolation. The nitrogen generally makes the compound basic and the compound exists in the plant as a salt. Thus, alkaloids are often extracted with water or mild acid and then recovered as crystalline material by treatment with base.

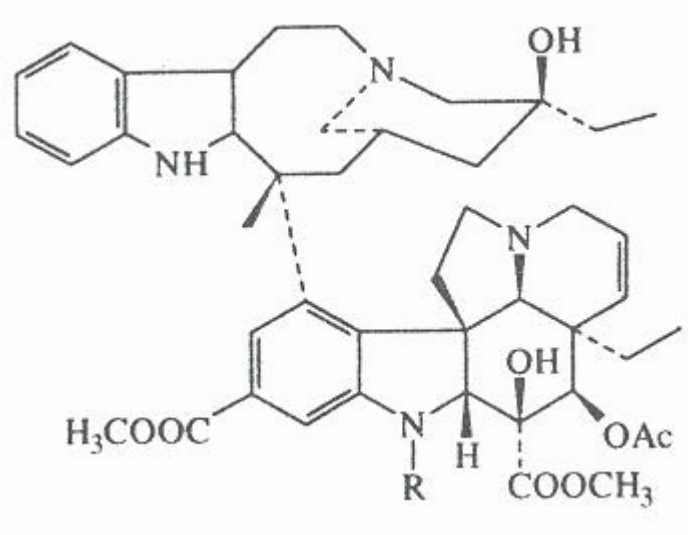
Alkaloids are a very heterogeneous group ranging from simple to complex compounds such as the pentacyclic structure of strychnine, the toxin of the *Strychnos potatorum* bark. Many alkaloids are terpenoid in nature whilst others are mainly aromatic compounds, for example colchicines. In plants they may exist in free state or as salts. Alkaloids show great variety in structure and virtually all the known nitrogen –ring systems occur (Trease and Evans, 1972).

1.2.1.1 Drugs derived from alkaloids

Historically plants have been a source of inspiration for novel drug compounds as plant derived medicines made large contributions to the human health and well being. Many drugs currently used clinically are derived from medicinal plants. Some of these drugs include the following;

1.2.1.2 Vinblastine and Vincristine

Early examples of the antileukemic alkaloids include vinblastine and vincristine which were both obtained from the Madagascan Periwinkle (*Catharanthus roseus*). Vinblastine and vincristine are thought to inhibit cell growth by disrupting the microtubules, causing the dissolution of cell mitotic spindles and the arrest of cells at metaphase (Decorti and Creasy, 1975). The two substances have been developed into commercial drugs and the major use of vinblastine being to treat patients with Hodgkin's diseases, renal, testicular, head and neck cancer whilst vincristine is widely used in combination with other anti-cancer agents in the treatment of acute lymphatic leukemia in children, certain lymphomas and sarcomas.



Vincristrine: R = CHO

Vinblastine: R = CH_3 (Decorti and Creasy, 1975).

Figure 1: Structures of the bis-indole alkaloids

1.2.1.3 Atropine

Atropine is a tropane alkaloid extracted from the deadly nightshade (*Atropa belladonna*) and other plants of the family Solanaceae. Use of *belladonna* herb was not clearly documented until the beginning of the sixteenth century. It has been used as a sedative and for checking secretion. Atropine is an anticholinergic agent that is a potent parasympatholytic, inhibiting actions of acetylcholine at postganglionic parasympathetic neuroeffector sites. It is a competitive antagonist of acetylcholine at smooth and cardiac muscles and various glandular cells. Use of the drug increases heart rate by slowing down some parts of the nervous system while simultaneously speeding up other parts. It relaxes bronchial smooth muscles, therefore reducing airway resistance and dead space. Other effects are an inhibition of salivary secretion and a reduction in motor activity in the stomach and small and large intestines. Side effects include confusion, dizziness, light-headedness, eye pain, and skin rash.

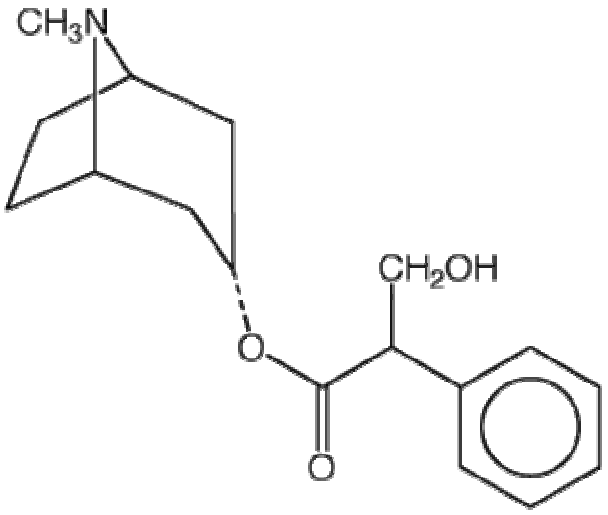


Figure 2: 2D Structure of atropine

(Wagner *et al.,* 1984).

1.2.1.4 Reserpine

Reserpine is an indole alkaloid antipsychotic and antihypertensive drug that has been used for the control of high blood pressure and for the relief of psychotic behaviors, although because of the development of better drugs for these purposes and because of its numerous side-effects, it is rarely used today.It is derived from *Rauwolfia* species. *Rauwolfia* has been used traditionally in India and other countries as an antihypertensive agent before the isolation of reserpine from *Rauwolfia* serpentine in 1952 (Curb *et al.,* 1988).

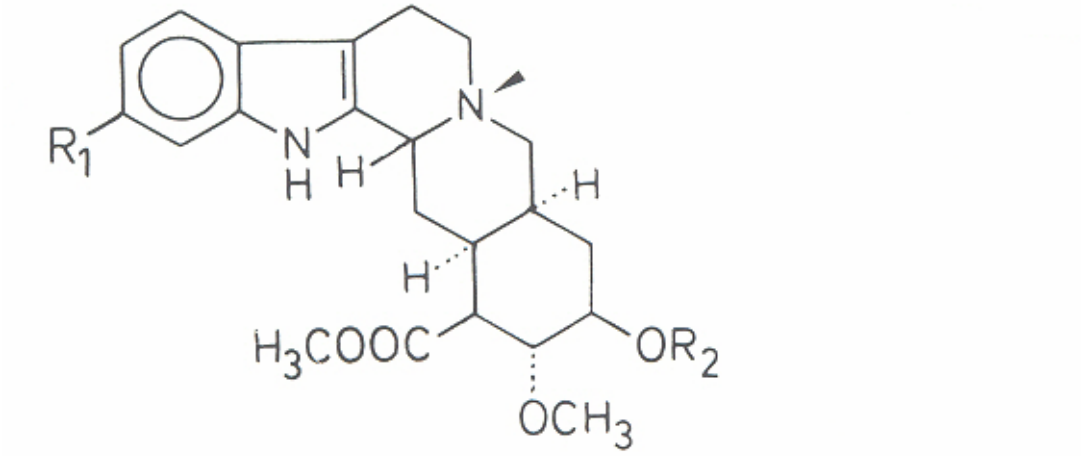


Figure 3:

Structure of Rersepine and Rescinnamine

(Wagner *et al.,* 1984).

The antihypertensive actions of Reserpine are a result of its ability to deplete catecholamines (among the others) from peripheral sympathetic nerve endings. These substances are normally involved in controlling heart rate, force of cardiac contraction and peripheral resistance. Reserpine almost irreversibly blocks the uptake (and storage) of norepinephrine (i.e. noradrenalin) and dopamine into synaptic vesicles by inhibiting the Vesicular Monoamine Transporters. Reserpine has reported side effects that include nasal congestion, nausea, vomiting, weight gain, gastric intolerance; gastric ulceration (due to increased cholinergic activity in gastric tissue and impaired mucosal quality), stomach cramps and diarrhoea are noted. The drug causes hypotension and bradycardia and may worsen asthma (Chobanian *et al.,* 2003).

1.2.1.5 Quinine and quinidine

Quinine and quinidine are used today as antimalarial and antiarrhythmic drugs respectively. They are derived from cinchona species. The cinchona bark has been used against fever since 1960. Colhicine; an amorphous yellowish alkaloid is currently used in the treatment of acute gout and arthritis. It is found in *Colhicum autumnale* which has been used as far back as 1952 by Arabian physicians in the treatment of gout and rheumatism. In Vienna (1973), it was also found to be of value in dropsy (oedema). For well over 3 centuries, these plants provided the only effective remedy for malaria (Van Wyk *et al.,* 1997). Quinine is now recognized as an indispensable and effective drug in the treatment of *Plasmodium falciparum* malaria resistant to 4-aminoisoquinolines and antifolates.

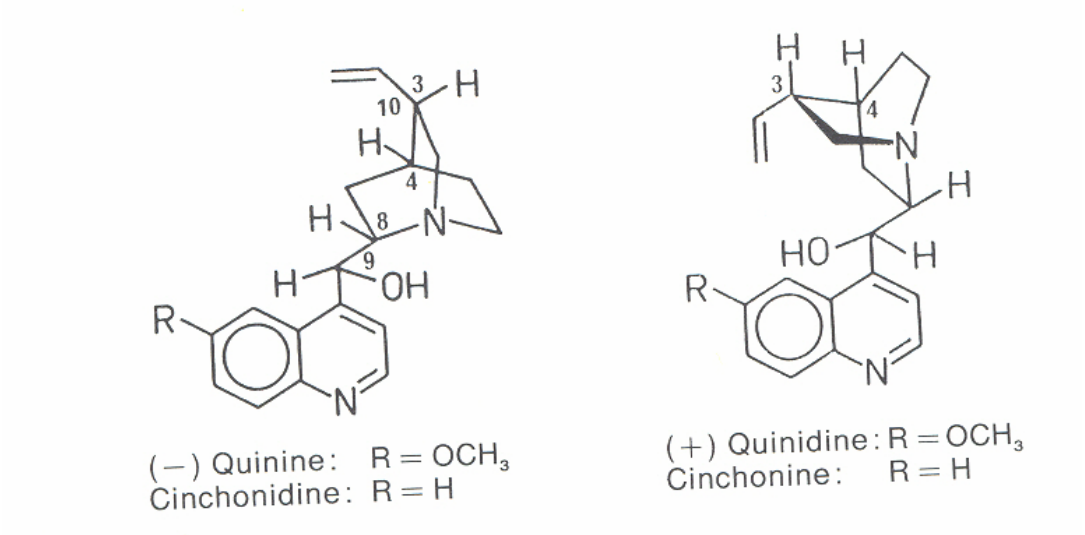


Figure 4: Quinine and Quinidine

(Wagner *et al.,* 1984).

1.2.1.6 Opium Alkaloids

Morphine (Fig 5) is the most abundant of opium's 24 alkaloids, accounting for 9 to 14% of opium-extract by mass. It is a highly-potent opiate analgesic drug and is the principal active agent in opium. Codeine is also an opiate of plant origin used for its analgesic, antitussive and antidiarrheal properties (Van Wyk *et al.,* 1997).

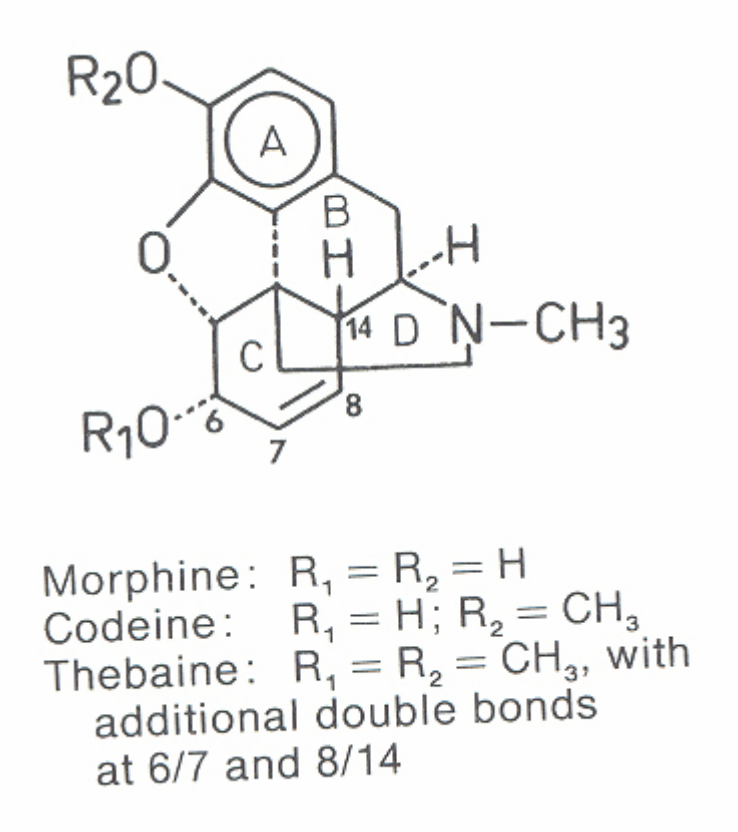


Figure 5: Structures of common drugs Morphine, Codeine and Thebaine (Wagner *et al.,* 1984).

1.2.2 Flavonoids

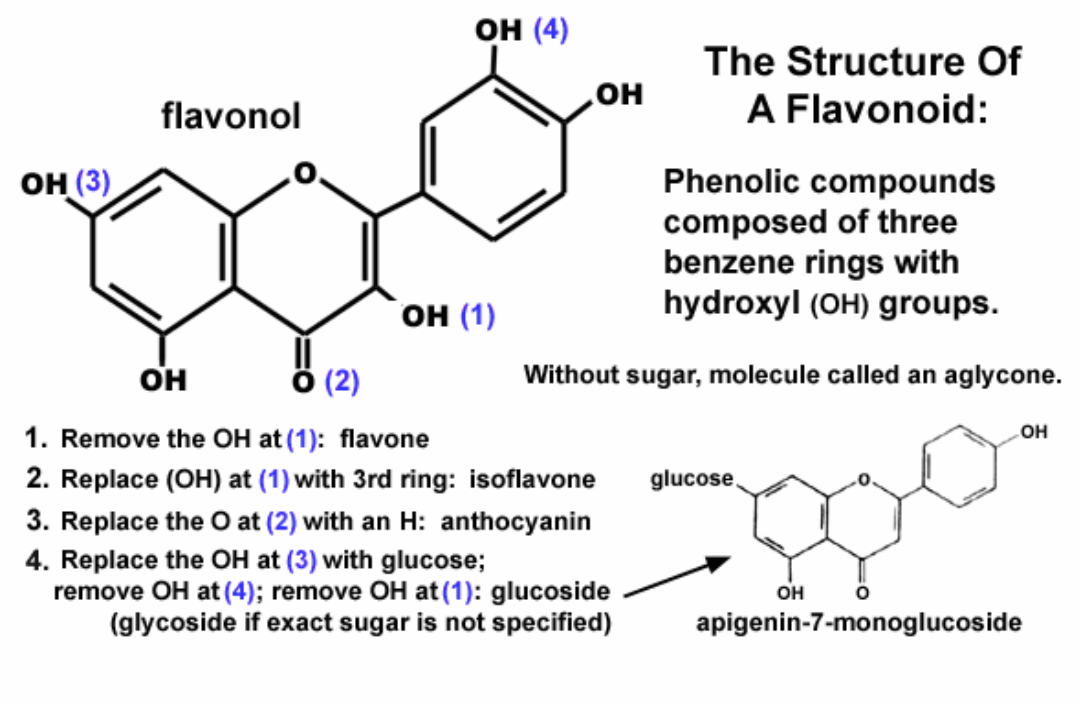


Figure 6: Structure of the parent flavonoid and possible substituent's
(Ogunleye and Ibitoye, 2003)

The flavonoids are structurally derived from the parent substance flavone. They are abundant in the Polygonaceae, Rutaceae, Leguminosae, Umbelliferae and Compositae families. Flavonoids are mainly water-soluble compounds and being phenolic they change colour when heated with base or ammonia. Flavones and flavonols are universal and isoflavones and biflavonyls are found only in a few families.

Flavonoids are water soluble polyphenolic molecules containing 15 carbon atoms. Flavonoids belong to the polyphenol family. Flavanoids can be visualized as two benzene rings which are joined together with a short three carbon chain. One of the carbons of the short chain is always connected to a carbon of one of the benzene rings, either directly or through an oxygen bridge, thereby forming a third middle ring, which can be five or six-membered. The flavonoids consist of 6 major subgroups: chalcone, flavone, flavonol, flavanone, anthocyanins and isoflavonoids. Together with carotenes, flavanoids are also responsible for the coloring of fruits, vegetables and herbs.

Flavonoids have antioxidant activity. They are becoming very popular because they have many health promoting effects. Some of the activities attributed to flavonoids include: anti-allergic, anti-cancer, antioxidant, anti-inflammatory, anti-viral, antithrombotic and vasoprotective effects.

The flavonoids quercetin is known for its ability to relieve hay fever, eczema, sinusitis and asthma. (Ogunleye and Ibitoye, 2003)

Epidemiological studies have illustrated that heart diseases are inversely related to flavonoid intake. Studies have shown that flavonoids prevent the oxidation of low-density lipoprotein thereby reducing the risk for the development of atherosclerosis.

The biological properties of flavonoids and the inverse association of several flavonoid-rich foods (including wine and tea) with coronary heart disease (CHD) risk have led to the hypothesis that dietary intake of flavonoids like apigenin, quercetin and luteolin- a bioflavonoid also present in smaller amounts in peppers) may protect against CHD. Several studies have examined the intake of various combinations of flavonoids, thought to reflect total flavonoid intake (Hertog *et al.,* 1993). *Terminalia arjuna*, an Indian medicinal plant prevents heart diseases.

1.2.3 Tannins

Tannins are generally defined as naturally occurring polyphenolic compounds of high enough molecular weight (ranging from 1000 to 5000) to form complexes with proteins of animal hides and prevent their decaying, thus converts hides to leather.

Many types of tannin are glycosides, for instance glucogallin. These are generally classified into two groups based on their structural types a) hydrolysable tannins which can be hydrolysed by acids or enzymes such as tannase into their component phenolic acids such as gallic and ellagic acids with their glucose moieties and b) condensed tannins (proanthocyanidins) constituting of molecules which are more resistant to breakage than hydrolysable tannins when treated with acids, condensed tannins decompose to form red insoluble compounds (IAEA Working Document, 1999) .

Tannins act as a defense mechanism in plants against pathogens, herbivores and hostile environmental conditions. Generally, tannins induce a negative response when consumed. These effects can be instantaneous like astringency or a bitter or unpleasant taste or can have a delayed response related to antinutritional/toxic effects. Tannins have been traditionally used for protection of inflamed surfaces of the mouth and treatment of catarrh, wounds, haemorrhoiods, and diarrhea, and as antidote in heavy metal poisoning (Ogunleye and Ibitoye, 2003). *Rubus villosus* (Blackberry root bark) is an example of a herb that contains tannins and is used as an astringent tonic for diarrhoea and dysentery.

1.2.4 Anthraquinones

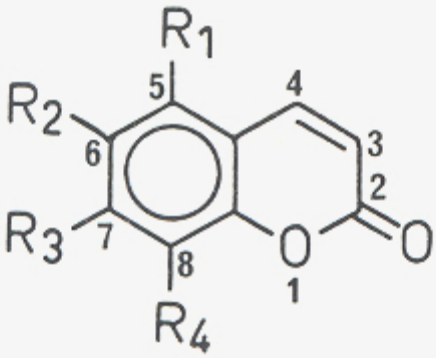
There are a great many varieties of anthraquinone derivatives, found in several plant families. They all share the same basic molecular configuration. They tend to be found in the form of their glycosides (the aglycone or active part combined with one or more sugar molecules) which, because of the variety of possible sugars, increases the range even further.
Anthraquinones were long detected in some plants such as rhubarb, cascara, senna and aloes. Anthraquinones derivatives are often orange-red compounds which may sometimes be identified easily. They are usually soluble in hot water or alcohol. Anthraquinones are described as compounds which have anticancer or antitumor activity and which are useful for inhibiting cancer cells and cells comprising tumors. Plant extracts containing anthraquinones are increasingly being used in cosmetics as well as in foods and pharmaceuticals. Recently, several anthraquinones and pre-anthraquinones have been isolated from the roots of Aloe (Dagne and Yenesew, 1994). Plants containing anthraquinones have also been used for millennia as dyestuffs and purgatives. This important commercial dual function led to an early isolation and characterization of the active principles, which were shown to be derived from, or related to, the substance anthraquinone.

From pharmacological investigation we know that the action of the anthraquinones is dependent on the presence in the gut of bile and on the fact that they are ingested in the glycoside form. The role of the bowel flora has also been firmly implicated (for example, it has been established that sennosides are hydrolysed to sennidins in a step-wise fashion via sennidin-8-monoglucosides, then reduced to rheinanthrone, a purgative active principle). The isolated aglycone is inactive if ingested although it is potently active if ingested intravenously. All this suggests that bile and the sugar moiety are both necessary for the absorption of the anthraquinone from the gut, but that it is the aglycone alone that is active. Once in the bloodstream the aglycone is absorbed into the internal machinery of as yet undefined target cells and processed there (probably to emodin in all cases). The anthraquinone laxatives essentially irritate the bowel wall, provoking increased muscle contractions and peristaltic movements. *Rheum officinale* contain large amounts of anthraquinones the active compounds, (emodin, chrysophanol and rhein) that have been specifically used to inhibit one of the carcinogenesis-related enzymes, cytochrome P450 (CYP) 1A1, and subsequently suppressed the mutagenicity of food-derived carcinogens (Mingzhong *et al.,* 2000)

1.2.5 Coumarins

The coumarin structure is derived from cinnamic acid via ortho-hydroxylation (a), trans-cis isomerisation of the side chain double bond (b) and (c), and lactonisation (d).Coumarins are a phytochemical with a vanilla like flavour with an oxygen heterocycle. Coumarins can occur either as free or combined with the sugar glucose (coumarin glycoside). They are found in several plants, including tonka beans, lavender, licorice, strawberries and apricots. Coumarins have blood-thinning, anti-fungicidal and anti-tumor activities. They should not be taken while using anticoagulants.

The parent structure of coumarins is as shown in fig 7 and many coumarins result from substitutions at different positions.



SIMPLE COUMARINS

R_1	R_2	R_3	R_4	
H	H	OH	H	Umbellifefone (7-Hydroxycoumarin)
H	H	OCH_3	H	Herniarin (7-Methoxycoumarin)
H	OH	OH	H	Aesculetin (6,7-Hydroxycoumarin)
H	H	OH	OH	Daphnoretin (7,8-Hydroxycoumarin)
H	OCH_3	OH	H	Scopoletin (6-Methoxy-7-hydroxycoumarin)
H	OCH_3	OH	OCH_3	Isofraxidin (7-Hydroxy-6,8-methoxycoumarin)
OCH_3	OH	OCH_3	H	Fraxinol (6-Hydroxy-5,7-methoxycoumarin)

Figure 7: Formulae of Constituents of Coumarin Drugs

(Wagner *et al.,* 1984).

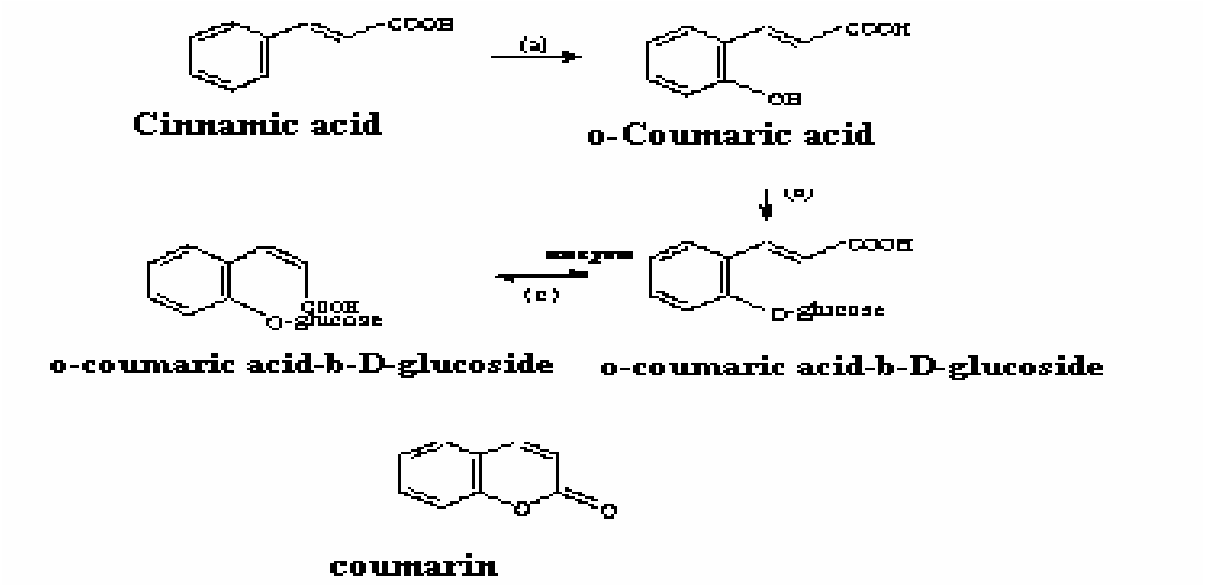


Figure 8: Biosynthesis pathway of coumarins

Coumarins increase the blood flow in the veins and decreases capillary permeability and can be toxic when used at high doses for a long period. They seem to work as a pesticide in the plants that produce them. The free radical scavenging and lipid peroxidation assays of some Korean medicinal plants (Fraxinus *rhynchophylla, Angelica dahurica, Evodia daniellii* and *Peucedanum japonicum)* revealed that five phenolic coumarins, scopoletin, aesculetin, fraxetin, umbelliferone and daphnetin possess considerable antioxidant activities (Phuong *et al.,* 2009).

1.2.6. Saponins

Saponins are phytochemicals which are natural detergents found in many plants, especially certain desert plants. They are also present in small amounts in some foods, such as soybeans and peas. Saponins consist of 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). Saponins have detergent or surfactant properties because they contain both water-soluble and fat-soluble components. They consist of a fat-soluble nucleus, having either a steroid or triterpenoid structure, with one or more side chains of water-soluble carbohydrates (sugars). Because of their surfactant properties, they are used industrially in mining and ore separation, in preparation of emulsions for photographic films, and extensively in cosmetics, such as lipstick and shampoo. Saponins have a bitter taste. Some saponins are toxic and are known as sapotoxin.

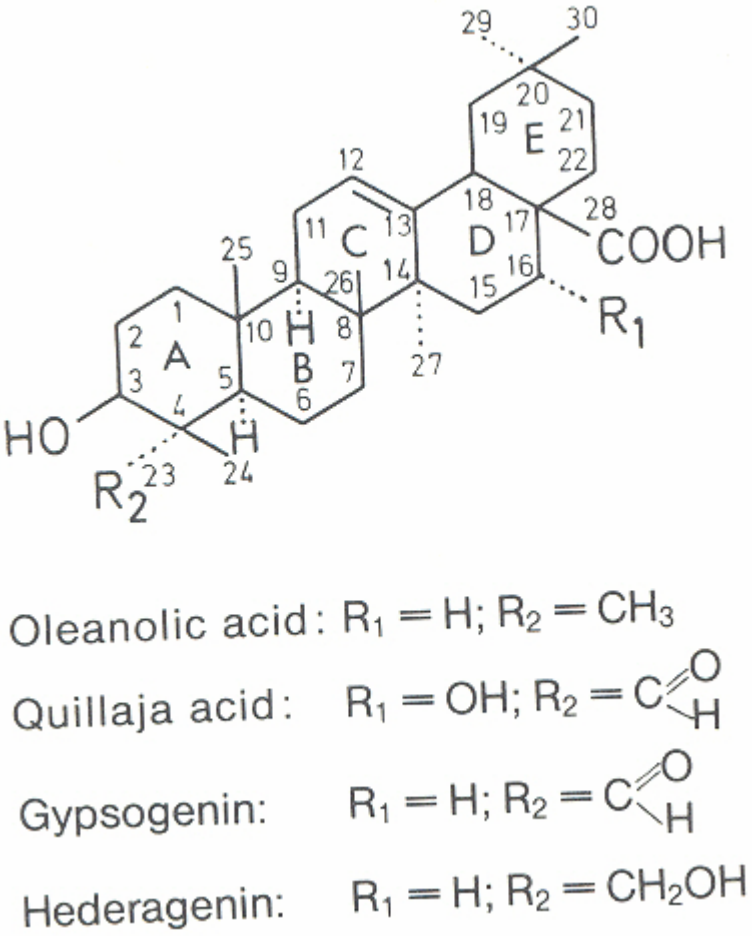


Figure 9: Parent structure of saponins and drug derivatives
(Wagner *et al.,* 1984)

The antifungal and antibacterial properties of saponins are important in cosmetic applications, in addition to their emollient effects. New applications for saponins in animal husbandry are being explored, especially the effect of saponins on protozoal diseases. Saponins form strong insoluble complexes with cholesterol. This has many important implications, including cholesterol-lowering activity in humans. The blood cholesterol-lowering properties of dietary saponins are of particular interest in human nutrition. This desirable effect is achieved by the binding of bile acids and cholesterol by saponins. Bile acids form mixed micelles (molecular aggregates) with cholesterol, facilitating its absorption. Cholesterol is continually secreted into the intestine via the bile, with much of it subsequently reabsorbed. Saponins cause a depletion of body cholesterol by preventing its reabsorption, thus increasing its excretion, in much the same way as other cholesterol-lowering drugs, such as cholestyramine (Cheeke R, 2005). Plants produce saponins to fight infections by parasites. When ingested by humans, saponins also seem to help our immune system and to protect against viruses and bacteria. The non-sugar part of saponins also has a direct antioxidant activity which may result in other benefits such as reduced risk of cancer and heart diseases.

In 1996 Xu *et al* identified some bioactive saponins in a Chinese medicinal plant *Mussaenda pubescens* (Rubiaceae). The plant has been used as a diuretic, antiphlogistic, diaphoretic and antipyretic agent, and has also been used to detoxify mushroom poisons and to terminate early pregnancy.

1.2.7. Cardiac Glycosides

Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia. These glycosides are found as secondary metabolites. Cardiac glycosides are found in a diverse group of plants including *Digitalis purpurea* and *Digitalis lanata* (foxgloves), Cardiac glycosides most often are used to treat severe heart failure and arterial fibrillation that can occur with congenital heart defects. The cardiac glycosides also represent an important class of useful, albeit somewhat dangerous, steroids. These compounds are characterized by the steroidal cardenolide aglycone bonded at the C-3 position to a sugar moiety which can range from a monosaccharide to a trisaccharide. The wellknown use of these compounds is in the preparation *digitalis*. This drug, which is used to treat congestive heart failure, super ventricular tachycardia, and several other heart conditions, is a cardiotonic agent which increases the tone of the heart muscle causing more effective emptying of the heart chambers.

1.2.8 Drugs derived from terpenes

1.2.8.1 Artemisinin

Another one of the remarkable success stories of anti-malarial compounds from plants is that of artemisinin from the Chinese plant qinghao. The wormwood *Artemisia annua* has been used for over 2000 years in China a febrifuge and in malaria therapy (Liu *et al.,* 1979). Artemisinin is a sesquiterpene lactone, with an endoperoxide group essential for its activity, and is found in the leaves and the flowering tops of the plant but not on the roots. Artemisinin has also proven itself as a safe and effective treatment for malaria in over two million patients, and now studies are showing that Artemisinin is effective against a wide variety of cancers as shown in a series of successful experiments. The most effective is leukemia and colon cancer. Intermediate activities were also shown against melanoma, breast, ovarian, prostate, CNS and renal cancer. Artemisinin contains two oxygen atoms linked together in what is known as an 'endoperoxide bridge', which react with iron atoms to form free radicals.

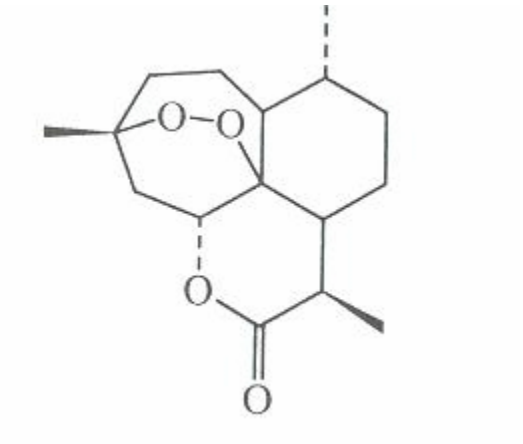


Figure 10: Artemisinin
(Liu *et al.,* 1979).

Artemisinin becomes toxic to malaria parasites when it reacts with the high iron content of the parasites, generating free radicals, and leading to damage to the parasite. By this same mechanism, Artemisinin becomes toxic to cancer cells which sequester relatively large amounts of iron compared to normal, healthy human cells.

1.2.8.2 Taxol

Taxol is a trade name for the generic chemotherapy drug Paclitaxel. Taxol Paclitaxel is a highly effective drug used against breast cancer. Taxol is classified as a "plant alkaloid," a "taxane" and an "antimicrotubule agent. It was originally extracted from the stem bark of the Pacific yew *Taxus brevifolius* (Taxaceae).

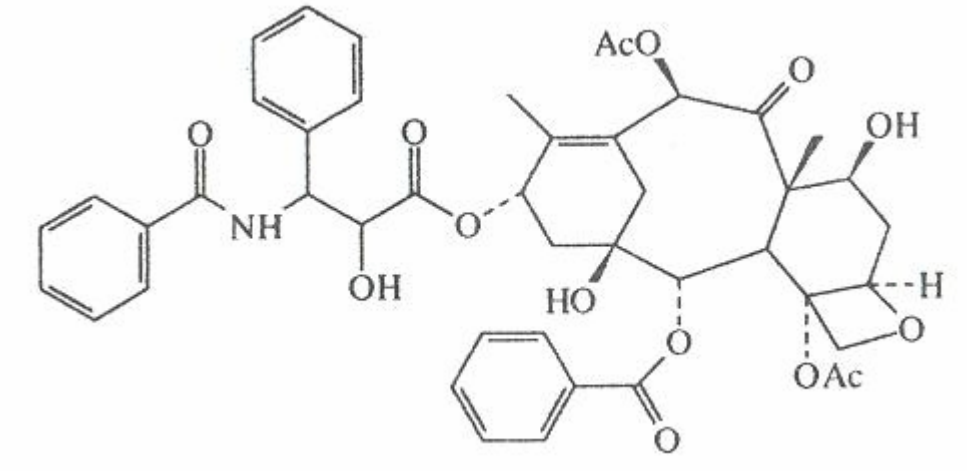


Figure 11: Taxol
(Liu *et al.,* 1979).

2.9.1 Analytical Techniques

The phytochemical investigations of plants may involve the following processes; Extraction of plant material, separation and isolation of constituents of interest, characterization of the isolated compounds, and investigation of the biosynthetic pathways, structure elucidations, bioactivity testing and quantitative evaluations. Different chromatographic techniques, analytical and spectroscopic instruments can be used for analysis.

In this investigation phytochemical analysis was performed using Thin Layer Chromatography (TLC) together with Ultra Violet (UV) spectrum and confirmatory tests. The use of TLC in the separation and identification of plant constituents is now a common technique. The advantages of TLC in the routine analysis of plant phytochemicals include: use of minimum apparatus, its relatively cheap, simple to use, minimum laboratory space required, its fast, excellent resolution of components and it is highly sensitive to drug constituents.

1.5 Pharmacological activities for assessing traditional medicinal plants

In order to fully characterise traditional medicines it is important to assess pharmacological activities that may be associated with the plant. Antioxidant activity is one of the activities that can be investigated as it has many implications in health care.
The human body is constantly under attack from free radicals. Free radicals are highly reactive molecules generated by the biochemical redox reactions that occur as part of normal cell metabolism and by exposure to environmental factors such as ultra-violet light, cigarette smoke, environmental pollutants like car exhaust fumes, gamma radiation and ozone. Toxic compounds can also result in the production of free radicals and these include anticancer drugs, anesthetics, analgesics etc.

Antioxidants are complex and diverse group of molecules that protect key biological sites from oxidative damage by free radicals. Thus antioxidants can be said to be antagonistic to the destructive effects of free radicals and thus afford the body protection from disease (Gutteridge, 1994). Antioxidant activity is normally associated with phenolic compounds like flavonoids and tannins. The antioxidant effect of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Shon *et al.*, 2003).

An antioxidant is any substance that when present in low concentrations compared to that of an oxidisable substrate significantly delays or inhibits the oxidation of that substrate (Percival, 1998; Young *et al*., 2001). The principle function of antioxidants is in delaying the oxidation of

other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals and they may reduce the oxidative damage to the human body (Namiki, 1990). New data are constantly gathered to show the role of oxidative stress and the involvement of reactive oxygen species (ROS) and the reactive nitrogen species (RNS) in the pathogenesis of degenerative diseases. The diseases are associated with the disturbance in the necessary balance between oxidation and reduction status in blood and in tissues, leading to oxidation of lipids, proteins and nucleic acids. Such oxidative damage is accompanied by changes in macromolecule structure and function and by the manifestation of clinical disorders such as cardiovascular diseases and cancer. Hence widespread research is being conducted aiming to investigate the possible effects and mechanisms of action of natural antioxidants in diseases.

A great number of aromatic, spicy, medicinal and other plants contain chemical compounds exhibiting antioxidant properties. The diverse classes of the compounds include polyphenols, tocopherols, alkaloids, tannins, carotenoids, terpenoids etc (Velioglu *et al*., 1998). The flavonoids and phenolic acids are particularly attractive as they are known to exhibit various beneficial pharmacological properties such as vasoprotective, anticarcinogenic, antineoplastic, antiviral, anti-inflammatory, as well as antiallergic and antiproliferative activity on tumour cells (Carr *et al*., 2000). Some of these properties have been related to the action of antioxidants, free radical scavengers, quenches or singlet and triplet oxygen, and inhibitors of peroxidation. Antioxidant activity of phenolic compounds is correlated to some structure-activity relationships, such as redox properties and the number and arrangement of hydroxyl groups (Cotelle *et al*., 1996).

Antioxidants are also useful in preventing the deterioration of pharmaceutical products. Deterioration of these usually results from oxidation of the active as well as the other ingredients upon atmospheric exposure, acidity, alkalinity, heat, light, moisture, bacterial and fungal contaminants. Among preparations prone to oxidation are vitamins, phenols, apomorphine, epinephrine, unsaturated fats and oils (Martindale, 1989). Antioxidants stabilize the pharmaceutical products undergoing a free-radical mediated chain reaction.

The aerobic organisms developed antioxidant defense mechanisms that arrest the damage caused by Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) entities. In the enzymatic mechanisms are included, for instance, superoxide dismutase, catalase, glutathione reductase and peroxidase, and nitric oxide synthase enzymes that fight the nitrogen free radicals produced by the nerve cells for example nitric oxide and nitrogen dioxide free radicals among others.

On the contrary, in the non-enzymatic mechanisms are comprised antioxidants and trapping agents such as ascorbic acid, α-tocopherol, β-carotene, glutathione, flavonoids, uric acid, cysteine, vitamin K, serum albumin, bilirubin, and trace elements as zinc and selenium, among

others (Chae *et al.,* 2004). Both processes can contribute to prevent the damage caused by oxidative reactions.

Vitamin E (consisting of various forms of tocopherols and tocotrienols) is an important fat-soluble, chain-breaking antioxidant. Besides working as an antioxidant, this compound possesses other functions with possible physiological relevance. The glutathione-dependent enzymes form another line of defense. Two important enzymes in this class are the free radical reductase and glutathione S-transferases (GSTs). The GSTs are a family of phase II detoxification enzymes. They can catalyze glutathione conjugation with various electrophiles. In most cases the electrophiles are detoxified by this conjugation, but in some cases the electrophiles are activated. Antioxidants do not act in isolation but form an intricate network. It is, for instance, known that vitamin E, together with glutathione (GSH) and a membrane-bound heat labile GSH-dependent factor work together.
The commonly used antioxidants for aqueous systems include sodium sulphate, sodium metabisulfate, sodium thiosulphate and ascorbic acid. For oil systems, ascobyl palmitate, hydroquinone, propyl gallate, butylated hydroxyanisole (BHA) and α-tocopherol are employed. Synergists, which increase the activity of antioxidants, are generally organic compounds that complex small amounts of heavy metal ions. These include EDTA derivatives, tartaric gluconic and saccharic acids. EDTA has also been used to stabilize ascorbic acid, penicillin, epinephrine and prednisolone (Remmington, 1980)

Antioxidants have apparently gained much attention of late owing to the realization that they are very effective in increasing the shelf life of products and affording man protection from diseases like cancer. Research into this area is therefore worthwhile for sound, flawless, cost-effective and safe use of antioxidants.

1.5.1 Role of Free Radicals in Health and Disease

The recent growth of knowledge of free radicals and reactive oxygen species (ROS) in biology is producing a medical revolution that promises a new age of health. In fact, the discovery of the role of free radicals in chronic degenerative diseases is as important as the discovery of the role of microorganism in infectious diseases (Bray, 1999). Although known as very harmful, there is at least one case however, in which organism employ radicals in a controlled way to achieve a useful purpose: the action of phagocytic cells. Free radicals are necessary in the immune system, prostaglandin biosynthesis and antibacterial cell activities. In the case of disturbed balance between formation of free radicals and antioxidant defense, in the cell we have oxidative stress and the free radicals can play a role in the development of various diseases. Overproduction of

ROS has been implicated in the etiology of host degenerative diseases and acute conditions such as trauma, stroke and infection and also in physical exercise and stress.

Free radicals also play a critical role in aging and the human body is in constant battle to keep from aging. Strong experimental evidence supports the free radical theory of aging. An increasing number of diseases and disorders as well as the aging process itself, demonstrate link either directly or indirectly to these reactive and potentially destructive molecules. Not much is known about the mechanism of aging and what determines lifespan. Leading theories attribute these to programs written in DNA and/ or to the accumulation of cellular and functional damage. Reduction of free radicals or decreasing their rate of production may delay aging and the onset of degenerative conditions associated with aging.

1.5.2 Carcinogenesis

Active oxygen species such as superoxide radicals and hydrogen peroxide have been associated with the induction of cancer. Although there is no definitive evidence that free radicals involvement is obligatory in these processes, it is clear that their presence in biosystems could lead to mutation, transformation and ultimately cancer (Simic, 1998). A carcinogen that can generate free radicals can in principle bring about the formation of modified bases as thymine glycols in cellular DNA. Critically damaging events can be abolished by free radical scavengers (Sun, 1990).

Besides induction oxygen radicals are involved in the promotion stage of carcinogenesis. The best studied tumour promoters are phorbol esters which stimulate inflammation (paradoxically can contribute to tumour promotion) of leucocytes to release superoxide. The final step in carcinogenesis is the invasive and metastatic spread to various body spaces and cavities. Free radicals promote metastatic growth by promoting tumour cell proliferation or oxidatively inactivating antiproteases. It is clear that free radicals are amongst the panoply of mechanisms leading to human cancers and this presents opportunities for intervention to prevent initiation, promotion or progression. Epidemiological evidence points out that antioxidants of natural origin are preventive in at least certain types of cancers. Exogenously added superoxide dismutase or catalse is significantly inhibitory towards tumour cell proliferation and viability.

1.5.3 Cardiovascular diseases

Heart diseases continue to be the biggest killer, responsible for about half of all deaths in developed countries. Polyunsaturated fatty acids occur as a major part of the low density lipoproteins (LDL) in blood. Oxidation of these lipid components plays a role in atherosclerosis. The three most important cell types in the vessel wall: endothelial cells, smooth muscle cell and macrophage can release free radicals which affect lipid peroxidation. With continued high levels

of oxidized lipids, blood vessel damage due to the reaction process continues and can lead to generation of foam cells and plaque, the symptoms of atherosclerosis. Oxidized LDL is atherogenic and is thought to be important in the formation of atherosclerotic plagues. Furthermore oxidized LDL is cytotoxic and can directly damage endothelial cells (De Whallel *et al.,* 1990).

1.5.4 Inflammation

Oxygen radicals have been implicated as host of common diseases which possess an inflammatory component. The diseases include rheumatoid arthritis, atherosclerosis, pulmonary emphysema cancer, inflammatory bowel syndrome and periodontal disease. A wide variety of oxidized biomolecules known to be specific products of free radical reactions have been detected in extracellular fluids from patients with these inflammatory diseases (Halliwell, 1999).the physiological functions which may be perturbed in inflammation include the oxidative modification of low lipoprotein and DNA damage repair. At sites of inflammation, increased free radical activity is associated with the activation of neutrophil NADH oxidase and/ or uncoupling of a variety of redox systems. Although the free radicals produced have the capacity to mediate tissue destruction, either alone or in concert with proteases, arguments have also been made that disturbances in the second messenger and regulatory activities of free radicals may also contribute significantly to the inflammation process.

1.5.5 Central Nervous System Injury

There is now extensive experimental support for the delay occurrence and pathophysiology importance of oxygen radical formation and cell membrane lipid peroxidation in the injured nervous system. If unchecked, lipid peroxidation is a geometrically progressing process that will spread over the surface of the cell membrane causing impairment of the phospholipids dependent enzymes, disruption of ionic gradients and if severe enough membrane lyses.

Central nervous tissue appears to provide an avid environment for the occurrence of oxygen radical generation and lipid peroxidation reactions due to high content of polyunsaturated fatty acids. There is now considerable biochemical and pharmacological data that supports such a connection between radical reactions and secondary CNS tissue injury. High dose pretreatment with Vitamin E has been shown to promote the chronic recovery of spinal cord injured cats (Anderson *et al*., 1998).

1.5.6 HIV and AIDS

It has been shown that oxidant stress can induce the expression and replication of the human immunodeficiency virus (HIV 1) in T –cell lines (Shreck *et al.,* 1991). Medical literature has shown that individuals who are HIV seropositive and those with AIDS have low levels of glutathione compared with uninfected control groups. As an antioxidant, glutathione provides the

vital defensive function of neutralizing the damaging effects of free radicals. Consequently, there is an invasion and growth of opportunistic microorganisms which has been the reason for the frequent pulmonary and gastrointestinal infections suffered by patients with AIDS. The role of antioxidants in preventing apoptosis and viral activation in HIV is well documented.

1.6 Antiinfective activity of plant metabolites

Microbiology is the study of microscopic forms of life. In its early period, microbiology as a science was concerned with the isolation, identification and control of microorganisms. Major advances in microscopy and biochemical techniques showed microorganisms to be useful models for the study of various processes of living systems especially human diseases. There are many other studies that have clearly shown that microorganisms perform many activities that are beneficial to humans for example microscopic forms of life manufacture antibiotics, vitamins and growth factors for humans, other animals and plants; decompose sewerage and solid industrial wastes and are essential to the formation of foods such as cheese, yoghurt and bread. Despite the established useful functions of microorganisms these may be best known as agents of food spoilage and causes of diseases. So far as is known, all primitive and civilized societies have experienced diseases caused by microorganisms, frequently with disastrous results (Wistreich and Lechtman, 1988).

Most plant metabolites have been found to have antimicrobial activity and there are some target microorganisms that are almost always present whenever there is an infection. In the current research six microorganisms were evaluated against the plant extracts and these included *Staphylococcus aureus* and *Streptococcus Group A* for gram positive bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* for gram negative bacteria and *Candida albicans* and *Aspergillus niger* for fungi. These microorganisms were chosen because they are associated with most diseases that were claimed to be cured by the plant extracts under investigation.

Table 2: Infections on Body Parts and the Associated Microorganisms

Body Part	Associated Microorganism
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*

1.5.1 *Staphylococcus aureus*

The gram positive bacterium is salt tolerant, non-motile and non-spore forming. It belongs to the micrococcus family. The bacterium divides randomly giving rise to irregular clusters of cells. It is a facultative anaerobe which grows rapidly on many types of media. It can grow at temperatures greater than 60°C and it can survive long periods of dryness (Norton, 1986). *S. aureus* infections cause significant morbidity and mortality and is the most common cause of hospital acquired infections. The constituents of its cell wall are important determinants of virulence (Norton 1986). *S. aureus* produces enzymes and toxins that have been implicated in the pathogenesis of its infections. It occurs as a commensal on the skin and nasal passages of healthy

humans and animals. Poor refrigeration and undercooked food allows he organism to proliferate and produce toxins. Most strains of *S. aureus* are susceptible to penicillin and methicillin, although some have developed resistance and with this in mind the project was done with the hope to find new antimicrobial drugs of plant origin.

1.5.2 *Streptococcus*

This group includes organisms of medical, dental and veterinary importance as well as starters used in the food and dairy industries, spoilage agents and saprophytes. Streptococci are gram positive bacteria that always divide in the same plane, forming pairs or chains; the individual cells may be oval or lanceolate. They are non-sporing, non-motile and some are capsulated. Most strains are aerobic or microaerophilic but there are anaerobic species. The bacterium is primarily involved in plaque formation and initiation of dental caries (Collins and Lyne, 1985).

The *Streptococcus Group A* species is β-haemolytic, and is so called haemolytic streptococci of scarlet fever, tonsillitis, puerperal sepsis and other infections of man. Some strains are capsulated and form large (3mm) colonies like water drops on the surface of the medium.

1.5.3 *Escherichia coli*

E. coli are gram negative, motile, petrichious, fimbricate and non-spore forming bacteria belonging to the family Enterobactericeae. It is a facultative anaerobe that grows readily on simple culture and synthetic media with glycerol or glucose as the sole source of carbon and energy (Sussman, 1985). *E. coli* has a thin cell wall with relatively porous peptidoglycan comprising 5-20% of cell wall weight. The principle reservoir of *E. coli* is the human intestinal flora and it gets into the external environment through excretion in faeces. Some enterotoxigenic strains are implicated in causing travellers diarrhoea in adults. Onset of symptoms of infection includes abdominal pains. Poor hygienic practices have led to contamination of baby food and even adult's food with faecal *E. coli* leading to severe outbreaks of diarrhoea in Zimbabwe (Eley, 1992).

1.5.4 *Pseudomonas aeruginosa*

P. aeruginosa is the quintessential opportunistic pathogen of humans that can invade virtually any tissue. It is also a leading cause of hospital-acquired (nosocomial) gram negative infections, but its source is often exogenous. The bacteria are non-sporing and grow on nutrient and usually MacConkey agars. Colonies on agar medium are large, flat spreading and irregular, grayish in colour. Bacteria are frequently isolated from human and animal material, from food and from environmental samples. The organisms are very resistant to antibiotics except polymyxin, gentamicin and carbenicillin (Collins and Lyne, 1985).

1.5.5 *Candida albicans*

This is yeast that forms ascospores and so belongs to the Ascomycotina family. This genus is of medical importance, *C. albicans* especially so. All Candida species form creamy white, smooth colonies on malt or peptone agar (Collins and Lyne, 1985). A rapid test for *C. albicans* is to inoculate the suspect yeast into sterile horse serum and incubate at 37°C and at the end almost all strains of *C. albicans* produce germ tubes, but not when the inoculum is heavy.

C. albicans is a member of the normal flora of the mucous membranes in the respiratory tract, gastrointestinal tract and the female genital tract. In these other locations it may proliferate excessively and result in some pathological conditions; however they rarely produce systemic progressive disease. Principle predisposing factors to *C. albicans* infection are diabetes mellitus, general debility, nutritional deficiency and disturbances in the normal flora with the absence of those bacterial components that usually keep Candida in check. Candida infection may be a secondary invader of the lungs where pre-existing disease is present, for example in cancer or tuberculosis.

1.5.6 *Aspergillus niger*

The genus forms colonies with colourless mycelium from which conidiophores arise. The colour which develops is due entirely to the spores, which also give the surface of the colony a powdery appearance. *A. niger* has round heads which are large enough to appear discrete to the naked eye. This, together with their black colour, makes them easy to recognize (Collins and Lyne, 1985).

1.6 Toxicology

Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose, or toxicology is simply pharmacology at a higher dose (Mclaughlin *et al*., 1982). Despite their advantages, several studies have established that some plant species are potentially toxic to humans and animals. Plant chemical compounds, toxic to humans and livestock, are produced as part of the plant's defense against being eaten by pests and herbivores or to gain an advantage over competing plants. Plant poisons are highly active substances that may cause acute effects when ingested in high concentrations and chronic effects when accumulated. In many cases of poisoning resulting from consumption of endogenous toxicants such as those in toxic vegetables and plants, death or prolonged and serious disabilities are reported. Most traditional herbs are relatively unpalatable and their digestibility may be limited hence toxic. Usually unpalatibility comes from allelochemicals in plants and these chemicals may be toxic. In addition, traditional medicines prepared from medicinal plants and sometimes from food plants are not always safe. Poisoning or toxic principles as relates to vegetables generally fall into various phytochemical groups, which include alkaloids, glycosides, oxalates, phytotoxins (toxalbumins), resins, essential oils, amino acids, furanocoumarins, polyacetylenes, protein, peptides, coumarins, flavonoids and glycosides. Toxicity is a relative concept that must be

considered in relation to the context in which these plants are used either as food or medicine. Like any therapeutic agent, when overdosed or incorrectly used medicinal plants also have the potential to induce adverse effects. The historic role of medicinal herbs in the treatment and prevention of disease, and their role as catalysts in the development of pharmacology do not, however, assure their safety for uncontrolled use by an uninformed public. Since most traditional medicinal plants are commonly consumed in Zimbabwe, a phytochemical screening of the plants needs to be carried out and especially to determine the toxicity tests. The toxicity results should be used to create awareness as to which plants are safe for consumption as food and medicines (Orech *et al.,* 2005)

Plants that are found to be toxic can be used as poisons, pesticides and in cancer treatment. Cancer is a big challenge to the world as suitable remedy is very costly and even impossible in some cases. On the other hand the conventional chemotherapeutic agents are growing resistance. Microbial infections are also creating health hazards for the multi-drug resistance bacteria. Scientists are now engaged to find potent remedy for cancer and other infectious diseases through the discovery of new and effective chemotherapeutic agents from plant, microbes and other suitable sources. Brine shrimp lethality bioassay is a primary assay to detect cytotoxic property of plant extract and for this, further studies are required to establish the cytotoxicity of the plant extracts against human cancer cell lines but predictions can be made.

There is a real need for reliable, general bioassays which can detect a broad spectrum of pharmacologic activities in higher plants and yet can be employed by natural product chemists, in-house at low cost, to guide phytochemical screening and fractionation. A possible approach to developing an effective bioassay for toxicity might be simply to screen for substances that are toxic to zoologic systems. Once such substances have been isolated, a battery of specific and more sophisticated bioassays could then be employed (Meyer *et al*., 1982). The aim of this method is to provide a frontline screen as the technique is easily mastered, costs little, and utilizes small amounts of test material. It appears that the Brine shrimp lethality test is predictive of cytotoxicity and pesticidal activity (Alkofahi *et al*., 1989). A positive correlation between brine shrimp toxicity and 9KB cytotoxicity have observed that ED50 values for general toxicities are about one tenth LC50 values in the brine shrimp test. A number of novel antitumour and pesticidal natural products have now been isolated using this bioassay (Alkofahi *et al*., 1989).

5.1 Anthology of Plants

From the list of endemic plants to Zimbabwe a brief background of all the plants under study and what is already known is given below.

5.1.1 Vangueria infausta

Figure 12: Vangueria infausta *leaves*Family: **Rubiaceae**

Common Names: Medlar(Eng), Mutsviru(Sh), Umviyo, Umthofu(Nd), Mububuzuka (T)
Status: Commonly used
Description: *Vangueria infausta* is a deciduous shrub or small tree that varies in height from 3-7m depending on habitat. It grows on all kinds of deep sand and is usually multistemmed. The bark is grayish to yellowish brown, smooth and peeling in irregular strips. The barks are covered with short, wooly hairs, especially when young. The flowers are small and form greenish-yellow clusters. The fruits are almost circular, glossy dark green when young and changing to a light brown colour when ripe. The fruits also have a pleasant sweet acid flavour. This tree can be found in woodlands, scrub, on kopjes or in sandy valleys. It is common in open, exposed grassland (Sigrid Leger 2000-2003). It is commonly found in the north, west, central and southern parts of Zimbabwe at most altitudes in the woodlands in shade (Breyer-Brandwick and Watt 1962)
Uses: Traditional medical practitioners mainly use the roots of the plant for treatment of different ailments. In Zimbabwe it is used mainly for treatment of abdominal pains, diarrhoea, dysmenorrhoea, inflammation of the naval cord and it stops vaginal discharge, malaria and pneumonia. The decoction of the root is in common use as a remedy for menstrual; troubles and the root is used as a roundworm remedy. Methods of administration vary depending on the type of sickness. In South Africa the root preparation is used to treat malaria. The ripe raw soft flesh fruit is eaten and tastes similar to an apple. When the fruits are dry they can also be soaked in water and then boiled, mashed and used as a kind of porridge (Gelfand *et al.,* 1985).
Known Research Findings: De Boer *et al* 2002 showed that *Vangueria infausta* extracted using ethyl acetate, methanol, cold water and boiling water has antimicrobial activity against a wide

range of microorganisms. In 2005 when some Tanzanian plants were screened for antibacterial and antifungal activity *Vangueria* was positive for the tests (de Boer *et al.,* 2002). Recently parasite lactate dehydrogenase assay method was developed for evaluating antimalarial compounds in the plant and it does show some antiplasmodial properties (Nundkumar and Ojewole 2002). Phytochemical screening of the active plant extracts revealed the presence of anthraquinones, flavonoids, seccoirridoids and terpenoids. The fruits do possess antioxidant activity. Vitamin C content of the fruit is 3.7 mg/100g. The leaf has given negative tests for haemolysis, alkaloids and tannins but positive ones for sterols (Breyer-Brandwick and Watt 1962)

5.1.2 *Erythrina abyssinica*


Figure 13: *E. abyssinica* leaves and flower

Family: Leguminosae

Common Names: Lucky bean tree (Eng), Mutiti, Munhimbitimbi, Mutate (Sh), Umgqogqogqo (Nd), Munhunguti (T)

Status: Endangered

Description: It is a deciduous shrub or tree up to 12m tall. It has orbicular leaves and long lived seeds which are however poisonous. *Erythrina* on blooming has some unusual bright red flowers with thread like filaments. This *Erythrina* with its brilliant red flowers has leaves that are edible and are crowded towards the ends of the branches with long petioles. The root is large and succulent and on drying forms an extremely light corky mass. The bark is grey, corky and rough. Young branches have dense brown hairs. Its light wood is used in fishermen's floats. It is one of the several plants producing red and black seeds called lucky bean which are stung into necklaces. The tree is well distributed in southern Africa and in Zimbabwe it is mostly common in the north, east, south and central parts. The tree is common to higher altitudes in wooded grassland. It can be propagated from the seed or truncheons.

Uses: This tree is widely used for its medicinal purposes. In Zimbabwe it is used to treat abdominal pains, gonorrhoea and wounds in mouth as well as for wasting in infants. The roots and leaves are mainly used and normally and an infusion taken by mouth is used. In Kenya it is

used for skin sores, ulcers and malaria whilst in East Africa it is used for treating inflamed eyes, gonorrhoea, malaria, syphilis and abdominal pains (Gelfand *et al.,* 1985). The seeds can be used as fish poison whilst the soft wood can be carved into curios.

Known Research Findings: Cyclooxygenase inhibiting and antibacterial activities of some South African Erythrina species were shown by Pillay *et al* 2001. Alkaloids were isolated by Lapiere 1951 whilst flavonoids were found by Moriyasu *et al* 1998. The seeds of Erythrina have yielded alkaloids erythralene, erysothione, erysorine, erysodine and erysothiopune (Breyer-Brandwick and Watt 1962).

5.1.3 Ximenia Caffra

Figure 14: *Ximenia caffra* leaves

Family: Olacaceae

Common Names: Sour plum (Eng), Munhengeni, Mutsvanzva (Sh), Umthunduluka (Nd), Munampeli (T)

Status: Commonly Used

Description: *Ximenia caffra* is a deciduous tree up to 6m tall with an untidy open crown. The bark is dark grey and rough, but pale green or brown on the younger branches. Branchlets are spine-tipped; leaves are alternate, elliptic, and blue-green and often hairy when young and turning to shiny green when getting older and vary in size. Sapwood is white and heartwood is hard and reddish brown. The root system is non aggressive and the flowers are small, sweet scented and creamy green. The tree is distributed in woodlands and grasslands and on rocky outcrops and sometimes on termites mounds (Coates 1989). The tree is common in mixed woodlands often on termite mounds, most frequent at medium to higher altitudes in the medium to better rainfall areas.

Uses: The ripe fruits are edible and contain a vitamin C content of 27%. It is high in potassium and contains proteins. The roots and leaves are normally used for medicinal purposes. A decoction from the leaves is used as a wash to soothe inflamed eyes. Infusions of the roots are used as a remedy for dysentery and diarrhoea. Powdered roots are applied to sores to speed up healing (Baloyi and Reynolds 2004). In Tanzania the leaf decoction is used for fever, syphilis

and diarrhoea whilst in South Africa it is also used as an eye remedy. The Kgatla use a decoction of the plant in cattle fertility rites while the Venda smoke the powdered root with horn shavings in a maize cob pipe to stop bleeding from the mouth and nose.

Known Research Findings: Antibacterial studies were done and *Ximenia caffra* was found to be active against 105 strains of bacteria from seven genera (Fabry *et al.,* 1998). Fabry *et al* 1998 also showed fungistatic and fungicidal activity of the plant.

5.1.4 Annona stenophylla

Figure 15:A*nnona stenophylla* leaves

Family: Annonaceae

Common Names: Dwarf custard apple (Eng), Muroro (Sh), Ububese (Nd)

Status: Commonly used

Description: It is a low rhizomatous shrub up to 1.5m tall, which grows on deep sandy soils and soft sandy areas. It starts to flower in November, producing yellow to dark orange flowers. The leaves are somewhat short usually narrow and hairy and its fruits are ripe from February onwards. The dark orange fruits are said to be almost heart shaped berries of about 3cm length, which contain many seeds. The flowers as well as the fruits have a strong pleasant smell (Sigrid Leger 2000-2003). The plant is common at medium to higher altitudes on vlei margins and in grassland subject to fire usually on sand. In Zimbabwe it is widely distributed in the north, west and central parts.

Uses: *Annona stenophylla* is used to treat gonorrhoea, syphilis and abdominal pains. Infusions, which are made with other plants, are taken by mouth. The roots provide a strong medicine for treating tooth pain and the infusion is cooled down before using to rinse the mouth and it is spat out (Gelfand *et al*., 1985).

Known Research Findings: Matazu and Gundidza 1996 have shown that *Annona stenophylla* contain antifungal activity. Plants belonging to the Annonaceae family have shown antidiabetic properties. There is no much literature documented on this plant.

5.1.5 Dicoma anomala


Figure 16: *Dicoma anomala* leaves

Family: Asteraceae

Common Names: Chifumuro (Sh), Ukhalimela (Nd)

Status: Very threatened

Description: It is a herb that has been widely used for its medicinal purposes. *Dicoma anomala* is a low lying bushy perrenial plant with tufted stems 40-300mm from a woody tap root. The leaves are alternate, subsessile, leathery, almost parallel sided, dull green above and grey hairy below. Flower heads are conical up to 10mm long surrounded by narrow, sharply pointed bracts, with purplish florets more or less hidden by silvery hairs. The plant is widely distributed in all parts of Zimbabwe and common among short grass in wooded grassland on sand.

Uses: The herb is used by traditional healers to treat abdominal pains, gonorrhoea, syphilis, wasting in infants, malaria, skin sores, ulcers and to drive away bad luck. Dicoma anomala is used as a remedy for dysentery, the decoction for intestinal worm infestations, diarrhoea and gall sickness. Southern Sothos use the decoction for venereal diseases and apply the powdered plant to sores and wounds. They also use the plant decoction as a purgative and as a colic and tooth ache remedy. The plant is also used to produce vomiting and a small piece chewed is used as a charm. The powdered root is sniffed in the nose for colds (Gelafand et al., 1985).

Known Research Findings: Dicoma anomala contains small amounts of volatile oil, crystalline glucosides and amorphous alkaloids and phytosterol. Steenkamp et al 2004 Dicoma anomala does have antibacterial activity against several bacterial strains and genera. Dicoma anomala species have also shown compounds known as germacranolides, which are closely related to lactones (Zdero 1989). The chemical composition of Dicoma was investigated and small amounts of volatile oil, colourless crystalline glucoside, small amounts of amorphous alkaloids, a phytosterol and other substances were isolatedfound out that.

5.1.6 *Pterocarpus angolensis*

Figure 17: *Pterocarpus angolensis* tree

Family: Leguminosae

Common Names: Bloodwood (Eng), Mubvamaropa, Mukwa (Sh), Umvagazi (Nd), Mukula (T)

Status: Threatened

Description: It is a common tree that grows up to 10m in different kinds of deep sandy soils. The leaves have 5 to 9 pairs of obovate leaflets, intensive green in colour. The flowers appear before the leaves from September to October in attractive, yellow panicles. The fruit is unique, in the middle it forms a circular ball shaped case covered with harsh bristles, which contain one single brown kidney shaped seed. The bark is blackish, rough and fissured. It produces red sap and its branches are greyish, drooping with indehiscent pods. The tree is common in the northern, west, central, eastern and southern parts of Zimbabwe at most altitudes in sandveld in woodland and wooded grassland and on granite kopjes. It is the only commercially harvested wood in Bushmanland and therefore an important tree (Sigrid Leger 2000-2003).

Uses: When the tree is cut or the bark is injured, a dark red sticky sap exudes from the wounds, which resemble human blood weeping from a wound. This sap is dried, pounded and mixed with oil to make an ointment. Old women apply the ointment to the whole body for skin care. The red sap is also used to treat severe coughs. The infusions taken by mouth can be used to treat diarrhoea, menorrhagia, backaches and bleeding gums or mouth ulcers in malnutrition. Other surveys in Zimbabwe have shown that it cures malaria, tuberculosis and lameness. In Zambia it is used to treat inflamed skin, bleeding gums and gonorrhoea (Gelfand *et al.,* 1985). The powdered leaf has been used to relieve backache by packing it into the rectum, the result being purging and haemorrhage from the bowl for 48 hours.

Known Research Findings: *Pterocarpus* was reported to yield a resinous kino which assays 76.7% of tannin and 0.55% of a new crystalline phenol muningin. Breyer-Brandwick and Watt 1962 also found flavones. Ndamba *et al* 1994 found that the extracts of *Pterocarpus angolensis* and other plants are used to treat schistossoma haematobium infections. In Spain *Pterocarpus angolensis* was found to be a potent antiinflammatory drug as it showed good positive results on initial screening.

5.1.7 Clausena anisata

Figure 18: *Clausena anisata* tree

Family: Rutaceae

Common Names: Horsewood, Maggot killer (Eng), Muvengahonye (Sh),

Status: Lower risk

Description: *Clausena anisata* is a shrub or small tree up to 6m high. Its leaves are pinnately compound with 10-17 alternate or sub-opposite leaflets and a terminal leaflet. The leaves are densely dotted with glands and have a strong scent when crushed. The scent has been likened to aniseed and opinions vary on its pleasantness. The flowers are small but attractive, white or cream in colour with orange-yellow stamens. The fruits are small, ellipsoid, about 13mm in diameter, shining blue-black drupes. Locally it is common in high rainfall areas and grows well on the margins of, evergreen forest.

Uses: This plant is commonly hung in houses or put on fires to keep away mosquitoes and evil spirits. The powdered roots, with lime and guinea grains are applied to rheumatic and other pains in Nigeria where also the leaves are considered anthelmintic. In East Africa Clausena is used for its odoriferous properties, especially under beds and toothbrushes are made from the twigs. Clausena is well known for its antidiabetic properties and is therefore widely used by traditional healers. It is also used for treating epilepsy and cancer. The leaves of the plant are normally used and methods of administration differ according to the type of sickness. In South Africa it is used to treat diabetes, in Ghana HIV-1, 2 and in Japan to treat cancer.

Known Research Findings: Investigations that have been done show that *Clausena anisata* has got a lot of antibacterial properties. Its leaves are crushed and applied to wounds infested with maggots for treatment (Gelfand *et al.,* 1985). The Zulus use the leaf as a parasticide and purgative and the root as an anthelmintic. The tree is used to treat toothaches and migraines in West Africa (Breyer-Brandwijk and Watt 1962). Ito *et al* 2000 has found new carbazole alkaloids which contain antitumour promoting activity. The alkaloids inhibit Epstein-Barr virus.

Extracts from *Clausena* have been shown to inhibit DNA and RNA viral replication or cytopathic effects of the viruses in the host cells. It also inhibited in vitro HIV infections in human T lymphocyte cells (Ayisi 2001). Ayisi and Nyadedzor 2003 obtained similar HIV results. In experiments done by Ojewole *et al* 2002 *Clausena anisata* was shown to produce hypoglycemic effects on rats. The leaf which is highly flammable yields 1.2-7.1% of a volatile oil whose chief constituent is anethole.

5.1.8 *Ziziphus mucronata*

Figure 19: *Ziziphus mucronata* leaves

Family: Rhamnaceae

Common Names: Buffalo thorn (Eng), Muchecheni, Chinanga (Sh), Umphafa (Nd), Muchechete (T).

Status: Commonly used

Description: This is a beautiful, indigenous deciduous tree found in most areas of South Africa and other countries. It is a small, many-branched tree up to 8m tall with a rounded crown, which grows on loamy sands. The leaves are three veined from the base and often; there is one straight and one hooked spine at the leaf axil. The flowers are said to be small and yellowish and form clusters. The fruit is almost circular, about 1.5cm in diameter and bright red when ripe. The fleshy drupes are rich in sugars and vitamins (Sigrid Leger 2000-2003). The plant is common at medium altitudes mainly in mixed woodland, in areas with medium to lower rainfall, also on termite mounds. In Zimbabwe it is mainly found in the northern, western, southern, central and eastern parts.

Uses: The Zulu take the powdered leaf and bark in water as an emetic in chest troubles. They also use hot infusions of the bark liberally for cough. The African, in general applies a poultice of the leaf to boils, carbuncles and other septic swellings of the skin. In Transvaal a decoction of the root is taken internally and a paste of the leaf is applied to tubercular glandular swellings. For pain of any sort, the African frequently applies a poultice of meal made with a decoction or of powdered baked root. Africans inhale the vapour and gargles with a decoction of the leaf and shoot for measles and scarlet fever (Breyer-Brandwick and Watt 1962). The roots can be used to

treat diarrhoea with blood in the stool or stomach ulcers. Ziziphus is a remedy for gonorrhoea. (Sigrid Ledger 2000-2003).

Known Research Findings: 90% methanol extracts were made and *Ziziphus mucronata* in the presence of metabolic activation showed mutagenic effects (Elgorashi *et al.*, 2003). The powdered leaf and bark in water is used as an emetic in chest troubles. Hot infusion of the bark is used for coughs. The root decoction is taken internally and a paste of the leaf is applied to tubercular glandular swellings. The bark has been shown to contain 12.2-15.7% tanning matter. (Palgrave 2002). The phytochemistry of the plant has not yet been exhaustively studied; current literature survey showed one other article indicating that the leaf extract contains low amounts of tannins (Aganga and Adogla-Bessa, 1999).

5.1.9 *Peltophorum africanum*

Figure 20: *Peltophorum africanum* leaves

Family: Fabaceae

Common Names: False black wattle, African wattle (Eng), Muzeze(Sh), Umsehla (Nd)

Status: Commonly used

Description: Semi-deciduous to deciduous trees of about 15m with a spreading, untidy canopy. They grow best in well drained soil. In older trees the bark is grooved and grey-brown; bark of young branches is smooth and grey. The leaves are acacia-like and silver-grey covered with fine hair; mature leaves yellowish at tip of branches. The leaves are twice compound with a pair of leaflets at the tip; alternate; up to nine pairs of pinnae each with 10-20 pairs of leaflets; leaf, stalk and rachis covered with reddish brown hairs. The trees have no thorns. Flowers form upright, showy sprays (150mm long) of bright yellow flowers with crinkled petals on the ends of branches; the stalk covered with reddish brown hairs. These are followed by clusters of thin, flat dark brown/black pods of about 100m, tapering to both ends (Sigrid Leger 2000-2003). The heart wood is brown and sometimes chocolate brown whilst the sap wood is pinkish and susceptible to blue staining by fungus. The plant is commonly widespread at most altitudes, in wooded

grassland and occasionally in woodland. In Zimbabwe it is found in almost all parts of the country.

Uses: This tree has many uses. Young leaves and pods are eaten by livestock. Flowers provide a high yield of nectar and pollen for bee-keeping. The timber can be used for furniture. The wood is good for fuel. It makes a good shade tree for both livestock and humans. There are also various medicinal uses recorded. Roots are used to heal wounds, toothache and throat sores; root, leaves and bark used to clear intestinal parasites and relieve stomach problems; bark relieves colic; stem and root used for diarrhoea and dysentery. It is also used to treat eyes, sore throats, joint pains, wounds, HIV/AIDS and venereal diseases. The Transvaal Sothos chew the fresh bark for relief of colic. Different administrations are used depending on the kind of sickness. Infusions are taken orally by mouth, whilst the powder is put onto the wounds (Gelfand *et al.,* 1985).

Known Research Findings: The extracts of Peltophorum africanum have been used in veterinary medicines as they show antibacterial activity (Bizimenyera et al., 2005). Bessong et al has shown the inhibitory properties against HIV type 1 reverse transcriptase and intergrase. The chemical compounds that have been found include berenin and catechin (fig 21) and a red coloured gallotannin. The root bark is said to contain tannins.

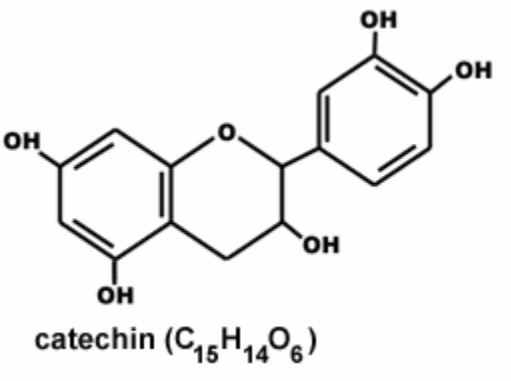


Figure 21: Structure of catechin (Bessong *et al.,* 2005)

5.1.10 Lannea edulis

Figure 22: *Lannea edulis* shrub

Family: Anacardiaceae

Common Names: Wild grape (Eng), Mushamba, Mutsombori (Sh), Intakubomvu, Umtsombole (Nd)

Status: Commonly used

Description: A small perrenial shrub, which grows from, branched underground stems 30-300mm high from large woody root-crown, or from a creeping rhizome. It occurs on deep sand and grows up to 20cm tall. The alternate pinnate leaves have 5 to 7 pairs of shiny leaflets which are variable in shape and size. The flowers are small, about 2mm long and yellow borne in a congested false spike or panicle and can be seen in September. The fruits are about 1cm long, green but turning red when ripe. They occur from September onwards and can be seen on the plant at same time as flowers (Sigrid Leger 2000-2003). The shrub is common in most Zimbabwean areas and is found in open woodland.

Uses: The fruit which is purplish-black in colour when ripe has a juicy pulp and is pleasantly sour. Mainly children eat the fruits. Usually they are not gathered, but only eaten while passing them during gathering or hunting. Traditionally the leaf infusion is used to treat stomachaches. The powdered leaves are applied on wounds for healing. A cold infusion of the roots of *Lannea edulis* is used for treating diarrhoea. Africans take a decoction of the roots particularly of its bark in frequent large doses for blackwater fever. The Lobedu use a cold infusion of the leaf as a local application to the eyelashes to loosen the dried pus in sore eyes (Gelfand *et al.,* 1985).

Known Research Findings: It has been found that the dichloromethane root extracts of *Lannea edulis* do contain some important phytochemical groups like alkylphenols, cardonols and dihydroalkylhexenones (Queiroz *et al.,* 2003). Lannea has also been shown to be able to induce frameshift mutations in Salmonella (Sohni *et al*., 1995). This means that it can be used in cancer therapy.

5.1.11 Turrea nilotica

Figure 23: *Turrea nilotica* leaves

Family: Meliaceae

Common Names: Bushveld honeysuckle-tree, Small mahogany, (Eng) Chipindura, Chirambagavakava, Chitungure, Mukandanyoka (Sh), Isidlamvundala (Nd),

Status: Commonly used

Description: *Turraea nilotica* is a deciduous shrub or small tree up to 6m with a greyish, corky bark. It has densely velvety hairs, particularly on the under surface of the leaves. The flowers have a distinct staminal tube, greenish-white, turning yellow with age, appearing before the leaves in dense clusters on the young branches. Leaves are alternate, shortly petiolate, widest above to about the middle, softly hairy with a rounded apex. The fruit is nearly spherical, thin and has a woody capsule. It can be distinguished from the other species by the smaller leaves being nearly glabrous. *Turraea nilotica* normally grows in miombo woodlands, wooded grassland and on termite mounds. In Zimbabwe it is widely distributed in the north, east, west, south and central parts of the country (Gelfand *et al.,* 1985).

Uses: The roots of *Turraea nilotica* are boiled in water and used for toothaches. In Tanzania *Turraea nilotica* is reported to be used by traditional healers for the treatment of oral candidiasis and fungal infections of the skin. The root of *Turraea* species is used by the Ikuzu for relief of abdominal pains and by Musoma district in Tanzania as an anthelmintic. The root cooked with maize is eaten as a cure for ulcers.

Known Research Findings: In previous studies strong antifungal activity was exhibited by extracts of Turraea nilotica. A chemical constituent niloticin was found (Fig 24) by Colegate and Molyneux 2007 and it is a proposed intermediate in the biosynthesis of limonoids. Not much work has been documented on this plant.

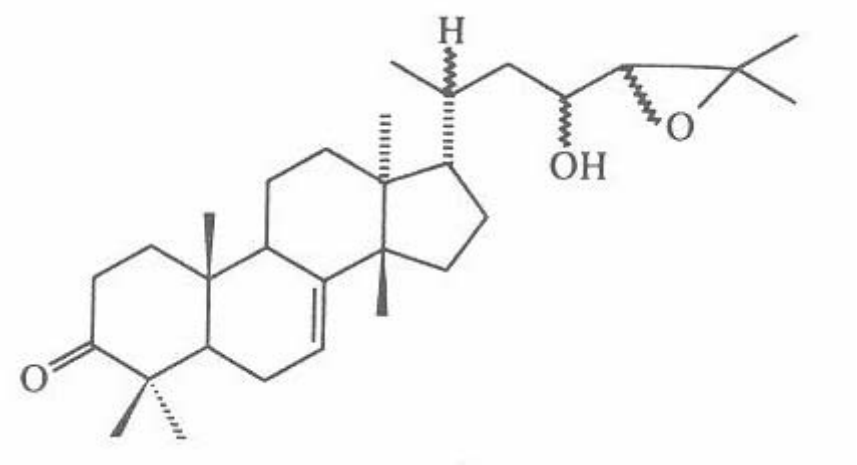


Figure 24: Niloticin (Colegate and Molyneux 2007

5.1.12 *Holarrhena pubescens*

Figure 25: *Holarrhena pubescens* leaves

Family: Apocynaceaea

Common Names: *Holarrhena antidysenterica* (Syn), Jasmine shrub (Eng), Muhatsu, Mukashumukono, Chigakuta (Sh), Umhatshu (Nd)

Status: Threatened

Description: Tropical Asian tree with hard white wood. It is an erect, evergreen shrub or tree up to 4m tall. Its bark is grey, deeply fissured, thick and corky. All parts produce a milky sap. The leaves are opposite, petiolate, glabrous to softly short-hairy, widest to about the middle, sometimes shorter and wider apex usually drawn out to a sharp point. Its flowers are white and tubular with spreading limb and the tube is over 10mm long very fragrant and showy. The fruit is paired, pendulous and the seeds have long silky hairs. The plant is widespread at the lower to medium altitudes on granite outcrops or on stony ground in medium to lower rainfall areas. In Zimbabwe the plant is mainly found in the north, east, west, central and southern parts. The seeds of the plant germinate readily and the plant is worth growing as a garden ornamental (Breyer-Brandwick and Watt 1962).

Uses*:* The bark of Holarrhena pubescens is used as a remedy for dysentry and diarrhoea. Milk in which the root has been boiled is used to wash a boy who is entering puberty. The preparation is also used as a remedy for snake bites and in the treatment of venereal diseases. In East Africa the bark is used as a febrifuge and as a tonic (Breyer-Brandwick and Watt 1962).

Known Research Findings: *Holarrhena pubescens* stem bark was tested for antibacterial efficacy against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* using the microdilution broth method as well as the disc diffusion method. The crude methanolic extract was active against all tested bacteria (Chakraborty and Brantner 1999). In 2001 Simonsen *et al* found that the plant has antimalarial activity as it was active against Plasmodium falciparum. Some alkaloids and steroidal compounds were found in the plant (Siddique *et al.,* 2001). The plant yields 1.2% dry weight of total alkaloids and is an effective amoebicide which is however, less active than emetine (Breyer-Brandwick and Watt 1962).

JUSTIFICATION OF STUDY

There is a vastland of medicinal plants which are widespread in Africa. They have generated so much interest because of their importance to human health. Just as their uses for the treatment of several infections have been increasingly recognised scientific ivestigations are also on the rise. The usefulness of medicinal plants holds tremendous promise in tackling the problem of rapid antimicrobial resistance by numerous microorganisms.

Microorganisms are reportedly adopting several mechanisms to evade the efficacy of antimicrobials, thereby causing the humongous problems of antimicrobial resistance. Coupled to the problem of antimicrobial resistance, is the prohibitive cost of antimicrobial agents, which may have ripple effects on affordability and effective health care delivery.

Consequent to the ongoing situations, the quest for alternative sources of treatment is inevitable and medicinal plants offer so such promise. Studies aimed at unraveling the efficacy of medicinal plants as well as structural elucidation of active compounds are therefore timely, important and relevant to health care delivery system especially of Zimbabwe.

The trumpcard of this study therefore is the scientific confirmation of the usefulness of selected medicinal plants used by herbalists in Zimbabwe for the treatment of bacterial and fungal infections an area so important and therefore greatly justified.

STATEMENT OF HYPOTHESIS

If medicinal plants contain phytochemical compounds, then they possess biological activity.

RESEARCH QUESTIONS

- Do medicinal plants contain phytochemical compounds?
- Do medicinal plants possess antiinfective activity (antibacterial/antifungal)?
- Do medicinal plants possess antioxidant activity?
- Are medicinal plants safe to use?

1.3 AIM OF STUDY

To investigate the activity of selected traditional medicinal plants for possible sources of anti-microbial drugs.

GENERAL OBJECTIVE

To identify and screen traditional medicinal plants for phytochemical and biological activities.

1.4 SPECIFIC OBJECTIVES

- To identify and review known literature on selected medicinal plants in Zimbabwe that are mostly used by traditional medical practitioners and are threatened with extinction.
- To isolate extracts by solvent extraction and to test them for phytochemical classes using chromatographic methods.
- To test extracts for antibacterial and antifungal activities
- To find the minimum inhibitory concentrations and the minimum bactericidal and fungicidal concentrations
- To determine their total phenolic content and antioxidant activity especially those presumably used for treating HIV/AIDS and related illnesses in selected parts of Zimbabwe.
- To test extracts for toxicity using Brine Shrimp Lethality Test

CHAPTER 2

2.0 MATERIALS AND METHODS

2.1 Chemicals, Reagents and Equipment

Ammonia AR (Batch 503542) Skylabs, Methanol Spectrophotometric grade (No M-3641) Sigma, TLC Plates (Batch 126 F-0130) T-6770, 250µm layer thickness, 2-25µm mean particle size, 20x20cm, Sigma, Atropine, University of Zimbabwe,School of Pharmacy, Toluene CP (Cat No 15, 500-4) Aldrich, Ethyl acetate, AR (Cat No 11,002-7) Aldrich, Diethyl amine AR (Batch 43410) Microlabs, Bismuth Nitrate (No B-9383) Lot 47F- 0698, Sigma

Potassium hydroxide, (Batch 19216) Saarchem, Sodium hydroxide, (Batch 1410507), Skylabs, 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, Digitonin (No D-5628) Lot 34F-0141 Sigma, Nutrient Broth (Batch 1066724) Art No C24 Biolab Diagnostics, Merck, Vanillin (S4551918) Merck, Germany, Dinitrobenzene (S05456) Merck, Sabouraud Dextrose Agar (Batch B001468) Biotec Laboratories, Whatman filter paper No 1 125mm (Cat No 1001125) Schleicher and Schuell

Potasium Iodide AR (Batch 69153) Skylabs , Potassium Chloride AR (Batch 1029052) Saarchem, Sodium Chloride AR (Batch 1028306) Saarchem, Dimethylsulphoxide AR (1.02952.2500) Merck , Sea Salt (LA 060670917) Baleine Germany,

The following chemicals were obtained from Associated Chemical Enterprises, RSA Benzene (Batch 6317/584); Ethanol AR (Batch 3875) and Hydrochloric acid AR (Batch 4504

The following chemicals were obtained from Saarchem Pvt Ltd, RSA

Chloroform AR (Batch 1010060); Methanol univAR (Batch 16229); Petroleum ether univAR (Batch 15060);

Ferric chloride (Lot 37F-3478); Acetonitrile (Lot 96F 3484) were sourced from Sigma, USA Thomas-Wiley Laboratory Mill Model 4

2.2 Plant Materials

2.2.1 Plant Selection Criteria

The choice of plants to study can be a real challenge considering the number of plants, which has not been investigated, both phytochemically and biologically. In this study plants from Matabeleland and Manicaland provinces which have a vast variety of plant species were chosen. The rational for choosing the plants was according to those which are reported by traditional healers to be useful in the treatment of prominent infectious diseases like HIV and AIDS, tuberculosis, sexually transmitted infections, herpes and those that are threatened with extinction.

A trip to Manicaland province (Chimanimani and Chipinge) was organized on the 28th of March and on the 6th of April 2007 to Matebeleland province (Matopos, Bulilima and Mangwe districts) where the bark, leaves and or roots of plant specimens were collected. Villagers and traditional healers helped as field guides in the search for the plants. The following plants were collected,

CHIMANIMANI - *Dicoma anomala*, Chifumuro (sh), Ukhalimela (nd)
Clausena anisata, Muvengahonye(sh Mjavikala (nd)

CHIPINGE - *Erythrina abyssinica,* Munhimbiti(sh) Umgqogqogqo (nd)
Lannea edulis, Mutsombori(sh), Intakubomvu (nd)

MATOPOS - *Pterocarpus angolensis*, Mubvamaropa(sh), Umbangu (nd)
Holarhena pubescens, Muhatsu(sh), Umhatshu (nd)

MANGWE - *Vangueria infausta*, Mutsviru (sh), Umthofu,umviyo (nd)
Ximeniacaffra, Mutengeni(sh),Umthunduluka(nd)
Ziziphus mucronata, Muchecheni (sh), Umphafa (nd)
Turrea nilotica, Mukandanyoka(sh)Isidlamvundala(nd)

BULILIMA - *Annona stenophylla,* Muroro (sh) Ububese (nd)
Peltophorum africanum, Muzeze (sh), Umsehla (nd)

2.2.2 Collection Procedure

Healthy plant specimens without traces of insect, fungi or mould attack were collected from the five districts (Chimanimani, Chipinge, Matobo, Bulilima, and Mangwe). Botanists at the National Herbarium identified the specimens and a sample for each plant was labeled and kept as a reference at the Department of Pharmacy University of Zimbabwe.

2.2.3 Plant Preparation

Leaves were detached from the twigs, barks peeled off and roots cleaned and cut into small pieces. The specimens were dried at the laboratory in shade at ambient temperature of around 25ºC, once dry; plant material was ground into fine powder either using a mortar and pestle or an electric grinder (Thomas-Wiley Laboratory Mill Model 4). The powder was placed in black containers, which were labeled and kept at room temperature in a dark place.

2.3 Extraction Procedures

2.3.1 Plant Extraction for Anti-infective Testing

Stored plant material (20g) was macerated in methanol for 24 hours on a shaker and filtered. The filtrate was concentrated under reduced pressure and the small amounts of extracts were then lyophilised by using a vacuum freeze dryer. Extractions were done in triplicate to ensure adequate and clean samples and the percentage yields were calculated. All extracts were bottled and kept in a refrigerator until testing. The extraction process took a long time since the number of samples was large and there is only one rotary vapour and freeze drier which takes small number of samples at a time.

Extraction yields of plant samples were done in triplicate and calculated as follows

Mass of bottle + sample extracted	x g
Mass of bottle	y g
Mass of sample extracted	z g

% yield (Mass of sample extracted÷Mass of weighed sample) ×100% and each value entered as the mean mass ± S.D of the triplicate measurement to 2 significant figures.

2.4 Antimicrobial Susceptibility Testing

2.4.1 Source of microorganisms

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

2.5 Preparation of Culture media

2.5.1 Sabouraud Dextrose agar Preparation

Fungi sensitivity tests were done on 4% Sabouraud Dextrose agar. 60g of the agar were suspended in 1L of distilled water. This was boiled with stirring until completely dissolved. Dissolved agar was poured into 20ml bottles and closed. The bottles were sterilized by autoclaving at 121°C, 15 P for 15 minutes and then cooled rapidly and stored in an oven at 40°C until use.

2.5.2 Nutrient broth preparation

Bacteria were grown in nutrient broth where 40g of powdered broth was weighed and suspended in 100ml of distilled water. The mixture was heated gently to dissolve the broth to clear. Broth was poured into 20ml bottles and sterilized by autoclaving at 121°C, 15 P for 15 minutes.

2.5.2.1 Sub-culturing

Fresh Nutrient broth (10ml) was inoculated with 0.1ml of each recently prepared fungal/bacterial suspension. Fungi were incubated at 20-25°C for 2-5 days and bacteria at 30-35°C for 1-24 hours.

The resulting suspensions were ready for use (when there was pure culture at the right turbidity).

2.6 Sensitivity Testing

The agar media was poured into petri dishes where 0.1ml of micro-organism had been seeded. The mixture was swirled and the agar was left to solidify. Lids of the petri dishes were kept closed as much as possible to prevent contamination. Using a borer of diameter 4mm four equidistant wells were punched with flaming. Plant extracts were then introduced and the plates were incubated within 15 minutes after applying the discs since the test is standardized under conditions where diffusion of the antibiotic and bacterial growth commence approximately at the

same time. The diameter of the zones of growth inhibition around each well were measured and recorded to the nearest mm using a ruler (Opara and Anasa, 1993).

2.7 Minimum Inhibitory Concentration Determination

The lowest concentration (highest dilution) of antibiotic preventing appearance of turbidity is considered to be the minimal inhibitory concentration (MIC) and at this concentration the antibiotic is bacteriostatic.

2.7.1 MIC Procedure

Sterile capped test tubes were numbered 1 to 12. Extract solution (2ml) was added to the first tube and 1ml of sterile broth to all the other tubes. One ml was transferred from the first tube to the second one. Using a separate pipette, the contents of this tube were mixed and 1ml transferred to the third tube. Dilutions in this manner were continued up to tube 11, being certain to change pipettes between tubes to prevent carryover of antibiotic on the external surface of the pipette. One ml was removed from tube 11 and discarded. The twelfth tube served as a control and received no plant extract. Several colonies of the culture to be tested were suspended to an appropriate turbidity in 5ml of Nutrient broth to give a slightly turbid suspension. The suspension was diluted by aseptically pipetting 0.2ml of the suspension into 40ml of Nutrient broth. One ml of the diluted culture suspension was added to each tube. The final concentration of the antibiotic was half the original. All tubes were incubated at 35°C overnight and examined for visible signs of bacterial growth. The highest dilution without growth was the MIC.

2.8 Minimun Bactericidal/Bacteriostatic Concentration Determination

This was performed as an adjunct to the MIC and is used to determine the concentration of the antibiotic that is lethal to the target bacteria/fungi in vitro.

2.8.1 MBC/MFC Procedure

Broth from the MIC broth tube (100ml) was aliquoted onto Nutrient agar / Sabouraud dextrose agar and spread. The plates were incubated at 35°C until the next day when they were examined for colony growth or lack of it.

No growth indicated that the antibiotic was bactericidal/fungicidal and growth indicated that it was bacteriostatic/fungistatic.

2.9 Extract Preparation for Phytochemical Screening

Different extraction methods and solvents (methanol) which extract most of the chemical group to be screened for were employed as reported by Wagner *et al.,* 1984, Harbone 1984, Trease and Evans, 2002 and Mojab *et al.,* 2003 and these are discussed below.

2.9.2 Alkaloids

Powdered drug (1g) was mixed thoroughly with 1 ml of 10% ammonia solution. The mixture was shaken and extraction was done with 5ml of methanol on a water bath at 60ºC for 5 mins. The filtrate was cooled and concentrated.

Atropine sulphate was used as a reference compound and was prepared in 1-% methanol solution. Thin layer chromatography was done on polyester silica gel plates using the solvent system in the ratios toluene (70): ethyl acetate (20): diethyl amine (10). Detection was done by visualising at 254nm where a pronounced quenching of fluorescence is shown and at 365nm where some alkaloids fluorescence blue or yellow in UV lights. With chemical treatment the Dragendorff reagent was used and brown or orange zones which appear immediately on spraying indicate the presence of alkaloids. The colour is not stable and can be enhanced by spraying further with 5% ethanolic sulphuric acid (Wagner *et al.,* 1984).

As a confirmatory test the alcoholic extract (corresponding to 2.5g of plant material) was evaporated to dryness and the residue was heated on a boiling water bath with 2N HCL (5ml). After cooling, the mixture was filtered and to the filtrate a few drops of Dragendorff reagent was added. The samples were observed for the presence of turbidity or precipitation. A (+) score was recorded if the reagent produced a slight opaqueness; a (++) score for a definite turbidity, but no flocculation and a (+++) score for a definite heavy precipitate or flocculation was produced (Mojab *et al.,* 2003).

2.9.3 Flavonoids

Powdered drug (1g) was extracted with 10ml methanol for 5 mins on a water bath at 60ºC and the clear filtrate was used for chromatography.

The reference compound quecertin was prepared as 0.05% solution in methanol.

The solvent system used for thin layer chromatography was in the ratio ethyl acetate (100): formic acid (11): glacial acetic acid (11): water (27) and visualization was at 254nm where all flavonoids cause a fluorescence quenching and at 365nm flavonoids fluoresce yellow, blue or green. Using chemical detection fast blue salt which give blue or violet zones was used.

The alcoholic extract was used for confirmatory tests where 5 ml was treated with a few drops of conc HCL and magnesium turnings (0.5g). The presence of flavonol was indicative if a red colour developed in 3 mins, orange for flavon and purple for flavanon.

2.9.4 Coumarins

Powdered drug (1g) was extracted by shaking with 10 ml methanol for 30 min on a water bath at 60ºC. The clear filtrate was used for thin layer chromatograhy using the solvent sytem ratios of

toluene (50): diethyl ether (50) saturated with 50ml of 10% acetic acid. 1% of coumaric acid was used as a reference compound. Detection was at 254nm where distinct fluorescence quenching is seen and at 365nm where blue or blue/green fluorescence is seen for single coumarins and yellow, brown or blue zones for furanocoumarins. The spray reagent used was 5% ethanolic potassium hydroxide which intensifies the zones as seen under UV light.

2.9.5 Saponins

Powdered drug (2g) was extracted by heating for 10minutes with 10ml of 70% ethanol. 20 to 25µl of this solution was applied for chromatography as reported by Wagner *et al.,* 1984. The solvent system used was in the ratio 64: chloromethane, 50: methanol and 10: water. The reference compound used was 0.1% of digitonin. The spray reagent vanillin-sulphuric acid was used and saponins formed mainly blue or blue to violet and yellowish zones under UV light. The confirmatory test was done according to the method by Mojab *et al.,* 2003 where the formation of persistent foam whose froth persists for 10 mins during shaking of 0.5mg plant extract with 10ml hot water for 10 seconds in a test tube was taken as evidence for the presence of saponins.

2.9.6 Tannins

Plant material (0.5g) was added to 10ml of distilled water, filtered and iron trichloride addded. Gallic tannins were shown by a blue-black precipitate whilst a greenish precipitate showed the presence of condensed tannins.

2.9.7 Anthracene derivatives

The extraction method, solvent system, detection method and reference compound *aloe* species was made as reported by Wagner *et al.,* 1984. Powdered drug (0.5g) was extracted with 5ml of methanol by warming for 5 minutes on a water bath. The clear filtrate was used directly for TLC with the reference compound of 0.1% *Aloe* species in methanol. The solvent system used was Ethyl acetate: Methanol: Water ratios (100:17:13). Detection without chemical treatment was at UV 254nm where all anthraquinone derivatives quench fluorescence or at UV 365 nm where they give a yellow or red-brown fluorescence. Using chemical reagent detection was done using ethanolic potassium hydroxide (5-10%) where anthraquinones show red fluorescencein UV at 365nm.

The confirmatory tests previously described by Sofowora, 1982 were used. Extract (500mg) was shaken up with benzene, filtered and the filtrate added to 5ml of 10% ammonia. The mixture was

shaken and the presence of red, pink or violet colour in the lower ammonical layer indicated the presence of free anthraquinones.

For combined anthraquinones 500mg of extract was boiled with sulphuric acid and filtered while hot. The filtrate was shaken with 5ml benzene and the benzene layer separated then 10% ammonia added (half the volume of benzene layer). Pink red or violet colour in the ammonical; phase (lower layer) indicated the presence of combined anthraquinones.

2.9.8 Cardiac glycosides

Powdered drug (1g) was extracted with 10ml of methanol for 5 minutes on a waterbath at 60°C. Then 10µl of filtrate was spotted on TLC plates. Petroleum ether in a 1:1 ratio was used as the solvent system. The extraction method, solvent system and detection method was done as reported by Wagner *et al.,* 1984. The reference compound digitonin (5mg) dissolved in methanol on a waterbath at 60°C was used.

The confirmatory test was done as previously described by Mojab *et al.,* 2003 where 500mg of methanolic extract was dissolved in pyridine and a few drops of 20% sodium hydroxide are added. A deep red colour that fades to a brownish-yellow colour indicated the presence of cardenolides (Legal test).

2.10 Plant Extraction for Antioxidant Activity Testing

Two-step extraction was done by shaking 2 g of the milled stored sample with 10 mL of 50% methanol for 2 h. The extracts were filtered and concentrated in a Buchi rotary evaporator (R-114) (Sibata Scientific Technology, Tokyo, Japan) at 64ºC. The methanolic extracts were prepared in triplicate.

2.11 Total phenolic content assay

The amount of total phenolic content was determined according to the method of Velioglu et al 1998, which used the Folin-Ciocalteu reagent with slight modifications. Aliquots of tannin-containing sample extracts (10µl) were made up to 1.00ml volumes with distilled water in test tubes. Folin C (500µl) and sodium bicarbonate (250µl) was then added. The tubes were vortexed and the absorbance read at 725nm after 40 mins. Tannic acid (0.5mg/ml) was used as a standard.

2.12 Radical Scavenging activity assay

The radical scavenging activity was measured following the methodology described by Brand-Williams *et al* (1995) where in the bleaching rate of a stable free radical, 2,2-diphenyl picrylhydrazyl (DPPH) was monitored at a characteristic wavelength in the presence of the

sample. A volume of methanolic DPPH solution (1990 µl) was added to the plant extract (10µl). The bleaching of DPPH was followed at 515nm for 30mins.

The DPPH Scavenging activity (%) was calculated as

[1-(Absorbance of sample/ Absorbance of control)] x100.

2.13 Toxicology/ Brine Shrimp Lethality Test

2.13.1 Hatching the brine shrimps

The brine shrimp (*Artemia salina*) toxicity bioassay test was conducted according to McLaughlin *et al* 1991. Artificial seawater was prepared by dissolving sea salt (38.0 g) in distilled water (1 L). The two compartments of the plastic chamber with several holes on the divider were used for hatching the brine shrimp eggs. The brine shrimp eggs were sprinkled into one compartment which was darkened, while the other compartment was illuminated. After 12 hours of incubation at room temperature (25-28ºC), nauplii (larvae) were collected by pipette from the lighted side whereas their shells were left in another side.

2.13.2 Bioassay

The test tubes used were washed and sterilized in an autoclave machine. 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 and placed in each vial containing 4.5 ml of brine solution. In each experiment,

0.5 ml. of the plant extract was added to 4.5 ml of brine solution and maintained at room temperature for 12 h under the light and surviving larvae were counted and recorded.

2.13.3 Lethality concentration determination

The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. LC_{50} values were obtained from the best-fit line plotted concentration versus percentage lethality. *Nerium oleander* was used as a positive control in the bioassay.

2.13.4 Statistical analysis

The percentage lethality was calculated from the mean survival larvae of extracts treated tubes and control. LC_{50} values were obtained by best-fit line method where the line that best represents the trend that the points in a scatter plot follows.

CHAPTER 3

3.0 RESULTS

3.1 Plant Extraction

The percentage yield of plant extracts (to 3.s.f) ranged from 4.62 ± 1.34% to 24.5± 0.870% depending on the initial amounts of plant material measured. The highest percentage yield was obtained from *P. africanum* root as shown in Table 3. The yields provided adequate samples for all the tests done.

Table 3: % Yield of Plant Samples

No	Plant Extract	Plant part	%Plant Sample Yield / g
1	*Annona stenophylla*	leaves	8.62 ±1.00
2	*Annona stenophylla*	roots	4.62 ±1.30
3	*Clausena anisata*	leaves	20.3±0.350
4	*Dicoma anomala*	tuber	14.0±0.830
5	*Erythrina abyssinica*	roots	23.6 ±2.37
6	*Horlarrhena pubescens*	leaves	17.0 ±2.23
7	*Horlarrhena pubescens*	roots	11.7 ±2.27
8	*Lannea edulis*	leaves	11.9 ±2.17
9	*Peltophorum africanum*	leaves	12.4 ±1.95
10	*Peltophorum africanum*	bark	23.4 ±3.15
11	*Peltophorum africanum*	roots	24.5±0.870
12	*Pterocarpus angolensis*	bark	9.85 ±0.530
13	*Pterocarpus angolensis*	roots	18.0 ±1.68
14	*Turrea nilotica*	roots	8.75 ±1.31
15	*Vangueria infausta*	leaves	10.2±0.770
16	*Vangueria infausta*	roots	14.9±0.930
17	*Ximenia caffra*	leaves	15.2 ±2.83
18	*Ximenia caffra*	roots	12.5 ±1.71
19	*Ziziphus mucronata*	leaves	11.8 ±1.10
20	*Ziziphus mucronata*	roots	13.4 ±2.10

3.2 Phytochemical Screening

Phytochemical screening was carried out using at least three methods. Preliminary screening was followed by confirmatory tests. This was necessary because of the varied sensitivities of the methods employed in detecting constituents that can occur with trace quantities in some cases.

The results of this study indicated that at least one phytochemical group was present in the plant extracts screened.

Table 4: Phytochemical and confirmatory test results of selected medicinal plants

No	Plant Name	Plant part	Alk	Flav	Sap	Coum	Anthr	Card	Tan
1	*Annona stenophylla*	leaves	+	-	+++	-	++	-	+
2	*Annona stenophylla*	roots	-	-	-	-	-	-	-
3	*Clausena anisata*	leaves	-	-	-	-	-	-	+++
4	*Dicoma anomala*	tuber	-	-	++	- -	-	-	+
5	*Erythrina abyssinica*	roots	++	++	+++	++	++	++	-
7	*Horlarrhenapubescens*	leaves	-	++	+	+	+++	++	++
6	*Horlarrhena pubescens*	roots	+++	-	+++	-	-	-	-
8	*Lannea edulis*	leaves	-	+	-	-	-	-	+++
9	*Peltophorumafricanum*	leaves	-	-	-	-	-	-	+++
10	*Peltophorum africanum*	bark	-	+++	+++	++	+ ++	++	++
11	*Peltophorum africanum*	root	-	+++	+++	+	++	+++	++
12	*Pterocarpus angolensis*	bark	-	-	+	-	-	-	++
13	*Pterocarpus angolensis*	roots	-	-	++	-	-	-	++
14	*Turrea nilotica*	roots	-	-	+	-	-	+	-
15	*Vangueria infausta*	leaves	-	+++	+++	-	-	-	++
16	*Vangueria infausta*	roots	-	-	+++	-	-	-	+
17	*Ximenia caffra leaves*	leaves	-	-	-	-	-	++	+++
18	*Ximenia caffra root*	roots	-	+	+	+	-	+++	+++
19	*Ziziphus mucronata*	leaves	-	+	+	-	-	-	++
20	*Ziziphus mucronata*	roots	-	-	+++	-	-	+	++

Key: Alk=Alkaloids,Flav=flavonoids,Sap=saponins,Coum=coumarins,Anthr=anthraquinones, Card=cardiac glycosides, Tan=tannins

+= trace quantities detected, ++=medium quantities detected, ++++=lots of quantities detected

As shown in table 4, the phytochemical distribution of compounds was as follows: 15% plant samples contained alkaloids, 25% anthraquinone derivatives and coumarins, 40% cardiac glycosides and flavonoids, 75% saponins and 80% tannins.

Saponins and tannins were most abundant phytochemicals found in this study. This can be explained by the fact that they fall under phenols and phenols constitute the largest group of plant secondary metabolites and are widespread in nature (Trease and Evans, 2002). *A.*

stenophylla roots did not contain any phytochemical constituent. For the rest of the plant extracts each plant extract contained at least one constituent. Eight of the extracts contained only 2 phytochemical constituents. The most constituents were found in 4 of the extracts that is *P. africanum* bark and leaves*, H. pubescens* leaves and *E. abyssinica* root. *X.caffra* root followed closely with 5 chemical constituents and this alone made these plants potentially more useful plants. Sample of the TLC plates that resulted from the runs made.

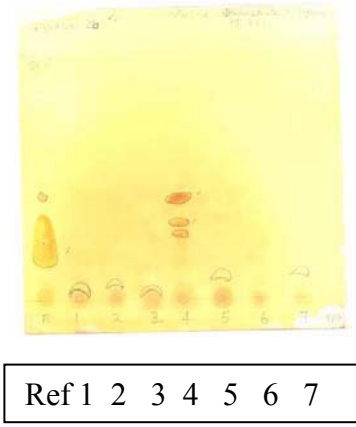


Figure 26: TLC plate 2 for alkaloid screening

Key: Ref=reference compound atropine, 1=*Pterocarpus angolensis* bark, 2= *Pterocarpus angolensis* root, 3=*Erythrina abyssinica* root, 4= *Holarrhena pubescens* root 5= *Holarrhena pubescens* leaves, 6=*Ziziphus mucronata* root, 7= *Ziziphus mucronata* leaves

Holarrhena pubescens root gave a positive result for alkaloids (Fig 26).

3.3 Antioxidant Activities

Phytochemicals, especially phenolics are suggested to be the major bioactive compounds for health benefits. Plant extracts, which contain different classes of polyphenols, are very attractive not only in modern phytotherapy but also in the food industry. Therefore, in this study, we investigated the total phenolic content and antioxidant properties of methanolic plant extracts of selected medicinal plants (Muchuweti *et al.,* 2006).

Table 5: Antioxidant Activity (% Inhibitions) and Total Phenolic Contents of Plant Extracts

No	Plant Extract	Plant part	DPPH %Inhibition	TAE mg/100mg plant
1	*Annona stenophylla*	leaves	83.3±1.70	0.122± 0.00509
2	*Annona stenophylla*	roots	81.2±0.141	0.0186±0.00424
3	*Clausena anisata*	leaves	80.9±0.778	0.110± 0.00375
4	*Dicoma anomala*	tuber	50.1±0.919	0.0301±0.00403
5	*Erythrina abyssinica*	roots	64.6±0.848	0.0601±0.00382
7	*Holarrhena pubescens*	leaves	83.2±0.0707	0.230±0.00537
6	*Holarrhena pubescens*	roots	82.9±0.0707	0.292±0.00283
8	*Lannea edulis*	leaves	93.9±0.00	0.257±0.00460
9	*Peltophorum africanum*	leaves	97.6±0.354	0.380±0.00495
10	*Peltophorum africanum*	bark	96.5±0.354	0.438±0.00424
11	*Peltophorum africanum*	root	96.4±0.00	0.324±0.00389
12	*Pterocarpus angolensis*	bark	96.5±0.141	0.231±0.00318
13	*Pterocarpus angolensis*	roots	91.4±0.990	0.306±0.00636
14	*Turrea nilotica*	roots	82.8±0.354	0.200±0.00764
15	*Vangueria infausta*	leaves	93.1±0.0707	0.00482±0.00255
16	*Vangueria infausta*	roots	39.7±0.212	0.0159±0.00233
17	*Ximenia caffra*	leaves	95.7±0.0707	0.271±0.00325
18	*Ximenia caffra*	roots	95.7±0.0707	0.332±0.00396
19	*Ziziphus mucronata*	leaves	84.6±0.0707	0.0586±0.00417
20	*Ziziphus mucronata*	roots	84.1±0.849	0.239±0.0629
21	*Beta carotene (standard)*		98.6±0.100	-

The Antioxidant activity %inhibition (to 3.s.f) reached nearly 100% for the standard b-carotene – 98.6 ± 0.100%, *P.africanum* leaves, bark, roots – 97.6±0.354%, 96.3±0.354%, 96.4±0.00% respectively and *P. angolensis* bark – 96.5±0.141% as shown in Table 5 showing the highest values. The lowest value was recorded in *V. infausta* leaves. The standard deviations were calculated to 3 significant figures and it measured the variability of the data sets which in this case were not that dispersed as shown by the small differences.

The total phenolic content was expressed as milligrams tannic acid per 100 mg plant sample using the Folin C method described earlier in the methodologies. Table 5 shows the different levels of the phenolic compounds present in the different plant extracts. A high total phenolic content was observed for *P.africanum* bark 0.438±0.00424 mg/100mg and the lowest for *V.infausta* leaves 0.0048±0.00255 mg/100mg TAE.

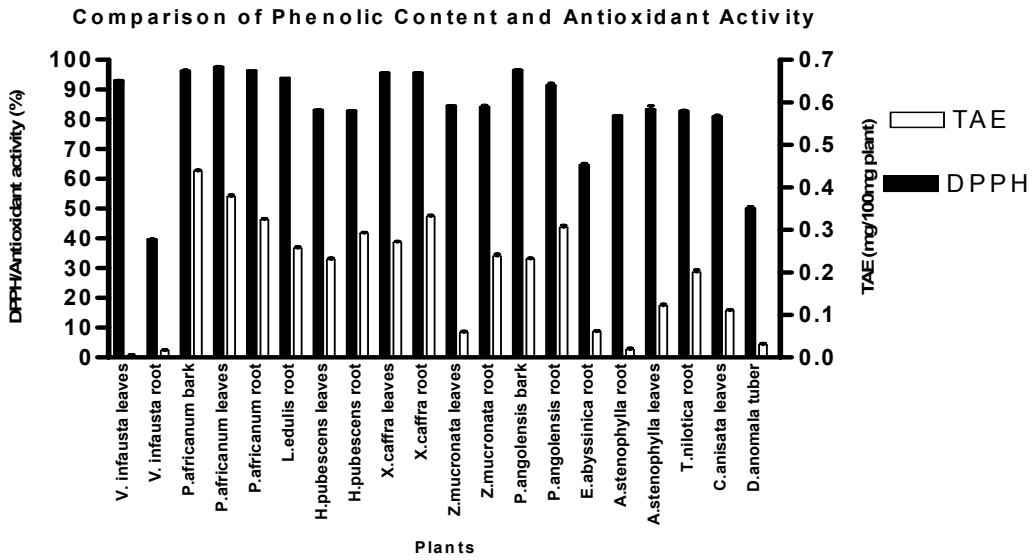


Figure 27: Comparison of the Total Phenolic Content and % Inhibitions of the Plant Extracts

Generally as the antioxidant activity decreased the amount of total phenolic compounds also decreased.

3.4 Toxicity Tests

The plants from the districts were screened for toxicity and implications for other uses and most were found to be safe to moderate safe. The toxic plants have potential for use as antitumour drugs. These preliminary observations give an early indication of safety when plants are used in traditional medicinal practice.

Table 6: Brine Shrimp Lethality Test results (LC_{50} µg/ml)

No	Plant Extract	Plant part	LC50 µg/ml	Toxicity
1	*Annona stenophylla*	leaves	1 190 ± 212	S
2	*Annona stenophylla*	roots	2 300 ± 276	S
3	*Clausena anisata*	leaves	533± 37.0	MS
4	*Dicoma anomala*	tuber	3 040 ± 1060	S
5	*Erythrina abyssinica*	roots	5 440 ± 0	S
7	*Horlarrhena pubescens*	leaves	2 260 ± 484	S
6	*Horlarrhena pubescens*	roots	2 260± 930	S
8	*Lannea edulis*	leaves	971 ± 86	MS
9	*Peltophorum africanum*	leaves	913 ± 7.32	MS
10	*Peltophorum africanum*	bark	882 ± 106	MS
11	*Peltophorum africanum*	root	1 060 ± 106	S
12	*Pterocarpus angolensis*	bark	478 ± 29.7	MS
13	*Pterocarpus angolensis*	roots	1 320 ± 266	S
14	*Turrea nilotica*	roots	701 ± 25.6	MS
15	*Vangueria infausta*	leaves	338 ± 23.4	MS
16	*Vangueria infausta*	roots	416± 28.3	MS
17	*Ximenia caffra*	leaves	1 020 ± 52.7	S
18	*Ximenia caffra*	roots	1 590 ± 752	S
19	*Ziziphus mucronata*	leaves	4 560± 1540	S
20	*Ziziphus mucronata*	roots	1 180 ± 144	S
21	*Neriium oleander* (+ control)	leaves	142± 68.2	T

Key: S = safe = LC_{50} values greater than 1000µg/ml, MS = moderately safe= LC_{50} values between 250 and 1000µg/ml, T =toxic = LC_{50} values of less than 50µg/ml

All the plants did show a high concentration (to 3.s.f) that was killing half the brine shrimp eggs. 40% of the plant extracts did show moderately safe results. Three plant extracts showing moderately safe results gave values that were quite low and were almost approaching the toxic level marked at 250mg/ml and these were, *V. infausta* root and leaves, 416± 28.3 and 338 ± 23.4µg/ml respectively and *P. angolensis* bark 478 ± 29.7µg/ml.

3.5 Antimicrobial Activity

The well plate diffusion method as outlined in the methodology was used to obtain the antimicrobial test results. The zone of inhibition was measured as the distance from the edge of the hole to the periphery of the bacterial/fungal inhibition, using a ruler. The zone was thus not

inclusive of the well diameter (4mm) and was taken as the radius not the diameter of the circle formed. Four readings were taken for each extract against a microbe and the average value and standard deviation was calculated. The results are as presented in table 7.

Table 7: Plants studied showing potential antibacterial activity

No	Plant (10mg/ml)	Plant part	Microorganism, zone of inhibition (radius, mm)			
			Sa Gm $^{+ve}$	***S Gp A*** Gm $^{+ve}$	***Ec*** Gm $^{-ve}$	***Pa*** Gm $^{-ve}$
1	Annona stenophylla Muroro(Sh), Ububese(Nd) Annonaceaea (CU)	leaves	1.00±0.00	1.50±0.58	-	-
2	Annona stenophylla Muroro(Sh), Ububese(Nd) Annonaceaea (CU)	roots	-	3.00±0.00	-	2.75±0.96
3	***Clausena anisata*** Muvengahonye(Sh) Rutaceae (LR)	tuber	-	-	-	1.25±0.5
4	***Dicoma anomala*** Chifumuro(Sh),Ukhalimela(Nd) Asteraceae (VT)	roots	3.25±0.5	4.25±0.5	-	-
5	***Erythrina abyssinica*** Munhimbiti(Sh), Umgqogqogqo(Nd) Faboideceae (T)	leaves	6.0±0	5.75±0.96	1.25±0.5	-
6	***Holarrhena pubescens*** Muhatsu(Sh), Umhatsu(Nd) Apocynacea (T)	leaves	1.25±0.5	3.0 ±0	-	-
7	***Holarrhena pubescens*** Muhatsu(Sh), Umhatsu(Nd) Apocynacea (T)	roots	-	1.0±0	-	-
8	***Lannea edulis*** Mutsombori(Sh), Intakubomvu(Nd) Annonaceaea (CU)	leaves	2.25±0.50	2.75±0.96	-	5.00±0.82
9	***Peltophorum africanum*** Muzeze(Sh), Umsehla(Nd) Fabaceae (CU)	leaves	2.00±0.00	3.75±0.50	-	-
10	***Peltophorum africanum*** Muzeze(Sh), Umsehla(Nd) Fabaceae (CU)	bark	3.00±0.00	4.75±0.50	4.0±0.82	-
11	***Peltophorum africanum*** Muzeze(Sh), Umsehla(Nd) Fabaceae (CU)	roots	4.25±0.50	4.50±0.58	10.0±0.82	9.75±0.96
12	***Pterocarpus angolensis*** Mubvamaropa(Sh), UmvagaziNd) Fabaceae (T)	bark	4.75±0.50	3.00±0.00	-	3.00±0.82
13	***Pterocarpus angolensis*** Mubvamaropa(Sh), UmvagaziNd) Fabaceae (T)	roots	4.00±0.00	3.75±0.50	10.75±0.50	3.00±0.82
14	***Turrea nilotica*** Mukandanyoka(Sh) Meliaceae (CU)	roots	5.00±0.00	3.50±0.58	4.50±0.58	-
15	***Vangueria infausta*** Mutsviru(Sh), Umthofu(Nd) Rubiaceae (CU)	leaves	-	1.75±0.5	1.5±0.58	3.25±0.5

No	Plant (10mg/ml)	Plant part	Microorganism, zone of inhibition (radius, mm)			
			Sa Gm $^{+ve}$	***S Gp A*** Gm $^{+ve}$	***Ec*** Gm $^{-ve}$	***Pa*** Gm $^{-ve}$
17	***Ximenia caffra*** Munhengeni(Sh), Umthunduluka(Nd) Olacaceaea (CU)	leaves	2.0±0	3.25±0.50	2.75±0.96	-
18	***Ximenia caffra*** Munhengeni(Sh), Umthunduluka(Nd) Olacaceaea (CU)	roots	5.00±0.82	5.50±0.56	10.75±0.50	5.75±1.71
19	***Ziziphus mucronata*** Muchecheni(Sh), Umphafa(Nd) Rhamnaceae (CU)	leaves	-	-	3.25±0.96	-
20	***Ziziphus mucronata*** Muchecheni(Sh), Umphafa(Nd) Rhamnaceae (CU)	roots	3.50±0.58	5.00±0.00	-	-
	Antimicrobial controls at 10µg concentrations					
	Ampicillin Gm $^{+ve}$		4.00	9.00		
	Amoxycillin Gm $^{+ve}$		5.00	4.00		
	Tetramycin Gm -^{ve}				11.00	12.00
	Gentamicin Gm $^{-ve}$				12.00	13.00

Key: Sa=Staphylococcus aureus, S GpA=Streptococcus Group A, Ec=Escherichia coli, Pa=Pseudomonas aeruginosa, Gm $^{+ve}$ =Gram positive bacteria, Gm $^{+ve}$ =Gram negative bacteria, -=no zone of inhibition resulted, lvs=leaves, rts=roots, brk=bark, Sh=Shona, Nd=Ndebele, CU= commonly used plant, T=Threatened plant, VT=Very threatened plant, LR=Lower risk

The zones of inhibition which indicate bacterial susceptibility taken as the radius ranged from 0 to more than 10mm. Plant extracts which showed zones of more than 6mm were ranked as highly active. Those that were between 4 and 6mm were of intermediate activity whilst those with less than 4 were of low activity and the plant extracts with no zone of inhibition were rendered inactive. The gram positive bacteria, *S. aureus* and *Strep. Group A* were more susceptible to the plant extracts as compared to the gram negative bacteria *E. coli* and *P. aeruginosa*. 17/20 extracts did give zones of inhibition against S. group A: this was the bacterial strain that had the highest number of extracts showing activity. This was followed by *S. aureus* which had 14/20 extracts giving zones of inhibition. 9/20 extracts inhibited the growth of *E. coli* whilst only 8/20 extracts inhibited the growth of *P. aeruginosa* and this gave the least number of extracts showing inhibition zones.

The largest zones of inhibition resulted from the plant extracts *X. caffra* root and *P. angolensis* root against *E.coli*; a reading of 10.75 ± 0.5mm. High zones were also recorded from *P. africanum* root against microorganisms *E. coli* and *P. aeruginosa*; readings of 10.0 ± 0.82mm and 9.75 ± 0.96mm respectively. Despite the fact that most extracts did show zones of inhibition against gram positive bacteria the highest of the readings were obtained from gram negative bacteria. The smallest zone of inhibition values were obtained from plant extracts *A.*

stenophylla leaves against bacterial strain *S. aureus* and *E. abyssinica* root against *Strep. group A*; both with a reading of 1.0 ± 0mm. Small values were also recorded from *A. stenophylla* leaves and *C. anisata* leaves against *S. aureus*; readings of 1.5 ± 0.58mm and 1.25 ± 0.5mm respectively.

Out of all the runs done for all the bacterial strains; 28 plant extracts showed no activity against the strains at different instances. The control antibiotics used for gram positive bacteria were amoxicillin and ampicillin which gave zones of inhibition of 5mm and 4mm respectively. Some of the plant extracts did show bigger zones of inhibition compared to these standards. For gram negative bacteria the controls tetracycline and gentamicin gave readings of 11mm and 12mm respectively and these readings were higher than all the plant extracts readings recorded. Most of the plant extracts under study did show activity in one way or the other.

Table 8: Plants studied showing potential antifungal activity

No	PlantsUsed	Plant part	Microorganism, zone of inhibition(radius, mm)	
			C.albicans	A.niger
1	Annona stenophylla Muroro(Sh), Ububese(Nd) Annonaceaea (CU)	leaves	-	-
2	Annona stenophylla Muroro(Sh), Ububese(Nd) Annonaceaea (CU)	roots	1.50±0.58	3.75±0.50
3	***Clausena anisata*** Muvengahonye(Sh) Rutaceae (LR)	leaves	1.25±0.5	-
4	***Dicoma anomala*** Chifumuro(Sh), Ukhalimela(Nd) Asteraceae (VT)	tuber	5.5±0.58	-
5	***Erythrina abyssinica*** Munhimbiti(Sh), Umgqogqogqo(Nd) Faboideceae (T)	roots	-	2.25±0.5
6	***Holarrhena pubescens*** Muhatsu(Sh), Umhatsu(Nd) Apocynacea (T)	leaves	3.0±0.82	1.0±0
7	***Holarrhena pubescens*** Muhatsu(Sh), Umhatsu(Nd) Apocynacea (T)	roots	-	-
8	***Lannea edulis*** Mutsombori(Sh), Intakubomvu(Nd) Annonaceaea (CU)	leaves	1.75±0.96	1.00±0.58
9	***Peltophorum africanum*** Muzeze(Sh), Umsehla(Nd) Fabaceae (CU)	leaves	-	
10	***Peltophorum africanum*** Muzeze(Sh), Umsehla(Nd) Fabaceae (CU)	bark	4.00±0.82	3.00±0.00
11	***Peltophorum africanum*** Muzeze(Sh), Umsehla(Nd) Fabaceae (CU)	roots	2.25±0.50	2.50±0.58
12	***Pterocarpus angolensis*** Mubvamaropa(Sh), UmvagaziNd) Fabaceae (T)	bark	-	-
13	***Pterocarpus angolensis*** Mubvamaropa(Sh), UmvagaziNd) Fabaceae (T)	roots	3.25±0.50	2.50±0.58
14	***Turrea nilotica*** Mukandanyoka(Sh) Meliaceae (CU)	roots	1.50±0.58	1.75±0.50

No	Plants Used	Plant Part	Microorganism, zone of inhibition(radius, mm)	
			C.albicans	A.niger
15	***Vangueria infausta*** Mutsviru(Sh), Umthofu(Nd) Rubiaceae (CU)	leaves	1.25±0.50	-
16	***Vangueria infausta*** Mutsviru(Sh), Umthofu(Nd) Rubiaceae (CU)	roots	2.0±0.82	1.5±0.5
17	***Ximenia caffra*** Munhengeni(Sh), Umthunduluka(Nd) Olacaceaea (CU)	leaves	1.75±0.50	1.25±0.50
18	***Ximenia caffra*** Munhengeni(Sh), Umthunduluka(Nd) Olacaceaea (CU)	roots	2.75±0.96	2.75±0.5
19	***Ziziphus mucronata*** Muchecheni(Sh), Umphafa(Nd) Rhamnaceae (CU)	leaves	-	-
20	***Ziziphus mucronata*** Muchecheni(Sh), Umphafa(Nd) Rhamnaceae (CU)	roots	2.75±0.50	3.75±0.50
21	AmphotericinB (Positive control at 10µg concentration)		6.35±0.5	6.75±0.58

Key: Sh=Shona, Nd=Ndebele, CU= commonly used plant, T=Threatened plant, VT=Very threatened plant, LR=Lower risk

As for the fungi more than three quarters of the extracts did show activity against *Candida albicans* and *Aspergillus niger*. The highest zone of inhibition was shown by *D. anomala* tuber against *C. albicans*; reading of 5.5 ± 0.58mm, *P. africanum* bark against *C. albicans*; reading of 4.0 ± 0.82mm and *Z. mucronata* root against *A. niger*; a reading of 3.75 ± 0.5mm. The lowest values were recorded from *C. anisata* leaves and *L. edulis* root against *A. niger*; readings of 1.00 ± 0mm and 1.00 ± 0.58mm respectively. The highest value was recorded for the positive control Amphotericin B 6.35±0.5 and 6.75±0.58mm.

The extracts that showed activity on antibacterial activity determinations were further tested for minimum inhibitory concentration (MIC) and minimum bactericidal/ fungicidal concentration (MBC/MFC) and the results are as shown in the tables 9 and 10. All the runs were done in duplicate and average values taken as the MICs.

Table 9: Minimum Inhibitory Concentration (MIC) in mg/ml

No	Plant Used	Part Used	S.A	S.G.A	E.C	P.A	C.A	A.N
1	Annona stenophylla Muroro(Sh), Ububese(Nd) Annonaceaea (CU)	leaves	>10	>10	-	-	-	-
2	*Annona stenophylla* Muroro(Sh), Ububese(Nd) Annonaceaea (CU)	roots	-	>10	-	10	>10	2.5
3	***Clausena anisata*** Muvengahonye(Sh) Rutaceae (LR)	leaves	-	-	-	-	>10	-
4	***Dicoma anomala*** Chifumuro(Sh), Ukhalimela(Nd) Asteraceae (VT)	tuber	>10	2.5	-	-	1.25	-
5	***Erythrina abyssinica*** Munhimbiti(Sh), Umgqogqogqo(Nd) Faboideceae (T)	roots	0.23	1.25	>10	-	-	5
6	***Holarrhena pubescens*** Muhatsu(Sh), Umhatsu(Nd) Apocynacea (T)	leaves	5	1.875	-	-	2.5	10
7	***Holarrhena pubescens*** Muhatsu(Sh), Umhatsu(Nd) Apocynacea (T)	roots	-	>10	-	-	-	-
8	***Lannea edulis*** Mutsombori(Sh), Intakubomvu(Nd) Annonaceaea (CU)	leaves	5	2.5	-	2.5	5	5
9	***Peltophorum africanum*** Muzeze(Sh), Umsehla(Nd) Fabaceae (CU)	leaves	5	2.5	-	-	-	-
10	***Peltophorum africanum*** Muzeze(Sh), Umsehla(Nd) Fabaceae (CU)	bark	>10	1.25	1.25	-	10	5
11	***Peltophorum africanum*** Muzeze(Sh), Umsehla(Nd) Fabaceae (CU)	roots	3.75	2.5	0.313	0.625	>10	5
12	***Pterocarpus angolensis*** Mubvamaropa(Sh), UmvagaziNd) Fabaceae (T)	bark	1.25	2.5	-	2.5	-	-
13	***Pterocarpus angolensis*** Mubvamaropa(Sh), UmvagaziNd) Fabaceae (T)	roots	1.25	1.25	0.625	>10	5	5
14	***Turrea nilotica*** Mukandanyoka(Sh) Meliaceae (CU)	roots	0.625	>10	2.5	-	>10	5

No	Plant/Part Used	Part Used	S.A	S.G.A	E.C	P.A	C.A	A.N
15	***Vangueria infausta*** Mutsviru(Sh), Umthofu(Nd) Rubiaceae (CU)	leaves	-	5	10	10	>10	5
16	***Vangueria infausta*** Mutsviru(Sh), Umthofu(Nd) Rubiaceae (CU)	roots	0.625	-	5	-	>10	10
17	***Ximenia caffra*** Munhengeni(Sh), Umthunduluka(Nd) Olacaceaea (CU)	leaves	5	5	10	-	10	5
18	***Ximenia caffra*** Munhengeni(Sh), Umthunduluka(Nd) Olacaceaea (CU)	roots	1.25	1.875	0.625	2.5	5	3.75
19	***Ziziphus mucronata*** Muchecheni(Sh), Umphafa(Nd) Rhamnaceae (CU)	leaves	-	1.25	>10	-	-	-
20	***Ziziphus mucronata*** Muchecheni(Sh), Umphafa(Nd) Rhamnaceae (CU)	roots	2.5	-	-	-	5	2.5

LR=Lower risk, S.A= *Staphylococcus aureus*, S.G.A=*Streptococcus Group A*, E.C=*Escherichia coli*, P.A= *Pseudomonas aeruginosa*, C.A= *Candida albicans*, A.N= *Aspergillus niger*

The lowest concentration (highest dilution) of antibiotic/plant extract preventing appearance of turbidity (growth of microorganism) is considered to be the minimal inhibitory concentration (MIC). In table 9, the plant extracts that showed the lowest concentration that prevented growth of *S. aureus* were *E. abysinica* (0.23mg/ml), *T. nilotica* root and *V. infausta* leaves both (0.625 mg/ml). The readings show that it only takes a little of the plant extract in order to be able to inhibit the bacteria. Plants that showed the highest concentrations that could not be determined as they were more than the neat solution were *A. stenophylla* leaves, *D. anomala* tuber and *P. africanum* bark; MIC value >10mg/ml.

The lowest concentration that inhibited growth of *Strep group A* bacteria was obtained from plant extracts *E. abyssinica* root*, P. africanum* bark and *P. angolensis* root; value of 1.25mg/ml. The MIC for four plants extracts *A. stenophylla* leaves and root, *H. pubescens* root and *Z. mucronata* could not be determined as it was above the neat solutions value of 10mg/ml. The concentration that inhibits all the bacteria could not be determined for eight of the extracts as readings went beyond 10mg/ml as well.

E.coli is a gram negative bacterium and 10 plant extracts which had shown activity on the zones of inhibition were further tested for MIC determinations. Two extracts, *Z. mucronata* leaves and *E. abyssinica* root gave MIC's greater than 10mg/ml and so the exact minimum inhibitory concentration could not be determined. *P. africanum* root gave the least MIC value of 0.313mg/ml meaning that even at low concentrations it can still inhibit the growth of *E. coli.*

Only 7 plant extracts showed activity against bacteria *P. aeruginosa*. The least MIC value was obtained from *P. africanum* root, 0.625mg/ml. only one extract *P. angolensis* root gave a value greater than the neat solution , >10 mg/ml and so the exact MIC value could not be determined. *P. aeruginosa* generally require more concentrated extracts to show activity.

14 plant extracts were tested for MIC against *C. albicans*. Six of the extracts did show MIC values greater than 10mg/ml and so the exact value of inhibition could not be determined. The extract that needed the least concentration in order to inhibit the fungus was *Dicoma anomala tuber*, a concentration of 1.25mg/ml. the rest of the extracts needed at least 5mg/ml.

Two plants extract *H. pubescens* and *V. infausta* gave 10mg/ml as the MIC value against *A. niger* and this was the value of the neat solution. The least concentration of inhibition was obtained from the extract *A. stenophylla* root 2.5mg/ml and Z. mucronata root.

Table 10: Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) in mg/ml

No	Plant Used	Part Used	S.A	S.G.A	E.C	P.A	C.A	A.N
1	Annona stenophylla Mururo(Sh), Ububese(Nd) Annonaceaea (CU)	leaves	>10	>10	-	-	-	-
2	Annona stenophylla Mururo(Sh), Ububese(Nd) Annonaceaea (CU)	roots	-	>10	-	>10	>10	10
3	***Clausena anisata*** Muvengahonye(Sh) Rutaceae (LR)	leaves	-	-	-	-	>10	-
4	***Dicoma anomala*** Chifumuro(Sh), Ukhalimela(Nd) Asteraceae (VT)	tuber	>10	>10	-	-	5	-
5	***Erythrina abyssinica*** Munhimbiti(Sh), Umgqogqogqo(Nd) Faboideceae (T)	roots	5	>10	>10	-	-	10
6	***Holarrhena pubescens*** Muhatsu(Sh), Umhatsu(Nd) Apocynacea (T)	leaves	>10	>10	-	-	10	>10
7	***Holarrhena pubescens*** Muhatsu(Sh), Umhatsu(Nd) Apocynacea (T)	roots	-	>10	-	-	-	-
8	***Lannea edulis*** Mutsombori(Sh), Intakubomvu(Nd) Annonaceaea (CU)	leaves	>10	10	-	10	>10	>10
9	***Peltophorum africanum*** Muzeze(Sh), Umsehla(Nd) Fabaceae (CU)	leaves	>10	10	-	-	-	-
10	***Peltophorum africanum*** Muzeze(Sh), Umsehla(Nd) Fabaceae (CU)	bark	10	>10	10	-	10	10
11	***Peltophorum africanum*** Muzeze(Sh), Umsehla(Nd) Fabaceae (CU)	roots	10	10	5	2.5	>10	10
12	***Pterocarpus angolensis*** Mubvamaropa(Sh), UmvagaziNd) Fabaceae (T)	bark	5	10	-	10	-	-
13	***Pterocarpus angolensis*** Mubvamaropa(Sh), Umvagazi(Nd) Fabaceae (T)	roots	10	10	5	>10	10	10
14	***Turrea nilotica*** Mukandanyoka(Sh) Meliaceae (CU)	roots	2.5	10	5	-	>10	10
15	***Vangueria infausta*** Mutsviru(Sh), Umthofu(Nd) Rubiaceae (CU)	leaves	-	>10	>10	>10	>10	>10
16	***Vangueria infausta*** Mutsviru(Sh), Umthofu(Nd) Rubiaceae (CU)	roots	5	-	10	-	>10	>10

No	Plant Used	Part Used	S.A	S.G.A	E.C	P.A	C.A	A.N
17	***Ximenia caffra*** Munhengeni(Sh), Umthunduluka(Nd) Olacaceaea (CU)	leaves	>10	>10	>10	-	>10	>10
18	***Ximenia caffra*** Munhengeni(Sh), Umthunduluka(Nd) Olacaceaea (CU)	roots	5	5	10	>10	10	5
19	***Ziziphus mucronata*** Muchecheni(Sh), Umphafa(Nd) Rhamnaceae (CU)	leaves	-	-	>10	-	-	-
20	***Ziziphus mucronata*** Muchecheni(Sh), Umphafa(Nd) Rhamnaceae (CU)	roots	10	10	-	-	10	5

Key: Sh=Shona, Nd=Ndebele, CU= commonly used plant, T=Threatened plant, VT=VerY threatened plant, LR=Lower risk, S.A= *Staphylococcus aureus*, S.G.A=*Streptococcus Group A*, E.C=*Escherichia coli*, P.A= *Pseudomonas aeruginosa*, C.A= *Candida albicans*, A.N= *Aspergillus niger*

The minimum bactericidal/fungicidal concentration determination was performed as an adjunct to the MIC and was used to determine the concentration of the antibiotic that is lethal to the target bacteria/fungi in vitro. The concentration values were more than the MIC values showing that the MIC does not necessarily inhibit all the microorganisms but a more concentrated dilution may inhibit all the organisms and no growth is observed. The minimum bactericidal/fungicidal concentration for most of the plants could not be determined as it was above the 10mg/ml for the neat extracts. *Peltophorum africanum* root against *P. aeruginosa* and *Turrea nilotica* against *S. aureus* gave the least concentration of 2.5mg/ml meaning only little quantities of the extract is required to totally inhibit all the microoganisms in the tube.

CHAPTER 4

4.0 Discussion

Medicinal plants constitute an effective source of both traditional and modern medicine, herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care. Over the years, the World Health Organization advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origins (World Health Organization, 1978).

4.1 *Annona stenophylla*:

Phytochemical screening: the leaves and the root extracts of the plant were investigated for different phytochemical compounds. The root extract did not give any chemical constituent screened for. The leaves indicated the presence of four chemical groups namely alkaloids, saponins, anthraquinones and tannins as shown in table 4. The results for the leaves do indicate that the part can be used as a potential anti-infective as different medicinal properties are associated with the chemical constituents found table 1 shows the uses. *Annona stenophylla* has been reported to treat gonorrhoea, syphilis and abdominal pains. Infusions, which are made with other plants, are taken by mouth. The roots provide a strong medicine for treating tooth pain and the infusion is cooled down before using to rinse the mouth and it is spat out (Gelfand *et al.,* 1985). The fact that *A. stenophylla* is used as an infusion may explain the absence of some other important chemical constituents especially in the root part for it to able to treat the ailments mentioned. In infusions synergism may take place and result in some other cures otherwise not expected.

Antioxidant activity: The plant's leaves gave a relatively high total phenolic content of 0.122±0.00509mg/100mg plant sample (table 5) whilst the root gave 0.0186±0.00424mg/100mg plant sample. The difference can be explained by the fact that the roots of the plant did not contain any of the screened phytochemical compounds of which the leaves contained four of the groups namely alkaloids, saponins, anthraquinones and tannins. The percentage inhibition of the leaves was 83.3% which is a high value that correlate with the high total phenolic content and the phytochemical compounds found. It took less than two minutes for the leaves *Annona stenophylla* to complete the DPPH reaction and this shows that it is a potential antioxidant. Previous studies at the University of Pretoria have reported antioxidant activity in the juicy parts of other species of *Annona*.

Antimicrobial activity: The leaf extract showed little activity against the gram positive bacteria, no activity against gram negative bacteria and fungi that it was tested against. Little activity shown can be justified by the presence of the phytochemical groups found in the leaf extract. The root extract showed medium activity against *Streptococcus Group A, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger*. The general activity is not justified by the fact that the root extract did not give any chemical constituent screened for in this research but other chemical compounds not screened for in this case could have given rise to the activity shown. The activity could also be due to low amounts of the phytochemical constituents which could have been present but below the detection limits for phytochemical analysis but enough for antiinfective activity inhibition. *Annona stenophylla* has been reported to treat gonorrhoea, syphilis and abdominal pains which go very well with the antimicrobial demonstrated in this research. Matazu and Gundidza 1996 have shown that *Annona stenophylla* contain antifungal activity and this result has been confirmed in the current studies as shown in table 8 where the root extract show zones of inhibition against the fungal strains tested. The MIC for the plant was high >10mg/ml for most of the microorganisms which shows that there is need for much extract to be administered in order to inhibit the microorganisms from growing. MIC values of ≤1mg/ml have been reported for other medicinal plants which show stronger activity (Steenkamp *et al.,* 2007).
Toxicity: the leaves of the plant are safe to use as shown by the low LC_{50} value of brine shrimp lethality. The root also showed a low LC_{50} value of 2 300±276 µg/ml table 6, which indicate that it is safe to use. The safety for use that was proven in the current study does justify the current wide usage of the plant by traditional medicinal practitioners. The toxicity results are also in accordance to previous studies done at the University of Pretoria where the isolated compounds of *Annona stenophylla* subsp. Nana roots showed little or no toxicity towards human lymphocytes.

4.2 *Clausena anisata:*

Phytochemical screening

The leaves of *C. anisata* were found to contain tannins as the only chemical constituent table 4. Previous work by Ito *et al* 2000 has found new carbazole alkaloids which contain antitumour promoting activity in *C. anisata*. Alkaloids in this work were not found and this could probably be because alkaloids are very difficult to screen for or because the environments in which the plants were growing is different and environment influences the types and amounts of secondary metabolites produced.

Antioxidant activity: the leaves of the plant gave a relatively low total phenolic content reading 0.110±0.00375mg/100mg of plant sample table 5. The amount of total phenolic compounds led to the good antioxidant activity value of 80.9±0.778% recorded which is supported by the fact that the tannins that were present as the only phytochemical group screened for could have given

rise to the activity as tannins fall under phenolic compounds which in turn are well known for contributing to antioxidant activity. Many antioxidant compounds, naturally occurring from plant sources, have been identified as free radical or active oxygen scavengers and *Clausena anisata* has been identified as one of the plant sources containing relatively low but good rate of scavenging activity (Jazet *et al.,* 2008)

Antimicrobial activity: The leaves of *C. anisata* were found to be active against gram positive bacteria alone and the fungal strains tested for. Activity against *A. niger* was very small. Literature survey revealed that the powdered roots, with lime and guinea grains are applied to rheumatic and other pains in Nigeria where also the leaves are considered anthelmintic. Clausena is well known for its antidiabetic properties and is therefore widely used by traditional healers (Ojewole *et al.,* 2002). In South Africa it is used to treat diabetes, in Ghana HIV 1, 2 and in Japan to treat cancer. Investigations have also shown that *Clausena anisata* has got a lot of antibacterial properties. Its leaves are crushed and applied to wounds infested with maggots for treatment (Gelfand *et al.,* 1985). The reported pharmacological activities are justified by the results of antimicrobial screening together with the phytochemistry of the plant. The activities reported do not correlate well with the findings in the current study as only one chemical group of compounds was found but the activity can be explained by the fact that not all the phytochemical compounds available were screened for and they could be the ones responsible for the activities. Literature for instance has it that the essential oils distilled from *Clausena anisata* previously showed good antifungal and antibacterial activity (Gundidza *et al.,* 1993). The absence of antibacterial activity in this study yet in others it was found could be because the bacterial strains were different and in this particular study the bacteria was resistant and also the concentrations of extract applied were less and in crude extract form whilst the report by Gundidza *et al.,* 1993 was on specific distilled and concentrated essential oils. The MIC value of (10mg/ml) indicates that a lot of the plant extract is needed to be able to inhibit microbial growth. This result is in agreement with other published results by van Vuuren and Viljoen 2006 whereby a value of 16mg/ml was found as the MIC against *Candida albicans*.

Toxicity: the results from the current study indicate that the leaves of the plant are moderately safe to use meaning they have to be used with great caution. The plant is currently being used and further in vivo tests can be taken to ascertain the safety of the general public using the plant. Previous studies in Southwestern Nigeria have shown that *Clausena anisata* at higher concentrations is toxic to aquatic life. The slight difference in these results is because low concentrations of 10mg/ml were used in this study and exposure period was up to 12 hours whilst in the study by Fafioye 2005 concentrations of more than 10mg/ml and exposure periods ranging from 4 to 48hours were used.

4.3 *Dicoma anomala:*

Phytochemical screening *D. anomala* tuber is widely used in Zimbabwe and it was screened for its phytochemical constituents and two groups of phytochemicals namely saponins and tannins were found to be present. *Dicoma anomala* in previous studies was found to contain small amounts of volatile oil, crystalline glucosides and amorphous alkaloids and phytosterol. *Dicoma anomala* species have also shown compounds known as germacranolides, which are closely related to lactones (Zdero 1989). The difference in the findings may be attributed to different methods of analysis as well as the environmental factors which affect the types of secondary metabolites that accumulate in the plant. The other types of constituents were also not screened for in this study and could have been positive. The groups found justify some of the uses that the traditional healers claim to use the plant for.

Antioxidant activity: *D. anomala* tuber gave a low total phenolic content reading of 0.0301±0.00403mg/100mg plant sample and an antioxidant inhibition of 50.1±0.919%. The small amounts of total phenolic compounds led to the low value of antioxidant activity as phenolic compounds contribute much to the antioxidant activity. It took 20minutes for the plant extract to decolourise the DPPH purple solution to the pale yellow colour which shows that the plant has potential to be used as an antioxidant although the level of potency is quite low compared to the other medicinal plants in this study. The results show that the small amounts of total phenolic content and the two phytochemical groups found in the plant sample contributed to the little activity shown.

Antimicrobial activity: The plant tuber did show the radius of zones of inhibition that were of medium activity against gram positive bacteria, and the highest zone of inhibition against *Candida albicans* 5.5±0.58mm microorganisms. These results indicate the tuber can be used to treat ailments whose causative microorganisms are mainly from gram positive bacteria. Reports from traditional healers and research publications do indicate that the tuber is widely used in the treatment of abdominal pains, gonorrhoea, syphilis, wasting in infants, malaria, skin sores, to drive away bad luck and ulcers. Southern Sotho's use the decoction for venereal diseases and apply the powdered plant to sores and wounds (Gelfand *et al.,* 1985). The phytochemical compounds (saponins and tannins) found in the study could be responsible for the antibacterial and antifungal activities shown by the plant extract. Steenkamp *et al* 2004 also found out that *Dicoma anomala* does have antibacterial activity against several bacterial strains and genera like Staphylococcus aureus and Streptococcus pyogenes and these include some of the ones tested in the current research. MIC value of 1mg/ml was reported by Steenkamp *et al* 2004 whilst in the current study a value of more than 10mg/ml was reported. The difference could have been caused by the original concentrations of the crude plant extract used whereby a more concentrated extract was used by the publisher.

Toxicity: the tuber of the plant gave one of the highest values regarded as safe under the toxicity tests. This observation is well supported by the information from the traditional healers that the tuber is used everywhere and in literature survey there was no mention of any levels of toxicity.

4.4 *Lannea edulis*:

Phytochemical screening: the leaf extract of the plant which was reported to be commonly used by traditional healers was screened for phytochemical constituents and two groups of flavonoids and tannins were found present. It has been found that the dichloromethane root extracts of *Lannea edulis* do contain some important phytochemical groups like alkyl phenols, cardonols and dihydroalkylhexenones Queiroz *et al* 2003. This research is therefore in line with other publications as flavonoids, and tannins are part of phenolic compounds which would need further investigations to establish the exact compounds.

Antioxidant activity: the extract of *Lannea edulis* gave one of the highest values of total phenolic content (0.257±0.00460) and a high antioxidant inhibition percentage of 93.9±0%) table 5. These results show that the high amount of total phenolic compounds influenced the antioxidant activity and this is as well supported by the fact that the extract had flavonoids as one of the phytochemical constituents a group that influences greatly in antioxidant activity. Activity-guided isolation of radical-scavenging compounds from the dichloromethane extract of the root bark of *Lannea edulis* led to isolation of two known bioactive alkyl phenols (cardonol and cardonol), and three new dihydroalkylhexenones were also isolated (Queiroz *et al* 2003). This shows that *Lannea edulis* contain phytochemical compounds that are responsible for antioxidant activity.

Antimicrobial activity: the root extract of the plant which was reported to be commonly used by traditional healers was active against all the microorganisms tested except for *E. coli*. A very high zone of inhibition was seen against *P. aeruginosa* a gram negative bacteria, reading of 5.0±0.82mm. From the literature surveyed not much work has been done on the antimicrobial activity of *L. edulis* but from the phytochemical results antimicrobial activity is justified because of the phytochemical groups present and thus in turn justifies the use of the plant in traditional medical practice.

Toxicity*:* the root extract was found to be moderately safe to use and therefore should be used with great caution as it could lead to undesirable side effects. Further in vivo tests can be done to ascertain the safety of the usage of the plant. Other scientists have reported the ability to induce mutagenecity in *Salmonella typhimurium* strains and so use of the extract should really be with due caution (Sohni *et al.,* 1995).

4.5 *Erythrina abyssinica*:

Phytochemical screening: the root of the plant was used in the screening tests. It did contain a lot of phytochemical constituents which include alkaloids, saponins, anthraquinones and cardiac glycosides. This wide range of constituents does justify its present uses as traditional medicinal plants as all the constituents are associated with pharmacological activity table 1. The results were in accordance with available literature of similar work that has been done. Alkaloids were isolated by Lapiere 1951 whilst flavonoids were found by Moriyasu *et al* 1998.

Antioxidant activity: the total phenolic content of the extract of *Erythrina abyssinica* was (0.0601±0.00382) and the antioxidant inhibition percentage was (64.6±0.849%) as shown in table 5. these values were quite low as compared to the other extract values. The low total phenolic content does explain the rather low antioxidant inhibition percent. Despite the numerous number of phytochemical compounds that the plant contain, its antioxidant activity is average and the reason could be that the total number of hydroxyl groups present in the aromatic constituents of a plant extract, in part, offers better antioxidant properties, it is presumed that compounds present in methanolic extracts belong to different classes of phenolics. These classes most likely have varying antioxidant strengths and that the synergistic effect polyphenolics with one another and/or components present in an extract may contribute to the overall observed antioxidant activity (Shahidi et al., 1994).

Antimicrobial activity: The antimicrobial results for the root of the plant indicated that the plant extract had very high activities recorded against *S. aureus* 6.0±0mm, *Strep. Group A* 5.75±0.96mm and low activity against the gram negative bacteria *E. coli* 1.25±0.5mm and no activity against *P. aeruginosa*. High activities were also recorded for the fungi as shown in table 8. Since the extract contained a lot of phytochemical constituents it was expected to show a lot of activity against the microorganisms used and this was fulfilled. Antibacterial activities of some South African Erythrina species were shown by Puillay *et al* 2001 and the results were in accordance with available literature of similar work that has been done. Reports by Thomik and Munjeri 1993 have indicated that *E. abyssinica* show activity against (*Staphylococcus aureus, Escherichia coil, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa*) and 2 strains of fungi (*Candida albicans, Aspergillus niger*) using the agar diffusion method. *Erythrina abyssinica* and *Warburgia salutaris* had activity against all of the bacteria comparable to between a third and half the activity of 1% Doxycycline. *Erythrina abyssinica* and *Warburgia salutaris* also showed notable activity against *Candida albicans*, and *Aspergillus niger* which was comparable to between 1/3 and 1/2 the activity of Ketoconazole. These results show great similarity in the microorganisms used and the antimicrobial activity to the current work that was done. According to the ethnopharmacology gathered the plant is used in Zimbabwe for treating abdominal pains, gonorrhoea, and wounds in mouth as well as for wasting in infants. These uses

are justified very well by the fact that the extract was able to inhibit *Strep. Group A* and *S. aureus* which are microorganisms found in mouth infections, throat, stomach and genitals.

Toxicity: the root of the plant was found to be safe to use LC_{50} value of 5 440±0µg/ml as shown in table 6. Other literature reports do point out that the seeds of the plant are poisonous but no toxicity reports have been made on the leaf and root extracts and therefore the results in the current study are supported by literature available (Breyer-Brandwick and Watt 1962).

4.6 *Holarrhena pubescens*:

Phytochemical screening: the leaves and the root extracts were screened for the phytochemical constituents and results indicate that the leaf extract contained six of the groups screened for and these include flavonoids, saponins, coumarins, anthraquinones and tannins and gave one of the highest numbers of groups' available table 4. The root extract contained two groups of chemical constituents that are alkaloids and tannins. Presence of these phytochemical constituents does indicate that the plant has got a lot of pharmacological activities. Some alkaloids and steroidal compounds were found in the plant by Siddique *et al* 2001 and this conforms to the results also found in this research. In addition to alkaloids more groups which have not been reported were found especially in the leaves. This suggests that the leaf extract should treat more ailments as it is associated with a lot of chemical groups.

Antioxidant activity: the root and the leaf extracts showed high total phenolic content as well as very high values of antioxidant activity as shown in table 5. The plant is therefore a potential antioxidant. The high phenolic content shows correlation with the antioxidant activity meaning that phenolic compounds contributed to the high antioxidant activity as they are well known for that. This is also supported by the phytochemical screening results which show a lot of compounds that fall under phenolic groups that were positive in the plant extract during screening. The high values of total phenolic content and antioxidant activity have also been shown in other published literature results of traditional medicinal plants (Muchuweti *et al.,* 2006).

Antimicrobial activity*:* The leaves and the root generally showed low to no activity against the microorganisms screened against. The leaves were active against both gram positive bacteria readings of 1.25± 0.5mm and 3.0±0mm against *S. aureus* and *Strep Group A* respectively. The leaves were also active against the fungi whilst the roots were only active against *Strep Group A* as shown in table 8. These results are in agreement with results published by Chakraborty and Brantner in 1999 where *Holarrhena pubescens* bark was tested for antibacterial efficacy against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus faecalis*, *Bacillus subtilis*,

Escherichia coli and *Pseudomonas aeruginosa* using the microdilution broth method as well as the disc diffusion method and the results were positive for the runs. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for the crude extract, the total alkaloids and the neutral fraction using microdilution broth method and showed remarkable activity against *S. aureus* (MIC=95 µg/ml). The MIC value was much lower than the one obtained in the current study (5mg/ml) and the difference could be because the samples used in the current study were not as concentrated as the ones used by Chakraborty and Brantner in 1999. The slight deviation of results could also be because of environmental factors as the plants studied are from different locations. The results f the study show that the leaves can be used to cure more ailments compared to the root. This is supported by the phytochemical results which showed that the leaf extract contained six of the groups screened for whilst the root extract contained only two groups of chemical constituents and presence of these phytochemical constituents does indicate the range of pharmacological activities.

Toxicity: Both the leaves and the roots gave safe values of the concentration killing half the brine shrimps which means they can be used safely to cure different ailments as pointed out by the traditional healers. No other reports on toxicity of the plant were found in the literature available.

4.7 *Peltophorum africanum*:

Phytochemical screening: The root, bark and leaf extracts of the plant were investigated for chemical constituents available as most traditional healers do use all parts of the plant for treatment of different ailments. The bark and root extracts gave six chemical groups which was the highest number of constituents recorded in the experiments table 4. The bark and root extracts gave flavonoids, saponins, coumarins, anthraquinones, cardiac glycosides and tannins whilst the leaf extract gave tannins as the only constituent. Previous studies have shown the presence of tannins and in addition a lot other groups have been found in current studies. The presence of all these groups indicates a lot of pharmacological activity associated, table 1.

Antioxidant activity: the highest total phenolic content and antioxidant activity was found in the root, bark and leaf extracts of the plant. The extracts also contained the most abundant phytochemical compounds some of which fall under phenolic compounds which contribute to antioxidant activity. The plant as a whole can be used as a potential antioxidant to cure the related diseases. Potential of neuroprotective antioxidant based therapeutics of the plant has already been reported by Bizimenyera *et al.,* 2007 and so the results of this current study are not unique but conform to the literature available.

Antimicrobial activity: Generally the root, bark and leaf extracts of the plant was among the best results from the investigations. Activity against all the microorganisms tested was reported from the plant and the root extract gave a zone of inhibition of 9.75±0.96mm against gram negative bacteria *P. aeruginosa* table 7. There are various medicinal uses documented for *P. africanum* roots and bark which in this case were found to be very active. The roots are used to heal wounds, toothaches, and throat sores, whilst the bark, roots and leaves are used to clear intestinal parasites and relieve stomach problems, diarrhea and dysentery. These uses are greatly justified as the microorganisms that were inhibited by the tested extracts are the same ones that are found in the outlined infections. *P. africanum* was one of the very best extracts which appeared in all three categories of gram-positive, gram-negative and fungal inhibiting extracts and this is well supported by the numerous phytochemical constituents that were found as they influence the antimicrobial activity and the size of the zones of inhibition. The extract gave inhibition zones that were equally as good as the standard antibiotics especially for gram-negative bacteria. The extracts of *Peltophorum africanum* have been used in veterinary medicines as they show antibacterial activity (Bizimenyera *et al.,* 2005). Zones of inhibition ranging from 4 to 8mm radius were previously reported and the result does not deviate much from the zones of 2 to 100mm radius in the current study. Studies have shown a Minimum Inhibitory Concentration of the plant to range between 1.5 to 12mg/ml and the current study showed concentrations between 1.25 and more than 10mg/ml (Samie *et al.,* 2005). This shows that the results found in the current study are in accordance with literature surveyed and this justify the wide use of *P. africanum* as a traditional medicine.

Toxicity: The plant is moderately safe to use as it gave LC_{50} values below 1000µg/ml. This means the plant has to be used with great caution and further in vivo tests are necessary as the plant is widely used and has shown great activity in all the tests it was screened for. Previous studies by Bizimenyera *et al.,* 2000 have otherwise proved that the extracts of *P. africanum* are not toxic to the brine shrimps.

4.8 *Turrea nilotica*:

Phytochemical screening: Saponins and cardiac glycosides were present in the root extract of *Turrea nilotica* and this indicates that it can be used for a number of pharmacological activities, table 1. From the literature surveyed a chemical constituent niloticin involved as an intermediate in the biosynthesis of limonoids was found by Kotschy and Peyr. Not much phytochemical constituents have been reported.

Antioxidant activity: The root of the plant gave moderate total phenolic content of 0.200±0.00764 and high antioxidant activity of 82.8±0.354% as shown in table 5. The result

shows that the amounts of the total phenolic compounds have contributed to the antioxidant activity. Flavonoids were found during the phytochemical screening exercise and these are a chief component of phenolic compounds a group that contribute a lot to the antioxidant activity and so the plant is a potential antioxidant just like most other medicinal plants that have been reported by Muchuweti *et al.,* 2006.

Antimicrobial activity: the root extract was active against all the bacteria it was screened against except for *P. aeruginosa* as shown in tables 7 and 8. Activity for all the microorganisms was moderate and this is justified by the number of phytochemical constituents in the plant. In previous studies by Thomik and Munjeri 1993 from the School of Pharmacy, University of Zimbabwe antifungal activity was exhibited to a lesser extent by extracts of *Turrea nilotica* where assays against 5 bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae, Pseudomonas aeruginosa*) and 2 strains of fungi (*Candida albicans*, *Aspergillus niger*) using the agar diffusion method was done. Not much work has been reported on the plant but the few articles are in accordance with the results of the current study.

Toxicity: the plant is moderately safe to use and use should be with great caution. Further in vivo tests can be done to ascertain the safety of the plant.

4.9 *Pterocarpus angolensis*:

Phytochemical screening: the root and bark extracts of the plant were screened for the presence of phytochemical constituents and both extracts showed the presence of saponins and tannins. *Pterocarpus* was reported to yield a resinous kino which assays 76.7% of tannin and 0.55% of a new crystalline phenol muningin. Breyer-Brandwick and Watt 1962 also found flavones. No flavones were found in current work and this could be because of the difference in the plant collection sites as environmental conditions influence the types of secondary metabolites produced. The other reason could be the detection limits of the method that was used in this study and therefore other investigations on the constituents can be done using more sensitive methods and techniques.

Antioxidant activity: one of the highest total phenolic content and antioxidant was found in the root and bark extracts of the plant. The extracts also contained the most abundant phytochemical compounds some of which fall under phenolic compounds which contribute to antioxidant activity. The high total phenolic compounds led to the high antioxidant activity as phenolic compounds contribute to the antioxidant activity and the values shown were as high as previously reported figures by Muchuweti *et al.,* 2006 for other traditional medicinal plants. The plant as a whole can be used as a potential antioxidant to cure the related diseases.

Antimicrobial activity: The root and bark extracts of the plant showed medium to high activity against the microorganisms screened against. Very high activity was recorded against *E. coli* a value of 10.75±0.5mm for the root. This result shows that the bark has got a lot of potential to be used as a remedy against all the diseases caused by E. coli the MIC also showed that only a little amount of the extract are required for activity to be demonstrated. A concentration of only 0.625mg/ml is required as the least amount to show inhibition whilst the minimum concentration required to kill all the microorganism is 5mg/ml. Pterocarpus is well known for its wide usage in medical practice and the ability to form zones of inhibition around microorganism justifies the uses. Antibacterial activity has been reported by previous researchers and this shows that the current research is in accordance with available literature.

Toxicity: the root extract was shown to be safe to use whilst the bark was shown to be moderately safe and should be used with great caution. The results of the bark extract correlate well with the literature results of *Pterocarpus angolensis* tested in the treatment of *Schistosoma haematobium* meaning at higher dosage the extract can be toxic (Nyazema *et al.,* 1994). Reports by McGaw *et al.,* 2007 have also shown that the bark of *Pterocarpus angolensis* is toxic to brine shrimps. The plant concentration used was however much concentrated (0-5mg/ml) than those used in the current studies (5-500µg/ml) and this may have led to the slight variation but suggestions of care in use are highlighted in both cases.

4.10 *Vangueria infausta*:

Phytochemical screening: The leaf and root extract were screened for and the phytochemical constituents found were tannins and saponins in both extracts and additionally flavonoids in the leaves. This shows that both extracts will perform similar functions due to similar chemical composition but the leaves could have additional functions because of the flavonoids present. Nundkumar and Ojewole in 2002 have done phytochemical screening of the leaf extract of *V. infausta* and revealed the presence of anthraquinones, flavonoids, seccoirridoids and terpenoids. The leaf has given negative tests for haemolysis, alkaloids and tannins but positive ones for sterols (Breyer-Brandwick and Watt 1962). The literature results in terms of flavonoids are similar but different in terms of tannins which were absent in literature. The presence of tannins in the current research could be because of different types of environment of growth of the plant under investigation. Presence could have been enhanced by good extraction methods which maximize the extraction of the tannins because on the rating of the tannins the root extract scored a single + which meant only trace amounts of the tannins were present whilst the leaf extract scored double + meaning medium amounts were present.

Antioxidant activity: Vangueria infausta leaves had the lowest total phenolic content (0.0048±0.00255) but had one of the highest antioxidant inhibition percent (93.1±0.0707%) as

shown in table 5. Although it is believed that the total number of hydroxyl groups present in the aromatic constituents of a plant extract, in part, offers better antioxidant properties, it is presumed that compounds present in methanolic extracts belong to different classes of phenolics. These classes most likely have varying antioxidant strengths and that the synergistic effect polyphenolics with one another and/or components present in an extract may contribute to the overall observed antioxidant activity (Shahidi et al., 1994). This may explain the anomalies that were experienced.

Antimicrobial activity: the leaf and root extracts of the plant were screened for antimicrobial activity and the root extract was active against all the microorganisms except for *Strep Group A* and *P. aeruginosa*. A very high reading was recorded against *S. aureus* 8.75±0.96mm and this was the highest of all values recorded against *S. aureus.* This shows that the extract can be used in the treatment of most diseases caused by the gram positive bacteria. Generally the root extract gave higher readings compared to the leaves and this shows that it is more potent as an antimicrobial agent and this is justified by the fact that the roots were reported by the traditional healers as the part they use mostly and literature from other researchers backs this up. De Boer *et al* 2002 showed that *Vangueria infausta* extracted using ethyl acetate, methanol, cold water and boiling water has antimicrobial activity against a wide range of microorganisms. In 2005 when some Tanzanian plants were screened for antibacterial and antifungal activity *Vangueria* was positive for the tests as well (de Boer *et al.,* 2002).

Toxicity: the leaf and the root extracts did show moderately safe to use results. They showed the closest values to the limit to toxicity range which means the plant has to be used with extreme caution and it may contains some levels of toxic compounds and follow up in vivo work will have to be done to ascertain the safety of the use of the plant. Work that has already been done by Nundkumar and Ojewole in 2002 has shown low levels of toxicity in the sense that the extract of Vangueria infausta supported the relatively low antiplasmodial activity.

4.11 *Ximenia caffra:*

Phytochemical screening: the root and leaf extracts of the plant showed positive results for flavonoids, saponins, coumarins, cardiac glycosides and tannins in the root and only cardiac glycosides and tannins in the leaf extract. The presence of the different types of chemical constituents indicate that the plant have got potential antimicrobial activity. From the literature surveyed there is not much so far reported on the phytochemical constituents of the plant.

Antioxidant activity: the plant showed one of the highest total phenolic content and antioxidant values as shown in table 5. The high total phenolic compounds led to the high antioxidant activity as phenolic compounds contribute to the antioxidant activity and the values shown were as high as previously reported figures by Muchuweti *et al.,* 2006 for other traditional medicinal

plants. This shows that the plant can be used as an antioxidant to curb diseases that result from lack of free radical scavenging compounds. The chemistry of the plant extracts do justify the high activity shown as phenolic compounds were found in phytochemical screening. The fruit of Ximenia caffra has been reported to contain high antioxidant levels (Ndhlala *et al.,* 2006)

Antimicrobial activity: the root of *X. caffra* showed high zones of inhibition against all the microorganisms it was screened against whilst the leaves did not show activity against one bacteria *P. aeruginosa*. The highest of all values was recorded against *E. coli* for the root extract 10.75±0.5mm. Previous antibacterial studies done showed that *Ximenia caffra* was active against 105 strains of bacteria from seven genera (Fabry *et al.,* 1998). Fabry *et al* 1998 also showed fungistatic and fungicidal activity of the plant and so these results are in accordance to what literature already has. *Ximenia caffra* showed interesting results as it featured in all the three categories of the anti-infective best results and this means that it holds great potential in the making of new antimicrobial agents. Ethno-pharmacologically the roots and the leaves of the plant are used and the roots in this study gave better results compared to the leaves a decoction of the leaves is used as a wash to soothe inflamed eyes of which most eye infections are with the microorganisms *S. aureus* and *Strep* which were inhibited by this extract in this study. In Tanzania it is used for fever and syphilis. All these uses are justified as *X. caffra's* extract demonstrated potential to inhibit all the microorganisms tested against it.

The minimum inhibitory concentration reached by *X. caffra* in the studies by Fabry *et al.,* 1998 ranged from 0.13 to 8 mg/ml whilst values in the current study ranged from 0.625 to 10mg/ml which shows the results are more or less the same as what has already been published. Their minimum bactericidal concentrations in the published results were all between 0.5 and >8 mg/ml whilst in the current study they were between 5 and >10 mg/ml which again is in the same range.

Toxicity: the root extract gave a LC_{50} value of 1 590±752µg/ml and the leaf extract 1 020±52.7µg/ml as shown in table 6. These results showed that the plant is safe to use and that is the reason why the plant is widely used by traditional healers without any problems reported so far. In a report by Moshi *et al.,* 2003 toxicity on brine shrimps of extracts of 34 plants traditionally used for the treatment of different diseases in Tanzania a different result was obtained. Most of the results indicated the possibility that some of the plant extracts may be toxic or contain useful cytotoxic compounds, which are not reported by the traditional healers. Ximenia caffra was reported to have an LC_{50} value 11.3 µg/ml and concluded to be potentially toxic. The difference in the results could be due to the different environments of where the plants were collected and the different extraction methods and exposure periods to the brine shrimps.

4.12 *Ziziphus mucronata*:

Phytochemical screening: the root and leaf extracts were screened for phytochemical constituents and the root extract showed the presence of saponins, cardiac glycosides and tannins whilst the leaf extract showed the presence of flavonoids, saponins and tannins as shown in table 4. The phytochemistry of the plant has not yet been exhaustively studied as current literature survey showed one article indicating that the leaf extract contains low amounts of tannins (Aganga and Adogla-Bessa, 1999). These phytochemical compounds are the ones responsible for the bioactivity shown by the plant.

Antioxidant activity: The total phenolic content of the leaf extract was quite low (0.0586±0.00417) table 5 as compared to the corresponding antioxidant activity of 84.6±0.0707%. This could have been because there are other compounds that lead to antioxidant activity besides the total phenols in it. The root extract gave a total phenolic content of 0.239±0.00630 but more or less the same inhibition percent as the leaves of 84.1±0.849% of which this result shows a correlation between the total phenolic content and the antioxidant activity meaning the phenolic compounds gave rise to the antioxidant activity. The plant is a potential antioxidant. These results are in accordance with other antioxidant activity results of other medicinal plants that have been investigated by Muchuweti *et al.,* 2006 where Warbugia Salutaris gave a low phenolic content value of (0.0540 ± 0.00799) but a high value of antioxidant activity (92.6 ± 1.00%) so the results do not deviate much from literature available.

Antimicrobial activity: The tree is well known for its delicious fruits. The root and leaf extracts were generally of moderate to low activity in terms of the inhibition zones formed around the microorganisms screened against. The root extract was more active against gram positive bacteria readings of 3.5±0.58 and 5.0±0mm for *S. aureus* and *Strep group A* respectively and for fungi readings of 2.75±0.5 and 3.75±0.5mm for *C. albicans* and *A. niger* respectively table 8. The leaf extract only showed activity against *E. coli*. More bacteria were inhibited by the root extract than the leaf extract as shown in table 7 and this could be because the phytochemical compounds of the root extract directly give rise to activity and therefore inhibit more microorganisms. Work that have been done previously also show that *Ziziphus mucronata* can inhibit microorganisms *Mycobacterium tuberculosis* and *Mycobacterium smegmatis* with minimum inhibitory concentrations ranging from 1.2 to 5mg/ml (Mativandlela *et al.,* 2008). The results are in agreement with those of the current study where the MIC values of the respective microorganisms ranged from 1.25 to 6mg/ml a result showing that the extract is very potent as it requires only small concentrations in order to exhibit activity.

Toxicity: the leaf and root extract are both safe to use. The leaves gave one of the safest margins and this is explained by the fact that herbivores feed on them without any known poisonous effects and also the plant has been used by traditional healers since time immemorial. A study by Taylor *et al.,* 2003 where medicinal plants from Southern Africa were tested for their safety has

proven that Ziziphus mucronata is safe to use and this study is in agreement with the results from the current study.

4.13 Phytochemical Screening

Phytochemical screening was carried out using at least three methods. Preliminary screening was followed by confirmatory tests. This was necessary because of the varied sensitivities of the methods employed in detecting constituents that can occur with trace quantities in some cases. The situation is made more complex by the fact that secondary metabolites have been shown to differ depending on age of plant, climatic conditions, soil, growing stage, season, diurnal and nocturnal variations within the same plant species (Sofowora, 1982).

Generally the phytochemical results indicate that only 15% of the plant extracts showed the presence of alkaloids with *Holarrhena pubescens* root giving the most definite result for the presence of alkaloids as it showed positive results for UV, TLC and the confirmatory test. These results do not deviate too much from literature, as a number of researchers have not reported the presence of alkaloids in plant extracts. Although it is estimated that alkaloids occur in perhaps 20% of higher plant families it should be borne in mind that alkaloid distribution is very uneven and many families lack them altogether (Harborne, 1984). Because they are such a large group of heterogeneous substances, it is difficult to identify an alkaloid from a new plant source without knowing approximately the type of alkaloid likely to be found. This was especially the case with the endemic plants where no previous related studies have been undertaken. In addition the wide range of solubility and other properties of the alkaloids make their detection difficult. The nature of biological active components can be enhanced by the extraction method hence the different extraction methods for the different groups. As most alkaloids are extremely toxic, plants containing them do not feature strongly in herbal medicine but they have always been important in the allopathic system where dosage is strictly controlled and in homeopathy where the dose rate is so low as to be harmless (Trease and Evans 2002). The absence of alkaloids in most of the plant extracts screened for justifies this fact as the pool of plants was selected from the commonly used traditional medicinal plants. Eight plant extracts showed positive results for the test of flavonoids (Table 4) and these were *Vangueria infausta* leaves, *Peltophorum africanum* bark and root, *Ximenia caffra* root, *Lannea edulis* leaves, *Holarrhena pubescens* leaves, *Ziziphus mucronata* leaves and *Erythrina abyssinica* root. 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.

Saponins were present in fifteen (75%) of the screened plant extracts which makes it the second abundant phytochemical group after tannins (80%) in this research. Only *Peltophorum africanum* leaves, *Ximenia caffra* leaves, *Lannea edulis* leaves, *Annona stenophylla* root and *Clausena anisata* leaves gave negative results for saponins. The leafy parts of the plants showed negative results, which shows that leaves mostly do not contain much saponin. Saponins have haemolytic properties and when injected into the blood stream is highly toxic. When taken by mouth, saponins are comparatively harmless which is why most of the plant extracts screened for are already in use yet they contain the high levels of saponins.

Coumarins were not very popular in the extracts. Five extracts (25%) contained coumarins and two methods were used to screen for the group. No method for confirming the coumarins is available in literature. Coumarin and its derivatives are principal oral anticoagulants and are water insoluble, which is why methanol was used for extraction. Five extracts (25%) did contain anthraquinones as confirmed by the tests. Anthraquinones are not very abundant in plants. In mammals they are known to exert a laxative effect and are relatively abundant in the Aloe genus in which they have been shown to possess antibacterial and antifungal properties. The anthranols present in rhubarb in winter are converted by oxidation to anthraquinones on the arrival of warmer weather (Faribairn, 1964).

Seven plant extracts showed the presence of cardiac glycosides and this was all confirmed by the UV, TLC and confirmatory tests. Tannins were the most abundant phytochemical group that was screened. Sixteen out of the twenty extracts (80%) did contain tannins. The four extracts which gave negative results were *Holarrhena pubescens*, *Annona stenophylla*, *Turrea nilotica* and *Erythrina abyssinica*. 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 sixteen extracts containing tannins only five extracts contained condensed tannins which is in contrast with literature which says condensed tannins are universal in occurrence in contrast to hydrolysable tannins whose distribution is very limited (Harborne, 1984). This result can be explained by the fact that the condensed tannins could have been hydrolysed to the hydrolysable gallic tannins during the extraction process.

These results clearly indicate that the plants under investigation are potential herbal plants that can be used for antiinfective activity. It has been documented that plant metabolites are responsible for the pharmacological activities that the extracts claim to have and Table 1 shows the different activities each of the chemical groups is responsible for (Akinyemi *et al*., 2005; Mojab *et al.,* 2003).

4.14 Antioxidant Activity

The DPPH assay was used to measure the antioxidant activity of the plant extracts. Unlike laboratory- generated free radicals, such as the hydroxyl radical and superoxide anion, DPPH radical has the advantage of being unaffected by certain side reactions, such as metal ion chelation, and enzyme inhibition, brought about by various additives. Caution must; however, be exercised when interpreting such results, as the reactions that DPPH radical elicits are not as simple and as straightforward. One cannot arbitrarily assume that the decrease in absorbance is solely attributed to the antioxidant donating a hydrogen atom or an electron to DPPH. Nevertheless, the 'DPPH test' is a commonly employed assay in antioxidant studies and offers a rapid technique in which to screen for antioxidant activity (Muchuweti *et al*., 2006).

The antioxidant values (percentage inhibition) of the crude methanolic extracts from the plant species were examined and compared with one another.

The percentage inhibition reached nearly 100% for the standard b-carotene – 98.6 ± 0.100%, *P. africanum* leaves, bark, roots – 97.6 ± 0.354%, 96.3 ± 0.354%, 96.4 ± 0.00% respectively and *P. angolensis* bark – 95.5 ± 0.141% as shown in table 5.

The lowest value was recorded in *V. infausta* 39.7± 0.212% roots, *E. abyssinica* root 64.6± 0.849%and *D. anomala* tuber 50.1±0.919%. The results show that the small amounts of total phenolic content in these three plant samples contributed to the little activity shown.

The plant extracts showed a time dependent scavenging of DPPH, which may be attributed to its hydrogen-donating ability. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced (Kuda *et al*., 2005). Eight of the plant extracts took less than two minutes to complete the radical scavenging activity reaction which shows their great potency in the use as antioxidants as they are fast acting. The extracts include *Turrea nilotica* root, *Annona stenophylla* leaves, *Ziziphus mucronata* root and leaves, *Ximenia caffra* leaves, *Holarrhena pubescens* leaves, *Peltophorum africanum* bark and *Clausena anisata* leaves. The rest of the plant extracts took about five to ten minutes to complete the reaction whilst four of the plants *Erythrina abyssinica* root, *Dicoma anomala* tuber, *Vangueria infausta* root and *Pterocarpus angolensis* root took at least twenty minutes. Results show that generally the leaves were more active in terms of scavenging activity compared to roots and barks. Most vegetables which have been studied for radical scavenging activity showed inhibition times of more than 120 minutes and this actually shows that traditional medicines are more potent antioxidants compared to vegetables.

Phenolic compounds are known as powerful chain breaking antioxidants (Shahidi and Wanasundara, 1992). So far, phenolics constitute one of the major groups of compounds acting as primary antioxidants. Therefore, it was reasonable to determine the total content in selected

medicinal plants. The content of phenolic compounds was expressed as milligrams tannic acid per 100 mg plant sample. The amounts of total phenolics in the studied medicinal plants are shown in Table 15. A high content was observed for *P. africanum* bark 0.438±0.00424 TAE and the lowest for *V.infausta* leaves 0.0048±0.00255TAE. Other low values were recorded for *V. infausta* root 0.0159±0.00233 TAE, *D. anomala* tuber 0.0301±0.00403 TAE, *C. anisata* leaves 0.110±0.00375 TAE*, E. abyssinica* root 0.0601±0.00382 TAE, *A. stenophylla* root 0.0186±0.00424 TAE and *Z. mucronata* 0.0586±0.00417 TAE. Most of the extracts did have few phytochemical groups containing phenolic compounds and also showed lower values of inhibition percentages although the relationship was not exactly in the same order as in the highest inhibition percentage for the lowest TAE in order to the highest, relationship was not very linear.

According to Pearson's correlation at 95% confidence interval, two tailed P value at (alpha=0.05), correlation was found to be significant (R^2= 0.5713). This was because the phenolic compounds found in the phytochemical profile were contributing to the antioxidant activity although it has been suggested that, besides the composition of the phenolics, other factors that involve different phenolic compositions or the presence of non-phenolic antioxidants such as ascorbate, α-tocopherol and β-carotene can play a major role in the antioxidant activity of plant materials which explains the few anomalies.

4.15 Antimicrobial Screening

Organic solvent extraction of plant extracts was done and after antimicrobial screening a number of interesting results surfaced. Of the bacterial strains used; *S. aureus* and *Strep. Group A* were the most susceptible pathogens and they are gram-positive. Gram-negative bacteria are known to be resistant to a lot of antimicrobial agents. The standard antibiotics which are currently in use that were used in this study did show different levels of inhibitions all times.

Generally five plants did show very good and interesting results against the gram-positive bacteria and the level of activity decreased in the following order: *E. abyssinica* root > *X. caffra* root > *P. africanum* root > *Z. mucronata* root > *V. infausta* roots can also be included as it gave the best and highly distinct value of inhibition against one of the bacteria and no action against the others.

Four plants gave good results against the gram-negative bacteria which are very resistant to most antibacterials. The level of activity decreased in the following order *P. africanum* root > *X. caffra* root > *P. angolensis* root > *T. nilotica* root. The extracts gave very high activities which were

very comparable to the standard antibacterials on the market. It was mostly the root part of the plants that were giving the highest activities but the barks were following closely.

More extracts were able to show activity against gram positive bacteria as compared to gram negative bacteria. This could be mainly because of the different cell wall components and these differences affect their susceptibilities to antibacterial agents. The outer peptidoglycan layer of *S. aureus* is not an effective permeability barrier as compared to the outer membrane of E. coli. The outer phospholipidic membrane of gram negative bacterial cell wall despite having porins which are transmembrane channels provides an apparent barrier to penetration of incoming antibacterial agents (Bryan, 1982). In gram positive bacteria all liposubstances are absent and antimicrobial molecules enter the cell wall easily and the degree of apparent barrier function of the outer membrane is strain specific (Bryan, 1982).

The top four plant extracts that gave good results against the fungi tested were in the following order of decreasing activity *P. africanum* bark > *X. caffra* root > *E. abyssinica* root > *P. angolensis* root. Generally some plants kept appearing in all the microorganisms tested and that shows they are highly active and interesting plants.

Although all the extracts were less active than the positive control in antifungal activity, they can possibly be modified to make them more active. Combinations of the active extracts could be further tested for synergistic effects. Synergism is currently being used in the treatment of endocarditis caused by Enterococcus faecalis. The organism can only be effectively controlled by using a combination of a cell-wall active antibiotic such as penicillin/ vancomycin with an aminoglycoside such as streptomycin/gentamicin that inhibits protein synthesis (Wang and Peterson 1984).

For Minimum inhibition concentrations the extracts that were bacteriostatic against the different microorganisms can be used to stop bacterial multiplication without necessarily killing and thus can qualify for further development into disinfectants. Lower MIC values were observed for the gram positive bacteria as compared to gram negative ones mainly because an increased volume of antibacterial agent that enters freely through the cell wall of gram positive bacteria results in build up of low concentration since concentration is indirectly proportional to volume. Thus, gram positives require a low concentration for its growth to be inhibited. In gram negative bacteria, less volume enters due to the outer membrane barrier and this decrease in the amount of antibacterial agent that enters the cell wall leads to an increase in the concentration required to inhibit growth of bacterial strains and also completely kill the cells.

A single traditional medicine is generally considered to be therapeutic against a number of infections caused by different types of microorganisms. Table 2 is an outline of the infections on different body parts and the microorganisms associated with the infection and this shows that any of the extracts that inhibited a certain microorganism can be potentially used as a

remedy to the infection associated with the microorganism Any of the plant extracts that showed a zone of inhibition against different microorganisms has a potential use in the outlined diseases associated with the microorganisms as listed in table 2.

4.16 Toxicity

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and antitumour properties. In the present study the brine shrimp lethality of extracts of twelve medicinal plants used in Zimbabwean traditional medicine to brine shrimp was determined using the procedure of Meyer *et al.,* 1982.

The LC_{50} values of the brine shrimp observed for extracts and of the medicinal plants and that of the positive control, *N. oleander* are given in table 6. It was observed that the degree of lethality was found directly proportional to the concentration of the extract. All the plant extracts were safe to use according to the concentration that killed half of the brine shrimps. This justifies why the plants are currently being used without any major reports of toxicity. Different literature papers have taken different levels of LC_{50} (µg/ml) as toxic/bioactive. According to Pisutthanan *et al*, 2004 extracts resulting in LC_{50} values less than 250µg/ml were considered significantly active /toxic and have potential for further investigation.

55% of the plant extracts did show moderately safe results which means they are safe to use but caution has to be exercised. Three plants did show results that were close to 250 µg/ml which was the lower limit for toxic plants, and the positive control *Nerium oleander* gave a reading of LC_{50} 142 ± 68.2 µg/ml and it is a known toxic plant. Vangueria infausta leaves 338 ± 23.4 µg/ml, *Vangueria infausta* root 416± 28.3 µg/ml, Pterocarpus angolensis bark 478 ± 29.7 µg/ml. These results show that none of the plants have got potential use as anticancer drugs. Most of the plants studied have not been screened for toxicity before.

CHAPTER 5

5.0 Conclusion

Out of the 12 plants 7 did show a wide range of activity in all the tests done. This is justified by the fact that the plants are already being used in traditional medicines for treatment of different ailments and this work was done to add value to the existing knowledge. It was really a major gain to able to ascertain the ability of the plants to inhibit the selected microorganisms as it then stands to reason that there is a lot of opportunity to further investigate the plants and use them in the formulation of new antimicrobial drugs.

Phytochemical Screening

The number of phytochemical constituents greatly influences the levels of biological activities shown as all the chemical compounds just by virtue of being present do have pharmacological implications table 1. The phytochemical screening results served to indicate the major chemical groups in the plants and the plants with most of the phytochemical compounds that could be of interest in further studies for instance *Peltophorum africanum*, *Ximenia caffra* and maybe *Holarrhena pubescens* for its alkaloidal content. In the study the major gain of phytochemical screening was that the broad groups of phytochemical compounds present were able to be detected and this followed the biological activities associated. It would be exciting to screen for all the other known compounds given the resources and from there it would be necessary to identify the actual compounds as the groups cover a lot of specific compounds which when separated show more activity.

Antimicrobial activity

Accordingly and justifiably, humankind has been searching for suitable therapy for nearly as long. The problem that has built up gradually over the last few decades has been that of bacterial resistance to existing antimicrobial agents. The emergence of previously unknown infections such as acquired immunodeficiency syndrome (AIDS) has made the search for new antiinfectives an absolute priority in the pharmaceutical industry.

Five plant extracts gave very good results against gram positive bacteria; the extracts in order of decreasing activity were *E. abyssinica* root, *X. caffra* root, *P. africanum* root, *Z. mucronata* root and *V. infausta* root. Those good against gram negative bacteria in order of decreasing activity were *P. africanum* root, *X. caffra* root, *P. angolensis* root and *T. nilotica* root whilst those good against fungi were *P. africanum* bark, *X. caffra* root, *E. abyssinica* root and *P. angolensis* root. *Z. mucronata*, *H. pubescens* and *V. infausta*. The big zones of inhibition that resulted could mean the overpowering of the problem of antimicrobial resistance especially by the gram negative bacteria as zones of inhibition together with Minimum inhibitory concentrations do indicate the

level of strength of the extracts. The antimicrobial activity results showed some traditional medicinal plants that can be further investigated for their high activity aand this was a major gain in this project.

Antioxidant activity

In this study, the methanolic extracts of the twelve plant species found in Zimbabwe were found to possess phenolics as well as antioxidant activity. The results gained in these assays provide simple data that make it possible to classify extracts according to their total phenolic content and antioxidant potential.

There is a need to characterise phenolic compounds present within each plant extracts, so as to assign different antioxidant activities, to ascertain whether phenolic structure affects antioxidant activity and also to determine whether synergism definitely occurs between certain phenolic compounds. The therapeutic value of the plant extracts may be partly because of their antioxidant activity. Further studies on the absorption and effects of phytochemicals present in the plant extracts on antioxidant status in animal models are needed to evaluate their potential health benefits.

Peltophorum africanum bark gave the best total phenolic content results whilst *X. caffra*, *P. africanum* leaves and *P. angolensis* gave the best antioxidant activity results. This shows the level of potential that medicinal plants have in antioxidant activity which is a major achievement in the project.

Brine Shrimp Toxicity Tests

In this study the Brine Shrimp Lethality test provided a simple platform to be able to quickly screen extracts for potential toxicity. Most of the extracts were shown as being safe to use and this is justified by the fact that most of the plants are already being used by traditional medical practitioners and some are used as vegetables and herbivores feed on their leaves. Some follow up work is worth for some of the extracts like *Clausena anisata, Lannea edulis, Peltophorum africanum, Turrea nilotica and Vangueria infausta* as they are moderately safe to use and should be used with caution. The major gain is that in this study it is possible to classify the medicinal plants as being very safe to use or moderately safe then caution can be exercised. Some in vivo tests at different concentrations could be done to ascertain safety of use of the plants.

Most of the active plant extracts featured in almost all the tests carried out and the plants *P. africanum*, *P. angolensis*, *E. abyssinica*, *X. caffra* and *Z. mucronata* are worth following up in further studies as they gave very good results.

5.13 Scope for Further Work

In view of the different biological activities exhibited by the best plant extracts P. *africanum*, *P. angolensis*, *E. abyssinica*, *X. caffra* and *Z. mucronata,* further work has to be done targeting the isolation, chemical characterization and identification of the active principles. Once this is done biological screens on the isolated compounds can be redone and in-vivo trial tests leading to drug development and formulations can be done. A recommendation from the study is for the responsible authority (Ministry of Environment and Tourism) to reinforce for the preservation of the endangered plant species through agro-forestry measures. As for the antioxidant activities isolation and identification of the various phenolic compounds present within each plant extract in order to assign antioxidant effects to individual compounds can be done using HPLC. Qualitative work to identify the exact compounds responsible for the various medicinal properties of the plant extracts will need to be done as not all flavonoids for example exert the pharmacological activities found. Toxicity tests can also be done in-vivo to assess whether the in-vitro tests really translate to in-vivo tests.

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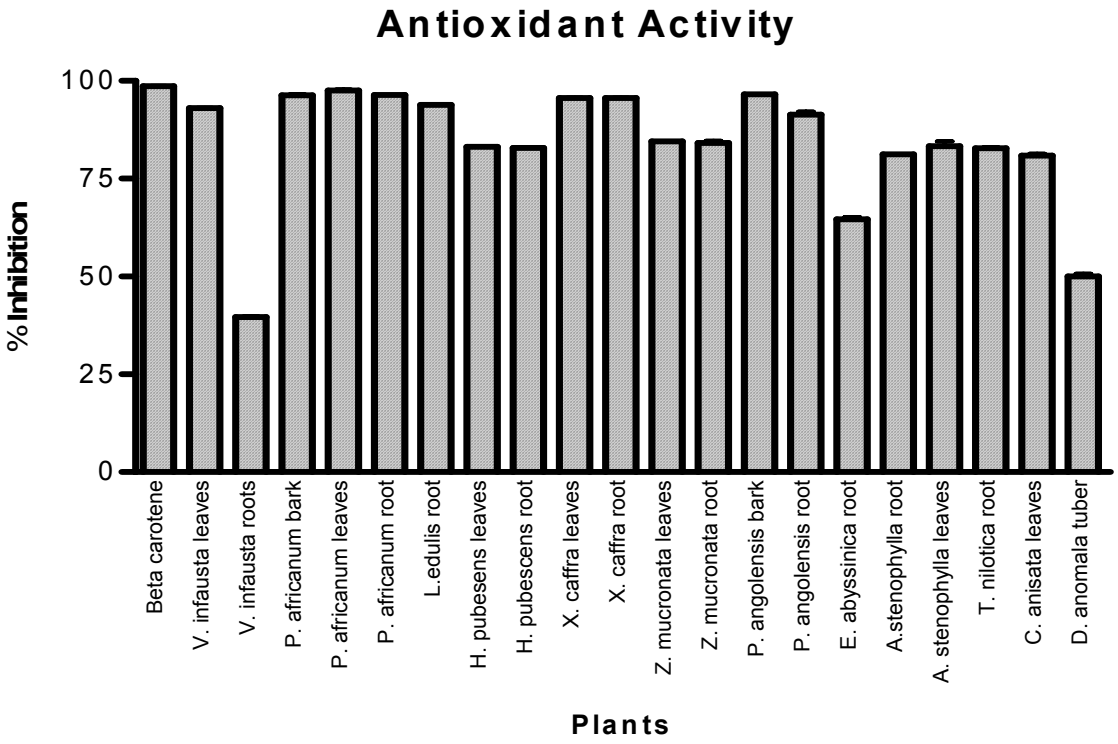
APPENDIX



Fi gure 23: Antioxidant Inhibition % of the Plant Extracts

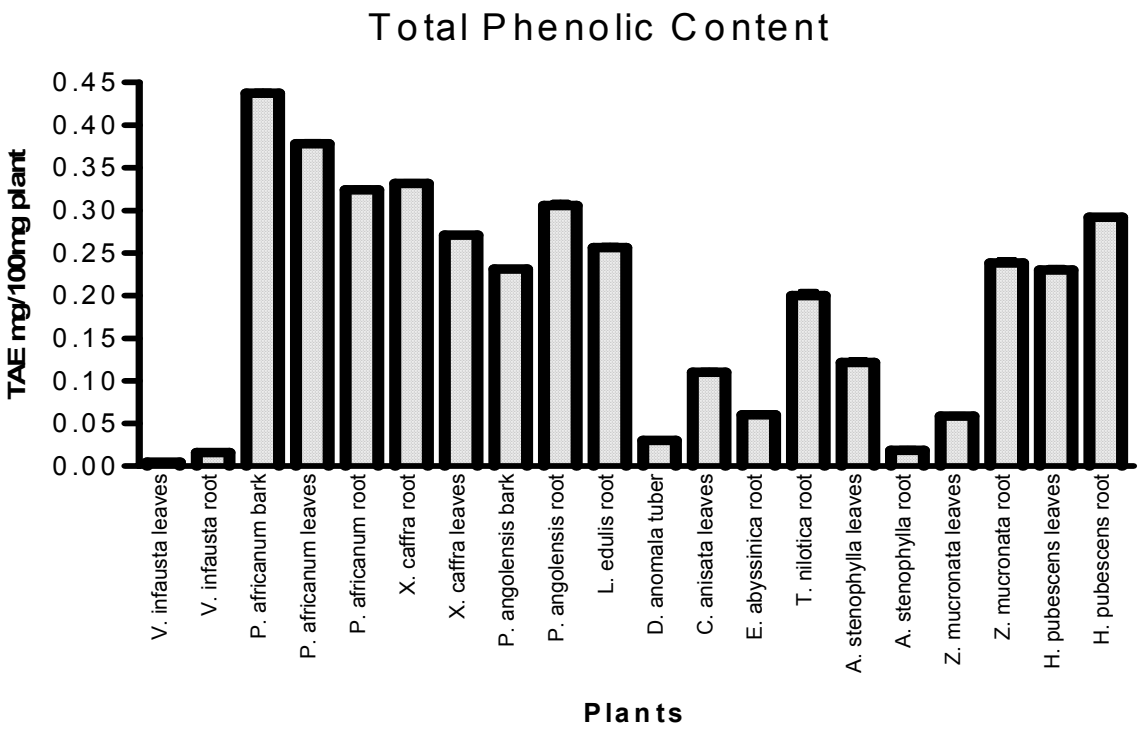


Figure 24: Total Phenolic Content of the Plant Extracts as TAE (Tannic acid equivalents)

Raw data for the duplicate runs of minimun inhibitory concentrations(MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC). Are as shown in the next pages

Table 11: Minimum Inhibitory Concentration (MIC) mg/ml against *S. aureus*

Plants/Part Used	Concentration mg/ml													
	10	**5**	**2.5**	**1.25**	**0.625**	**0.313**	**0.156**	**0.078**	**0.396**	**0.198**	**0.099**	**0.0 ctrl**	**MIC**	**MBC**
Annona stenophylla leaves	+ +	+ +	+ +	+ +	++	++	++	++	++	++	++	++	>10	>10
Dicoma anomala tuber	+ +	+ +	+ +	+ +	++	++	++	++	++	++	++	++	>10	>10
Erythrina abyssinica root	- -	- -	- -	- -	- -	- +	+ -	++	++	++	++	++	0.23	5
Horlarrhena pubescens leaves	- -	- -	+ +	+ +	++	++	++	++	++	++	++	++	5	>10
Lannea edulis root	- -	- -	+ +	+ +	++	++	++	++	++	++	++	++	5	>10
Peltophorum africanum bark	+ +	+ +	+ +	+ +	++	++	++	++	++	++	++	++	>10	>10
Peltophorum africanum leaves	- -	- -	+ +	+ +	++	++	++	++	++	++	++	++	5	10
Peltophorum africanum root	- -	+ -	+ -	+ +	++	++	++	++	++	++	++	++	3.75	10
Pterocarpus angolensis bark	- -	- -	- -	- -	++	++	++	++	++	++	++	++	1.25	5
Pterocarpus angolensis root	- -	- -	- -	- -	++	++	++	++	++	++	++	++	1.25	10
Turrea nilotica root	- -	- -	- -	- -	--	++	++	++	++	++	++	++	0.625	2.5
Vangueria infausta root	- -	- -	- -	- -	--	++	++	++	++	++	++	++	0.625	5
Ximenia caffra leaves	- -	- -	+ +	+ +	++	++	++	++	++	++	++	++	5	>10
Ximenia caffra root	- -	- -	- -	- -	++	++	++	++	++	++	++	++	1.25	5
Ziziphus mucronata root	- -	- -	- -	+ +	++	++	++	++	++	++	++	++	2.5	10

Key: ++=No inhibition on both runs, +-=Ihibition on one run and no inhibition on the second run, --= Inhibition on both runs

Table 12: Minimum Inhibitory Concentration (MIC) mg/ml against *Strep. Group A*

Plants/Part Used	Concentration mg/ml													
	10	5	2.5	1.25	0.625	0.313	0.156	0.078	0.396	0.198	0.099	0.0 ctrl	MIC	MBC
Annona stenophylla leaves	+ +	+ +	+ +	+ +	++	++	++	++	++	++	++	++	>10	>10
Annona stenophylla root	- +	+ +	+ +	+ +	++	++	++	++	++	++	++	++	>10	>10
Dicoma anomala tuber	- -	- -	- -	+ +	++	++	++	++	++	++	++	++	2.5	>10
Erythrina abyssinica root	- -	- -	- -	- -	++	++	++	++	++	++	++	++	1.25	>10
Horlarrhena pubescens leaves	- -	- -	+ -	- +	++	++	++	++	++	++	++	++	1.875	>10
Horlarrhena pubescens root	+ +	+ +	+ +	+ +	++	++	++	++	++	++	++	++	>10	>10
Lannea edulis root	- -	- -	- -	+ +	++	++	++	++	++	++	++	++	2.5	10
Peltophorum africanum bark	- -	- -	- -	- -	++	++	++	++	++	++	++	++	1.25	>10
Peltophorum africanum leaves	- -	- -	- -	+ +	++	++	++	++	++	++	++	++	2.5	10
Peltophorum africanum root	- -	- -	- -	+ +	++	++	++	++	++	++	++	++	2.5	10
Pterocarpus angolensis bark	- -	- -	- -	+ +	++	++	++	++	++	++	++	++	2.5	10
Pterocarpus angolensis root	- -	- -	- -	- -	++	++	++	++	++	++	++	++	1.25	10
Turrea nilotica root	+ +	+ +	+ +	+ +	++	++	++	++	++	++	++	++	>10	10
Vangueria infausta leaves	- -	- -	+ +	+ +	++	++	++	++	++	++	++	++	5	>10
Ximenia caffra leaves	- -	- -	+ +	+ +	++	++	++	++	++	++	++	++	5	>10
Ximenia caffra root	- -	- -	+ -	+ -	++	++	++	++	++	++	++	++	1.875	5
Ziziphus mucronata root	- -	- -	- -	- -	++	++	++	++	++	++	++	++	1.25	10

Key: ++=No inhibition on both runs, +-=Ihibition on one run and no inhibition on the second run, --= Inhibition on both runs

Table 13: Minimum Inhibitory Concentration (MIC) mg/ml against *E. coli*

Plants/Part Used	Concentration mg/ml													
	10	5	2.5	1.25	0.625	0.313	0.156	0.078	0.396	0.198	0.099	0 ctrl	MIC	MBC
Erythrina abyssinica root	+ +	+ +	+ +	+ +	+ +	++	+ +	+ +	+ +	+ +	+ +	++	>10	>10
Peltophorum africanum bark	- -	- -	- -	- -	+ +	++	+ +	+ +	+ +	+ +	+ +	++	1.25	10
Peltophorum africanum root	- -	- -	- -	- -	--	--	+ +	+ +	+ +	+ +	+ +	++	0.313	5
Pterocarpus angolensis root	- -	- -	- -	- -	--	++	++	+ +	+ +	+ +	+ +	++	0.625	5
Turrea nilotica root	- -	- -	- -	+ +	+ +	++	+ +	+ +	+ +	+ +	+ +	++	2.5	5
Vangueria infausta leaves	- -	+ +	+ +	+ +	+ +	++	+ +	+ +	+ +	+ +	+ +	++	10	>10
Vangueria infausta root	- -	- -	+ +	+ +	+ +	++	+ +	+ +	+ +	+ +	+ +	++	5	10
Ximenia caffra leaves	- -	+ +	+ +	+ +	+ +	++	+ +	+ +	+ +	+ +	+ +	++	10	>10
Ximenia caffra root	- -	- -	- -	- -	--	++	+ +	+ +	+ +	+ +	+ +	++	0.625	10
Ziziphus mucronata leaves	+ +	+ +	+ +	+ +	+ +	++	+ +	+ +	+ +	+ +	+ +	++	>10	>10

Key: ++=No inhibition on both runs, +-=Ihibition on one run and no inhibition on the second run, --= Inhibition on both runs

Table 14: Minimum Inhibitory Concentration (MIC) mg/ml against *P. aeruginosa*

Plants/Part	Concentration mg/ml													
Used	**10**	**5**	**2.5**	**1.25**	**0.625**	**0.313**	**0.156**	**0.078**	**0.396**	**0.198**	**0.099**	**0.0 ctrl**	**MIC**	**MBC**
Annona stenophylla root	- -	+ +	+ +	+ +	++	+ +	+ +	+ +	+ +	+ +	+ +	++	10	>10
Lannea edulis root	- -	- -	- -	+ +	++	+ +	+ +	+ +	+ +	+ +	+ +	++	2.5	10
Peltophorum africanum root	- -	- -	- -	- -	--	+ +	+ +	+ +	+ +	+ +	+ +	++	0.625	2.5
Pterocarpus angolensis bark	- -	- -	- -	+ +	++	+ +	+ +	+ +	+ +	+ +	+ +	++	2.5	10
Pterocarpus angolensis root	+ +	+ +	+ +	+ +	++	+ +	+ +	+ +	+ +	+ +	+ +	++	>10	>10
Vangueria infausta leaves	- -	+ +	+ +	+ +	++	+ +	+ +	+ +	+ +	+ +	+ +	++	10	>10
Ximenia caffra root	- -	- -	- -	+ +	++	+ +	+ +	+ +	+ +	+ +	+ +	++	2.5	>10

Key: ++=No inhibition on both runs, +-=Ihibition on one run and no inhibition on the second run, --= Inhibition on both runs

Table 15: Minimum Inhibitory Concentration (MIC) mg/ml against *C .albicans*

Plants/Part	Concentration mg/ml													
Used	**10**	**5**	**2.5**	**1.25**	**0.625**	**0.313**	**0.156**	**0.078**	**0.396**	**0.198**	**0.099**	**0.0 ctrl**	**MIC**	**MFC**
Annona stenophylla root	+ +	+ +	+ +	+ +	++	++	++	++	++	++	++	+ +	>10	>10
Clausena anisata leaves	+ +	+ +	+ +	+ +	+ +	++	++	++	++	++	++	+ +	>10	>10
Erythrina abyssinica root	- -	- -	- -	- -	+ +	++	++	++	++	++	++	+ +	1.25	5
Horlarrhena pubescens leaves	- -	- -	- -	+ +	+ +	++	++	++	++	++	++	+ +	2.5	10
Lannea edulis root	- -	- -	+ +	+ +	+ +	++	++	++	++	++	++	+ +	5	>10
Peltophorum africanum bark	- -	+ +	+ +	+ +	+ +	++	++	++	++	++	++	+ +	10	10
Peltophorum africanum root	+ +	+ +	+ +	+ +	+ +	++	++	++	++	++	++	+ +	>10	>10
Pterocarpus angolensis root	- -	- -	+ +	+ +	+ +	++	++	++	++	++	++	+ +	5	10
Turrea nilotica root	+ +	+ +	+ +	+ +	+ +	++	++	++	++	++	++	+ +	>10	>10
Vangueria infausta leaves	+ +	+ +	+ +	+ +	+ +	++	++	++	++	++	++	+ +	>10	>10
Vangueria infausta root	+ +	+ +	+ +	+ +	+ +	++	++	++	++	++	++	+ +	>10	>10
Ximenia caffra leaves	- -	+ +	+ +	+ +	+ +	++	++	++	++	++	++	+ +	10	>10
Ximenia caffra root	- -	- -	+ +	+ +	+ +	++	++	++	++	++	++	+ +	5	10
Ziziphus mucronata root	- -	- -	+ +	+ +	+ +	++	++	++	++	++	++	+ +	5	10

Key: ++=No inhibition on both runs, +-=Ihibition on one run and no inhibition on the second run, --= Inhibition on both runs

Table 16: Minimum Inhibitory Concentration (MIC) mg/ml against *A. niger*

Plants/Part	Concentration mg/ml													
Used	**10**	**5**	**2.5**	**1.25**	**0.625**	**0.313**	**0.156**	**0.078**	**0.396**	**0.198**	**0.099**	**0.0 ctrl**	**MIC**	**MFC**
Annona stenophylla root	- -	- -	- -	+ +	++	+ +	+ +	+ +	+ +	+ +	+ +	++	2.5	10
Erythrina abyssinica root	- -	- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	++	5	10
Holarrhena pubescens leaves	- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	++	10	>10
Lannea edulis root	- -	- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	++	5	>10
Peltophorum africanum bark	- -	- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	++	5	10
Peltophorum africanum root	- -	- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	++	5	10
Pterocarpus angolensis root	- -	- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	++	5	10
Turrea nilotica root	- -	- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	++	5	10
Vangueria infausta leaves	- -	- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	++	5	>10
Vangueria infausta root	- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	++	10	>10
Ximenia caffra leaves	- -	- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	++	5	>10
Ximenia caffra root	- -	+ -	+ -	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	++	3.75	5
Ziziphus mucronata root	- -	- -	- -	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	++	2.5	5

Key: ++=No inhibition on both runs, +-=Inhibition on one run and no inhibition on the second run, --= Inhibition on both runs