Chapter 1

INTRODUCTION

1.1 Background

1.1.1 Utilization of herbal drugs

Plants have adapted to the diverse habitats of the world through their physical and biochemical modifications**,** and they have been used traditionally as a source of treatment for various ailments for thousands of years throughout the world among all human races (Ellis, 1986). Human populations have adapted largely through the generations and application of knowledge – ecological, practical and theoretical. Today traditional societies throughout the world possess a wealth of such knowledge which they have accumulated during prolonged interaction with the natural world, and which remains fundamental to their physical, spiritual and social well being (Cotton, 1996).

Because of the fear of diseases and death every cultural group has responded by developing a medicinal system and making use of natural products to cure various ailments, but where undoubtedly plants play a major role (Ellis, 1986). According to Finimh (2001), some of these traditions and medicinal practices may seem strange and magical, others appear rational and sensible, but all of them are attempts to overcome illness and suffering, and enhance quality of life.

Many traditional healers still use plants in their crude form (herbal remedies), although Western technologies have transformed some plant products in more palatable forms like tablets, capsules and syrups. Extracts from some of the medicinal plants being used by traditional healers have been found to contain properties that inhibit the growth of bacteria, viruses and other microbes (Ndubani and Hojer, 1999).

Chapter 1 ***Introduction***

The recognition and validation of traditional medicine and the search for plant derived drugs could lead to new strategies to control various diseases (Gessler *et al*., 1994). The industrial uses of medicinal plants are mainly in traditional medicines, health foods and in pharmaceuticals. The global herbal market and industry have been growing rapidly in recent years and therefore, today medicinal plants are of utmost importance (WHO, 1987). Medicinal plants have also become the focus of discovering new drugs and an integral component of research developments in the pharmaceutical industry (Rabe and Van Staden, 1997). Such research focuses on the isolation of and direct use of active medicinal constituents of plants, semi-synthetic drugs and pharmacologically active compounds. As a result the pharmacology industry has invested vast resources into screening the active constituents of medicinal plants from all over the world (Finimh, 2001; Ulubelen *et al*., 1988).

Of the 250 000 species of higher plants known to exist on earth, only a relative handful have been thoroughly studied for all aspects of their potential therapeutic value in medicine. About 25% of prescribed drugs used in modern medicine today originated from the plant kingdom (Balick, 1990). Approximately one-third of the top selling drugs in the world are natural products or their derivatives' often with ethnopharmacological background (Table 1.1). The practice of traditional medicine is wide spread throughout the whole world. In China about 40% of the total medicinal consumption is attributed to traditional tribal medicines and it is estimated that in the mid-nineties, receipts of more than 2,5 billion US dollars ($) have resulted from the sales of herbal medicines in Thailand. The same applies to Japan where herbal medicinal preparations are more in demand than mainstream pharmaceutical products (Lemma, 1991).

Moreover, natural products are widely recognised in the pharmaceutical industry for their broad structural diversity as well as their wide range of pharmacological activities. New medicines have been discovered with traditional, empirical and molecular approaches (Harvey, 1999; Strohl, 2000). The traditional approach makes use of material that has been found by trial and error over many years in different cultures and systems of medicine (Cotton, 1996).

Another approach is by bioassay-guided fractionation of plant extracts isolation of biologically active molecules. Plants investigated for their medicinal properties have yielded many useful compounds such as quinones, flavonoids, lectins, alkaloids, glycoproteins, terpenoids, coumarins, glycosides and glycones. Examples include drugs such as morphine, quinine and ephedrine that have been in widespread use for a long time and more recently derivatised synthesized compounds such as the antimalarial artemisinin. Among the successful clinical agents derived from plants are 'benzodiazepines' for insomnia and anxiety attacks, 'atenolol' for the treatment of hypertension, 'salbutamol' for asthma etc. (Phillipson, 2001).

1.1.2 Medicinal plant-industry in Africa

Africa is known to be a rich source of medicinal plants and some pharmaceutical agents have been produced from local plant species. In contrast with western medicine, which is technically and analytically based, traditional African medicine takes 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 (Anyinam, 1987; WHO, 1987). Traditional African medicine is a holistic discipline involving extensive use of herbalism combined with aspects of spirituality. Traditional rural African communities have relied upon the spiritual and practical skills of the Traditional Medical Practitioners (TMP's) whose botanical knowledge of plant species and their ecology as well as scarcity are invaluable.

In most traditional African societies there are individuals such as herbalists, spiritualists, traditional practitioners, etc. who have the responsibility of providing relief from different diseases and social problems (Duke, 1985). It is often argued that traditional healers operate close to the people and that they are very helpful in many rural communities where modern medicine is not readily available (Cunningham, 1988). In the past, traditional healers were considered to be more effective against psychosocial illness. It is now known that traditional healers use a vast array of

medicinal plants for infections such as colds, coughs and inflammatory diseases (Shale *et al*., 1999). Indigenous medicinal plants are used by more than 60% of South Africans in their health care needs or cultural practices (Table 1.2). Approximately 3,000 species are used by an estimated 200,000 indigenous traditional healers (Van Wyk *et al*., 1997). Due to urbanization, a large informal trade business has been established with medicinal plants. Unfortunately, commercial exploitation threatens to deplete plant populations, resulting in many species being considered vulnerable to extinction and being lost from their natural habitat (Mander *et al*., 1995). It is estimated that 70% of South Africans consult traditional healers known as 'inyanga'. These traditional healers use many traditional medicines derived from plants for various ailments (Table 1.3). Parts of the plants can be applied directly to wounds and cuts or prepared as powders that are used like snuff or in the form of smoke or fumes, and also as infusions (tinctures).

Among a few medicinal plants being cultivated for international trade industry are *Warburgia salutaris* (pepperbark tree), *Siphonochilus aethiopicus* (African ginger), *Aloe ferox* (Cape aloes), *Agathosma* sp. (buchu), *Harpagophytum procumbens* DC (devil's claw), *Pelargonium sidoides* DC (Umkcaloabo) and *Xysmalobium undulatum* (Uzara), (George, 1997) (Table 1.2). Buchu and devil's claw are cultivated commercially, but is also harvested in the wild, which can lead to over utilization of natural habitat. *Aloe ferox* Miller is sustainably harvested from the wild. A large number of species containing chemical components have the potential to play a role in the medicinal market on a global scale. At present bio prospecting is done in all plants in South Africa to determine among other things its pharmaceutical potential (Mander *et al*., 1995).

Table 1.1. The world's 25 best selling pharmaceuticals in 1991 (Phillips and Drew, 1992)

Position 1991	Product	Therapeutic Class	Sales $m
1	Ranitidine	H2 antagonist	3,032
2	[a]Enalapril	ACE inhibitor	1,745
3	[a]Captopril	ACE inhibitor	1,580
4	[a]Diclofenac	NSAID	1,185
5	Atenolol	β-antagonist	1,180
6	Nifedipine	Ca^{2+}	1,120
7	Cimetidine	H_2 antagonist	1,097
8	[a]Mevinolin	HMGCoA-R inhibitor	1,090
9	[a]Naproxen	NSAID	954
10	[a]Cefaclor	β-lactam antibiotic	935
11	Diltiazem	Ca^{2+} antagonist	912
12	Fluoxetine	5HT reuptake inhibitor	910
13	Ciprofloxacin	Quinolone	904
14	Amlodipine	Ca^{2+}	896
15	[a]Amoxycillin/ clavulanic acid	β-lactam antibiotic	892
16	Acyclovir	Anti-herpetic	887
17	[a]Ceftriaxone	β-lactam antibiotic	870
18	Omeprazole	H^+ pump inhibitor	775
19	Terfenadine	Anti-histamine	768
20	[a]Salbutamol	β_2-agonist	757
21	[a]Cyclosporin	Immunosuppressive	695
22	[a]Piroxicam	NSAID	680
23	Famotidine	H_2 antagonist	595
24	Alprazolam	Benzodiazepine	595
25	[a]Oestrogens	HRT	569

[a]Natural product derived

Table 1.2 Selection of indigenous medicinal plants used in the South Africa (Mabogo, 1990)

Species	**Family**	**Popular name**
*Agathosma betulina (*Bergius) Pill.	Rutaceae	Buchu
Agathosma crenulata (L.) Pill.	Rutaceae	Buchu
Aloe ferox Miller	Asphodelaceae	Aloe
Artemisia afra Jacq. ex Willd.	Asteraceae	Wormwood
Balanite maughamii Delile	Balanitaceae	Torchwood
Bersama tysoniana Oliv	Melianthaceae	White ash
Boophane disticha (L.f.) Herbert.	Amaryllidaceae	Tumbleweed
Bowiea volubilis Harv.	Hyacinthaceae	Climbing lily
Cassine papillosa (Hochst.) Kuntze	Celastraceae	Common saffron
Clivia miniata Regel.	Amaryllidaceae	Bush lily
Cryptocarya latifolia Sond.	Lauraceae	Broad leaved quince
Curtisia dentata (Burm.f.) C.A. Smith	Cornaceae	Assegaai
Dioscorea sylvatica (Kunth) Ecklon	Dioscoreaceae	Elephant's foot
Eucomis autumnalis (Mill.) Chitt.	Hyacinthaceae	Wild pineapple
Gunnera perpensa L.	Gunneraceae	Wild rhubarb
Harpagophytum procumbens DC.	Pedaliaceae	Devil's claw
Ocotea bullata (Burchell) Baillon	Lauraceae	Stinkwood
***Pelargonium sidoides* DC.**	**Geraniaceae**	**Umkcaloabo**
Pittosporum viridiflorum Sims	Pittosporaceae	Cheesewood
Rapanea melanophloeos (L.) Mez	Myrsinaceae	Cape beech
Scilla natalensis Planch.	Hyacinthaceae	Blue hyacinth
Siphonochilus aethiopicus (Schweinf.) B.l. Birtt	Zingiberaceae	African ginger
Stangeria eriopus Nash	Stangeriaceae	Natal grass cycad
Warburgia salutaris (Bertol.f.) Chiov.	Canellaceae	Pepperbark tree

Table 1.3 Uses of medicinal plants (Balick, 1990)

Species	Common name	Uses
Aloe Vera	Aloe	Wounds, Burns, laxative
Angelica arcangelica	Angelica	Promotes menstrual flow
Piminella anisum	Anise	Promotes digestion, antispasmodic
Cinnamomum campora	Campor	Antiseptic, antispasmodic
Eugenia caryophyllata	Clove	Antiseptic, antibacterial, mind & body stimulant
Taraxacum officinale	Dandelion	Diuretic, antibiotic, digestive
Eucalyptus globules	Eucalyptus	Antiseptic, antifungal, expectorant
Allium sativum	Gallic	Antibiotic, expels worms, antiplasmodic, diaphoretic
Zingibar officinali	Ginger	Diaphoretic, circulatory stimulant, antiseptic, coughing, anti-inflammatory
Ginkgo biloba	Ginkgo	Anti-inflammatory, antiplasmodic, anti-asthmatic, anti-allergenic
Jasminum grandiflorum	Jasmine	Anti-spasmodic, aromatic
Lavandula officinalis	Lavender	Relieves muscle spasms, antiseptic, antibacterial, antidepressant
Citrus limon	Lemon	Antiseptic, anti-rheumatic, antibacterial, antioxidant, reduces fever
Commiphora molmol	Myrrh	Stimulant, antiseptic, anti-inflammatory, astrigent, antispasmodic, expectorant
Olea europaea	Olive	Digestive, diuretic, anti-inflammatory
Petroselinum crispum	Parsley	Digestive, diuretic, anti-inflammatory
Mentha piperita	Peppermint	Carminative, relieve muscle spasms, increase sweating, antiseptic
Piper nigrum	Pepper	Antibacterial, digestive, antiseptic, reduces fever
Raphanus sativus	Radush	Digestive, mild laxative
Rauvolfia serpentina	Rauvolfia	Antidepressant, lower blood pressure
Rheum palmatum	Rhubarb	Laxative, constipating, antibacterial, eases stomach pains
Rosa gallica	Rose	Aromatic, antidepressant, anti-inflammatory, sedative
Sesamum indicum	Sesame	Digestive, aromatic, antispasmodic
Malaleuca alternifolia	Tea Tree	Antiseptic, antibacterial, anti-viral, antifungal
Valeriana officinalis	Valerian	Sedative, relaxant, relieves muscle spasms, lower blood pressure

1.1.3 Future of traditional medicine

Despite the increasing use of medicinal plants, their future is being threatened by complacency concerning their conservation. Each year large numbers of medicinal plants are destroyed through over-exploitation by herbalist, medicinal plant traders and also through conversion of forests to agriculture (Balick, 1990). In developed countries like France, Britain, Norway, Sweden, Switzerland, Denmark, Canada, USA, China and Japan, reserves of herbs and stocks of medicinal plants are diminishing and in danger of extinction as a result of growing trade demands for cheaper health care products in preference to more expensive target-specific drugs and biopharmaceuticals. The results of over exploitation, non-sustainable harvesting techniques, loss of growth habitats and unmonitored trade of medicinal plants have put the future of traditional medicine under threat (Hoareau and Da Silva, 1999).

Scientific validation of traditional medicinal plants is essential in order to benefit humankind. Many medical scientists still find it hard to accept that natural medicines can be good as pharmaceutical therapeutics. The increase in scientific research on traditional medicine will probably change this thinking (Finimh, 2001). Herbal remedies play a fundamental role in the traditional medicine in rural areas of South Africa. More and more people utilize traditional medicine for their major primary health care needs. Today, there is growing interest in natural and traditional medicines and scientists all over the world are looking for new cures in collaboration with traditional healers (Sindambiwe *et al*., 1999).

1.2 Tuberculosis

1.2.1 Epidemiology

Despite all the advances in the treatment of tuberculosis (TB), this disease continues to be one of the major health problems facing mankind particularly in the developing

countries, with India accounting for nearly 30% of the global burden. According to the World Health Organisation (WHO, 1997), global tuberculosis report for 2003, the incidence rate of TB has shown a 0.4% increase per year. Tuberculosis kills approximately 2 million people each year and the global epidemic is growing and becoming more dangerous. The breakdown in health services, the spread of HIV/AIDS and the emergence of multidrug-resistant (MDR) TB are contributing to the worsening impact of this disease. In 1993, the WHO took an unprecedented step and declared tuberculosis a global emergency. It is estimated that between 2002 and 2020, approximately a billion people will be newly infected, more than 150 million people will get sick, and 36 million will die of tuberculosis. Today, *M. tuberculosis* is responsible for more morbidity in humans than any other bacterial disease. *M. tuberculosis* infects 1,7 billion people per year which is equal to 33% of the entire world population. Since 1985, the number of TB cases has risen every year. This rise is largely attributed to the emergence of HIV/ AIDS infections that has occurred during the same period of time.

TB was the cause of the "White Plague" of the 17th and 18th centuries in Europe. During this period nearly 100% of the European population was infected with *Mycobacterium tuberculosis* and 25% of all adult deaths were caused by *M. tuberculosis* (Fadda and Rowe, 1984). In the USA in Maryland, new TB cases rose from 262 in 2001 to 306 in 2002, mainly among the elderly in Montgomery, Prince George's counties and in Baltimore (Mitnick *et al.*, 2003). The national tuberculosis rate fell to a record low in 2002, according to recent statistics, in Virginia in the USA. Cases of the contagious disease continues to climb, and officials are warning that more money and stronger initiatives are needed for this kind of public health threat. The latest report by the federal Centers for Disease Control and Prevention put the county's TB rate at 9.8% cases for every 100,000 residents. That's almost twice the national rate of 5.2% increase cases and represents more active cases than in all but 14% cases in U.S. cities. Although TB cases fell in 2002 to an all-time national low in the Washington region, of 15,078 since reporting began in 1953, where cases spiked, according to the federal report released in 2003.

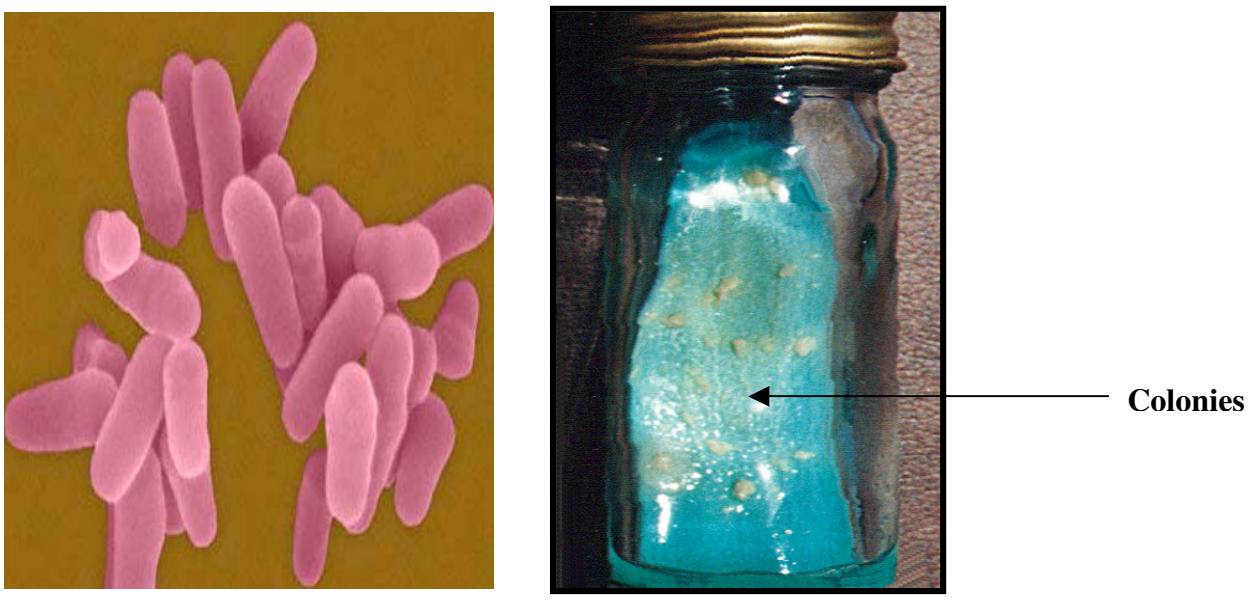
The current threat in TB treatment lies on the fact of emergence of strains resistant to two most effective antituberculosis drugs, isoniazid (IND) and rifampicin (RIF). The prevalence of Multi-drug resistant TB (MDR-TB) is the highest (14.1%) in Estonia in USSR. There are a number of countries that have made remarkable progress in expanding population coverage with cure rates whereas, South Africa battles with more than 188 000 new TB cases per year (Bloom, 2002). The Medical Research Council's National Tuberculosis Research Programme estimates that South Africa will see the number of TB sufferers approaching 300 000 in 2003. Taking into account the link between TB and HIV, it is even more disturbing to note that almost 50% of those suffering from TB will also be HIV positive. Statistics show that in the absence of HIV infection, only about 10% of TB-infected individuals get sick with TB during their lifetime. In people who are co-infected with HIV and TB, about 50% will develop TB. TB also probably accelerates the progression of the HIV (Lodha and Kabra, 2004).

1.2.2 *Mycobacterium tuberculosis*

M. tuberculosis is the etiologic agent of tuberculosis in humans. *M. tuberculosis* is a fairly large non-motile rod-shaped bacterium (Figure 1.1a). The rods are 2 – 4 μm in length and 0,2 – 0,5 μm in width. The cell wall structure of *M. tuberculosis* contains peptodoglycan and lipids which consists of major components; mycolic acids which are α-branched lipids in cell walls, make up 50% of the dry weight of the mycobacterium cell envelope and are very strong hydrophobic molecules that form a lipid shell around the organism (Goren, 1990). Cord factor is a glycolipid (trehalose dimycolate) found in the cell wall that induces replication *in vitro*, resulting in serpentine cords of organisms. The role of the cord in the pathogenesis of tuberculosis is still under investigation, however, it is thought to be important because it inhibits and induces secretion of TNF-alpha by macrophages (Brennan, 1988). *M. tuberculosis* is an obligate aerobe and is always found in the well-aerated upper lobes of the lungs. The bacterium is a facultative intracellular parasite, usually of macrophages, and has a slow generation time, 15 – 20 hours, a physiological

characteristic that may contribute to its virulence. *M. tuberculosis* is not classified as either a Gram-negative or Gram-positive bacteria because it does not have the biochemical characteristics of both. If a Gram stain is performed on *M. tuberculosis*, it stains very weakly Gram-positive or not at all. *Mycobacterium* species, along with members of a related genus *Norcardia*, are classified as acid-fast bacteria due to their impermeability by certain dyes and stains. One acid-fast staining method for *M. tuberculosis* is staining with carbon-fuchsin (a pink dye) and decolourising with acid alcohol. The smear is counterstained with methylene blue or certain other dyes (Figure 1.1b; Fadda and Rowe, 1984; Dunigan *et al*., 1995).

Infection with *M. tuberculosis* occurs by inhalation of small (1 - 10 microns) droplets containing only a few live tubercle bacilli. The primary focus of infection is usually therefore, the middle or lower zones of the lung. The bacilli are readily taken up by lung macrophages but survive and grow to form the primary focus of infection and from there, entering the local lymphatic and then throughout the body via the blood and lymphatic system. This stage of disease is usually clinically silent or associated with mild fever and in most cases immunity develops within a few weeks and the patient becomes tuberculin positive (Girling *et al*., 1989).



(a) (b)

Figure 1.1 ***M. tuberculosis***

(a) Rods of *M. tuberculosis* Magnification: x 6.250 (based on a 35 mm slide *image* of 24 mm in the narrow dimension (Courtesy: SEM/ 97229A).

(b) Colonies of *M. tuberculosis* on Lowenstein-Jensen medium

1.3 Other bacterial infections

Bacteria are the oldest, the simplest, unicellular organisms with the most numerous forms of life and are responsible for much more than just disease. Bacteria existed long time about 3.5 billion years ago, until recently, the term bacteria was used for all microscopic prokaryotes. There are thousands of different kinds of bacteria; most of them are harmless to humans. About two thousand species of bacteria have been identified; it is also possible for bacteria to reproduce as often as every twenty minutes (Crockett, 1994). A bacterium's structure is a prokaryotic cell consisting of a capsule, cell wall, cell membrane and cytoplasm; there are no intracellular organelles. Bacteria are found almost everywhere even in the Dead Sea. They are important for their role of decomposition, leading to the release of carbon to the atmosphere that

plants use and also essential nutrients into the air and soil. With no carbon dioxide there would be no photosynthesis (Flannery, 1997). Some bacteria form symbiotic relationships in the gastrointestinal tract of animals as well as humans, assisting with digestion. Bacteria even produce essential vitamins, such as vitamin B12, which is essential for humans. Vitamin B12 is the most important vitamin produced by *Pseudomonas denitrificans*. Deficiency of which leads to pernicious anaemia. Other important producers are species of the genus *Propionibacterium*. The other important vitamin made commercially by micro-organisms is Riboflavin. Most riboflavin is produced by a fungus called *Ashbya gossifyii*, however, bacteria can also produce it. Doctors and scientists have figured out how to use dead or weakened bacteria to prevent other bacterial diseases (vaccination). A number of drugs, hormones or antibodies are obtained from bacteria (Schlessinger, 1990).

Bacteria are classified according to shape (bacilli, cocci and spiral), ability to form spores, nutritional requirements and reaction to the Gram stain. The Gram stain is a purple dye called crystal violet, which is washed out by the application of alcohol or acetone from Gram-negative cells. Bacteria that are not discoloured by the alcohol or acetone washed are the Gram-positive (Cullimore, 2000). Man is a host to a variety of pathogenic bacteria, protozoa and viruses. Bacteria colonize a particular part of a person's body and remain there, often without causing illness. Some may even help the body function, as in the case of intestinal bacteria. 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. Strep throat, cholera, pneumonia, diarrhoea and whooping cough are some of the diseases, which are caused by the Gram-positive and Gram-negative bacteria (Table 1.4; Stanier *et al*., 1976).

Respiratory infections can be divided into upper respiratory tract infections (URTI's); including tonsillitis, sinusitis and otitis media, which is the inflammation of the area behind the eardrum (tympanic membrane) producing pus, fluid, causing coughs and the lower respiratory tract infections (LRTI's) including bronchitis and

pneumonia. Bacterial infections are usually proceeded by viral respiratory tract infections. Lower respiratory infections are caused by secondary infections by pathogens that are inhaled from the environment, spread from blood, or breathed in from the upper respiratory tract such as from microorganisms causing bronchitis; *Haemophilus influenza*, *Moraxella catarrhalis* and *Streptococcus pneumonia* (Benjamin *et al*., 1991).

In this study, the selected plants have been investigated for their antibacterial activity against the types of bacteria that cause acute bronchitis including *S. pneumonia*, *M. catarrhalis* and *H. influenza*.

Table 1.4. Gram-positive and Gram-negative bacteria associated with upper and lower respiratory infections

Organism	**Disease**
Enterococcus faecalis	Sore throat, coughs
Enterobacter sp.	Fever
Haemophilus influenza	Bronchitis, pneumonia
Klebsiella pneumonia	Pneumonia
Proteus mirabilis	Fever, chest pains, pneumonia
Pseudominas aeruginosa	Pneumonia
Serratia marcescens	Coughs, fever
Staphylococcus aureus	Nose, throat, pneumonia
Moraxella catarrhalis	Bronchitis, pneumoniae, ear infections,
Streptococcus pneumonia	Bronchitis, pneumonia, sinus and ear infections

1.3.1 *Haemophilus influenza*

H. influenza is a Gram-negative coccobacilli bacterium, found in low numbers as indigenous micro flora of the upper respiratory tract, an opportunistic pathogen causing pneumonia, epiglottitis, bronchioitis and meningitis. This bacteria is not the cause of influenza, influenza is caused by a virus. *H. influenza* have been categorised as a, b, c, d, e and f on the basis of antigenic differences in their capsular material. The case-fatality rate for invasive *H. influenza* disease is 2% to 5%. Invasive diseases caused by *H. influenza* type b can affect many organ systems.

The most common types of invasive diseases are bronchitis, meningitis, epiglottitis, pneumonia, arthritis, and cellulites. Type b is the one most often associated with serious diseases in children, possesses a capsule that is a polymer of ribose and ribotil phosphate (commonly called PRP). *H. influenza* is an obligate human parasite that is passed from person to person by way of the respiratory route. It is reported that 30 – 50% of all children carry the bacillus in the nasopharynx (Brook, 2002)). All organisms in the genus *Haemophilus* are morphologically similar (small 0.3 x 2µm rods), though they are pleomorphic and often vary from coccoid to long, filamentous or distorted forms. They are among the most highly adapted and fragile parasitic and pathogenic bacteria with fastidious nutrient requirements. Most grow well on "chocolate agar" (i.e. infusion agar, with about 10% blood, heated to 90ºC for 10 minutes) though some require special nutrition (Fuerst, 1983).

1.3.2 *Moraxella catarrhalis*

The genus *Moraxella* is named after Morax, a famous French ophthalmologist-bacteriologist, who first isolated the type species *Moraxella laculata* (Boom *et al*., 1992). *M. catarrhalis* is a Gram-negative, aerobic oxidase + diplococcus that was discribed for the first time in 1886 with the name '*Mikrokokkus catarrhalis'*. The organism also has been known as *Micrococcus catarrhalis*, *Neisseria catarrhalis* and *Branhamella catarrhalis*. In the twentieth century, *M. catarrhalis* was considered a saprophyte of the upper respiratory tract with no significant pathogenic consequence (Karlidag, *et al*., 2002). *M. catarrhalis* causes bronchitis and pneumonia in children and adults with underlying chronic lung disease and occasionally is a cause of bacteremia or meningitis, especially in patients who are immunocompromised (Benjamin *et al*., 1991). It colonizes the throat of about 5 percent of healthy children and a higher percentage of adults with chronic lung disease.

M. catarrhalis, has also been associated with nasocomial infections. The most significant infections caused by *M. catarrhalis* are upper respiratory tract infections (0.2 – 8.1%), including otitis media and sinusitis in children and lower respiratory

tract infections in adults. Infections with *M. catarrhalis* in adults are more common if underlying conditions are present, especially in elderly persons. (Girst *et al*., 1979).

1.3.3 *Streptococcus pneumonia*

S. pneumonia formely named, 'diplococcus pneumoniae', and commonly called the pneumococcus, was isolated in 1881 by Pasteur and was later shown to be the major cause of lobes of pneumoniae in humans (infecting one or more lobes of the lung). *S. pneumonia* is Gram-positive bacteria with a well-formed capsule. The organisms are characterized by a coccus appearance, a thick cell wall and aerobic action on glucose.

This bacterium is commonly found in the nose and throat. This organism is one of the commonest seen in community-acquired pneumonia, accounting for up to 25% of these infections. *S. pneumonia* has a high fatality rate, being a frequent cause of death in children and the elderly (Benjamin *et al*., 1991). *S. pneumonia* is considered invasive when it is found in the blood, spinal fluid or other normally sterile sites. It infects the upper respiratory tract and can cause pneumonia, lining of the brain and spinal cord (meningitis), bones (osteomyelitis), joints (arthritis), ears (otitis media) and sinuses (sinusitis and bronchitis).

1.3.4 Gram-positive and Gram-negative bacteria

Both Gram-positive and Gram-negative bacteria contain peptidoglycan in the cell wall, but in Gram-negative, various other layers protect it and so the stain cannot reach inside to cause the colouration, the outer membrane is a lipid bilayer consisting of lipopolysaccharides. On the inside of the peptidoglcan layer is the cell membrane, which contains proteins. Additionally phospholipids, protein, lipoprotein and a small amount of peptidoglycan are present. These always face the outside and are involved in increasing the barrier for molecules entering the cell. The space between the peptodoglycan layer and the outer membrane is called the periplasm, containing many different proteins. Hydrophobic and larger molecules cannot pass through and this is

how the Gram stain is prevented from reaching the peptidoglycan layer to colour it (Ainsworth and Sussman, 1968). In Gram-positive bacteria there is a very big variation in structure and composition.

The peptidoglycan is on the outside of the Gram-positive bacteria and the stain can easily reach it. The peptidoglycans, which are sometimes also called murein, are heteropolymers of glycan strands, which are cross-linked through short peptides. There have also been some minor variations recorded in composition in some groups. Thus, in *Mycobacterium* and *Nocardia* the *N*-acetyl moiety of the muramic acid is replaced by the oxydised form *N-glycolyl*. The amino acid composition of both the cross-linking as well as the stem polypeptides can vary extensively with different groups. These differences form the basis for the taxonomy of these organisms (Martha *et al*., 1997). On the side-chains are carried the bases for the somatic antigen specifity of these organisms. The chemical composition of these side chains both with respect to components as well as arrangement of the different sugars determines the nature of the somatic or antigen determinants, which are such important means of serologically classifying many Gram-negative species (Abraham *et al*., 1993).

The difference between Gram-positive and Gram-negative bacteria is in the permeability of the cell wall to these 'purple coloured iodine-dye complexes' when treated with the decolourising solvent. The cell wall of Gram-positive bacteria, such as *B. subtilus, S. pyogenes* and *S. aureus*, retain gentian violet. In contrast, Gram-negative bacteria, such as *E. coli* and *V. cholerae*, which do not have a comparable cell wall, do not retain gentian violet (Cullimore, 2000).

1.4 Fungal infections

Fungi differ from bacteria in possessing a higher number of chromosomes within a well-defined nuclear membrane, mitochondrion, and an endoplasmic reticulum. Like plants they have definite cell walls, but these are usually composed of chitin, glucan, chitosan, mannan and other components in various combinations, rather than

cellulose. They lack chlorophyll, so they live either on dead organic material as saprophytes, or on living organic matter as parasites. The cells may live separately (yeasts) or more commonly, they form long multicellular filaments or hyphae, which may contain cross-walls or septa (Ansteid *et al*., 1999). A mass of hyphae is a mycelium. Many species have both yeast and mycelial forms which are dependent on the cultural conditions, a process known as dimorphism (Alexopoulos *et al*., 1996). The classification of fungi is based on the form of their sexual reproductive apparatus, but there is a large group, containing most of the human parasites, which have never been known to undergo sexual reproduction (Ellis *et al*., 1994). The following are the main four classes of fungi:

1. Zygote fungi (zygomycota): There are about 600 different species of zygote fungi. Most of them are terrestrial and live in soil or decaying plant material. One of the most common types of zygote fungi is bread mould (*Rhizopus stolonifer*), some of the zygote fungi form mycorrhizae (*R. nigricans*) with plants for them to live on. *Rhizopus* soft rot is a disease caused by these types of fungi, the hyphae secrete pectinolytic enzymes that break down the middle lamellae of infected tissue and causes a soft, watery rot. The fungi lack cutinases and, therefore, can enter host tissue only through wounds.

2. Sac fungi (ascomycota): There are over 60 000 different species of sac fungi and they therefore have a wide range of habitats and characteristics, members of the sac fungi are important in digesting resistant materials such as cellulose and collagen. Yeasts (*Saccharomyces*), molds, morels and truffles (*Claviceps)* are examples of sac fungi. They produce sexual spore in saclike ascii and most carry out their sexual stages in a fruiting body known as an astrocarp. Asexual spores are produced in long chains or clusters at the end of the hyphae and they are dispersed with the wind. Sac fungi contain toxic alkaloids, including lysergic acid diethylamide (LSD). When infected rye is made into bread, the toxins are ingested and cause vomiting, muscle pain, feeling hot or cold, hand and foot lesions, hysteria and hallucinations.

3. Club fungi (basidiomytes): There are about 25 000 species of club fungi, most of them have a club- shaped basidium which gives them their name, for example, the mushroom, puffballs, bracket fungi, birds nest fungi, and stinkhorns. These fungi are extremely important in the decomposition of organic matter. They reproduce asexually. They cause some serious plant diseases such as rusts and smuts.

4. Imperfecti fungi (deuteromycotina): Imperfect fungi are mycelial fungi that produce by means of conidia that are generally produced on free or aggregated conidiophores on the substrate surface. These fungi lack a sexual or a perfect stage. They have lost their ability to reproduce sexually. They have developed parasexual reproduction in which clear fusion occurs but not meiosis proper.

Different organs in the human body are infected by different fungal species (Table 1.5). Systematic mycoses is a respiratory disease caused by fungal pathogens. Coccidioidomycosis is caused by *Coccidioides immitis*, infecting the pulmonary spaces. Histoplasmosis is caused by the fungus *Histoplasma capsulatum*, causing mild lung infections. Blastomycosis is caused by the fungus *Blastomycoses dermatitidis* infecting the human by the airborne route. *Aspergillus, Penicillium, Cladosporium, Fusarium*, *Paecilomyces*, dust-borne *Zygomycetes*, and dust-borne. *Alternaria* species are often associated with lower respiratory infection, bronchitis and chronic pulmonary disease. Toxicity by inhalation can be 40 times greater than by ingestion (Kowalski and William, 1998).

Table 1.5. Fungal pathogens associated with upper and lower respiratory infections

Organism	**Disease**
Malassezia furfur	Skin
Microsporum species	Skin, hair
Epidermophyton species	Skin, hair, nails
Fusarium species	Lungs, nose, throat
Penicillium species	Lungs, sinus
Blastomyces dermatitidis	Lungs, ear infections, throat
Cryptococcus neoformans	Lungs, nose, Central Nervous System
Aspergillus species	Respiratory system, bone marrow failure
Rhizopus species	Lungs, nails, skin, ear infections

1.4.1 *Aspergillus niger*

The *Apergillus* fungus (sac fungi), causes different types of pulmonary disease: inhalation of spores may result in bronchial asthma or acute allergic alveolitis. The fungus can colonize old tuberculous or bronchiectatic cavities, in which it may invade the lung tissue to produce a haemorrhagic and necrotozing pneumonia. The last condition tends to occur in immunodeficient or immunodepressed individuals, where the fungus may also invade the walls of the pulmonary vein, causing local thrombosis, and becoming disseminated by the blood stream: which can result in the infection of the heart valve (MacSween and Whaley, 1992).

Aspergillus species are common aspergillosis causing agents in humans and in animals. *A. niger* is one of the frequent agents of otomycosis and of pulmonary "fungus ball" (Ammari *et al*., 1993). Pulmonary cavities in the upper lobe of human lung and chronically obstructed paranasal sinuses can permit the growth of large mycelial masses, called fungus balls. In the lung, a fungus ball (1 – 2cm) may reach several centimetres in diameter. Aspergillosis is a rare complication of AIDS. It is usually seen in patients with advanced disease (Ioachim, 1989). Allergic fungal sinusitis (infection of the sinus) is a unique, probably under-diagnosed condition similar to the lower airway disorder, allergic bronchopulmonary aspergillosis (Schonheyder, *et al*., 1988). Characteristic features of fungal sinusitis are signs or symptoms of chronic sinusitis and infection of the lungs. Other fungal pathogens associated with this condition including A*spergillus*, are the C*urvularia*, *Drechslera, Bipolaris, Exserohilium, Alternaria*, *Helminthosporium* and *Fusarium* species (Pursell, 1992).

A. fumigatus is the most common cause of all forms of aspergillosis, with *A. flavus* being the second, and *A. niger* being the third. They are probably the most important contaminants of man-made and naturally occurring organic material. They are also able to produce mycotoxins when they grow as contaminants in food. These *Asperigillus* species are associated with asthma, bronchitis, hypersensitivity

pneumonitis (alveolitis) and cystic fibrosis, and have been found to be dwelling in walls, carpets, mattresses and wooden window frames of some houses (Kauffman, *et al*., 1995).

1.4.2 *Fusarium oxysporum*

Infection caused by *F. oxysporum* (sac fungi), is called 'fusariosis', which usually occurs in immunocompromised individuals (Booth, 1971). *Fusarium* attacks cells in humans much the way it attacks cells in plants through the secretion of mycotoxins. These mycotoxins dissolve the cell walls, and the fungus is then free to absorb the cell's contents, enter the cell cavity, reproduce and continue the process of attacking other cells. Many of these mycotoxin-producing species have estrogenic, emetic and feed refusal syndromes (Gastaldi *et al*., 1994). Human infection usually occurs as a result of invasion of the organisms through the body surfaces, thus causing skin infection, respiratory tract infections in patients suffering from tuberculosis, bronchitis or arthritis. *F. oxysporum* produces 70% mortality rate in immunocompromised patients (Monier *et al*., 1994). *F. oxysporum*-infections are rare but devastating infections caused by this common fungus. It is more commonly known as a fungus that destroys crops. However, immunocompromised patients are increasingly at risk for contracting an infection. Most of the initial infections are as a result of inhalation of the spores and involves the respiratory tract infections of the lungs (Bennett, 1995).

1.4.3 *Rhizopus stolonifer*

Rhizopus (zygote fungi) species, are strictly terrestrial fungi, their spores often floating around in the air and are either saprophytes or weak parasites of plants and plant products on which they cause soft rots or moulds. *Rhizopus* species may cause mucorosis in immune compromised individuals. The Zygomymycetes have well-developed mycelia without cross walls and produce non-motile spores in sporangia. *Rhizopus* species are frequently found in house dust, soil, fruits, nuts and seeds.

Exposure to large numbers of *rhizopus* spores has reportedly caused respiratory complications. *Rhizopus* can be an allergen and opportunistic pathogen for immunocompromised individuals, especially those with diabetic ketoacidosis, malnutrition, and severe burns or in some cases, the common cold (Lunn, 1977). In most cases the air-borne diseases caused by *R. stolonifer* are associated with sinus problems and upper respiratory tract infections (Anstead, *et al*., 1999). The sites of infection are the lungs, nasal sinus passages, brain, eyes and skin. This mold produces mycotoxins, which can be inhaled and ingested (Campbell and Sewart, 1980).

1.5 Literature review

Over the past decade there has been a proliferation of literature on the antibacterial, antifungal and antiviral properties of plant extracts. There are several reports on *in vitro* inhibition of mycobacterium by medicinal plants. Ten of 408 ethanolic extracts of plants such as *Actaea spicata*, *Angustura vera*, *Cinnamonium camphora*, *Piper cubeba*, *Guauacum officinale*, *Ipomea purga*, *Rhamnus cathartica* inhibited growth of *M. tuberculosis* H37Rv at dilutions of 1 in 160 to 1280 and a high proportion of the other extracts inhibited growth at lower dilutions (Grange and Dawe, 1990). It was found that *M. tuberculosis* was also sensitive towards the Rwandese medicinal plants, *Pentas longifolia*, *Tetradenia riparia* and *Bidens pilosa*. The active compound isolated from the leaves of *T. riparia* was tested against *M. tuberculosis* and showed activity at 100 µg /ml (van Puyvelde *et al*., 1994). *Hydrocotyle asiaticum* inhibited growth of *M. tuberculosis* at a dilution of 1:20 (Grange and Dawe, 1990).

A number of plants have been cited in the literature as being used for medication against various bacterial and viral infections or as containing biologically active compounds. Research conducted by Noristan, Pretoria, suggests that from a total number of about 300 plants screened, at least 31% show marked analgesic, anti-inflammatory and anti-infective properties (Theunis *et al*., 1992). Out of 100 medicinal plants of Rwanda, 30% of the plants tested showed activity against *B. subtilis* and *S. aureus* (Boily and Van Puyvelde, 1986). A significant antibacterial

activity was displayed by a novel diterpene diol isolated from *Iboza riparia* (De Kimpe *et al*., 1992). The zones of inhibition produced by water and methanolic extracts of *Bridelia ferruginea* ranged from 4 to 20 mm when tested against *S. aureus*, *E. coli*, *K. pneumonia S. pyrogenes* etc. Organic extracts of *Helichrysum crispum* inhibited the growth of *M. tuberculosis* and *P. aeruginosa* (Salie *et al*., 1996).

Plant extracts from *Artemisia aucheri*, *A. scoparia*, *Carthamus oxyacantha*, *Francoeuria undulate*, *Tripleurospermum disciforme* and *Xathium spinosum* have been found to be with antifungal activity (Salehi-Surmaghi and Amin, 1993). Plants have been endowed with therapeutic virtues both in legend and in scientific literature and are being used in treating various ailments such as coughs, colds, other pathogenic bacterial and viral infections. The use of antimicrobials from natural vegetation has a great impact in human health care of undeveloped countries. Herbal medicine has been used for centuries in rural areas by local healers and has been improved in industrialized countries. A number of substances used in modern medicine for the treatment of serious diseases have originated from research on medicinal plants (Theunis *et al*., 1992).

1.6 *Pelargonium reniforme* Curtis and *Pelargonium sidoides* DC.

The traditional use of the South African plant species; *Pelargonium reniforme* Curtis and *Pelargonium sidoides* DC. in treatment of various acute and chronic infections like bronchitis, sinusitis, tonsillitis, tuberculosis and rhinopharyngitis has been reported (Watt and Breyer-Brandwyk, 1962). However, the activity of extracts has not been scientifically validated as yet against Gram-negative bacteria; *M. catarrhalis,* the fungal pathogens; *A. niger, F. oxysporum* and *R. stolonifer* and *M. tuberculosis*.

1.7 Scope of thesis

1.7.1 Antibacterial, antifungal and antituberculosis activity of *P. reniforme* and *P. sidoides*

We intend to investigate the crude acetone and ethanol extracts of *P. sidoides* and *P. reniforme* against *H. influenza*, *M. catarrhalis*, *S. pneumonia*, *A. niger*, *F. oxysporum*, *R. stolonifer*. Acetone, ethanol and chloroform extracts of these two plants will also be tested for activity against *M. tuberculosis*.

1.7.2 Bioassay guided fractionation of *P. sidoides*

Bioassay guided fractionation of the best extract will be conducted and an attempt will be made to isolate and purify the active principle.

1.8 Structure of thesis

Chapter 1 describes the potential of medicinal plants for various ailments.

The selection, description and phytochemical constituents of the plant species selected for the present study is reported in **chapter 2**.

The detection of antibacterial assay of crude extracts of *P. reniforme* and *P. sidoides* against bacteria that cause bronchitis and the other infections are described in **chapter 3**.

Chapter 4 reports on the antifungal activity of crude acetone and ethanol extracts of *P. reniforme* and *P. sidoides* by using the agar dilution method.

Chapter 5 reports on the antimycobacterial results of *P. reniforme* and *P. sidoides* by the BACTEC radiometric method for susceptibility testing of *M. tuberculosis*.

In **Chapter 6** evaluation of different extracts from *P. sidoides* are discussed.

In **Chapter 7** bioassay guided fractionation of *P. sidoides* is discussed.

In **Chapter 8** evaluations of the isolated compounds from butanol extract of *P. sidoides* is discussed.

Chapter 9 comprises of general discussion and conclusion, summarizing the motives of the entire research, the importance of *P. reniforme* and *P. sidoides* as traditional medicine and recommendations from the findings.

Chapter 10 includes references.

Chapter 11 people contributing towards this project have been acknowledged.