LITERATURE REVIEW

1.1 Introduction

Herbal medicine has a long history in the treatment of several kinds of disease (Holm *et al*., 1998). Their use for the treatment of disease has been practised by man for many years and is still being widely practised even today (Kokwaro, 1993). For many years, people have developed a store of empirical information concerning the therapeutic values of local plants before orthodox medical practice appeared. Through periods of trial, error, and success, these herbalists and their apprentices have accumulated a large body of knowledge about medicinal plants. According to Iwu *et al.* (1999) the first generation of plant drugs were usually simple botanicals employed in more or less their crude form. Several effective medicines used in their natural state were selected as therapeutic agents based on empirical study of their application by traditional societies from different parts of the world.

Following the industrial revolution, a second generation of plant drugs emerged based on scientific processing of the plant extracts to isolate ″their active constituents″. Plant materials remain an important component in combating serious diseases in the world; for the therapeutic approach to several pathologies. Interest in medicinal plants has been overwhelming in the recent times especially as an important source of medication/health care. Currently, the global market for medicinal plants has been estimated to be around US $62 billion and the demand is growing rapidly (Indian Council of Medical Research, 2003). It is globally recognised that medicinal plants

play a significant role in providing health benefits to human beings. The World Health Organization (2000) has estimated that 80 % of the inhabitants of the world rely mainly on traditional medicines for their primary health care needs, and it may be presumed that a major part of traditional healing involves the use of plant extracts or their active principles**.**

Infectious diseases account for approximately one-half of all deaths in tropical countries (Iwu, 1999). Medicinal plants have been traditionally used for different kinds of ailments including infectious diseases. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties. Historically, plants have provided a good source of anti-infective agents. The isoquinoline alkaloid, emetine, obtained from the underground part of *Cephaelis ipecuanha*, and related species, have been used for many years as an amoebicidal drug for the treatment of abscesses due to the spread of *Escherichia histolytica* infections. Quinine, an alkaloid that occurs naturally in the bark of the *Cinchona* tree, is another important drug of plant origin with a long history of usage against malaria. The higher plants have made important contributions in areas beyond anti-infective, such as cancer therapies. Scientists from divergent fields are investigating plants with an intention to discover valuable phytochemicals. Laboratories all over the world have found literally thousands of phytochemicals which have inhibitory effects on all types of microorganisms *in vitro* (Cown, 1999).

1.2 The value of plants used in ethnomedicine for drug discovery

Medicinal plants provide a rich source of raw materials for primary health care in Africa and other parts of the developing world. According to Fabricant & Farnsworth (2001) the goals of using plants as sources of therapeutic agents are: 1) to isolate bioactive compounds for direct use as drugs; 2) to produce bioactive compounds of novel or known structures as lead compounds for semi synthesis to produce patentable entities of higher activity and/ or lower toxicity**;** 3) to use agents as pharmacologic tools; 4) to use the whole plant or part of it as a herbal remedy. Notable examples were quinine from *Cinchona pubescens*, reserpine from *Rauvolfia serpentine* and taxol from *Taxus spp*. Various other plant based drugs are listed in Table 1.1. The sequence for development of pharmaceuticals usually begins with the identification of active lead molecules, detailed biological assays, and the formulation of dosage forms. This is followed by several phases of clinical studies designed to establish safety, efficacy and the pharmacokinetic profile of the new drug (Iwu *et al.*, 1999).

During the last few decades, there has been a resurgence of interest in plants as source of medicines and of novel molecules for use in the elucidation of physiological/biochemical phenomena. There is the worldwide **g**reen revolution, which is reflected in the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs. Furthermore, underlying this upsurge of interest in plants is the fact that many important drugs in use today were derived from plants or from starting molecules of plant origin: digoxin/digitoxin, the vinca alkaloids, reserpine and tubocurarine are some important examples (Iwu *et al.*, 1999).

Table1.1 Drugs from plants (Ali & Azhar, 2000)

Drug	**Disease**	**Plant species**	**Family**
Ajmaline	Arrhytmia	*Rauvolfia* spp.	Apocynaceae
Vinblastine	Hodgkin's disease	*Catharanthus roseus*	Apocynaceae
Strophanthin	Congestive heart failure	*Strophanthus gratus*	Apocynaceae
Deserpidine	Hypertension	*Rauvolfia canescens*	Apocynaceae
Rescinnamine	Hypertension	*Rauvolfia serpentina*	Apocynaceae
Reserpine	Hypertension	*Rauvolfia serpentina*	Apocynaceae
Proscillaridin	Cardiac malfunction	*Drimia maritima*	Liliaceae
Protoveratrine	Hypertension	*Veratrum album*	Liliaceae
Colchicine	Gout	*Colchicum autumnale*	Liliaceae
Demecolicine	Leukemia, limphomata	*Colchicum autumnale*	Liliaceae
Atropine	Ophthalmology	*Atropa belladona*	Solanaceae
Scopolamine	Motion sickness	*Datura stramonium*	Solanaceae
Ipratropium	Bronchodilator	*Hyoscyamus niger*	Solanaceae
Hyoscyamine	Anticholinergic	*Hyoscyamus niger*	Solanaceae
Stigmasterol	Steroidal precursor	*Physostigma venonosum*	Fabaceae
Dicoumarol	Thrombosis	*Melilotus officinalis*	Fabaceae
Psoralen	Vitiligo	*Psoralea corylifolia*	Fabaceae

Table1.1 (continued)

Drug	Disease	Plant species	Family
Physostigmine	Glaucoma	*Physotigma venenosum*	Fabaceae
Morphine	Analgesic	*Papaver somniferum*	Papaveraceae
Noscapine	Antitussive	*Papaver somniferum*	Papaveraceae
Cocaine	Analgesic, antitussive	*Papaver somniferum*	Papaveraceae
Papaverine	Antispasmodic	*Papaver somniferum*	Papaveraceae
Quinidine	Cardiac arrhytmia	*Cinchona pubescens*	Rubiaceae
Quinine	Malaria prohylaxis	*Cinchona pubescens*	Rubiaceae
Emetine	Amoebic dysentery	*Cephaelis ipecacuanha*	Rubiaceae
Ipecac	Emetic	*Cephaelis ipecacuanha*	Rubiaceae
Aspirin	Analgesic, inflamation	*Filipendula ulmaria*	Rosaceae
Benzoin	Oral disinfectant	*Styrax tonkinensis*	Stracaceae
Camphor	Rheumatic pain	*Cinnamomum camphora*	Lauraceae
Ephedrine	Bronchodilator	*Ephedra sinica*	Ephedraceae
Eugenol	Toothache	*Syzygium aromaticum*	Myrtaceae
Papain	Attenuates	*Carica papaya*	Caricaceae
Picrotoxin	Barbiturate antidote	*Anamirta cocculus*	Menispermaceae
Picrotoxin	Glaucoma	*Pilocarpus jaborandi*	Rutaceae
Sennoside A, B	Laxative	*Cassia angustifolia*	Fabaceae

Table1.1 (continued)

Drug	Disease	Plant species	Family
Teniposide	Bladder neoplasms	*Podophyllum peltatum*	Berberidaceae
Xanthotoxin	Vitiligo	*Ammi majus*	Apiaceae
Caffeine	Stimulant	*Camellia sinenis*	Theaceae
Theophylline	Diuretic, asthma	*Camellia sinenis*	Theaceae
Digitoxin	Atrial fibrillation	*Digitallis purpurea*	Scrophulariaceae

Laboratories around the world are engaged in the screening of plants for biological activity with therapeutic potential. The potential of higher plants as sources for new drugs is unexplored (Hostettman *et al*., 1996). Among more than 250 000 species of higher plants, only about 5-10 % has been investigated chemically for the presence of biological active compounds (Balandrin *et al*., 1993; Ayensu and De Filipps, 1978).

1.3 Antiviral compounds from plants

Many antiviral agents have been isolated from plant sources and have been partly or completely characterised. An antiviral may be defined as a product that is able, *in vitro* or *in vivo*, to directly or indirectly reduce the infectious viruses in the host cell. The discovery of antiviral agents from plants and other natural sources has assumed a sense of urgency (Hudson & Towers, 1991). Table 1.2 summarizes isolated antiviral compounds studied, as well as their targets of action**.** Since a retrovirus, designated Human Immunodeficiency Virus (HIV), was isolated and identified as the etiologic

agent of the Acquired Immune Deficiency Syndrome (AIDS), numerous compounds have been evaluated for their inhibitory effects on HIV replication *in vitro* (Ito *et al*., 1987). Effective therapies for HIV infection are being sought far and wide, in the natural world as well as in laboratories (Cown, 1999). For example, benzylisoquinoline alkaloid, 'papaverine', has been shown to have a potent inhibitory effect on the replication of several viruses including HIV.

A traditionally used tuber found growing along the banks of the Zambezi River and used commonly throughout southern Africa has become a popular traditional treatment for HIV-related illnesses. It is widely called the 'African potato', but the botanical name is *Hypoxis hemerocallidea* (formerly *H. rooperii*) and it has been traditionally used as food and medicine. The tuber is reported to help maintain or increase CD4-cells and boost cellular immunity in the body. Traditional health practitioners in southern Africa use it for managing HIV infections, cancer, TB, influenza, arthritis, psoriasis and common cold (Bodeker, 2003).

1.4 Mozambican traditional medical practice

In Mozambique, as in most developing countries, human health services are still very poor and are compounded by many people living in rural areas several kilometers from a health center. Modern health services have not been provided to the greater part of the rural areas of the country. What are available to this sector of the population are their own indigenous medicines, especially the folk herbal medicines. These remedies are fairly well accepted, easily available and bear at minimal cost.

Table 1.2 Compounds isolated from higher plants with antiviral activity against animal or human viruses[a] (Vanden Berghe *et al*. 1993)

Plant derived compounds	**Origin**	**Target (s)**
Methylgallate	*Sapium sebiferum*	Herpes simplex
Gallotannins	*Spondias mombia*	Coxsackie B virus Herpes simplex virus
Tetragalloyl quinic acids	*Turkish and Chinese galls*	HIV reverse transcriptase
Quinovic acid glycosides (triterpenes)	*Uncaria tomentosa*	Vesicular stomatitis virus
Quinovic acid glycosides (triterpenes)	*Guettarda platypoda*	Rhino virus type1 B
Glycyrrhizin	*Glycyrrhiza radix*	Polypeptide phosphorylation, HIV
Castanospermine (alkaloids)	*Castanospermum australe*	Cytomegalo virus HIV
5, 7, 4'- Trihydroxy- 8-methoxyflavone and others	*Scutellaria baicalensis*	Influenza A virus
Isoflavonic glycoside	*Ulex europaeus*	Herpes simplex virus Polio virus
Triterpenes	*Euptelea polyandra*	Epstein Barr virus activation
Gossypol (polyphenols)	Cotton seed	HIV reverse transcriptase
Dextro-odorinol (alkaloids)	*Aglaia roxburghiana*	Ranikhet disease virus
Alkaloids	Amaryllidaceae	*Herpes simplex virus*
Citrusinine I (acridone alkaloid)	Citrus	*Herpes simplex virus* Cytomegalo virus
Alkaloids	*Chelidonium majus*	Adenovirus 12 and 5 *Herpes simplex virus*
Sesquiterpene glycosides	*Calendula arvensis*	Vesticular stomatitis virus Rhinovirus type I B

Aloe emodin (Anthroquinones)	*Aloe barbadensis*	Enveloped virus (virucidal)
Hypericin and pseudohypericin	Species of *Hypericum*	Retroviruses
Hypericin	*Hypericum triquetrifolium*	Herpes simplex virus Influenza A virus
Lignins	*Pinus parviflora*	Influenza A virus
α-(-) Peltatin (lignans)	*Amanoa oblongifolia*	Sindbis virus Murine cytomegalo virus
Lectins	*Narcissus pseudonarcissus* *Listeria ovata*	Cytomegalo virus
Plant proteins	*Gelonium multiflorum* *Dianthus caryophyllus*	HIV
Trichosanthin and other proteins	*Trichosanthes kirilowii*	HIV
Fulvoplumierin (iridoids)	*Plumeria rubra*	HIV reverse transcriptase
Allicin (sulfur compounds)	*Allium sativum*	Virucidal activity
Prunellin (sulfated polysaccharides)	*Prunella vulgaris*	HIV
Phloroglucinol derivates (polyphenols)	*Mallotus japonicus*	Herpes simplex virus
Catalpol (iridoids)	*Picrorrhiza kurroa*	Hepatitis B virus
Epilupeol (triterpenes)	*Vicoa indica*	Ranikhet disease virus

[a] All compounds were isolated or studied after 1987.

The traditional use of medicinal plants in Moçambique has been well documented (Yansen & Mendes, 2001). However, the effectiveness of these plants has not been scientifically evaluated. There is a lack of scientific validation and no documented evidence of efficacy is found in particular with reference to use against microbial and viral complaints. The present study was undertaken to test a few medicinal plants

collected in Mozambique for their activity against a variety of human pathogens namely: Gram-positive and Gram-negative bacteria, Human Immunodeficiency Virus (HIV) and *Mycobacterium tuberculosis.*

1.5 Hypothesis and motivation of study

Natural product research continues to provide a tremendous variety of lead structures, which are used as templates for the development of new drugs by the pharmaceutical industry. Many of the plants studied have shown very promising activity in the area of antiviral agents (Table 1.2). Also many species of plants have been found to be active against a wide variety of micro-organisms. Among the more than 250 000 species of higher plants, only a small percentage of about 5-10 % have been phytochemically investigated (Nahrsted, 2002; Ayensu and De Filipps, 1978) and an even smaller fraction has been submitted to biological or pharmacological screenings (Hostettmann, 1991). The plant kingdom still represents an enormous reservoir of new molecules to be discovered. There should be an abundance of drugs remaining to be discovered from plants.

The discovery of new antibacterial, anti-HIV and antituberculosis compounds from herbal remedies would assist in the development of new preparations to combat infectious diseases. Infectious diseases, TB and HIV cases are quite prevalent in Mozambique, particularly in rural areas where an astounding number and variety of plants are used by communities to treat these diseases without prior scientifically determined information. In this study, the antibacterial, antituberculosis and anti-HIV activities of the medicinal plants collected in Mozambique were examined. The

evaluation of these plants for biological activity is necessary, both to substantiate the use of these plants by healers, and also as a possible lead for new drugs or herbal preparations. This study will provide valuable information for further isolation of bioactive compounds from the studied plant species.

1.6 Objectives of the study

The primary objectives of this study were to investigate the antimicrobial and antiviral properties of medicinal plants collected in Mozambique by in *vitro screening* and secondly to isolate bioactive compounds from selected plants with antituberculosis, anti-HIV and antibacterial activity.

The specific objectives of this study were to:

- Determine antibacterial, antitubercular and antiviral (anti-HIV) activities of the crude extracts of selected medicinal plants from Moçambique.
- Isolate, identify and determine the structures of the active principles from the one or two samples which exhibit potent antimicrobial activity.
- Determine the antibacterial, antitubercular and anti-HIV activity of the purified compounds.
- Determine the cytotoxicity of selected extracts and purified compounds.
- Establish a scientific basis for the use of these plants.

1. 7 Scope of this thesis

The importance of plant- based drugs has been discussed in **Chapter 1**.

In **Chapter 2** the antibacterial activity of acetone extracts of 22 Mozambican medicinal plants against Gram-positive and Gram-negative bacteria species, using the agar diffusion methods has been reported. **Chapter 2** further describes the antimycobacterial activity of 10 selected medicinal plants against *Mycobacterium tuberculosis***.**

In **Chapter 3** the *in vitro* activity of Mozambican medicinal plants against the human immunodeficiency virus has been documented. Determination of activity against HIV was based on inhibition of the enzymes α-Glucosidase, β-Glucuronidase and Reverse transcriptase (RT).

The isolation and identification of compounds from *Lippia javanica* and *Hoslundia opposita* is described in **Chapter 4** and **5**, respectively**.**

Chapter 6 describes the antibacterial activity of isolated compounds from *Lippia javanica* and *Hoslundia opposita*. **Chapter 7** describes the antimycobacterial bioassay of compounds isolated from *Lippia javanica* and *Hoslundia opposita*. **Chapter 8** documents the antiviral activity of isolated compounds from *Lippia javanica* and *Hoslundia opposita*. In **Chapter 9** the cytotoxicity of *Lippia javanica* and *Hoslundia opposita* plant extracts and bioactive isolated compounds is discussed.

Finally **Chapter 10** summarises the entire project, the importance of medicinal plants folkloric use and entails the recommendations from the findings of this study.

1.8 References

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ANTITUBERCULOSIS AND ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANTS FROM MOZAMBIQUE

Abstract

Twenty two medicinal plants selected through a literature survey in Mozambique were investigated using the agar diffusion method for their antibacterial activity. Five Gram-positive and five Gram-negative bacterial species were used. Acetone extract of *Lippia javanica* showed inhibitory activity against Gram-positive bacteria, at a concentration of 0.125 mg/ml. The minimal inhibitory concentrations (MIC) of six other plant extracts were found to be 0.5 mg/ ml. Only extracts of *Adenia gummifera* and *Momordica balsamina* were found to have activity against Gram-negative bacteria at a concentration of 5.0 mg/ ml. Acetone extracts of ten plants species used for respiratory diseases were also tested against *Mycobacterium tuberculosis* using the BACTEC radiometric method. Four extracts showed activity against *M. tuberculosis* at 0.5 mg/ml.

2.1 Introduction

Man is host to a variety of pathogenic bacteria, protozoa and viruses. Persons who are deficient in the production of circulating antibodies are highly susceptible to respiratory infections by Gram-positive bacteria. Persons who are deficient in T-cell

functions, however, tend to succumb to infections by fungi and viruses, as well as to bacteria which grow predominantly intracellularly (Stanier *et al*., 1958). The pathogenicity of some of the bacterial species is significant because of their resistance to known antibiotics. The emergence of methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci and multiresistant Gram-negative bacteria has become a serious issue (Rao, 1998). In an earlier study it was found that 36 strains of *Bacillus cereus* were highly resistant to lincomycin, polymyxin B and penicillin G-cephalosporin (Arribas *et al*., 1988). Fifty methicillin-resistant strains of *S. aureus* were isolated at a hospital in Osaka between 1986 and 1990 of which a few were also found to be resistant to streptomycin and kanamycin (Kondo *et al*., 1991).

Tuberculosis (TB), an airborne lung infection, is becoming an epidemic in some parts of the world. It kills about 1 million children each year and it is estimated that between now and 2020, nearly 1 billion more people will be infected, 200 million people will get sick and 70 million will die from TB if control is not strengthened (World Health Organization, 1997). Moreover, TB has also been recognised as one of the most frequent opportunistic infections in persons suffering from the human immunodeficiency virus (HIV), particularly in Africa. Given the alarming incidence of drug resistance to strains of bacteria, there is a constant need for new and effective therapeutic agents (Bhavnani and Ballow, 2000).

Plants contain numerous biologically active compounds, many of which have been shown to have antimicrobial properties (Cowan, 1999). Ethnobotanical data are useful in the search for new antimicrobial agents and several bioactive compounds have been isolated from medicinal plants (Penna *et al.,* 2001).

In this study 25 medicinal plant species from Mozambique, were investigated for their antimicrobial activity. The plants selected are used for various infections,uberculosis

related symptoms such as chest pain, cough, etc. by Mozambicans. The effectiveness of these plants has not been scientifically evaluated. There is a lack of scientific validation and there is no documented evidence of efficacy particularly with reference to their use for antimicrobial complaints.

2.2.1 Materials and methods

2.2.2 Plant material

Different parts of the plants, (Table 2.1) were collected in 2002 from the south and central parts of Mozambique (Maputo, Chókwe, Massingir, Manica and Zambezia) Figure 2.1. The plants were identified at the HGWJ Schweickerdt herbarium of the University of Pretoria (PRU) and also at the herbarium of the South Africa National Biodiversity Institute, Pretoria (PRE). Voucher herbarium specimens have been submitted at the herbarium of the University of Pretoria.

2.2.3 Preparation of plant extracts

Various solvents have been used to extract plant metabolites. In this study acetone solvent was used for plants extraction. Acetone is very useful extractant because dissolve many hydrophilic and lipophylic components, is miscible with water, is volatile and has a low toxicity to the bioassay (Eloff, 1998).

Acetone extracts of each air-dried plant sample were prepared by stirring 50 g of the powdered plant material in 500 ml acetone for 48 hours. The extracts were filtered and concentrated to dryness at reduced pressure.. The resultant residue was later dissolved in acetone to a concentration of 100.0 mg/ ml.

Figure 2.1 Map of Mozambique with the location of the collected medicinal plants

Table 2. 1 Selected Mozambican medicinal plant investigated for antibacterial, antitubercular and anti-HIV activities

Plant species	Plant part collected	Voucher specimen	Medicinal uses	References
Adenia gummifera (Harv.) Harms (Passifloraceae)	Roots Leaf and stem	SM92062	Decoctions are administered for Malaria and Leprosy. Menorrhagia and infertility Biliousness Seediness or depression Decoctions bath is used for malaria. Emetic and as a cosmetic pigment on the	(Mabogo, 1990) (Watt & Breyer-Brandwijk, 1962) (Bryant, 1966) (Watt & Breyer-Brandwijk, 1962) (Gerstner, 1938)
Adenium multiflorum Klotzsch (Apocynaceae)	Plant	SM92063	The latex is widely used as an arrow poison in Limpopo (South Africa) and Mozambique	(Neuwinger, 1996)
Aloe marlothii A. Berger (Liliaceae)	Shoots	SM92064	Shoot decoction are used for stomach troubles Leaf and root decoctions are administered orally or as enemas for roundworm infestations. Chewed roots are used in enemas for babies. Leaf sap is applied to mothers' breasts to hasten weaning	(Watt & Breyer-Brandwijk, 1962) (Gerstner, 1939) (Hutchings, 1996)
Aloe parvibracteata Schönlond	Roots	SM92065	Used as dye source	(Van Wyk *et al*. 2000)

Table 2. 1 (continued)

Plant species	Plant part collected	Voucher specimen	Medicinal uses	References
Cassia abbreviata Oliv. (Fabaceae)	Root Bark and root	SM92066	Infusion for relief of toothache. It is used as dysentery and diarrhea remedy. Used for malaria	(Watt & Breyer- Brandwijk, 1962)
(*Catharanthus roseus* (L.) G.Don Apocynaceae)	Leaves Flowers Milk sap Root Root+leaves	SM92067	Rheumatism, menorrhagia Galactogogue Arthritis, gout, cancer Tea for blood cleansing Insect bites and warts Used as a diabetes remedy Venereal diseases For toothache, liver congestion Scury skin complaints Tonics, haemostatics vermifuges Used as purgative, emetics and depuratives	(Hutchings, 1996, Watt & Breyer-Brandwijk, 1962) (Watt and Breyer-Brandwijk, 1962) (Hutchings, 1996) (Watt and Breyer-Brandwijk, 1962) (Mabogo, 1990) (Watt & Breyer-Brandwijk, 1962)
Cissus quadrangularis L. (Vitaceae)	Leaves and pounded stems Stem leaves	SM92068	Burns and wounds Saddle sores on animals Gastro-intestinal complaints Washes for febrile pain and malaria Induce milk flow in cattle. In ointments for backache and body pain	(Oliver- Bever, 1986) (Dalziel, 1937). (Bhat *et al*. 1990) (Hedberg & Staugard, 1989;

Table 2. 1 (continued)

Plant species	Plant part colleted	Voucher specimen	Medicinal uses	References
Coccinia rehmannii Cogn. (Cucurbitaceae)	Tuber	SM92069	Used as pot-herb The fruit is edible.	(Watt & Breyer-Brandwijk, 1962)
Elephantorrhiza elephantina (Burch) Skeels (Fabaceae)	Roots	SM92070	Infusion used as an enema for dysentery and diarrhoea Fever, chest and stomach complaintþ‘ as love charms Intestinal disorders and syphilis Infertility in women and as aphrodisiacs For children who menstruate at an early age and to wipe the anus of a child with bloody diarrhoea.	(Bryant, 1966) (Gerstner, 1938) (Jacot Guillarmod, 1977) (Gelfand *et al*., 1985) (Hedberg & Staugard, 1989)
Gladiolus dalenii Van Geel (Iridaceae)	Root Corms	SM92071	Infusions of root are administered to sterile women. Corms are placed in seed- gourds as fertility charms to ensure a good harvest. The infusions of corms are administered as emetics for chest ailments thought to have been caused by sorcery, and are also taken as love charm emetics.	(Gerstner, 1941) (Hulme, 1954)

Table 2. 1 (continued)

Plant species	Plant part collected	Voucher specimen	Medicinal uses	References
Hemizygia bracteosa (Benth.) Briq. (Lamiaceae)	Leaves	SM92072	Repellent for mosquitoes	
Hoslundia opposita Vahl (Lamiaceae)	Leaves	SM92073	inter alia snake bite, conjunctivitis, epilepsy, chest pain, yellow fever, stomach troubles, and mental disorders. Infusions as a purgative, diuretic, febrifuge, antibiotic and antiseptic.	(Ayensu & De Filipps, 1978) (Onayade *et al*., 1989)
Lippia javanica (Burm.f.) Spreng. (Verbenaceae)	Leaves	SM92074	Infusions as tea to treat coughs, colds, fever and bronchitis. Influenza, measles, rashes, malaria, stomach problems and headaches Strong infusions are used topically for scabies and lice The leaves are sometimes smeared on the body as a protection against dogs and crocodiles. Treatment of HIV	(Van Wyk & Gericke, 2000; Smith, 1966; Watt & Breyer-Brandwijk, 1962 and Hutchings, 1996) (Smith, 1966; Watt and Breyer-Brandwijk, 1962; Hutchings, 2003, Hutchings & Van Staden, 1994) (Doke and Vilakazi, 1972) Hutchings, 2003)
Litogyne gariepina. (DC.) Anderb. (Astereaceae)	Leaves	SM92075	Unspecified parts are used for fevers Includes use as anthelmintic	(Doke & Vilakazi, 1972)

Table 2. 1 (continued)

Plant species	Plant part collected	Voucher specimen	Medicinal uses	References
Melia azedarach L. (Meliaceae)	Leaves	SM92076	The plant has been widely used in various countries as emetic and cathartic Anthelmintic. It is used as a tonic and antipyretic The decoction of the bark is used as a lotion on ulcers, syphilitic The trees is poisonous to animals	(Watt & Breyer-Brandwijk, 1962)
Maerua juncea Pax (Capparaceae)	Leaves	SM92077	Respiratory problems	Personal communication
Momordica balsamina L. (Cucurbitaceae)	runners Roots Leaves	SM92078	Cold infusion or decoctions of the runners are used to soothe squeamish stomachs Infusions of roots are used for intestinal complaints Infusions of leaves are administered as anti-emetics Bitter stomachic, purgatives and to reduce fever	(Bryant, 1996). (Hulme, 1954) (Mabogo, 1990) (Watt & Breyer-Brandwijk, 1962)
Ocimum americanum (Lamiaceae)	Leaves	SM92079	Used for hemorrhage of the nose inhale the smoke from burning the dried leaf	(Watt & Breyer-Brandwijk, 1962)

Table 2. 1 (continued)

Plant species	Plant part	Voucher specimen	Medicinal uses	References
Plectranthus fruticosus L' Hérit (Lamiaceae)	Leaves	SM92080	Cough and chest complaints	
Pseudolachnostylis maprouneifolia Pax (Euphorbiaceae)	Stem bark Roots	SM92081	Used for HIV treatment Smoke from burning roots is inhaled to treat pneumonia Bark extracts are used to treat diarrhea and venereal disease	Van Wyk *et al*., 2000; Palgrave, 1981)
Rhoicissus revoilli Planch. (Vitaceae)	Roots	SM92082		
Rhoicissus tomentosa (Lam.) Wild & R.B. Drumm (Vitaceae)	Roots	SM92083	Milk decoctions of roots are given as anthelmintics to calves	(Watt & Breyer-Brandwijk, 1962)
Salvadora australis Schweick. (Salvadoraceae)	Leaves	SM92084	Cough Smoke from burning leaves is inhaled to stop nosebleeds	(Arnold & Gulumian, 1984)
Salvadora persica L. (Salvadoraceae)	Leaves	SM92085	Cough	
Senna italica Mill. (Caesalpinaceae)	Leaves	SM92086	Used for burns and wounds	(Wat & Breyer-Brandwijk, 1962)

2.2.4 Antibacterial bioassay

Five Gram-positive bacteria, *Bacillus cereus* (ATCC 11778), *B. subtilis* (ATCC 6051), *B. pumilus* (ATCC 7061), *Staphylococcus aureus* (ATCC 12600), *Enterecoccus faecalis* (ATCC 292192) and five Gram-negative bacteria, *Enterobacter cloacae* (ATCC 13047), *Escherichia coli* (ATCC 11775) *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 33584) and *Serratia marcescens* (ATCC 1380) were tested for susceptibility to plant extracts. The bacteria were obtained from the Department of Microbiology and Plant Pathology, University of Pretoria. Each organism was maintained on a nutrient agar slant and was recovered for testing by growing them in fresh nutrient broth (No. 2, Biolab) for 24 hours**.** Before streaking, the culture was diluted to 1:10 with fresh sterile nutrient broth. The minimum inhibitory concentration (MIC) of the extracts was determined using the agar dilution method (Jorgensen *et al.,* 1999). The tested concentrations were 5.0, 2.5, 1.0, 0.5, 0.25, 0.125 and 0.062 mg/ml. Plant extracts were added to 5 ml of nutrient agar medium in Petri dishes and swirled carefully before congealing. The organisms were streaked in radial patterns on agar plates containing plant extracts (Figure 2.2), incubated at 37^{o}C and observed after 24 hrs (Mitscher *et al*., 1972). Plates containing only nutrient agar and 1% acetone without the plant extracts served as controls. In addition two plates containing streptomycin sulfate at concentrations of 100.0, 50.0 and 10.0 µg/ml served as positive controls. The MIC was regarded as the lowest concentration of the extracts that did not permit any visible growth when compared with that of the controls.

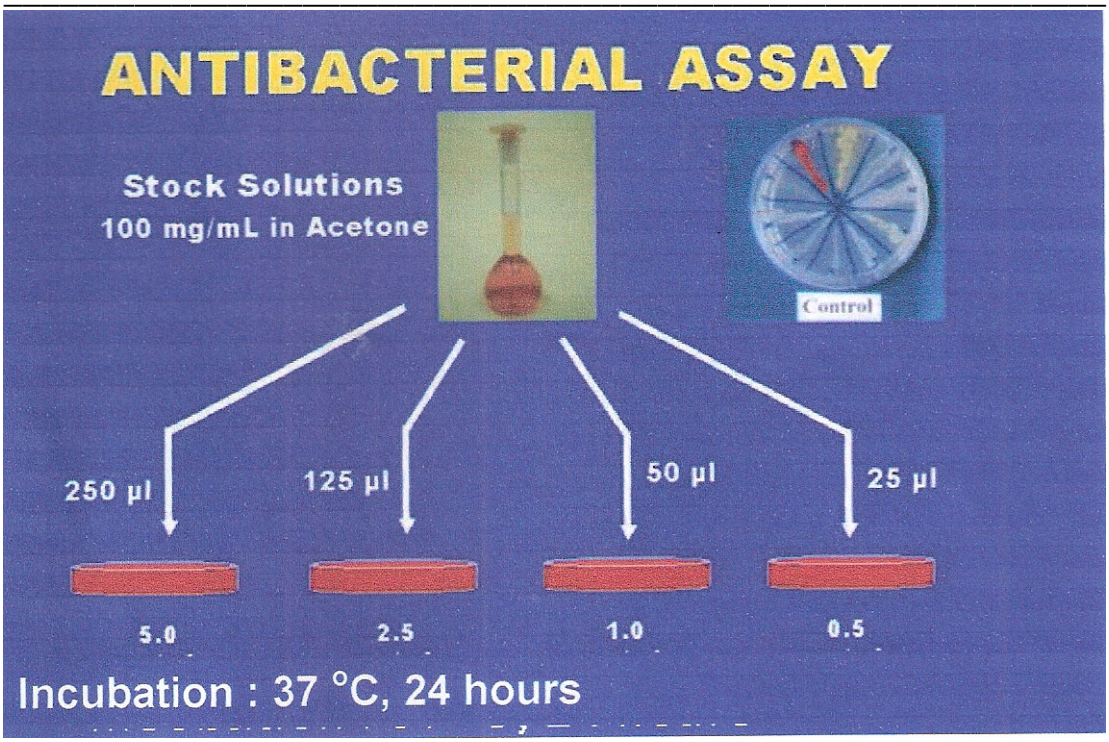

Figure 2.2 Antibacterial assay procedure

2.2. 5 Antimycobacterial bioassay

Among the 22 species, ten plants which showed good antibacterial activity (C*assia abbreviata, Elephanthorrhiza elephantina, Hemizygia bracteosa, Gladiolus dalenii, Hoslundia opposita, Lippia javanica, Litogyne gariepina, Melia azedarach*, *Rhoicissus tomentosa* and *Salvadora australis* used for respiratory diseases) were further tested against a drug sensitive strain of *Mycobacterium tuberculosis* H37Rv, (ATCC 27294), considered to be gram positive, using rapid radiometric respiratory technique with the BACTEC apparatus as described by Middlebrook *et al.*(1977). Solutions of the plant extracts were prepared by maceration of a requisite amount of the extracts in a known volume of dimethyl sulphoxide (DMSO) to obtain a

concentration of 100 mg/ml and stored at 4°C until used. Subsequent dilutions were done in DMSO and added to 4 ml of BACTEC 12B (7H12 medium) broth to achieve the desired final concentrations of 5.0, 2.5, 1.0, 0.5 and 0.25 mg/ml, together with PANTA (Becton Dickinson & Company), an antimicrobial supplement. Control experiments showed that the final amount of DMSO (1%) in the media had no effect on the growth of *M. tuberculosis*. BACTEC drug susceptibility testing was also done for the standard anti-TB-drugs, streptomycin (Sigma Chemical Co, South Africa) and ethambutol at concentrations of 6.0 and 7.5 µg/ml, respectively, against the H37Rv strain. The homogenized cultures (0.1 ml) of *M. tuberculosis*, yielding $1x10^4$ to $1x10^5$ colony-forming units per millilitre (CFU/ml), were inoculated in the vials containing the extracts as well as in the control vials (Lall and Meyer, 1999; Heifets *et al*., 1985). Three extract-free vials were used as controls (medium + 1% DMSO): two vials (V1) were inoculated in the same way as the vials containing the extracts, and the other (V2) was inoculated with a 1:100 dilution of the inoculum (1:100 control) to produce an initial concentration representing 1% of the bacterial population ($1x10^2$ to $1x10^3$ CFU/ml) found in the vials containing extracts.

The MIC was defined as the lowest concentration of the extract that inhibited more than 99% of the bacterial population. When mycobacteria grow in 7H12 medium containing ^{14}C-labeled substrate (palmitic acid), they utilize the substrate and $^{14}CO_2$ is produced. The amount of $^{14}CO_2$ detected reflects the rate and amount of growth occurring in the sealed vial, and is expressed in terms of the Growth Index (GI). Inoculated bottles were incubated at 37°C and each bottle was assayed every day to measure GI at about the same hour until cumulative results were interpretable. The

difference in the GI values of the last two days is designated as ΔGI. The GI reading of the vials containing the test plant extract was compared with the control vial (V2). Readings were taken until the control vials, containing a 100 times lower dilution of the inoculums than the test vials, reached a GI of 30 or more. If the ΔGI value of the vial containing the test plant extract was less than the control, the population was reported to be susceptible to the extract. Each test was replicated three times.

2. 3 Results and discussion

2.3.1 The antibacterial bioassay

Most of the plant extracts inhibited the growth of the Gram-positive microorganisms (Table 2.2). The minimum inhibitory concentration of eight plants (*Cassia abbreviata, Elephanthorrhiza elephantina, Hemizygia bracteosa, Hoslundia opposita, Momordica balsamina, Rhoicissus tomentosa* and *Salvadora australis*) against Gram-positive bacteria was found to be 0.5 mg/ml. Among the 22 acetone extracts tested, two were found to have activity against Gram-negative bacteria at a concentration of 5.0 mg/ml (*Adenia gummifera* and *Momordica balsamina*). *Rhoicissus revoilli* inhibited *E. cloacae*, a Gram-negative strain, at a concentration of 2.5 mg/ml. The resistance of Gram-negative bacteria to plant extracts has been documented previously (Meyer and Afolayan, 1995). These observations are likely to be the result of the differences in cell wall structure between Gram-positive and Gram-negative bacteria. It has been stated that the outer membrane of Gram-negative bacteria acts as a barrier to many environmental substances, including antibiotics (Tortora *et al*., 2001).

The reference antibiotic, streptomycin sulfate inhibited the growth of all the bacterial species tested in this study at 10 µg/ml except, *Pseudomonas aeruginosa* and *Serratia marcescens* which were inhibited at 50 µg/ml and 100 µg/ml respectively.

Gram-positive bacteria were found to be susceptible to extracts of *Lippia javanica* at concentration of 0.125 mg/ml similar to the reports of other researchers previously (Matingo and Chagonda, 1993). These results confirm the findings of other researchers where it was found that acetone extracts of *C. abbreviata*, showed significant inhibition against *B. pumulis*, *B. subtilis* and *S. aureus* at 0.5 mg/ml (Kambizi and Afolayan, 2001). Similar to the reports of the other researchers previously Matingo and Chagonda, (1993).

Khan *et al.* (2001) reported that a previous evaluation of antibacterial activity of the dichloromethane fraction of the stem bark of *Melia azeradarach* showed inhibition at the highest levels used. In another study, extracts of the leaves of *Salvadora persica* L. were found to have an antimicrobial effect on *Streptococcus faecalis* (Almas, 1999, Almas 2001). The antibacterial properties of *Hemizygia* species has already been reported by Kato *et al.* (1996).

Table 2.2. Antibacterial activity of Mozambican medicinal plants

	MIC [a] (mg ml^{-1})									
Plant species	Ba.(+)	Bp (+)	Bs (+)	Sa (+)	Ef (+)	Ecl (-)	Ec (-).	Kp (-)	Pa (-)	Sm (-)
Adenia gummifera	1.0	1.0	1.0	1.0	1.0	5.0	5.0	5.0	5.0	5.0
Cassia abbreviate	0.5	0.5	0.5	0.5	0.5	na[b]	na[b]	na[b]	na[b]	na[b]
Catharanthus roseous	5.0	5.0	5.0	5.0	5.0	na[b]	na[b]	na[b]	na[b]	na[b]
Cissus quadrangularis	5.0	5.0	5.0	5.0	5.0	na[b]	na[b]	na[b]	na[b]	na[b]
Coccinia rehmannii	5.0	5.0	5.0	5.0	5.0	na[b]	na[b]	na[b]	na[b]	na[b]
Elephanthorrhiza elephantine	0.5	0.5	0.5	0.5	0.5	na[b]	na[b]	na[b]	na[b]	na[b]
Hemizygia bracteosa	0.5	0.5	0.5	1.0	1.0	na[b]	na[b]	na[b]	na[b]	na[b]
Hoslundia opposita	0.5	0.5	0.5	0.5	0.5	na[b]	na[b]	na[b]	na[b]	na[b]
Lippia javanica	0.125	0.125	0.125	0.125	0.125	na[b]	na[b]	na[b]	na[b]	na[b]
Litogyne gariepina	2.5	2.5	2.5	2.5	2.5	na[b]	na[b]	na[b]	na[b]	na[b]
Gladiolus dalenii	5.0	na[b]	5.0	5.0	5.0	na[b]	na[b]	na[b]	na[b]	na[b]
Maerua juncea	1.0	1.0	1.0	1.0	1.0	na[b]	na[b]	na[b]	na[b]	na[b]
Melia azedarachta	5.0	na[b]	5.0	5.0	5.0	na[b]	na[b]	na[b]		
Momordica balsamina	0.5	0.5	0.5	0.5	0.5	5.0	5.0	5.0	5.0	5.0
Ocimum americanum	2.5	2.5	2.5	2.5	2.5	na[b]	na[b]	na[b]	na[b]	na[b]

Table 2.2 (continued)

	MIC [a] (mg ml^{-1})									
Plant species	Ba.(+)	Bp (+)	Bs (+)	Sa (+)	Ef (+)	Ecl (-)	Ec (-).	Kp (-)	Pa (-)	Sm (-)
Plectranthus fruticosus	2.5	2.5	2.5	2.5	2.5	na[b]	na[b]	na[b]	na[b]	na[b]
Pseudolachnostylis maprouneifolia	5.0	5.0	5.0	5.0	5.0	na[b]	na[b]	na[b]	na[b]	na[b]
Rhoicissus revoilli	1.0	1.0	1.0	1.0	1.0	2.5	na[b]	na[b]	na[b]	na[b]
Rhoicissus tomentosa	0.5	0.5	0.5	0.5	0.5	na[b]	na[b]	na[b]	na[b]	na[b]
Salvadora australis	0.5	0.5	0.5	0.5	0.5	na[b]	na[b]	na[b]	na[b]	na[b]
Salvadora persica	2.5	2.5	2.5	2.5	2.5	na[b]	na[b]	na[b]	na[b]	na[b]
Senna italica	2.5	2.5	2.5	2.5	2.5	na[b]	na[b]	na[b]	na[b]	na[b]

Ba (+) = *Bacillus cereus*, Bp (+) = *Bacillus pumilis*, Bs (+) = *Bacillus subtilis,* Sa (+) = *Staphylococcus aureus,* Ef (+) = *Enterococcus faecalis,* Ecl (-) = *Enterobactercloacae,* Ec (-) = *Esherichia coli*, Kp (-) = *Klebsiella peneumonia,* Pa (-) = *Pseudomonas aeruginosa,* Sm (-) = *Serratia marcescens*

(+) or (-) = Gram reaction

MIC[a], minimal inhibitory concentration

na[b], not active at the highest concentration (5.0 mg ml^{-1}) tested

2. 3.2. The antimycobacterial bioassay

Four of the plant species tested (C*assia abbreviata, Hemizigya bracteosa, Lippia javanica* and *Melia azedarach*) were observed to be active against the H37Rv. (ATCC 27294) strain of TB at a concentration of 0.5 mg/ml which was the lowest concentration used in this study (Table 2.3). *Gladiolus dalenii, Rhoicissus tomentosa* and *Salvadora australis* showed weak antituberculosis activity. According to a previous report on the antitubercular activity of another *Lippia* species (*Lippia turbinata*) complete inhibition of the growth of *M. tuberculosis* was observed by MeOH-CH_2CL_2 extracts obtained from the aerial parts (Timmermann *et al*., 2001). This can explains the wide use of *Lippia* species for respiratory treatment disorders (Pascual *et al.,* 2001).

Table 2.3 Effect of plant extracts on the growth of the sensitive strain (H37Rv) of *Mycobacterium tuberculosis*

Plant species	MIC[a] (mg ml^{-1})	ΔGI[b] values of plant extracts		ΔGI values of the control vials
Cassia abbreviata	0.5	9.3 ± 7.5	(S)	26.5 ± 4.7
Elephantorrhiza elephantina	1.0	45.0 ± 16.1	(R)	26.5 ± 4.7
Gladiolus dalenii	2.5	27.7 ± 5.8	(S)	26.5 ± 4.7
Hemizigya bracteosa	0.5	22.0 ± 1.0	(S)	26.5 ± 4.7
Hoslundia opposita	1.0	9.5 ± 0.7	(S)	26.5 ± 4.7
Lippia javanica	0.5	19.7± 5.1	(S)	26.5 ± 4.7
Litogyne gariepina	1.0	27.7± 28.9	(S)	26.5 ± 4.7
Melia azedarach	0.5	10.3 ± 5.8	(S)	26.5 ± 4.7
Rhoicissus tomentosa	2.5	8.0 ± 3.6	(S)	26.5 ± 4.7
Salvadora australis	2.5	105 ± 7.8	(R)	26.5 ± 4.7

[a]minimal inhibitory concentration, [b]ΔGI values are average ± standard deviation, S, susceptible; R; resistant

2.3.3 Conclusion

The evaluation of plants used in traditional medicine is necessary. In this investigation, a number of plants exhibited promising activity against a variety of bacteria and *Mycobacterium tuberculosis*. It is concluded that the demonstration of inhibitory activities of the tested plants revealed their value in traditional medicine and supports the enormous role of medicinal plants in primary health care.

The results corroborate the importance of ethnopharmacological surveys in selection of plants for bioactivity screening. The results obtained represent a worthwhile expressive contribution to the characterization of the antibacterial and

antimycobacterial activities of plant extracts of traditional medicine plants from Mozambican flora.

Subsequently, bio-guided fractionation will be conducted on plants showing potential activity to identify the active compounds

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