# BIOACTIVITY OF TRADITIONAL MEDICINAL PLANTS USED IN THE TREATMENT OF TUBERCULOSIS IN THE FREE STATE, SOUTH AFRICA

By

Mandla Victor Hlongwane

Student number: 2008106706

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Supervisor: Dr L.V. Komoreng

Declaration

I, Mandla Victor Hlongwane, declare that the masters's Degree research dissertation or interrelated, publishable manuscripts/published articles, or coursework Master's Degree mini-dissertation that I herewith submit for the Master's Degree qualification in Botany at the University of the Free State is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.

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Dedication

This work is dedicated to all the members of my family, and a special dedication goes to my lovely daughter “Sisanda Luhle Hlongwane” and her mother Nompumelelo Kubheka.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| CDC | Centre for disease control |
| TB | Tuberculosis |
| WHO | World health organisation |
| MDR-TB | Multidrug-resistant tuberculosis |
| LTBI | Latent tuberculosis infection |
| HIV | Human Immunuedeficiency Virus |
| TBM | Tuberculosis meningitis |
| DOT | Directly observed treatment |
| TAM | Traditional African medicine |
| SIRMIP | Swaziland Institute for Research in Traditional Medicinal and Indegenous Food Plants |
| TLC | Thin layer chromatography |
| DOTS | Directly observed treatment short course |
| ACE | Acetylcholinesterace |
| DMSO | Dimethyl sulfoxide |
| MIC | Minimum inhibitory concentration |
| INT | *p*- Iodonitrotetrazolium Violet |
| WHA | World health assembly |
| TM | Traditional medicine |
| TMPs | Traditional medicinal plants |

## ABSTRACT

Many medicinal plant species were once used as a primary source of all medicines in the world and they continue to provide humankind with new remedies. Tuberculosis (TB) is one of the dreaded diseases that have been managed using medicinal plants. TB continues to be one of the airborne diseases that cause more deaths in the world than any other infectious diseases. The TB bacteria (*Mycobacterium tuberculosis*) has become resistant to the orthodox drugs used to treat TB diseases. Most strains of TB which have become resistant to all major anti-TB drugs have emerged.

In the present study, eight medicinal plant species (*Dicoma anomala*, *Xysmolobium undulatum*, *Hermania depressa*, *Lotinonius lanceolata*, *Senecio harveianus*, *Lentsweni*, *Eucomis automnalis* and *Drimia depressa*) that are traditionally used in the Free State province for the treatment of respiratory infections were collected for investigation. An ethnobotanical survey was conducted from January to June 2014 in consultation with the traditional healers and herbalists of the Free State Province. Plants were collected, extratced and tested for phytochemicals, antibacterial, antifungal and antimycobacterial activity. The phytochemical carried out revealed some of the secondary metabolites being absent in some of the plants and present in others. *X*. *undulatum* and *E*. *automnalis* revealed the presence of saponins only. *Lentsweni* and *H*. *depressa* revealed the presence of tannins, saponins and terpenoids, whereas *L*. *lanceolata* and *S*. *harveianus* revealed the presence of tannins, saponins, flavonoids and cardiac glycoside.Antibacterial activity was tested using four bacterial strains; two Gram-positive strains (*Bacillus pumilus* and *Staphylococcus aureus*) and two Gram-negative strains (*Klebsiella pneumoniae*and *Escherichia coli*), while the antifungal activity was tested against two fungal species namely *Candida albicans* and *Trichophyton mucoides*. The test for antimycobacterial activity was done against the causative agent of tuberculosis (*M*. *tuberculosis*).

Extracts exhibiting low or no antibacterial activity were *D*. *anomala* (methanol and aqueous extracts), *X*. *undulatum* (acetone and aqueous extracts), *Lentsweni* (methanol extract), *E*. *automnalis* (ethanol and methanol extracts). Good antibacterial activity was observed with the acetone and ethanolic extracts of *D*. *anomala* having the highest activity against *K*. *pneumonia* (0.130 mg/ml) and *E*. *coli* (0.781 mg/ml), with *B*. *pumilis* and *S*.

*aureus* having the best antibacterial activity (MIC value of 0.098 mg/ml). Promising results were detected with the ethanolic extracts prepared from *X*. *undulatum*, *L*. *eriantha* and *D*. *depressa* against all the bacterial strains with MIC values of 1.563 mg/ml.

The antifungal activity of the acetone, ethanol, methanol and aqueous extracts prepared from the eight selected medicinal plant species displayed the best activities against *C*. *albicans* and *T*. *mucoides*, with sMIC values ranging between 0.098 to 0.781 mg/ml.

The best antimycobacterial activity was detected with all lipophilic extracts prepared from *S*. *harveianus* with MIC values of 0.195 mg/ml. *H*. *depressa* organic solvents extracts exhibited good activity against *M*. *tuberculosis* with MIC values of 0.78 mg/ml.

The selected medicinal plants used for the treatment of respiratory ailments in the Free State Province have demonstrated significant activities, which may better explain and justify their frequent use by the traditional healers. These results might give some leads for further analysis in order to develop new pharmaceutical drugs derived from plants.

# CHAPTER 1

# GENERAL INTRODUCTION

## 1.1. BACKGROUND

The Centre for Disease Control (CDC) describes tuberculosis (TB) as a disease that is caused by a bacterium known as *Mycobacterium tuberculosis* (CDC, 2014a). TB is a potentially fatal, contagious disease that affects almost any part of the body, but is mainly an infection of the lungs (CRAMER AND FREY, 2006). It can also affect the central nervous system (meningitis), lymphatic system, circulatory system, genitourinary system, bones and joints (MANN, 2008). TB is one of the airborne diseases that cause more deaths in the world than any other infectious diseases. The causative agent of TB, *M. tuberculosis*, is transmitted mainly by airborne particles that are 1 to 5 micrometers (MARTINEZ-JIMENEZ *et al*., 2013). TB is estimated to cause at least three million deaths per year worldwide (WHO, 2008), and TB is believed to be one of the major public health problems and the major cause of deaths among people living with HIV than any other disease in South Africa. Based on a study undertaken by the World Health Organization (WHO), South Africa is ranked fourth on the list of 22 high-burden TB countries in the globe (WHO, 2013). In the year 2012, an estimate of 8.6 million people all over the world became infected by *M. tuberculosis* and became ill with tuberculosis, and 1.3 million died from it (WHO, 2013). The estimate included 410 000 women and 74000 children (WHO, 2013). Nonetheless, it is estimated that about one third of causes of TB are still either not diagnosed or not reported. Even when people with suspected TB are identified, the disease is often diagnosed and treated very late (WHO, 2013). What does this imply? It implies that, TB continues to cause permanent or long-term damage if not treated early.

The TB bacteria can become resistant to the orthodox drugs used to treat TB disease. Strains of TB resistant to all major anti-TB drugs have emerged. Drug resistance emerges due to the improper use of antimicrobials in chemotherapy of drug-susceptible TB patients. There are two main forms of drug resistant TB, which can be determined in

a laboratory using special tests; these are multidrug-resistant TB (MDR-TB) and extensively drug- resistant TB (XDR-TB).

MDR-TB is the type of TB that is resistant to at least two of the best anti-TB drugs such as the isoniazid and rifampicin (BRAUN *et al*., 2013; CDC, 2014b). These two antibiotics are considered first-line drugs and are recommended for treatment of all persons with TB disease. While MDR-TB is curable, its treatment is complex and requires expert management and frequent monitoring. Such treatment can last up to two years with more expensive second-line drugs, which also have more side effects (BRAUN *et al*., 2013). XDR-TB, also known as Extremely Drug-Resistant TB, is defined as the TB that is resistant to isoniazid and rifampicin, plus resistant to any fluoroquinolone and at least one of the three injectable second-line drugs (capreomycin, kanamycin and amikacin) (CDC, 2014b). XDR-TB is emerging as an even more ominous threat as set is resistant to the drugs used to treat MDR-TB (BRAUN *et al*., 2013).

### 1.2. THE DIFFERENT TYPES OF TB

There are two TB-related conditions that are known to exist; latent TB infection and TB disease (CDC, 2014a; CDC, 2014b).

#### 1.2.1. Latent TB infection

Latent TB infection (LTBI) is acquired when a person has breathed in the TB bacteria into his/her lungs from the air droplets coughed by a person who has active TB disease.The TB bacteria can live in the body without making the infected person ill or sick. In other words, *M. tuberculosis* is present in the body but does not multiply and/or does not show any symptoms or radiographic evidence of active TB disease. In most cases, the immune systems of people infected with TB bacteria fight the bacteria and stop them from multiplying (CDC, 2014a; CDC, 2014b). Indeed, patients with LTBI usually do not feel sick, they tend not to have any visual or diagnosable TB symptoms

hence, cannot spread the TB bacteria to people in close contact or in a place with poor ventilation. People having this inactive or the quiescent *tubercle bacilli* (Figure 1a) in their body when tested (skin test), the results of the skin test come out positive although they tend to have normal chest X-rays (Figure 1b) as this is not expected in the case of TB. Health practitioners prescribed that people with LTBI do not have to take any precautions to stop the spread of TB, they can continue with their daily activities as usual. However, there is a high risk of TB disease within the first 2 years of becoming infected, after which, there is about 5-10% chance of developing TB disease. If one suspects that s/he has latent TB, the person can take a TB skin test (CDC, 2014b) where small amounts of TB extract will be injected under the skin on the person's forearm. An immune reaction to the extract will result in swelling that can be detected within 2 to 3 days. Sometimes the test may be given too soon after exposure to TB for an immune system's response to develop. Therefore, a second skin test is usually given at 8 to 10 weeks after exposure (CDC, 2014b).

CDC (2014b) estimated that about 90% of people who are infected with TB develop this LTBI infection. In some people, *M. tuberculosis* overcome the defenses of the immune system and begin to multiply; the person will shift from having LTBI to have the TB disease (CDC, 2014a; CDC, 2014b).

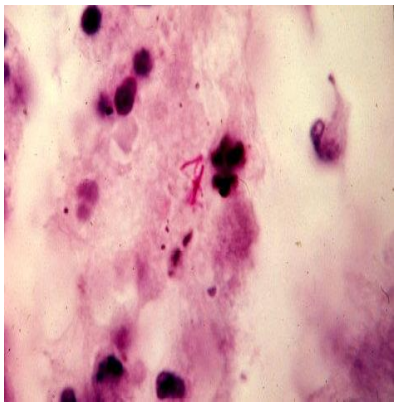

**Figure 1a: *Tubercle bacilli***

Source: TUBERCLE BACILLUS, 2015

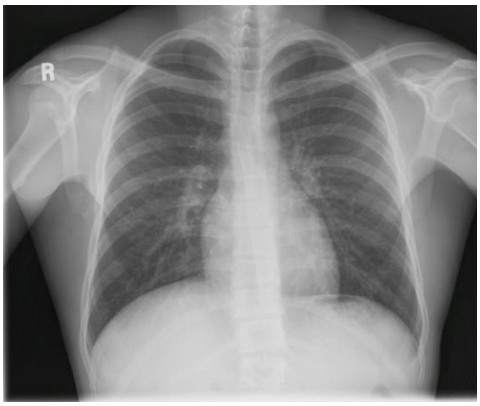


**Figure 1b: Normal Chest X ray**

Source: NORMAL X-RAY, 2015

### 1.2.2. TB disease

Though people with LTBI do not feel sick as the TB bacteria is yet not active, there are situations where the person's own immune system will be weakened and become unable to fight TB bacteria from multiplying due to conditions such as organ transplant, HIV infection, silicosis, kidney disease, diabetes, cancer, low body weight, abuse of alcohol or drugs and steroids medications. This, leads to the development of active TB disease. TB disease or active TB disease is one of the airborne/ respiratory diseases that occurs when *M. tuberculosis* have started to multiply and become numerous enough to overcome the body's immune system (MUGISHA *et al*., 2006). Persons with active TB disease usually feel ill, and in certain circumstances, they are able to pass the *tubercle* bacteria on to other people. TB disease attacks people at all levels and ages; this includes babies, pre-school children and the elderly as they have weakened immune system than healthy adults. Several studies undertaken by HONG (2001) and CDC (2008) reported that people who have had close and long contact with people who have active TB of lungs or throat are prone to TB infection.

TB disease can also infect other parts of the body, including brain, kidneys and spine (MUGISHA *et al*., 2006). The symptoms of TB disease are very variable and they depend on the part of the body which has been infected, and that determines the type of TB. Generally, symptoms for active TB disease include weakness or feeling very tired, losing weight with no expectations and or trials, lack of appetite, chills, fever (a high temperature of 38°C or above) and having night sweats (MUGISHA *et al*., 2006). TB may infect any part of the body, but it most commonly occurs in the lungs. This is known as pulmonary TB. If TB is in other parts of the body it is commonly called extrapulmonary TB, also known as disseminated or military TB. The disease usually progresses by spreading from the lungs to locations outside the lungs (extrapulmonary sites) (CRAMER AND FREY, 2006). The main tissues or organs that TB may affect include the lymph node, bones, brain, abdominal organs, etc.

*1.2.2.1. Lymph node TB*

Lymph node TB, also known as tuberculosis lymphadenitis, is usually presented as a gradually increasing painless swelling of one or more lymph nodes throughout the body. The swollen lymph nodes are often noticeable in the neck area, although they can be in the groin. This type of disease is common in children and women. In immunocompetent children, the lymph node TB is often caused by a typical mycobacterium called *M. scrofulaceum* and other non-tuberculous mycobacterium (NTM). TB infection of the lymph nodes in the neck is sometimes referred to as Scrofula or TB adenitis (VOHRA *et al*., 1997).

*1.2.2.2. Skeletal TB (bone and joints)*

Skeletal TB is a TB that occurs in the bones and joints. Skeletal TB accounts for 10 to 35% of cases of extra pulmonary tuberculosis and, overall, for almost 2% of all TB cases (WATTS AND LIFESO, 1996; PETO *et al*., 2009). The most common form of skeletal TB is Pott disease or TB Spondylitis, a disease of the spine; this entity comprises approximately half of musculoskeletal TB cases (VOHRA *et al*., 1997). The most common initial symptom of bone TB is the pain, but it depends on the bone or joint that is affected. There may also be curving of the affected bone or joint, as well as loss of movement in the affected bone or joint. The affected bone may also be weakened and may fracture easily.

*1.2.2.3. Meningitis TB*

The inflammation of the meninges covering the brain and spinal cord is caused by infection with mycobacteria, which in most cases, is usually *M. tuberculosis* and is referred to as tuberculosis meningitis (TBM) (MARAIS *et al*., 2010). TBM is one of the most severe forms of tuberculosis and causes substantial morbidity and mortality in adults and children (PRASAD *et al*., 2000). Usually, TBM does not start with classic symptoms; the condition is characterized by headaches, fever, and convulsions. It is

diagnosed clinically, with confirmation by microscopy and culture of cerelorospina fluid (PRASAD *et al*., 2000).

*1.2.2.4. Gastrointestinal or abdominal TB*

Gastrointestinal TB is the infection of abdominal organs, peritoneum and abdominal lymphatic with *M. tuberculosis* organism (SHEER AND COYLE 2003; PULIMMOD *et al*., 2011). Gastrointestinal TB usually occurs at any location in the gastrointestinal tract (i.e. ileocaecum, colon, liver, spleen, peritoneum and lymph nodes) (PULIMOOD *et al*., 2011). The symptoms and signs of gastrointestinal TB are listed in Table 1.

**Table 1: Symptoms and signs of gastrointestinal tuberculosis**

| **Symptoms** | **Signs** |
|---|---|
| Fever | Fever or high temperature |
| Abdominal pain | Abdominal tenderness |
| Weight loss | Abdominal mass |
| Anorexia | Ascites |
| Diaphoresis | Lyamphadenopathy |
| Diarrhea | Hepatomegaly |
| Constipation | Peritoneal signs |
| Hematochezia | Jaundice |
| Nausea and vomiting | |

**Source: SHEER AND COYLE (2003)**

## 1.3. CAUSES OF TB

TB is caused by a *Mycobacterium* species that spreads from person to person through microscopic droplets released into air. Any person with an untreated active TB infection can spread the disease either by sneezing or by coughing; even talking can release the bacteria into surrounding air. In that way, people breathing this air will then become infected. *Mycobacterium* species settle in the air sacs and passages of the lungs, while in most case they will be contained by the immune system.

## 1.4. SYMPTOMS OF TB

Usually, if a person is infected with inactive TB there will not be symptoms. They usually develop slowly so the symptoms might not begin until months or even years after initial exposure to the bacteria. Symptoms only appear when TB infection becomes active. They develop bit by bit and might take many weeks before one notices that something is wrong. Symptoms are mild and not specific and may include a productive and prolonged cough that lasts for more than 3 weeks (PULIMMOD *et al*., 2011). It can start as a dry irritating cough that tends to continue for months and gets worse. In time, the cough produces green to yellowish phlegm (sputum), which may also be blood stained (haemoptysis). Systematic sysmptoms include a high temperature (fever), chills, night sweats, loss of appetite, weight loss, fatigue, dyspnoea, and chest pain. If left untreated, complications such as fluid collection between the lungs often develop and this makes the person breathless (SHEER AND COYLE, 2003; PULIMMOD *et al*., 2011).

The symptoms of TB mostly depend on where the infection had occurred. A study undertaken by WEJSE *et al*. (2008) indicated that the occurrence of additional symptoms depends on where the disease has spread. For example, if TB spreads to the lymph nodes, it can cause swollen glands at the sides of the neck or under the arms. In some other cases, when TB spreads to the bones and joints it can cause pain and swelling of the knee or hip. Genitourinary TB can cause pain in the flack with frequent urination, pain or discomfort during urination, and blood in the urine (WEJSE *et al*., 2008). Brain TB can cause meningitis with symptoms such as headache, nausea,

vomiting, convulsions, drowsiness and a change in behavior (SHEER AND COYLE, 2003).

## 1.5. TRANSMISION OF TUBERCULOSIS

The TB germs are put into the air when a person with an active TB disease of the lungs or throat coughs, sneezes, speaks or sings (HONG, 2001; CDC, 2008). *M. tuberculosis* is transmitted through the air, not by surface contact (CDC, 2005; CDC, 2014b) and it spreads from person to person. The TB disease spreads by aerosol containing *tubercle bacilli*. Each droplet is 1 to 5 micrometers in diameter and contains 1 to 3 bacilli. The transmission of the TB disease occurs when a person inhales droplet nuclei containing *M. tuberculosis*, and the droplet nuclei traverse the mouth or nasal passages, upper respiratory tract, and bronchi to reach the alveoli of the lungs (CDC, 1992), where they are taken up by macrophages. Within these cells, the bacilli multiply and then spread through the lymph vessels to nearby lymph nodes. At this point the bacilli may either remain alive but quiescent or they may cause active disease. A person with untreated active TB can infect on average 10 to 15 people per year (HONG, 2001).

### 1.5.1. FACTORS THAT DETERMINE THE PROBABILITY OF *M. TUBERCULOSIS* TRANSMISSION

Studies carried out by CDC (1992 and 2005) reported four factors that determine the probability of *M. tuberculosis* transmission. These are susceptibility, infectiousness, environment and exposure.

#### *1.5.1.1. Susceptibility*

A variety of observable studies indicated that certain populations appear to exhibit unusual susceptibility (the immune status of the exposed individual) to TB, and it is likely that, to a certain degree, this susceptibility has genetic basis (CDC, 2005).

Susceptibility to a TB disease is more likely to appear to people who might suffer from HIV, diabetes mellitus, silicosis, cancer of the head and neck, hematologic and reticuloendothelial diseases, intestinal bypass or gastrectomy, chronic malabsorption syndromes, low body weight, organ transplanted those who have the immunocompromised system (CDC, 1992). However, there are still some conditions which are associated with high risk of TB disease infection to people, these include people who have had close and long contact with people who have active TB of lungs, and people from areas of the world where TB is a high burden or common. Further observations also state that people who are addicted to alcohol, injection drug users and the homeless people are more susceptible to developing active TB disease. Studies also indicated that staff and residents of nursing homes, shelter, hospitals and jails could be at high risk of TB infection (CDC, 1992; CDC, 2005).

*1.5.1.2. Infectiousness*

The infectiousness of the person with TB is directly related to the number of *Tubercle bacilli* that the infected patient expels into the atmosphere. Patients expelling many of the *Tubercle bacilli* (droplet nuclei) are more infectious than people expelling few or no bacilli at all (CDC, 1992; CDC, 2005). Factors associated with infectiousness from TB patient include clinical, which is characterized by the presence of cough that lasts for a period of three weeks or longer (CDC, 1992; CDC, 2005). Other factors such as the respiratory tract diseases, which involves the larynx, are said to be highly infectious. A patient failing to take prevention measures such as covering the mouth when coughing, and failure of the patient to adhere to the prescribed treatment duration (CDC, 2005; CDC, 1992) increases the spread of TB. The second factor being the procedure, where a patient is undergoing a cough-inducing or aerosol-generating procedure (CDC, 1992; CDC, 2005), for example bronchoscopy, sputum induction, administration of aerosolized medication. The third factor is radiographic and laboratory where the cavitations on the chest radiograph is tested with positive culture for the *M. tuberculosis* and positive AFB sputum smear results (CDC, 1992; CDC, 2005).

*1.5.1.3. Environmental factors*

There are several factors that affect the concentration of *M. tuberculosis* in the environment; the first factor being the concentration of infectious droplet nuclei, which indicates the number of droplet nuclei expelled into the air.The higher number of droplet nuclei increases the probability of *M. tuberculosis* being transmitted to other people (CDC, 1992; CDC, 2005)**.** The second factor is space; if the space is too limited, unaffected people are at risk of being infected. For example, areas with poor ventilations result in insufficient dilution or removal of infectious droplet nuclei (CDC, 1992; CDC, 2005)**.** The third factor is air circulation in which the environment or the air is contaminated with the infectious droplet nuclei.

1.5.1.4. *Exposure*

Transmission of *M. tuberculosis* is also accelerated by the proximity and the length of exposure. Duration of exposure to a person with infectious TB is one of the factors that determine the transmission of the *M. tuberculosis* (CDC, 1992; CDC, 2005). The longer the duration of exposure, the higher the risk for the transmission of TB. The second factor being frequency of exposure to infectious persons.The more frequent the exposure to an infectious person the higher the risk of transmission (CDC, 1992; CDC, 2005)**.** The third factor being physical proximity to infectious person; transmission mostly occurs when infected people get close (closer proximity) to uninfected individuals, this makes uninfected individuals to be at a high risk of being infected.

## 1.6. DIAGNOSIS AND MANAGEMENT OF TB

The diagnosis of TB is made based on laboratory test results. TB is generally diagnosed through a chest X-ray and/or a tuberculin skin test, followed by phlegm (sputum) test. Until today, many countries still rely on a long-used method for TB diagnosis called sputum smear microscopy. With reference to the study conducted by WHO (2013), the sputum “smear” samples are wisely collected in a health facility or in the community and

the samples are then sent to a laboratory for analysis. New rapid TB test, known as Xpert MTB/RIF, is a fully automated diagnostic molecular test, which has the potential to revolutionize and transform TB care and control. Such test simultaneously detects *M. tuberculosis* DNA and rifampicin (RIF) drug resistance and provides accurate results in less than two hours. The costs of the XpertMTB/RIF, tests are not yet widely available, initially because of their relatively high cost. As the costs are reduced, more countries acquire and use the tests. TB testing will, therefore, become increasingly responsive to patients needs for quick diagnosis and immediate treatment (WHO, 2013). If patients are diagnosed with active TB disease, they will then be treated with a standard six-month course of four anti-TB drugs. Since the TB-treatment has to be taken exactly as prescribed and every day over such a long period, some patients are offered support from health workers or trained volunteers. The health workers observe the people/person taking the treatment, hence called directly observed treatment (DOT) (WHO, 2013). In the early 1900s, the decline of TB mortality in both Europe and North America was essentially driven by two parallel streams including a series of public health measures and socioeconomic development resulting in improved quality of life (BOCCIA *et al*., 2011), most especially in nutrition and housing conditions. There is a consensus that further actions are needed, both to develop better biomedical tools, delivery and social support mechanisms, and to tackle the root causes of TB, including poverty and other socioeconomic determinants of health. However, after the introduction of antibiotics (Isoniazid, Streptomycin and Rifampicin) an integrated approach switched towards a curative focus and led to the modern TB control framework based on early case detection and successful treatment (WHO, 2013).

# CHAPTER 2

# PROBLEM STATEMENT, AIMS AND OBJECTIVES

## 2.1. INTRODUCTION

According to VAN WYK *et al*. (1997), medicinal plants were once a primary source of all medicines in the world and they continue to provide humankind with new remedies. With the use of traditional medicine, there is limited data that has been scientifically proven and, currently, traditional systems of medicine continue to be widely practiced by the indigenous people of South Africa. There have been a lot of interpretations and definitions about the use and practice of herbalism and use of traditional medicinal plants. WHO (2000) defined traditional medicine as “including diverse health practices, approaches, knowledge and beliefs incorporating plant, animal and/or mineral based medicines, spiritual therapies and exercises, applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness”**.** Thus, plant species form an integral part of healing processes of traditional medicines used in the treatment or prevention of various ailments by the traditional healers. Broad knowledge of medicinal plants, usage and concentration of the healing properties can be found from traditional healers, including other knowledgeable people who may have inherited the information from one of their elders.

The population in the world has increased and as a result there is an imbalance or an inadequate supply of drugs. According to WHO (2000), about 80% in 4 billion populations cannot afford the products of Western Pharmaceutical Industry or they lack access to essential medicine, hence, they have to rely upon the use of traditional medicines, which are mainly derived from plant materials, for their primary healthcare**.** This fact is well documented in the inventory of medicinal plants, listing over 20 000 plant species (WHO, 2000). Medicinal plants have played an essential role in the development of human culture, i.e. in religion and different cultural practices. For example, some communities within South Africa still practice the burning of *Helichrysum* species. Some of the local traditional healers in the Phuthaditjhaba community use

*Helichrysum* species (*impepho*) for cultural practices (MATLAKALA, *pers comm*.). In South Africa, healing using traditional medicine is widely practiced and approximately 70% of the South Africans regularly use medicinal plants based medicine for their primary healthcare (DEVIENNE AND RADDI, 2002)**.**

It has been found that most plant species have an important medicinal value, such that they play a vital role in human health. These medicinal values or qualities of plants are due to the chemicals found within the plants (MAZID *et al*., 2011)**.** Plants synthesize many compounds, known as primary metabolites, which are critical to their existence (MAZID *et al*., 2011)**.** These primary metabolites include proteins, fats and carbohydrates that serve a variety of purposes indispensable for sustenance and reproduction, not only for them, but also for other organisms that feed on them (humans and animals) (DEVIENNE AND RADDI, 2002). A study has shown that carbohydrate digestion and widespread compounds such as polyphenolics and phytates, form part of the traditional diets that may have more specific constituents offering protection against chronic diseases such as diabetes (MAZID *et al*., 2011).

There are also dazzling arrays of additional components that plants synthesize, these are called secondary metabolites. Secondary metabolites are known to be “antibiotic” in a very broad sense.They protect plants against fungi, bacteria, animals, and even from other plants (MAZID *et al*., 2011). Some phytochemicals, which are known to be secondary metabolites with other phenolic compounds, are known to have pronounced biological and physiological consequences in microbes (TEMIKOTAN *et al*., 2013). In South Africa, many people use plants as medicines due to the chemical constituents present in plants. A study carried by *Temikotan et al*. (2013) highlights the man’s motivation to characterize plants secondary metabolites, which is often driven by commercial interest, as they have been a valuable source of drugs, pesticides and chemicals important in the food production industries.

Research has shown that every plant species contains chemicals that affect some of the micro-organisms or animals negatively, thus, strongly supporting the interpretation that secondary metabolites play a vital role in combating diseases and deterring herbivores (MAZID *et al*., 2011)**.** Plants have been a rich source of medicines due to the

host bioactive molecules they produce, most of which evolved as chemical defenses against infections (MAZID *et al*., 2011). Although some of the plants, including the compounds extracted from plant species, are toxic, some of the communities residing in rural areas completely rely on the healing properties of traditional medicinal plants provided by herbalists and traditional healers. Moreover, as for many Africans living in rural communities where there is irregular income and rising medical costs, the therapeutic herbalism has become a dependent way of healthcare (STANLEY, 2004). The underdeveloped and developing countries are characterized by high rate of unemployment and prohibitively high cost of treatments. The side effects of several allopathic drugs and the development of resistance to currently used drugs, has led to an increased emphasis on the use of plant materials as a source of medication for a wide variety of human's ailments. Since the methods of preparation and administration of medicinal plants are usually provided by traditional herbalists, WHO (1999) recommended that tests and documentations of the different standards defining the identity, purity, and potency of plants used medicinally in the form of monograph should be conducted. Hence, a quick evaluation on the quality, standardization, safety and efficacy of traditional medicinal plants will prove the claims made by herbalists.

## 2.2. MEDICINAL PLANTS USE AND IMPORTANCES

### 2.2.1. The use of medicinal plants in the world

There is a vast number of medicinal plants in the world. A study undertaken by TYLER (1993) reported that about 13000 plant species have been employed as traditional medicines by various cultures around the world. A list with over 20 000 medicinal plants has been published (SMALL AND CATTING, 1999), and likely a much larger number of the world's flowering plants has been used medicinally. In USA, almost 1800 medicinal plant species are commercially available (MULLER AND CLAUSON, 1998). Looking at the value of medicinal plants with a global eye, there is a growing demand for traditional herbs due to their effective biological activity. Most human societies throughout the

world have accumulated a vast body of indigenous knowledge over centuries on the use of medicinal plants (PALA *et al*., 2010). About 80% of the developing countries still depend on the use of medicinal plants for their healthcare (PALA *et al*., 2010). When one looks at the current pharmaceutical drugs, about 25% of drugs contain plant-derived ingredients and there is a growing interest in green products in the national industries (PALA *et al*., 2010)**.**

Countries like China, Cuba, India, Sri Lanka, Thailand and few other countries have endorsed the official use of traditional system of medicine in their healthcare programmes (PALA *et al*., 2010). As the fact remains, large sections of the population in developed and developing countries still rely on traditional medicine. However, the World Health Assembly (WHA) has adopted a number of resolutions, which then draw attention to the use of medicinal plants (ZHANG, 1998), with Ayurveda being one of the most ancient systems of medicines in the world (SHETH, 2005). India is one of the countries in the world that have a richest heritage in utilization of Ayurveda and herbal medicines, which are supported by neutraceuticals (SHETH, 2005). The herbal medicine and Ayurveda have been practiced and used all over the world for many years but have only recently started getting legal acceptance in many countries in the world as alternative system of medicines (SHETH, 2005).

Today, India is known to be a “Botanical Garden of the world” as it is the largest producer of medicinal products. Measuring the total value of imports of natural materials of plant origin for the pharmaceuticals and cosmetics industry, it amounts to about 53.5 million US dollars (MAZID *et al*., 2012) and India was one of the largest suppliers by far with 10,055 tons of plants and 14 tons of vegetable, alkaloids with other derivatives (JHA, 1995). A study that was conducted by SHETH (2005) indicated that only 10% of more than 25000 plants, which are of medicinal importance, are used for their medicinal value. Currently, the global market of herbal and Ayurvedic medicines is estimated to be more than 100 billion US dollars, while the Europe accounts for only 40%, Japan 20%, and USA 10% (SHETH, 2005). As reported by the World Bank, the trade in medicinal plants, botanical drugs products and raw material is growing at an annual growth rate between 5 to 15% (AKANDA, 2013). With the Global Pharmaceutical market risen to

550 billion US dollars in the year 2004 and 900 billion in the year 2008 (AKANDA, 2013), all the Asian countries account for only 30% of the global market (SHETH, 2005). With advances in technology, the world has developed medicines that act fast, which are potent and able to treat and provide symptomatic relief (SHETH, 2005). Attention has now been shifted from relief to prevention and cure. The United State of America has shown more support and has promoted the use of food supplement and/or neutraceuticals (SHETH, 2005).

Currently, the knowledge of medicinal plants is evolving in many countries. This is shown by numerous researches conducted by many institutes in South Africa and all over the world. According to JHA (1995), there is a distinct possibility that the curative properties required to treat various ailments lie within the herbal medicinal system. Today, new important anti-infectives are being discovered from microbial, plant, and animal sources. Evidence is shown by an antimalarial agent, *Artemisinin*, which was isolated from the Chinese medicinal plant called *Artemisia annua* (AKANDA, 2013). The active constituent was isolated and identified as the sesquiterne endoperoxide artemisinin, and this was due to its lipophilic structure (AKANDA, 2013). This makes the effectiveness of the herbal medicine to be globally recognized.

### 2.2.2. The use of medicinal plants in Africa

Traditional African medicine (TAM) is a holistic discipline that involves the extensive use of indigenous herbalism together with aspects of African spirituality (HELWIG, 2005). The traditional knowledge related to the health of both humans and animals existed in almost all countries of Africa. As traditional medicine is considered as a solid amalgamation of dynamic medicinal known-how and ancestral experience (CUNNINGHAM, 1997), interest from them was gained and is becoming more and more widely recognized in the development of policies, the media and scientific literature. In the African continent, traditional healing and remedies made from plants play essential roles in the health of millions of life (CUNNINGHAM, 1997). Plants have a long history of use in the African continent in treating different diseases and complaints. In certain

African countries, up to 90% of the populations still rely exclusively on plants as sources of medicine (ROBERTS, 1990). In countries like Ghana, Mali, Zambia and Nigeria, herbal medicine is used as the first-line treatment for 60% of children with high fever resulting from malaria (WHO, 2002). CARPENTIER *et al*. (1995) discovered an increasing demand of traditional medicine in treating rheumatic and neurological complaints in Burkina-Faso. In Ghana, about 70% of the population depends primarily on traditional medicine (ROBERTS, 1990). About 27 million South Africans (usually the black South Africans) use traditional medicine to treat a variety of ailments (MANDER, *et al*. 2007; LEKOTJOLO, 2009). MAKUNDI *et al*. (2006) reported that traditional healthcare has contributed very significantly to the treatment of *degedege* (convulsions) in rural Tanzania. Moreover, in some instances, patients use traditional medicine simultaneously with modern medicine in order to alleviate sufferings associated with diseases and illnesses. AMIRA AND OKUBADEJO (2007) reported that a significant number of hypertensive patients receiving conventional treatment at the tertiary health facility in Lagos, Nigeria, also used complementary and alternative medicine therapies.

In Swaziland, the practices of traditional medicines are an immemorial mode of primary healthcare as in many parts of the African continent (AMUSAN *et al*., 2007). A large proportion of the Swazi population rely on medicinal plants species for their primary healthcare. About 85% of the Swazis depend on traditional medicinal plants for their medical care (AMUSAN *et al*., 2005). About 8000 medicicinal plant species has been reported to be used in Swaziland (AMUSAN *et al*., 2005). People in Swaziland have popularized herbal medicine for socio-cultural reasons. Herbal medicine is now regarded as a holistic system, which is used in treating almost every part of a human body.

Despite the popularity of herbal medicine and its importance in Swaziland, information about the system is not readily available (AMUSAN *et al*., 2005). Knowledge about the practice is acquired through oral tradition from one generation to the next, hence, there is an ongoing research in Swaziland conducted by the University of Swaziland (MAKHUBU *et al*., 2002). One of the Swaziland research institutes, known as Swaziland Institute for Research in Traditional Medicinal and Indigenous Food Plants (SIRMIP)

provides a forum on Traditional Medical Practitioners (TMPs), orthodox medical practitioners, natural and social scientists, lawyers, agriculturalists, nutritionists and policy makers to tackle multifaceted research agenda inherent in nutrition and traditional medicine (MAKHUBU *et al*., 2002).

Within the Lesotho community, the practice of herbal medicine has existed since ancient times, but the concepts and practices of traditional healing are not yet well known. People from the Lesotho believe that traditional medicine is not only used to cure illnesses but can be used in almost any situation (MOTEETEE AND VAN WYK, 2011). The Basotho people consider good health to be both physical and spiritual, hence, ancestral spirits form an integral part of their lives (MOTEETEE AND VAN WYK, 2011).

Because of the extreme complex socio-cultural fabric in a country like Mozambique, there is an unquestionable mixture of medicinal subculture each with its own characteristics and structures. With approximately 5500 plant species in Mozambique, in which some of them offer a variety of products which are used by its people, plant resources are used as food, medicine, building material and fuel wood (BANDEIRA *et al*., 2001). The use of traditional medicine for healing purposes by the Mozambicans accounts for 70 percent or more of basic healthcare (BANDEIRA *et al*., 1999). Mozambique provides few species to the international market as compared to other African countries.

In Africa, the importance of traditional healing and traditional medicine plays a vital role in the health of millions. Inadequate accessibility to Western drugs to treat and manage illnesses in middle and low-income households may have contributed to the widespread use of traditional medicine. The widespread use of traditional medicine in Africa may have attributed to its accessibility (ABDULLAHI, 2011). For example, in Ghana for every traditional healer there are 224 people as compared to one medical doctor for close to 21000 people. In Swaziland the same applies where for every healer there are 110 people whilst for every medical doctor there are 10 000 people (Table 2) (GREEN, 1985; HOFF AND MASEKO, 1986). Traditional healers prove to be an influential group

in primary healthcare and an integral part of the African culture, and are required for the health of its people.

**Table 2: Sample ratio of TMPs compared with the ratio of medical doctors to the population**

| **Countries** | **Ratio Of Traditional Practitioners To Population** | **Ratio Of Medical Doctors To Population** |
|---|---|---|
| Kenya, Urban (Mathare)<br>Rural (Kilungu) | 1: 833<br>1: 143-345 | 1: 987<br>1: 70 000 |
| Zimbabwe | 1: 600 | 1: 6 250 |
| Swaziland | 1: 100 | 1: 10 000 |
| Nigeria (Benin City)<br>National Average | 1: 110<br>No data | 1: 16 400<br>1: 15 740 |
| South Africa (Venda area) | 1: 700- 1 200 | 1: 17 400 |
| Ghana | 1: 200 | 1: 20 000 |
| Uganda | 1: 700 | 1: 25 000 |
| Tanzania | 1: 400 | 1: 33 000 |
| Mozambique | 1: 200 | 1: 50 000 |

**Source: (ABDULLAHI, 2011)**

Many stereotypes exist for the individual traditional healers and their medicines that are collectively called *Muti*. *Muti* is a word derived from medicinal plant and it refers to medicines that are traditionally sourced from plants, minerals and animals. *Muti* is often associated and adequated with body parts used for witchcraft in the African continent (HASSAN *et al*., 2009). Many sensational stories of human killing as to obtain *muti* exist (HASSAN *et al*., 2009). This may be true and may happen occasionally, but it is done by deranged individuals who have twisted beliefs of traditional healing and are similar to serial killers in the western psyche (HASSAN *et al*., 2009). These atrocities are not truly indicative of what traditional healing is. True traditional healing uses plant, mineral and

animal products to bring about both physiological and psychological effects in a person (HASSAN *et al*., 2009). TAM involves diviners, midwives and herbalists (HELWIG, 2005). In Africa, the healers are addressed as *Babalawo*, *Adahonse* or *Abianibok* in Nigeria; and *Tangoma* or *Tinyanga* among South Africans (HELWIG, 2005).

### 2.2.3. The use of medicinal plants in South Africa

Traditional medicine features in the lives of thousands of South Africans every day. MANDER (1998) reports that South Africa has more than 100 000 practicing traditional healers. In South Africa, the value of medicinal plants contributes to both the health and livelihood of many indigenous populations. There has recently been a growing interest from large pharmaceutical companies on the use of medicinal plants for primary healthcare needs. Medicinal plants are viewed by the pharmaceutical industry as a source of qualified lead in the identification of bioactive agents in the production of synthetic modern drugs (CUNNINGHAM, 1997). Hence, almost all national and international Universities together with most of the major herbal-based pharmaceutical companies are showing constant interest on medicinal plants.

Traditional healers play a crucial role in building the health system among South Africans, with the Traditional Healers Organization being the biggest traditional healer umbrella organization in the country. It counts 69 000 full-time or registered traditional healers in Southern Africa as its members, with about 25 000 of those residing in South Africa (RICHTER, 2003). In South Africa, medicinal plants contribute to both the healthcare and livelihood of many indigenous populations (VAN WYK *et al*., 2009)**.** Most people in South Africa do not consider traditional medicine an inferior alternative to the western medicine but is thought to be desirable and necessary for treating a range of health problems that western medicines do not treat satisfactorily (Mander et al., 2007a). This is proved by a study conducted by MANDER *et al*. (2007b), which showed that about 80% of clinic patients in Durban (one of the cities in SA) use traditional medicines for their primary healthcare. These patients indicated that they use the herbal

medication by choice and not as a result of lack of access to western medicine and the cost issues associated thereof.

Hence, if we look at the market, the trade in traditional medicines in South Africa is a large and growing industry. It forms part of a multimillion-rand 'hidden economy' in South Africa (CUNNINGHAM, 1989). According to MANDER *et al*. (2007b), there are 27 million consumers of traditional medicine in South Africa and the trade of these medicines contributes to an estimated cost of R2.9 billion to the national economy**.** Moreover, the authors have shown that 72% of the black African population is estimated to use traditional medicines, accounting for some 26.6 million consumers (MANDER *et al*., 2007b). These consumers are from a diverse range of categories such as age, education levels, religions and occupations. Table 3 below highlights on users of medicinal plants based on the education levels. The statistics in Table 3 show that consumption of traditional medicine is a common practice across most sectors of the African population, from the rural villages to the highly developed suburbs.

**Table 3: Level of education of medicinal plants consumers collected from one of the healers in Durban**

| **Education level** | **% of respondents surveyed at healers practice** |
|---|---|
| No School | 7.8 |
| Up to grade 7 | 31.0 |
| Up to grade 10 | 26.0 |
| Up to grade 12 | 26.0 |
| Tertiary qualification | 8.7 |

**Source: (MANDER *et al*., 2007b)**

The importance of medicinal plants in the South African society remains an inherent part of many cultures. Due to the high number or level of plant species which are ethno-

botanically significant in South Africa, there are 22 000 species of vascular plants, of which 80% are endemic (LOW AND REBELO, 1996). Approximately 3000 species are of medicinal value and are used by the indigenous TMPs (VAN WYK *et al*., 1997 and 2009). The demand for medicinal plants is increasing at an alarming rate, with a growing consumer population and no available suitable alternatives and/or substitutes. A very large and growing industry exists in the harvesting of medicinal plants, processing and selling of herbal and natural medicinal products made from them. In South Africa, the average frequency of traditional medicine use per consumer is 4.8 times per year, with an average mass of 157 g of plant material per treatment (MANDER et al., 2007b). A study of the trade in medicinal plants in the Eastern Cape Province undertaken by DOLD AND COCKS (2002) documented a minimum of 166 medicinal plant species that were traded at various study sites (Table 4), providing 525 tonnes of plant material valued at approximately R27 million annually. Of the species documented, 93% were harvested unsustainably as they were either partially or entirely removed, resulting in the death of the plant. The use and trade of plants for medicine is no longer confined to traditional healers, but has entered both the informal and formal entrepreneurial sectors of the South African economy, resulting in an increase in the number of herbal gatherers and traders (CUNNINGHAM, 1989; DAUSKORDT, 1990; COCKS *et al*., 2004; WIERSUM *et al*., 2006).

**Table 4: Total number of respondents from each stakeholder group at different city centers in the Eastern Cape Province**

| City center | Street trader | Traditional healers | Store owners | Clinic patients | Total |
|---|---|---|---|---|---|
| King William's Town | 14 | 9 | 4 | 20 | 47 |
| East London | 9 | 18 | 4 | 25 | 56 |
| Port Elizabeth | 21 | 11 | 3 | 30 | 65 |
| Uitenhage | 4 | 11 | 3 | 20 | 36 |
| Umtata | 9 | 13 | 2 | 20 | 44 |
| Queenstown | 0 | 9 | 2 | 23 | 34 |
| Total | 57 | 69 | 18 | 138 | 282 |

**Source (DOLD AND COCKS, 2002)**

A study carried out by WILLIAMS (2006) and WALDHEIM (2008) estimated about 20000 tonnes of medicinal plants are traded each year in South Africa with a street value of approximately R270 million. An estimated 20 000 tonnes of indigenous plants are harvested from grasslands, forests, woodlands and thickets in eastern South Africa every year, with only a few tens of tonnes (maximum 50 tonnes per annum) being cultivated (MANDER *et al*., 2007b). Extinction and the rapidly dwindling wild stock of certain species of medicinal plants are prompting changes in the medicinal plants market, thus creating great opportunities for commercial cultivation of medicinal plants. Nevertheless, since cultivation only focuses on plants that are being traded in informal markets, few species are being produced (WILLIAMS, 2006; WALDHEIM, 2008). Cultivation initiatives and new management programmes are obligatory regimes to conserve biodiversity and project threatened species since formal and traditional conservation measures have been unsuccessful (DOLD AND COCKS, 2002). The need for these regimes is acknowledged in developing countries throughout the world (DE BEER AND MCDERMOTT, 1996; LEAKEY *et al*., 1996; RUIZ-PEREZ AND ARNOLD, 1996).

## 2.3. CONSERVATION OF MEDICINAL PLANTS

Given the demand for a continuous and uniform supply of medicinal plants and the accelerating depletion of forest resources, increasing the number of medicinal plants species in cultivation would appear to be an important strategy for meeting a growing demand (UNIYAL *et al*., 2000). Demand for a wide variety of wild species is increasing with growth in human needs, numbers and commercial trade (SCHIPPMANN *et al*., 2002). With the increased realization that some wild species are being over-exploited, a number of agencies are recommending that wild species be brought into cultivation systems (WHO, IUCN AND WWF 1993; LAMBERT *et al*., 1997; BAH, 2002).

Most people in South Africa have a low standard of living and the population is continuing to grow rapidly so. There is a decline in supply of indigenous medicinal plants due to over-exploitation (GUPTA *et al*., 2010). This might be due to population growth, urbanization and industrialization. According to ELOFF (1988), WHO (2002) and TEMIKOTAN *et al*. (2013), it is of prime importance to preserve our heritage and the ongoing utilization of medicinal plants. Unrestricted collection of medicinal plant species from the wild is currently resulting in an over-exploitation of natural products or resources in southern Africa and throughout the world. This will soon result in extinction of important plant species. Medicinal plant parts which are frequently used in southern African regions include leaves, stems, barks, roots, bulbs and rhizomes. Barks and the undergrounds parts are found to be the most frequently used plant parts for medicinal purposes (JAIN *et al*., 2012). Consequently, the slow-growing forest trees, shrubs and herbaceous plants used for healing purposes are threatened by over-exploitation and are thus recognized by healers as becoming scarce (CUNNINGHAM, 1991). It, therefore, seems that the problem of tree ring-barking and the extinction of commonly used plant species is a problem in southern Africa. One of the possible strategies to solve this problem is adulteration.

Adulteration is a process whereby plant parts are replaced with other active plant parts of the same plant species for the same functions (PRAKASH *et al*., 2013). Adulteration may also be defined as mixing or substituting the original material with other spurious, inferior, defective, spoiled, useless parts of the same or different plant (PRAKASH *et al*.,

2013). Hence, adulteration was brought as an alternative to substitute the most-frequently used part of the slow growing plant.

According to JAIN *et al*. (2012) plant part substitution could possibly fulfill sustainable harvesting. Plant part substitution maintains the good health of patients, as well as biodiversity. This practice is widely practiced by large pharmaceutical industries as most products are derived from medicinal plant species. Most traditional healers today also practice the method of adulteration for the most frequently used plant species, for example, instead of using leaves from the frequently used plant species the bark from the plant can also be used to treat the same illness. According to CUNNINGHAM (1990), forest trees are highly vulnerable to excessive exploitation, mainly because the mature bark which are the most commonly used plant part in southern Africa. Ring barking has been recognized as the most destructive harvesting practice as it means that the debarked tree has no chance of survival (ZSCHOKE *et al*., 2000). There is evidence from recent data to show that leaf and fruit harvesting does not damage plants in the same way as debarking (CUNNINGHAM, 1988). Slow growing bulbous and tuberous plants, which are frequently used in traditional Zulu medicine like *Bowiea volubilis*, *Eucomis autumnalis* and *Scilla natalensis* (Hyacantaceae), represent another group of plants that are particularly threatened by over-exploitation and recognized by the healers as becoming scarce (CUNNINGHAM, 1991).

Again, the main problem is the destructive harvesting of the underground parts of these plants. The same applies to bulbous and tuberous plants where the stems or leaves could be harvested instead of the underground parts (ZSCHOCKE *et al*., 2000). An evaluation of differences and similarities between various parts of the same plant with respect to chemical composition and pharmacological properties was conducted by ZSCHOCKE *et al*., (2000). Moreover, preliminary results into four of the most important South African medicinal plants *E. automnalis* (Hyacanthaceae), *Siphonochilus warburgia* (Zingiberaceae), *Ocotea bullata* (Lauraceae), and *Warburgia salutaris* (Canellaceae)- reported to have the same phytochemical and pharmacological similarities on different plant parts (ZSCHOCKE *et al*., 2000). This practice requires more scientific research where extracts of various plant parts will be compared

chemically using the Thin Layer Chromatography (TLC) analysis and pharmacologically in terms of biological activity.

## 2.4. MANAGEMENT OF TB USING MEDICINAL PLANTS

The practice of traditional medicines remains culturally strong in the African countries, especially the orient and amongst a minority of enthusiasts in the Western society. South Africa has a rich heritage of indigenous knowledge on the use of medicinal plants. Herbal remedies from these plants have contributed to the reduction of excessive mortality, mobility, and disability brought about by diseases such as diabetes, HIV/AIDS, malaria, TB and other microbial infections (LAWAL *et al*., 2014). TB is a fearful disease in developing nations, particularly in the Asian and African continents, probably due to inadequate means for the management and treatment of the disease (LAWAL *et al*., 2014).

Due to the resistance developed by *M. tuberculosis* against both the first-line and second line drugs (GUPTA *et al*., 2010), there is an urgent need for MDR-TB and XDR-TB drugs that will combat TB disease all over the world (SINGH, 2007). A study which was conducted by a global laboratory network, CDC and the WHO, has reported that virtually untreatable TB was due to the extensive drug resistance, which was present in every region of the world (CDC, 2006)**.** Based on the study carried out by CDC (2006), all patients with MDR-TB, will eventually die within an average of 25 days from the first time of establishment. These deadly strains are most likely to further jeopardize TB control, if they are left untreated, with the possibility of killing a large number of people living with HIV\AIDS throughout the southern Africa regions.

Tuberculosis control programmes currently emphasize the Directly Observed Treatment Short Course (DOTS) strategy, promoted by the WHO and the International Union against TB and lung disease. South Africa adopted the WHO's DOTS strategy in all nine provinces (Department of Health, 2011). Key tenets of plan are standardized treatment of 68 months for all infectious patients; with directly observed therapy for at least the initial two months (WHO, 2005). However, previous studies by NEEDHAM *et*

*al*. (1998) AND RUSSELL (2004) noted that rural patients often delay TB treatment as they cannot afford to travel to treatment centers (DOTS clinics) daily to have a health worker watch them take their drugs. KANDEL *et al*. (2008) discovered that of the 255 TB patients who came for treatment at Mbekweni Health Centre in the King Sabata Dalidyebo district in the Eastern Cape Province of South Africa, 121 had interrupted their treatment. Reasons for interruption included change of living place, side effects of the drug, lack of knowledge about the treatment course, physical disability (either too sick or old) to collect treatment, clinic too far and drug not available in the clinic (KANDEL *et al*., 2008).

A number of studies evaluated the usefulness of using traditional healers as TB supervisors (COLVIN *et al*., 2001, CDC, 2006). COLVIN *et al*. (2001) reported that 25 traditional healers offered to volunteer in the study, and thus attended training workshops on the management of TB in the Hlabisa community of Kwa-Zulu Natal. The results indicated that most of the patients who were treated by the traditional healthcare practitioners expressed a high level of satisfaction with their care (FLOYD *et al*., 1997; CDC, 2006).

The medicinal properties of plants have been well known since time immemorial and plants offer a new source of potent antimicrobial agents in the form of secondary metabolites (COWAN, 1999). Antimycobacterial activity has been reported in a number of higher vascular plants (BUWA AND AFOLAYAN, 2009; GREEN *et al*., 2010; SEMENYA AND MAROYI, 2013). The *in vitro* efficacy tests of plants used in the traditional treatment of TB were successfully screened against *M. tuberculosis* in Kwa-Zulu Natal, Eastern Cape, Western Cape, Northern Cape, Free State and Mpumalanga Provinces (GREEN *et al*., 2010).

Aqueous extracts of five plants from the study conducted by GUPTA *et al*. (2010) were tested for antimycobacterial activity against MDR isolates of *M. tuberculosis.* Other plants, which were reported to possess anti-tuberculosis activity included *Adhatoda vasica* (GUPTA AND CHOPRA, 1993; GRANGE AND SNELL, 1996), *Allium cepa* (JAIN, 1993; RATNAKAR AND MURTHY, 1996) and *Aloe vera* (GOTTSHALL *et al*., 1949; BRUCE, 1976; REYNOLDS AND DWECK, 1999). *Acalypha indica* was also

screened due to its use in the treatment of respiratory disorders (HIREMATH *et al*., 1993). The plant displayed activity against the MDR strain.

## 2.5. PROBLEM STATEMENT

Respiratory diseases are among the major human killers in the world (MANN *et al*., 2007). Respiratory tract infections include diseases such as flu, common cold, bronchitis, sinusitis, pneumonia, bronchiolitis, and TB. TB has been reported to be one of the infectious bacterial diseases. It is a notifiable disease causing significant morbidity and mortality worldwide, most especially in the developing countries (CRONJE AND BARKER, 2006). According to a study undertaken by MATIVANDLELA *et al*. (2008), between the years 2000 and 2020 almost 20 million individuals will fall victims of TB and 35 million will unfortunately die. Global Tuberculosis Report 2013 released by WHO revealed that TB disease killed 1.3 million people worldwide in 2012, while India alone accounted for 26 percent of total TB drugs globally. The report further reveals that the majority of TB cases worldwide in 2012 were in the South-East Asia (29%), Africa (27%) and Western Pacific (19%) regions (MATIVANDLELA *et al*., 2008). TB largely affects developing countries, especially those in Asia and Africa. Over 95% of TB deaths occur in low and middle income countries, and it is among the top three causes of death for woman aged 15 to 44. According to WHO (2012), 530 000 children became ill with TB and 74 000 HIV negative children died of TB.

South Africa is ranked the third highest in the world in terms of TB burden (0.4-0.59 million), after India (2.0-2.5 million) and China (0.9-1.2 million) (WHO, 2011). Today, the number of TB cases is increasing at an alarming rate in South Africa, and this might be due to the human immune deficiency virus (HIV) (Cronje and Barker, 2006). According to NEL *et al*. (2013), HIV is fueling the TB epidemic, with more than 70% of TB patients living with HIV. Approximately 1% of the South African population develops TB disease every year. The number of cases detected for all forms of TB has steadily increased from 148 164 in 2004 to 401048 in 2010 (NEL *et al*., 2013). A deadly combination of HIV and TB is cutting a wrap through South Africa. South Africa is the epicenter of the

HIV pandemic and as the epidemic has matured the number with advancing HIV-related diseases has grown (PADAYATCHI *et al*., 2010). Within HIV patients, most of them are likely to be infected by TB. There is a wide variation in HIV and TB prevalence across age, race, gender, socio-economic status and geographical location. It is estimated that 80% of the South African population is infected with the TB bacillus; however, not everyone who is infected will progress to active TB disease.

TB is the most commonly notifiable disease in South Africa and the fifth largest cause of death (LALL AND MEYER, 1999; GREEN *et al*., 2010; MADIKIZELA *et al*., 2013). The number of recorded TB deaths in South Africa has increased from 22 000 in 1997 to 67 000 in 2003 (MADIKIZELA *et al*., 2013). According to a study undertaken by GREEN *et al*. (2010), South Africa has the seventh highest number of people with TB in the world and the second in Africa. In addition, the country has the fifth highest burden of drug-resistant TB cases in the world (NATURAL DEPARTMENT OF HEALTH, 2011). The Free State Province is one of the Provinces with high numbers of TB patients.

In an article published by Sowetan Live, it was stated that thousands of people are suffering from TB in the Free State though it is a manageable disease in South Africa (SELEKE, 2013). The Province's senior manager for TB treatment and control, revealed that the Mangaung Metropolitan area was leading the pack with 6520 registered TB patients, followed by Lejweleputswa District with 5825 patients, Thabo Mofutsanyane with 3912 patients, Fezile Dabi registered 3147 patients, and Xhariep District had 1432 patients (SELEKA, 2013). The author further states that Lejweleputswa District had registered 24 cases of children with TB, followed by Thaba Nchu District that had 14 cases. According to SELEKA (2013), TB is a serious disease that is associated with socio-economic hardships, and it needs a holistic approach. Poverty is one of the major contributors to poor health through food insecurity, which in turn is linked to HIV and TB acquisition and poor treatment adherence. The challenges which the Free State Province is facing are complicated and ranging from poverty, illiteracy, low educational attainment levels, unemployment, brain drain and poor health outcomes (PUUKKO *et al*., 2012). There is seriously an urgent need for an alternative medicine or treatment that is cheaper and readily available. Several researchers, such as, LALL AND MEYER

(1999), MATIVANDLELA *et al*. (2008); BUWA AND AFOLAYAN (2009); MADIKIZELA *et al*. (2013); MASOKO AND NXUMALO (2013), have successfully studied some medicinal plants used against TB and other respiratory ailments in South Africa. Little or no information is available on medicinal plants used by the people of the Free State Province in the treatment of TB and related respiratory ailments. This study is aimed at making a significant literature contribution in filling that gap.

## 2.6. AIMS AND OBJECTIVES

### 2.6.1. Aim

The broad aim of this study was to screen the traditional medicinal plants used by the traditional healers and herbalists of the Free State Province for the presence of antimicrobial properties.

### 2.6.2. Objectives

The specific objectives were to:

- Conduct an ethnobotanical survey of plants used by the traditional healers of the Free State Province in the treatment of tuberculosis,
- Screen the plants used by the traditional healers and herbalists of the Free State Province in the treatment of TB for the presence of secondary metabolites,
- Screen traditional medicinal plants used in the treatment of TB for antimycobacterial activity,
- Screen traditional medicinal plants used against TB for antibacterial activity, and
- Screen traditional medicinal plants used against TB for antifungal activity.

# CHAPTER 3

# PLANT SELECTION, COLLECTION, IDENTIFICATION AND EXTRACTION

## 3.1. INTRODUCTION

Natural resources provide us with new lead molecules for the development of drugs used against various ailments. The multidisciplinary approach of combining botanical, ethnobotanical and biological techniques has led to new drug discovery from the plants (NEWMAN *et al*., 2000). It has been estimated that less than 10% of the large diversity of 25000–500000 plant species on earth have been studied chemically and pharmacologically for their medicinal properties (FARNSWORTH AND SOEJARTO, 1991). The estimate was true for the tropical flora, as it recorded only 1% of the species that have been studied for their pharmacological potential (GURIB-FAKIM, 2006).

Plant-derived compounds have been, and even today are still, important as models (lead compounds) for medicine. To date, about 50 drugs come from plant species, as plants continue to be a potent source of lead components (LOUISA, 2013). Natural products have pharmacological or biological activity that can be of therapeutic benefit, particularly in the treatment of various diseases. As such, natural products are the active components of many traditional medicines. Traditional medicine comprises knowledge system that developed over generations within various societies before the era of modern medicine. Practices known as traditional medicines include Ayurveda, herbal, traditional Chinese medicine, traditional African medicine, Ethiopian traditional medicine, Siddha medicine, Perso-arabic traditional medicine, ancient Iranian medicine, Irani-tebb or Irani traditional Medicine, Islamic medicine, and traditional Korean Medicine (SLIKKERVEER, 1990; ACHARYA AND SHRIVASTAVA, 2008; ACHARYA AND ANSHU, 2008). Examples of medicines successfully derived from natural products include most antibiotics, the acethylcholineesterace (ACE) inhibitors, many anticancer agents, the immunosuppressant, cyclosporine, rapamycin, and the antiparasitic avermectins (HARVEY AND WATERMAN, 1998). This discovery of new biological agents from natural resources has been an essential quest of mankind since prehistoric

times simply because they are relatively easyto collect, they exhibit potential for sustainable use, and because of structural and biological diversity of their constituents (BUWA, 2006).

Medicinal plants use has accumulated most especially in the tropical parts of the world as most people have used plants for millennia. Herbal medicines include herbal materials, herbal preparation and finished products that contain parts of plants or other plant materials as active ingredients (MAHOMMODALLY, 2013). In many parts of Africa, medicinal plants are the most easily accessible resources available to the community, and they are the preferred option for some patients. For a number of these people, traditional healers and herbalists offer counseling, treatment and information to patients and their families in a personal manner since they have an understanding of their patient's background (MOKAILA, 2001; GURIB-FAKIM, 2006; GURIB-FAKIM AND MAHOMOODALLY, 2013). Plants are dispensed in the form of crude extract such as tinctures, teas, powders, and other herbal formulations, which now serve as the basis of novel drug discovery (BUWA, 2006).

In Africa, the ethnopharmacological and botanical knowledge on the uses of medicinal plants is often passed down orally from generation to generation. This distribution of information is in danger of disappearing since it is often kept a secret until the last minutes of death of the traditional healer when they eventually call on someone to inherit the information or the call (KOKWARO, 1976). Moreover, there is a rapid loss of natural habitats of some of the plants used in traditional medicine due to anthropogenic activities and due to an erosion of valuable traditional knowledge (MAHOMMODALLY, 2013). There is, therefore, a need for documentation of medicinal uses of African plants, together with the traditional systems.

There are several ways used in the selection of plants which may be used in searching for new medicinal plants and their active compounds. These include ethnopharmacological approach, phylogenetic approach and random sampling. Concerning ethnopharmacological approach, medicinal plants are often of substantial importance for finding new potential medicinal plants or new ways of using already known plants (FYHRQUIST, 2007). According to LIN *et al*. (1999), about 74% of the

pharmacologically active compounds isolated from plants were discovered after the ethnomedical uses of the plants. The second most important way of discovering new medicinal plants and lead components is the phylogenetic approach. In this approach, a number of closely related plant species which are assumed to contain related chemical compounds (Chemotaxonomy) are screened for their biological effects (COTTON, 1996; VUORELA *et al*., 2004). In random sampling, collecting plant samples from certain habitats with high species diversity can be beneficial for finding novel chemical entities (NCEs). However, this method is somewhat time-consuming and requires hard work (FYHRQUIST, 2007).

This chapter was, therefore, aimed at collecting and documenting information on medicinal plants used against TB and related ailments in the Free State, South Africa.

## 3.2. MATERIALS AND METHODS

### 3.2.1. Plant collection

An ethnobotanical survey was conducted from January to June 2015, information was compiled through general conversations with traditional healers and herbalists while structured questionnaires were used to obtain additional information about the methods of treatment (Figure 2). The questionnaire was prepared in both English and Sesotho. The main aim and vision of the interviews were explained to the participants and their consent to publish the findings was obtained before questioning. Tokens were also paid to the participants for their time and knowledge shared. Moreover, it was agreed that this research would not be for commercial purposes, but to serve as enlightenment to the South African community at large, particularly people living in the Free State. The knowledge of plant parts used and the mode of preparation, administration, the source of plant materials, and efficacy of herbal drugs were also obtained from the herbalists and the traditional healers. Plants were then collected with the assistance of the traditional healers and herbalists. Voucher specimens (HLON01 to HLON08) for each of the collected plant species were prepared and deposited at the


**Figure 2: Consultation with one of the traditional healers who sells *muti* at the local shoping complex in Phuthaditjhaba**

herbarium of the University of the Free State (QwaQwa campus) and were scientifically identified and aunthenticated by Dr L.V. Komoreng and Dr E.E.J Sebien.

### 3.2.2. Plant material preparation

The accessibility of plants from the surrounding mountains, centralizing the University of the Free State (QwaQwa campus), allowed an immediate processing of fresh plant parts, prior to extraction. The collected plant material was cleaned by washing with distilled water. Plant material was then chopped and dried in an oven at 45°C until dryness, where after, it was ground to fine powder using a blender. The material was then stored in sealed glass jars in the refrigerator until further processing.

### 3.2.3. Preparation of plant extracts

Thirty grams of powdered plant material was extracted with 300 ml of methanol, ethanol, acetone and water, respectively. Plant extracts were filtered through Whatman No.1 filter paper discs, dried and continually weighed until a constant weight was obtained. The water extracts were dried in a hot water bath at a temperature of 40°C by allowing the liquid to evaporate. Dry plant extracts were stored at approximately 4°C until further use. The schematic presentation of the preparation of plant extracts is shown in Figure 3.

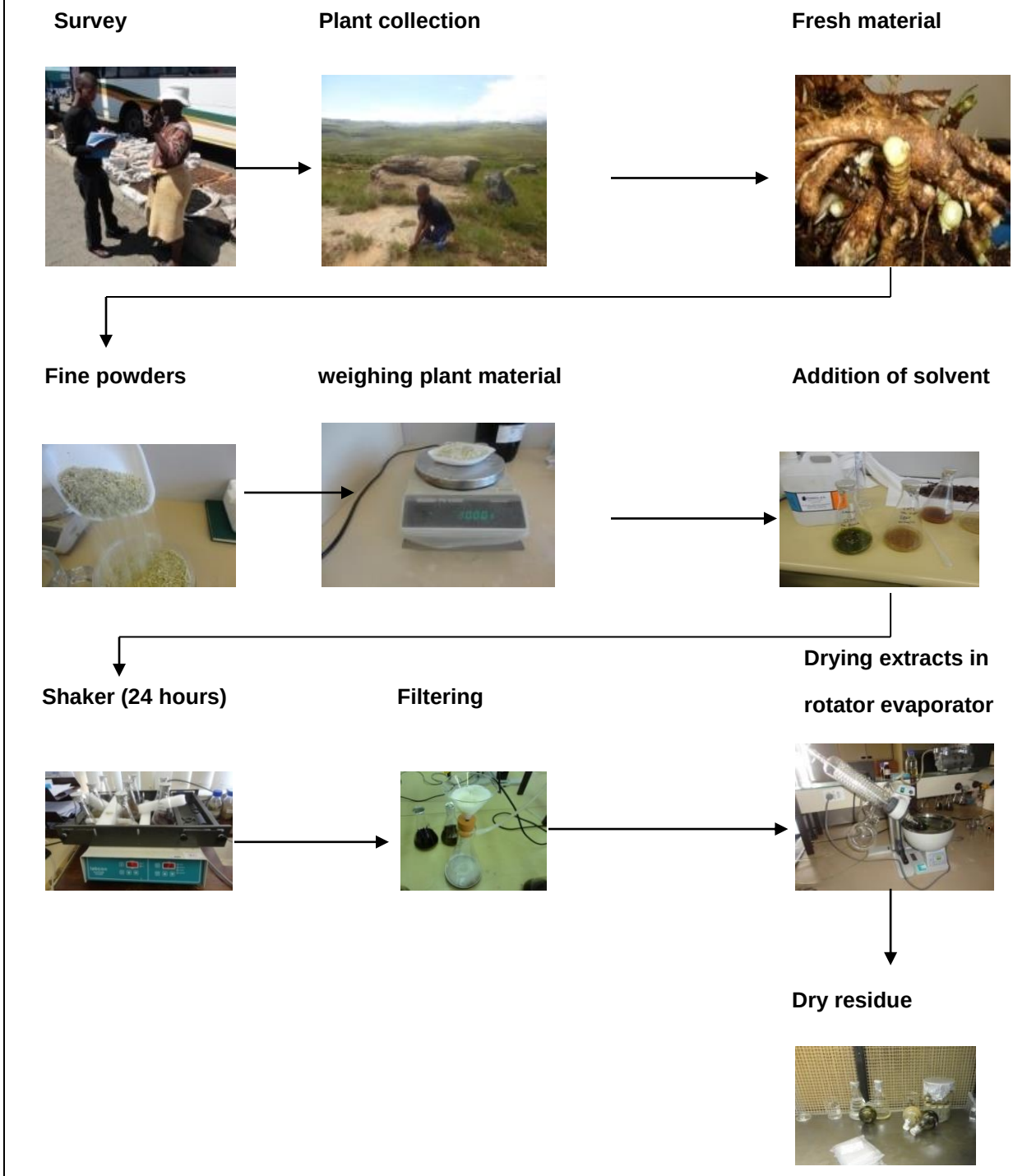


**Figure 3: Shows the schematic representation of the collection of information, preparation and extraction of collected plant material.**

## 3.3. RESULTS AND DISCUSSION

A total number of 11 traditional healers and herbalists were interviewed. The people who provided the information on traditional medicinal plants used against TB were the elders ranging between the ages of 46 to 55 years old. The interviewed traditional healers work as full-time and part-time traditional doctors, whereas others are street vendors. One of the traditional healers collects and sells the herbs or medicinal plants, whereas the other interviewed traditional healers collect medicinal plant species from the wild and cultivate some of the plants in their home gardens to bring them closer.

Table 5 shows the list of the plant species collected during the survey. The documented information included the families to which the plants belong, botanical names and common names, parts of the plant used, and the mode of preparation. Collectively, eight plant species belonging to 5 families were said to be effective against TB and related ailments, with Asteraceae and Hyacinthaceae having 2 species each. Table 6, shows the distribution of species according to their number of occurrences in the family. Medicinal plant species which were documented in this study are *Xysmalobium undulatum* L*., Drimia depressa* Baker*., Eucomis automnalis* Mill., *Dicoma anomala* Sond*., Senecio harveianus* MacOwan., *Lotononis lanceolata* E.Mey., *Hermania gerrardii (*N.E.Br) and *Lentsweni* (unidentified specimen). The bulbs, leaves and roots were the most frequently used plant parts in the preparation of traditional remedies.

During the ethnobotanical survey, it was discovered that decoction, using water and alcohol, of roots, stems, bulbs and leaves was one of the methods commonly used by traditional healers in preparation of the remedies. At some point, infusion of different parts of the plants is prepared and administered by a patient. Half a cup of the prepared remedies are taken three times a day, but this will depend on the strength of the illness. Four different solvents, namely acetone, ethanol, methanol and water, were selected based on the literature review. VLACHOS *et al*., (1996) reported that solvents such as methanol, followed by acetone are the most effective solvents for extracting antibacterial compounds from plants.

### 3.3.1. *Dicoma anomala* Sond.

The genus *Dicoma,* belonging to the Asteracea family, consists of about 50 species of herbs, shrubs and small trees (BECKER *et al*., 2011). *D. anomala*, commonly known as *hloenya* or *mohasetse* in Sesotho has stiff, sharp-pointed bracts, large flower heads with mauve-white florets surrounded by many white bristles (Figure 4). *D. anomala* is a prostrate, decumbent or erect perennial herb with an underground tuber (ONDERSTALL, 1996). The stems of *D. anomala* are thinly covered with hairs. The plant possesses simple leaves, which are positioned alternate around the stem, stalkles, linear or narrowly lanceolate, the upper surface glabrous while the lower surface is white-hairy (ONDERSTALL, 1996; HUTCHINGS *et al*., 1996). Flowering of the plant occurs from November to July, with peak flowering in February and March. *D. anomala* is widely distributed in sub-saharan Africa, resulting in pronounced morphological variety. It grows in the summer rainfall areas and in stony grasslands, hillsides or flat grassland. In South Africa, it is widely distributed in Limpopo, North-west, Gauteng, Mpumalanga, Free State, Northern Cape and KwaZulu-Natal (HUTCHINGS *et al*., 1996).

The plant is widely utilized by people due to the medicinal properties it possesses. Boiled plant parts of *D. anomala* used for cough and respiratory ailments, while the powdered plant material can be used for sores and wounds. The root decoction of the plant is administered orally or as enemas to children believed to be suffering from blood disorders. Root decoction can also be used as a purgative for intestinal worms, colic, diarrhea, dysentery, toothache, as an ingredient for sterilization medication and for haemorrhoids (RETIES AND HERMAN, 1997).

### 3.3.2. *Xysmalobium undulatum* L.

The genus *Xysmalobium* consists of about 40 species, which all are endemic to Africa. Approximately 24 species are found in the southern Africa and are mainly associated with grassland (BRANDWIJK, 1927). *X. undulatum,* commonly known as Milk bush in English and in southern Sotho is known *as poho-tsehla* is a robust geophytic herb that grows from 0.5-2.0 m tall (EVANS, 1926). The wave-leaved *Xysmalobium* is well known feature of the verges of most of the highways in the grassland biome during November and December. *X. undulatum* sprouts annually from rootstock in spring and dies back in winter (POOLEY, 2005). The plant is characterized by stalked inflorescences developing in the axils of the leaves (Figure 5). *X. undulatum* is widely distributed in the eastern parts of southern Africa. It can be found in all nine provinces of South Africa including Namibia, Botswana, Lesotho and Swaziland (WATT AND BREYER-BRANDWIJK, 1962). The roots of *X. undulatum* are widely used in Africa and throughout the world as medicine. The powdered root of *X. undulatum* is used to treat wounds and abscesses. Traditionally, the plant is used as traditional medicine to treat headaches. Dry powdered roots and extracts of the roots are apparently an excellent remedy for painful menstrual cramps and have antispasmodic action. The root has been used widely for treating indigestion, malaria and fever, including typhoid (WATT AND BREYER-BRANDWIJK, 1962; POOLEY, 2005).


**Figure 4: *Dicoma anomala* (Sond)**


**Figure 5: *Xysmalobium undulatum* (L.)**

### 3.3.3. *Hermannia depressa* N.E.Br

The genus consists of 154 species which have a distribution mainly across the *Flora of Southern Africa* area. In South Africa alone, there are 141 species of *Hermannia,* of which 81 are endemic to South Africa (DYER, 1954). *H. depressa*, commonly known as a doll's rose in English, *seletjane* or *phathe-ya-ngaka* in SeSotho, belongs to the Malvaceae family in the subfamily of sterculioideae (Mallow family) which is a very diverse and attractive group of plants with highly ornamental flowers, fitting a variety of habitats and growth forms (VAN ROOYEN AND STEYN, 1999). *Hermannia* is the little porcelain bell of the Southern African veld. It is a genus of small shrubs, ranging from upright to sprawling prostrate shrubs (Figure 6). The plant species is characterized by the presence of minute glandular or star-like hairs on the leaves and stems (GERMISHUIZEN AND MEYER, 2003). The flowers are dropping, from short erect stalks, and vary in colors between orange, pinkish, mauve and yellow-cream (WATT AND BREYER-BRANDWIJK, 1962). Leaves are alternate and entire, lobed or incised. Most *Hermannia* species possess a thick woody stem and roots, forming an underground stem, which enables the plant to survive dry periods and fires. However, the plant is most likely to be distributed in grassland to highveld.

In South Africa the plant is used to treat and manage various ailments including heartburn and an antidote for food poisoning; provide remedy for colic, used as an emetic, charm against witchcraft, used by traditional healers to make the divining bones indicate illness and treatment required, increases the properties of other medicines when mixed with them and gives them a red colour (MOFFET, 2010). Many members of the genus are used medicinally for coughs and internal aches, as stimulates or purgatives, to soothe wounds and cuts. In some areas, the leaves are infused in a tea, and used to purify blood, whereas root infusion was used by early European colonial settlers against epilepsy (MANNING AND GOLDBLATT, 1996). *H. depressa*, mixed with other plant species can also be used for diarrhea (MOFFETT, 2010).

### 3.3.4. *Drimia depressa* (Bak. Jessop)

The genus *Drimia* belongs to the Hyacinthaceae family with approximately 700-900 species in about 70 genera. The plant is characterized by wide, wary-edged leaves and bulb, which is the underground structure of the *D. depressa* (Figure 7). *D. depressa* commonly known as *Moretele* in Sesotho possesses significant ethno-medicinal uses such as antiulcerous, antinematodal, antitumorous, anthelmintic, and antiarthrities. It is also used to cure skin diseases like warts, abscesses, boils, cardiac diseases, antidote to scorpion sting. The bulb and leaves of the *D. depressa* are widely used in South Africa for medicinal purposes (GAO *et al*., 2011), e.g. as a diuretic for cleaning the bladder and treating diseases of the uterus. It is also known for its most powerful good luck charm. It is used by traditional healers, and the chiefs were formerly “vaccinated” with *D. depressa* to protect them from harm (MOFFETT, 2010).


**Figure 6: *Hermannia depressa* N.E.Br**

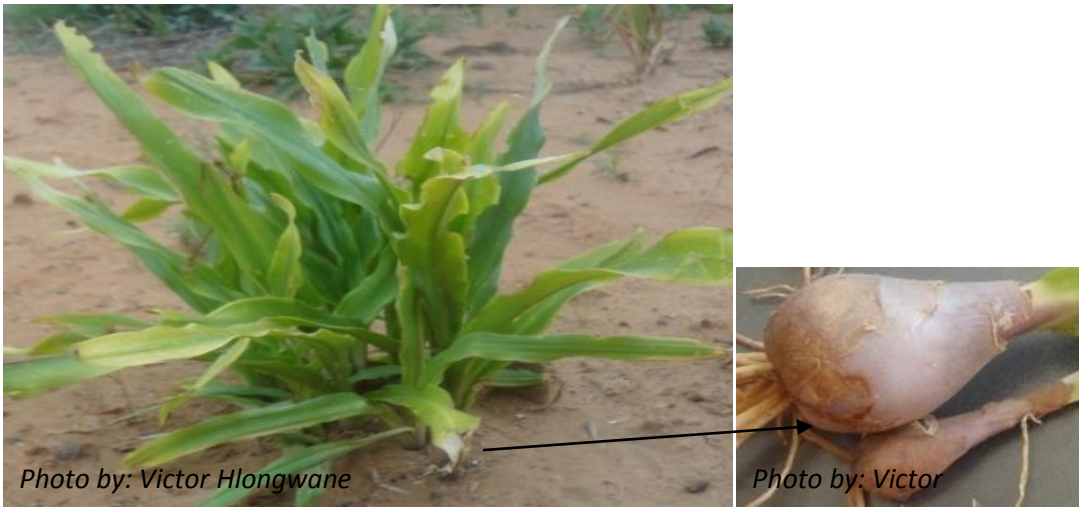


**Figure: *Drimia depressa* Bak. Jessop. Arrow shows the bulb of *D. depressa***

### 3.3.5. *Senecio harveianus* MacOwan

*S. harveianus*, commonly known as *Kgotodiya* in Sesotho belong to the Asteraceae family. There are about 133 species that are considered as weeds from approximately 1500 species that belong to the genus *Senecio*. *S. harveianus* is a dominant invasive species in part of the Western Europe and is one of the most rapidly spreading introduced plant species in Europe (EPPO, 2006). Some similar species of *S. harveianua* include *S. douglasii, S. lautus, S. lythroides, S. malacitanus* and *S. inaequidens* (HEGER AND BÖHMER, 2006). *S. harveianus* is a perennial herbaceous or woody shrub that grows up to 100 cm tall, spherically shaped, rising from a shallow taproot (Figure 8). It can grow in rural areas, rockey outcrops and sand dunes. *S. harveianus* can also grow under temperate and Mediterranean climates (EPPO, 2006). The plant can be opportunistic in having the ability to colonise a wide range of habitat including the temperate deciduos forests, temperate steppes and Mediterranean sclerophyllous forests and sclerophyllous shrub (EPPO, 2006). Reportedly, the leaves of *S. harveianus* are used as food in specific population of Southern Africa (HEGER AND BÖHMER, 2006). There is no information recorded about the use of this plant species for any ailments, whereas information gathered during the interviews mentioned that the plant can be used for coughs, tuberculosis and back pains.

### 3.3.6. *Eucomis autumnalis* (Mill.) Chitt.

*Eucomis autumnalis* is a member of the Hyacinthaceae family, formerly part of the Liliaceae (Lily family). The genus *Eucomis* consists of approximately eleven species that occur in southern Africa. *E. autumnalis*, commonly known as a pineapple lily in English and *umathunga* or *mathithibala* in isiZulu, is a deciduous, summer growing bulbous plant (Figure 9). The plant has a large bulb of about 8-10 cm in diameter, ovoid in shape, and it gives rise to a rosette of large, broad soft-textured, fleshy, wary-edge leaves. The inflorescence is a dense cylindrical raceme on a stout stalk crowded with up to ±125 starry yellowish-green flowers with a tuft of leaf-like bracts at the tip. *E.*

*autumnalis* is divided into three subspecies, most clearly distinguishable by the structure of the peduncle which is either club-shaped or cylindrical.

The plant bulb is known to be toxic but the plant is used medicinally in South Africa. Decoction of the bulb in water or milk is usually administered as enemas for the treatment of low backache, to assist in post-operative recovery, and to aid in healing fractures (DU PLESSIS AND DUNCAN, 1989).

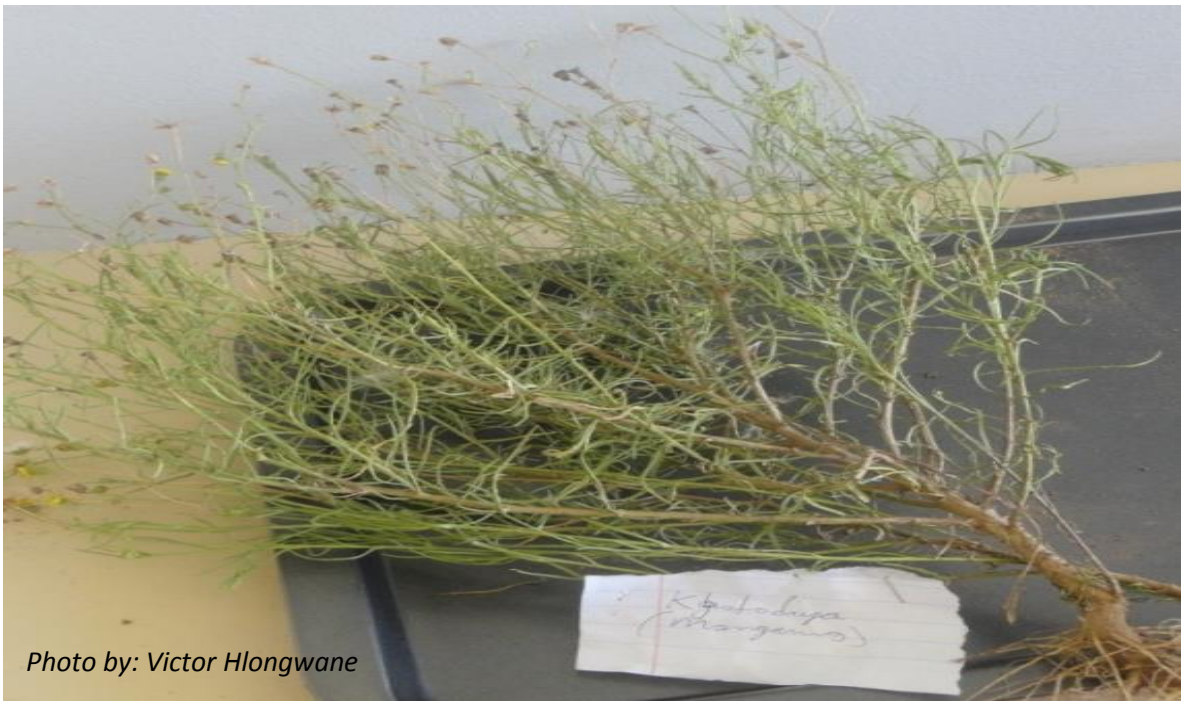


**Figure 8: *Senecio harveianus* MacOwan**


**Fugure 9: *Eucomis automnalis* (Mill.) Chitt. Arrow ashows Bulb of *E. automnalis***

3.3.7. *Lotononis lanceolata* (E.Mey.) B.

*L. lanceolata,* belonging to the fabaceae family, is commonly known as *kgonati* in Sesotho. This plant species is a herbaceous perennial plant with woody root stock. *L. lanceolate* is mostly characterized by few flowers in dense terminal cluster which is surrounded by leaves. *L. lanceolate* is a twining or original erect perennial legume with a taproot conspicuous up to 40 cm deep (Figure 10). The plant is medicinally used by traditional healers to cure anthrax. Herbalists, in treating fevers and contagious diseases, can use the plant alone or with *Aster bakerianous*. It is also used as an enema or drunk as a decoction to treat diarrhea (MOFFETT, 2010).


**Figure 10: *Lotononis lanceolata* (E.Mey.) B.**

**Table 5: List of medicinal plants used by traditional healers of the Free State Province in the management of TB**

| Family | Botanical name (Voucher number) | Local name | Uses | Plant part used | Mode of preparation |
|---|---|---|---|---|---|
| Asteraceae | *D. anomala* (HLON01) | *Hloenya* | Stomach pains, tuberculosis, wounds, blood pressure and diabetes, and colds | Leaves | Infusion leaves in water or chewing the leaves of *D. anomala* |
| Hyacinthaceae | *D. depressa* ( HLON02) | *Moretele* | Stomach pains, fever and colds, headache, and coughs. | Bulb | The bulb of the *D. depressa* can be infused for few days |
| Hyacinthaceae | *E. automnalis* ( HLON03) | *Mathithibala* | Stomach pains, enema for low back ache, coughs, piles, gonorrhea, and colds | Bulb | Infusion of the bulbs drunk for stomach pains |
| Sterculiaceae | *H. depressa* ( HLON04) | *Seletjane/ phate-yangaka* | Heartburn, antidote for food poisoning, tuberculosis and used as charm for whitchcraft. | Roots | Mixed with other plants and boiled |
| Fabaceae | *L. lanceolata* ( HLON05) | *Kgonatha* | Fevers, contagious diseases and TB. | Tuber | Decoction of the tuber and drunk while is still warm |

**Table 5 continued**

| Family | Botanical name (Voucher number) | Local name | Uses | Plant part used | Mode of preparation |
|---|---|---|---|---|---|
| Unknown | [a]*Lentsweni* (HLON06) | Unknown | Fevers, headache, period pains and coughs. | Whole plant | Soaking the plant part used and the extracts can be drunk there after |
| Asteraceae | *S. harveianus* (HLON07) | *Kgotodiya* | Fevers, colds, coughs, and chronic diseases. | Whole plant | Decoction of the plant parts used |
| Apocynaceae | *X. undulatum* (HLON08) | *Poho-tsehla* | Colic, diarrhea, dysentery, hysteria and tuberculosis | Whole plant | Steeping parts used, applying powdered material to wounds |

**Table 6: Species distribution according to their families**

| **Families** | **Number of species** |
|---|---|
| Asteraceae | 2 |
| Hyacinthaceae | 2 |
| Apocynaceae | 1 |
| Fabaceae | 1 |
| Sterculiaceae | 1 |
| [a]*Lenstweni* | Unidentified |

## 3.4. CONCLUSION

In conclusion, this study has highlighted a vital role of the medicinal plants in the treatment of various ailments, most particularly respiratory ailments. Due to the growing interest on ethnobotanical studies, it was very important to collect information about the knowledge of medicinal plants and their use. And document the retrieved information for future uses and knowledge. During the survey, one of the traditional healers stated that she collects some of the herbs from the wild and cultivate them in her back yard so that they can be readily available when she wants to use them. This provides sustainable use and conservation of traditional medicinal plants used in the treatment of TB and related ailements.

# CHAPTER 4

# PHYTOCHEMICAL AND PHARMACOLOGICAL SCREENING

## 4.1. INTRODUCTION

Bacteria and fungi are the vectors responsible for a variety of diseases for humans, animals and plant species (VERASTEGUI *et al*., 1996). However, due to the use and misuse of antimicrobial drugs, an emergence of bacterial resistance to antibiotics has become a common phenomenon, which is a major problem. The emergence and spread of antibiotic resistant bacteria has necessitated the search for novel and more effective antibacterial drugs (ALY AND BAFIEL, 1997).

Due to the fact that most microorganisms have developed resistance against many common antimicrobial agents, infectious diseases continue to be one of the greatest health challenges throughout the world. The increasing problem of multi-drug resistant microorganisms has led to researches to try and develop new strategies to fight antimicrobial resistance (HOJGARD, 2012). The strategies for the most effective antibacterial agents focus on plants; this is due to the long historical experience and the fact that there has been a good portion of the world's population, most particularly in developing countries, who primarily relied on plants for the treatment of both infectious and non-infectious diseases (BUWA, 2006). Medicinal plants have formed a basis of healthcare throughout the world since the earliest days of humanity, and are still widely used and have considerable importance in international trade. Recognition of their clinical, pharmaceutical and economic value is still growing. Plants, as starting materials for synthesis of drugs or as models for pharmaceutically active compounds, are important for pharmaceutical research and drug development. Plant extracts have been used traditionally to treat a number of infectious diseases including those caused by fungi, bacteria, protozoa and viruses (MEYER *et al*., 1996; VERASTEGUI *et al*., 1996; BOKHARI, 2009). Medicinal plants are valuable natural resources and regarded as potentially safe drugs. Medicinal plants have been tested for various biological activities (ALY AND BAFIEL, 1997; AMER *et al*., 2008).

### 4.1.1. Phytochemical analysis

Plants, and the evolutionarily more recent subdivision of flowering plants (angiosperms), have colonized the vast majority of the terrestrial surface, courtesy of rich levels of specialization and intricate relationships with other organisms (KENNEDY AND WIGHTMAN 2011). They make an exponentially larger contribution to terrestrial biomass by volume and weight than all other forms of life combined (PIMENTEL AND ANDOW 1984). However, as stationary autotrophs, plants have been able to cope with a number of challenges, including engineering their own pollination and seed dispersal, local fluctuations in the supply of the simple nutrients that they require to synthesize their food, and the coexistence of herbivores and pathogens in their immediate environment. Plants have therefore evolved secondary biochemical pathways that allow them to synthesize a raft of chemicals, often in response to specific environmental stimuli, such as herbivore-induced damage, pathogen attacks, or nutrient deprivation (REYMOND *et al*., 2000; HERMSMEIER *et al*., 2001). Some of the roles of secondary metabolites are relatively straight forward; for instance, they play a host of general, protective roles (e.g. as antioxidant, free radical-scavenging, UV light-absorbing, and anti-proliferative agents) and defend the plant against microorganisms such as bacteria, fungi, and viruses.

Medicinal plants are useful for healing as well as in curing human diseases because of the presence of phytochemical constituents (WADOOD *et al*., 2013). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. They are naturally occurring in medicinal plants, leaves, vegetables and roots that have defense mechanism. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents, whereas secondary compounds have terpenoid, alkaloids and phenolic compounds (KRISHNAIAH *et al*., 2007). Several plant species have been reported to be used for their antimicrobial traits, which are due to the secondary compounds (NASCIMENTO *et al*., 2000). These products are known by their active substances such as the phenolic compounds, which are part of the essential oils (JANSEN *et al*., 1987) and the tannins (SAXENA *et al*., 1994).

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in plant. Metabolites such as alkaloids, steroids, tannins, and phenol compounds are synthesized and deposited in specific parts or in all parts of the plants. The plants secondary products exert their action by resembling endogenous metabolites, legends, and hormones which are signal transduction molecules and that have beneficial medicinal effects on human (CIOCAN AND BARA, 2007).

### 4.1.2. Classes of secondary metabolites

Secondary metabolites can be classified into:

#### *4.1.2.1. Tannins*

Tannins are part of the polyphenols that are obtained from various parts of different plants (PAREKH AND CHANDA, 2007). Studies have shown that tannins possess the potential for antiviral (LIN *et al*., 2004) and antibacterial activity (AKIYAMA *et al*., 2001; FUNATOGAWA *et al*., 2004). Plants containing tannins have also been studied for their effects against cancer (PAREKH AND CHANDA, 2007). In general, tannin is a descriptive name for a group of polymeric phenolic substances, which are capable of tanning leather or precipitating gelatin from solution, a property known as astringency (CIOCAN AND BARA, 2007). They are divided into two groups, hydrolyzed tannins which are based on gallic acid (Figure 11a), and condensed tannins which are derived from flavonoid monomers (Figure 11.b).

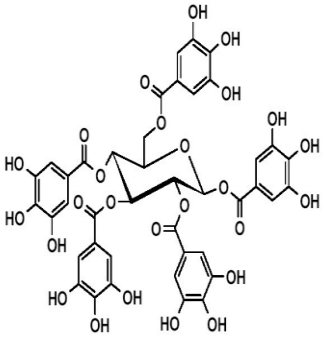

**Figure 11a: Hydrolyzed tannins**

Source: HAGERMAN, 2002a

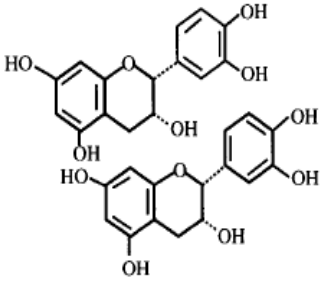

**Figure 11b: Condensed tannins**

Source: HAGERMAN, 2002b

*4.1.2.2. Saponins*

Saponins are found in plants containing glycosides that form soapy lathers when mixed and agitated with water. They are of high-molecular-weight glycosides, sugar moiety linked to a triterpene or steroids aglycone (KREDY, 2010). Saponins are defined based on their surface activity, most saponins have detergent properties; they give stable foams in water, showing hemolytic activity (KREDY, 2010). Saponins are toxic and they have a bitter taste. Saponins are glycosylated triterpenoids, steroids, or steroidal alkaloid molecules that occur constitutively in many plant species (PRICE *et al*., 1987; HOSTETTMANN AND MARSTON, 1995; OSBOURN, 1996; MORRISSEY AND OSBOURN, 1999). Some plant species containing saponins have been screened to evaluate antibacterial activity against bacterial pathogens (KREDY, 2010). Most plants displayed that saponins possessed activity against a broad range of microorganisms including bacteria, filamentous fungi and yeast (FIRN, 2010; DOUGHARI, 2012).

*4.1.2.3. Flavonoids*

Flavonoids are defined as polyphenolic compounds that are ubiquitous in nature and are categorized according to their chemical structure. They include flavonoids, flavones, flavonones, isoflavones, catechins, anthocyanides and chalcones (SILVER AND BOSTIAN, 1990). Plants containing flavonoids have aroused considerable beneficial effects on human health. According to SILVER AND BOSTIAN (1990), flavonoids have antiviral, anti-allergic, antiplatelet, anti-inflamatory, antitumor and antioxidant activities. Flavonoids are found to disrupt microbial membrane, however they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms (CIOCAN AND BARA, 2007). The activity of plants containing flavonoids might be probably due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell walls (SILVER AND BOSTIAN, 1990). Increasingly, plants rich in flavonoids are mostly adapted in the subject of medical research. For many years, physicians and lay healers in attempts to treat human diseases (SILVER AND BOSTIAN, 1990) have used products that contain flavonoids as the principal

physiologically active constituents. Flavonoids are also known to be synthesized by plants in response to microbial infection. There are suggestions that because flavonoids are widely distributed in edible plants and beverages and previously been used in traditional medicine, they are likely to have minimal toxicity (CUSHNIE AND LAMB, 2005).

#### *4.1.2.4. Alkaloids*

Alkaloids are found primarily in plants and are especially common in certain families of flowering plants. About 3000 different types of alkaloids have been identified in more than 4000 plant species (BORDE *et al*., 2014). However, the function of alkaloids in plants is not yet understood. Some of the studies have shown that they are simple waste products of plant metabolic processes, though evidence suggests that they may serve specific biological functions. Alkaloids are one of the most diverse groups of secondary metabolites, which are found in living organisms and have an array of structure type, biosynthetic pathway, and pharmacological activities (BORDE *et al*., 2014). Many alkaloids have been used for hundreds of years in medicine.

#### *4.1.2.5. Steroids*

Plant steroids or steroid glycosides are one of the most naturally occurring plant phyto-constituents that have found therapeutic applications of arrow poisons or cardiac drugs (FIRN, 2010). Steroids can be divided into four different types, i.e. (i) sterols, which are alcoholic, (ii) sterolin, are glycosides of sterols and insoluble in water, (iii) steroidal saponins, and (iv) nitrogen containing steroidal saponins (GUPTA *et al*., 1996). Steroids are often present in tissue culture as phytosterols with either sitosterol or stigmasterol being the dominant sterol. The two most commonly available steroids are cholesterol and ß-sitosterol (DAND, 1970). Approximately, two third of the raw material for chemical synthesis of the steroids hormones produced has depended on diosgenin obtained from plant species (GUPTA *et al*., 1996). Besides, other plants sterols such as sitosterol,

stigmasterol, campesterol solasodine and hecogenin are considered highly promising precursors. Most roots and leaves of plant containing steroids are abortifacient, aphrodisiac, diuretic, nervine tonic, laternative, narcotic, sedative, ostringet, growth promoter and anthelmintic (GUPTA *et al*., 1996). Such plants also have the antiarthritic, antibacterial, antidote for scorpion sting, antistress, antitumour and anticancer activities (DAND, 1970). Studies based on antibacterial activity and antifungal activity have indicated that crude extracts containing steroids displayed significant activities against various pathogenic strains (GUPTA *et al*., 1996; CHATTOPADHYAY *et al*., 2001; TALEB-CONTINI *et al*., 2003).

*4.1.2.6. Terpenoids*

Terpenoids may be defined as natural products that possess a structure, which is considered to be divided into several isoprene units. Terpenes form the main building block of any plant, resin or essential oils and they contribute to the scent, flavor, and colors. Terpenoids are synthesized from acetate units, and as such they share their origins with fatty acids (HOSTETTMANN AND MARSTON, 1995). Terpenes differ from fatty acids in that they contain extensive branching and are cyclized. They are active against bacteria, fungi, viruses and protozoa. Some are even known to have medicinal value. Terpenoids represent the largest class of secondary metabolites that serve as protective agents against various pathogens including insects, bacteria, and fungi (NASSAR *et al*., 2010). Terpenes can serve as potential anticancer drugs by direct cytotoxicity activity against cancer cells or by modulating the tumor development (BURKILL, 1996 AND KINTZIOS, 2003). Terpenoids exhibit various important pharmacological activities such as antiinflamatory, anticancer, antiviral, and antibacterial activities (MAHALO AND SEN, 1997).

*4.1.2.7. Cardiac glycosides*

In general, cardiac glycosides are defined as the condensation products of sugar (this include polysaccharides) with a host of different varieties of organic hydroxyl (Occasional thiol) compounds (invariably monohydrate in character) (DOUGHARI, 2012). Glycosides are neutral in reaction and can be readily hydrolyzed into its components with fermentes or mineral acids. Cardiac glycosides are compound containing glycosides (sugar) that act on the contractile force of the cardiac muscle. Glycosides are found as secondary metabolites in several plants. From ancient times, humans have used cardiac glycosides containing plants and their crude extracts as arrow, ordeal, homicidal, suicidal and rat poisons, heart tonics, diuretics and emetics. To date, purified extracts or synthetic analogues of cardiac glycosides have been adapted for the treatment of congestive heart failure and cardiac arrhythmia (SING AND RASTOGI, 1970). Though cardiac glycosides can be beneficiary, cardiac glycosides-containing plants have also caused accidental fatal poisoning in humans (MCVANN *et al*., 1992), with reports of deaths after oleander twigs (the principal cardiac glycosides being oleandrin and nerrin) were used as skewers for cooking meat (RADFORD *et al*., 1986) and accidentally ingested by a child (BREWSTER, 1986).

*4.1.2.8. Anthraquinones*

Anthraquinones are the derivatives of phenolic and glycosidic compounds (DOUGHARI, 2012). Surely, they are derived from anthracene giving variable oxidized derivatives such as anthrapenes and anthranols (MAURYA *et al*., 2008; Firn, 2010). Anthraquinones are commonly found as glycosides in living plant. Most athraquinone derivatives, usually in the form of glycosides or rhamnosides, form part of the active components in a number of crude drugs having purgative properties (GARCIA-SOSA *et al*., 2006). The sugar that is present to the anthraquinones, makes it a prerequisite for the pharmacological effects, since the sugar moiety increases the solubility of the molecule and facilitates its transport to the site of action. A study which was carried by

AGGARWAL AND SHISHODIA, 2006 indicated that anthraquinone derivatives are known to possess antibacterial and antifungal activity.

### 4.1.3. Antibacterial activity

Bacteria are amongst the most abundant microscopic organisms with a relative simple and primitive form of prokaryotic type. However, some of the bacteria are somehow beneficial to humans, at the same time they are harmful at some instances. Most pharmacological industries have produced a number of new antibiotics in the previous decades; resistance to these drugs by microorganisms has increased at an alarming rates. Thus, in general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (NASCIMENTO *et al*., 2000).

The bacterial cell wall is designed to provide strength, rigidity, shape, and to protect the cell from osmotic rupture and mechanical damage (HAJIPOUR *et al*., 2012). With reference to their structure, components, and functions, the bacterial cell wall can be divided into two main categories. Danish bacteriologist Christian Gram has categorized bacteria based on their cell wall staining technique, known as the Gram staining, which differentiated bacteria into two groups, viz. Gram-positive and Gram-negative. This classification was based on whether these microscopic organisms do or do not stain with Gram's stain. Gram-positive bacteria are known to retain the crystal violet color and also resist decolorization with acetone or alcohol hence they appear to be in a deep violet in color (HAJIPOUR *et al*., 2012). Concerning their structural composition, the cell wall is relatively simple structure. It forms no appreciable barrier to the entry of antibiotics (HAJIPOUR *et al*., 2012). Whereas, the Gram-negative bacteria lost the crystal violet color. The Gram-negative bacterium is counter stained by saffranin and hence appears to be red in color. Gram-negative microorganisms have a more complex cell structure. Having a look from the outwards of the plasma membrane, Gram-negative bacteria consists of (i) a periplasmic space containing enzymes and other components; (ii) a 2 nm peptidoglycan layer, which is often linked to outward projecting

lipoprotein molecules (iii) an outer membrane consisting of a lipid bilayer with protein molecules and complex lipopolysaccharides on its outer surface (BUWA, 2006). The study further elaborates that both the Gram-positive and Gram-negative bacteria consists of different structural, chemical and functional composition (BUWA, 2006). RANG AND DALE (1987) suggested that these differences play a vital role, with regards to the action of the bacteria to respond to antibiotics.

Antibacterial agents can be bacteriostatic or bactericidal. A bacteriostatic agent is a biological or chemical agent that prevents the growth of bacteria. Its mechanism of action involves blocking a specific metabolic pathway in the bacteria (FINBERG *et al*., 2004). This kind of antibacterial agent inhibits and prevents the growth of susceptible bacteria, i.e. it keeps them in the stationary phase of growth. However, bactericidal agent kills bacteria. In reality, this type of antimicrobial agent does not inhibit the growth of bacteria. Although antimicrobial agents have saved many lives and eased the suffering of millions of people, poverty, poor sanitations, inadequate access to drugs, poor and inadequate healthcare systems, civil and bad governance in developing countries have tremendously limited the benefits of these agents in controlling infectious diseases (MONDELLO *et al*., 2006). The ermegence of multidrug-resistant isolates in TB, acute respiratory infections and diarrhoea has had its greatest toll in developing countries. The emergence of multidrug resistance in human and animal pathogenic bacteria, as well as undesirable side effects of certain antibiotics, has triggered immense interest in the search for new antimicrobial drugs of plant origin (MONDELLO *et al*., 2006).

Several studies have been conducted in different regions of the African countries to prove such efficiency of the medicinal plants (IKRAM AND INAMU, 1984; ALMAGBOUL *et al*., 1985; KUBA *et al*., 1993; SHAPOVAL *et al*., 1994; ARTIZU *et al*., 1995; IZZA *et al*., 1995).

### 4.1.4. Antifungal activity

Fungi are abundant in the environment, and infections due to fungal pathogens become more common. The fungal infection occurs in almost every part of the body (this includes liver, brain, skin, eye, kidneys and nails) and other subcutaneous tissues in both humans and animals (BOKHARI, 2009). Other fungal species tend to affect every part of the body most especially when they are active. Infections caused by fungi are widely distributed all over the world, with various degrees, and more common in men than in women. Fungal diseases of man were recognized in some of the earliest medical literature (BUWA, 2006).

Although several species of fungi are potentially pathogenic in humans, *Candida* (particularly *Candida albicans*) is the organism responsible for most fungal infections. *Candida* species can live within the human body continuing inducing problems. It can be found in up to 70% of healthy individuals at any given time (SALTERELLI, 1989; HIBINA, 2009; CHENG, 2012). However, when the balance of normal bacteria is upset as a result of antibiotic treatment or the immune system of the host is weakened due to treatment with systematic corticosteroids, *Candida* can proliferate (MURZYN, 2010). It may, however, invade and cause infections that may lead to death in humans with weakened immune system (SALTERELLI, 1989). Anyone can acquire a fungal infection, but the elderly, critically ill, and individuals with weakened immune system due to diseases such as HIV/AIDS or use of immunosuppressive medications have a higher risk of getting infected (CHEN *et al*., 2008; KHAN *et al*., 2009; HSU, 2011; BADDLEY, 2011). In the past few decades, there was an increase in the incidence of the fungal infections worldwide (SUPREETHA *et al*., 2011).

Patients who are treated for TB tend to develop a deadly fungal infection that is often untreated as a result of a recurring TB (MILLER AND TAINTER, 1944). A study undertaken by WHO (2000) reported that more than 1 million of people develop chronic pulmonary aspergillosis after being treated for TB each and every year. Such cases most often occur in countries with high rates of TB, including India, China, Indonesia, Bangladesh and the Philippines (MILLER AND TAINTER, 1944). People with treated TB are most likely to gain other infections from the opportunistic invaders. These fungi

invade debilitated or immunocomprommised individuals and those with pre-existing, diseased and scamed lung parenchyma. *Aspergillus* is an airborne fungus that everyone breaths in daily without getting sick. An infection, which is caused by the fungi, is known as aspergillosis, which is a common mold that lives either indoors or outdoors. Currently, about half of those who develop the disease die within five years (MILLER AND TAINTER, 1944).

The development of antifungal agents has logged behind that of antibacterial agents (DIXON AND WALSH, 1996). This is a consequence of cellular structure of the organisms involved. Fungi are eukaryotic and, consequently, most agents that are toxic to fungi are also toxic to the host. Recent studies have shown that there are very few antifungal agents that are available and licensed for use in veterinary practice or human being treatment (BOKHARI, 2009). The use of systemic drugs is off limit to treat man/animal due to their high toxicity level and problem of residues intended for human consumption (ARAUJO *et al*., 2009). There are different treatments, which have been recommended to control fungal infections. In general, pharmacological treatment may include antifungal agents (ALY, 1997 AND AGWA *et al*., 2000). Antifungal drug resistance has become an increasing problem with the development of a larger compendium of antifungal agents. Given the rise in *candida* infections (HSU, 2011) and their increasing resistance against existing antifungal drugs (PFALLER, 2012), novel therapies for the prevention and management of these infections are needed (MAILANDER-SANCHEZ, 2012). Recently, the use of natural products has emerged. Substances and extracts isolated from different natural resources, especially plants, have always been a rich arsenal for controlling the fungal infections and spoilage (MOGHADAMTOUSI *et al*., 2014).

Evidence of plants, herbs and their compounds, which are said to possess the antimicrobial properties and antitoxin properties have been documented since the late $19^{th}$ centuries (BOKHARI, 2009). Natural products are safe to humans and the ecosystem as compared to the chemical antifungal compounds, and they can be easily used by the public since they have been used for thousands of years to enhance flavor and aroma of food as well as economic value (SHELEF *et al*., 1980; SHELEF, 1993).

For example, due to extensive use of turmeric in food products, various researches have been conducted in order to investigate the use of turmeric and curcumin to control fungal related spoilage and fungal pathogens. A study undertaken by MOGHADAMTOUSI *et al*. (2014) showed that turmeric had inhibitory activity against fungal contaminations at 0.8 and 1.0 g/L. Moreover, in a study done by UNGPHAIBOON *et al*. (2005), the methanol extract of turmeric demonstrated antifungal activity against *Cryptococcus neoformans* and *C. albicans* with MIC values of 128 and 256 µg/ml, respectively. Curcumin was found to be more effective compared to fluconazole (an antifungal agent) in a study conducted by MARTINS *et al*. (2009).

According to TSAO AND YIN (2000), the combinations of curcumin with existing fungicidal agents can provide more significant effect against systematic fungal infection like candidemia and candidosis. These synergic effects showed that medicinal plants in combination with different fungicide materials can significantly elicit synergistic activity to enhance the efficacy of existing antifungal strategies.

### 4.1.5. Antimycobacterial activity

*Mycobacterium tuberculosis* is a pathogenic bacterial species and a causative agent of most TB cases (KASSIM AND RAY, 2004). Mycobacteria have waxy cell walls due to the presence of mycolic acid, hence, they are Gram-resistant. They are acid fast, aerobic, non-motile, pleomorphic rods distantly related to the Actinomycetes (PATEL *et al*., 2011). Most Mycobacteria are found in habitats such as water or soil. However, a few are intracellular pathogens of animals and humans. *M. tuberculosis*, along with *M. bovis, M. africanum,* and *M. microti* all cause the TB disease and are members of the TB species complex. Each member of the TB complex is pathogenic, but *M. tuberculosis* is pathogenic to humans while *M. bovis* is usually pathogenic to animals (GRANGE, 2001). The *M. tuberculosis* complex can be cultivated on common liquid and solid culture media. Mycobacteria have an unusual outer membrane which is approximately 8 nm thick, despite being considered Gram-positive. The *Mycobacteriam* cell wall, in principal, consists of an inner layer and an outer layer that surround the

plasma membrane (HETT AND RUBIN, 20008). The outer compartment consists of both lipids and proteins (DRAPER, 1971, BRENNAN AND NIKAIDO, 1995; BRENNAN, 2003; DRAPER *et al*., 2008). The outer membrane and the mycolic acid arabinoglactan-peptidoglycan polymer from the cell wall, constitutes an efficient permeability barrier in conjunction with the cell inner membrane (ZHENG *et al*., 2008). The tough cell wall prevents passage of nutrients into and excreted from the cell, therefore giving it the characteristics of slow growth rate. Due to its slow growth, a statement of negative culture cannot be made after an incubation time of 6-8 weeks. *M. tuberculosis* divides every 15-20 hours, which is extremely slow as compared to other bacteria which have division measured in minutes. *E. coli,* for example, can divide every 20 minutes. *M. tuberculosis* is a small bacillus that can stand weak disinfectants and can survive in a dry state for weeks. The mycobacterial cell wall is impermeable to a number of compounds, which makes it a key virulence factor. The complexity of the cell wall represents a challenge to the organism, thus, requiring specialized mechanisms to allow cell division to occur (HETT AND RUBIN, 2008).

Mycobacteria are inherently resistant to numerous antibiotics. Much of this resistance is attributable to unusual cell wall. Mycobacteria have unusual impermeable cell walls that are thought to be advantageous in stressful conditions of osmotic shock or desiccation as well as contributing to their considerable resistance to many drugs. The *M. chelonae* cell wall is 30 times less permeable to hydrophilic molecules than *E. coli* while *M. smegmatis* was found to be about 20 times less permeable than *E. coli* (MITAL *et al*., 2006).

Expectations to develop new effective anti-TB drugs that will bring various outcomes in reducing the total expenditure and treatment of resistance by a single dosage regiment have been set (MITAL *et al*., 2006). In pursuit of achieving such goals, researchers have to put efforts on the development of novel structural moieties having antimycobacterial properties. Medicinal plants, also known to be traditional therapies, offer a great hope to fulfill these needs. According to LALL AND MEYER (2001), few plants have been tested against mycobacterium and anti-TB activity. Several studies have been conducted to evaluate some medicinal plants for their antimycobacterial

activity against different strains of *Mycobacterium* species (LALL AND MEYER, 1999; LALL AND MEYER, 2001; ELDEEN AND VAN STADEN, 2007; BUWA AND AFOLAYAN, 2009; GUPTA *et al*., 2010; GREEN *et al*., 2010; TABUTI *et al*., 2010; NARWADIYA *et al*., 2011; SEMENYA AND MAROYI, 2013; LAWAL *et al*., 2014; NGUTA *et al*., 2015).

## 4.2. MATERIALS AND METHODS

### 4.2.1. Phytochemical screening

The presence of phytochemical constituents such as tannins, saponin, flavonoids, steroids, terpenoids, cardiac glycosides, anthraquinones and alkaloids was determined using the standard procedures described by HARBONE (1973), TREASE AND EVANS (1989) AND SOFOWORA (1993). The test for the presence of secondary metabolites was based on the visual observation of colour change or through the formation of the precipitate after the addition of the specified reagent(s).

#### *4.2.1.1. Test for Alkaloids*

In the test for alkaloids, about 0.5 g of the powdered extracts was stirred in 5ml of 1% HCl aqueous solution on a steam bath (water bath) for 5 minutes. The obtained mixture was then filtered using Whatman's No 1 filter paper. Four drops of the Dragendoff's reagent was added into 1 ml of the filtrate. The precipitate revealed the presence of alkaloids.

#### *4.2.1.2. Test for Anthraquinones*

In testing for the anthraquinones, 5 g of the powdered plant material was dissolved into 10 ml of benzene; the mixture was shaken until a homogenous mixture was obtained. Mixture was then filtered. The filtrate was then shaken with 5 ml of chloroform. The formed chloroform layer was then pipetted into another test tube and 1 ml of dilute

ammonia was added. The observed resulting solution with a pink colour revealed the presence of anthraquinones.

*4.2.1.3. Test for the cardiac glycosides (Keller-Killani test)*

About 0.5 g of the plant material was dissolved in distilled water and filtered. Five millilitres of the extract was then treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. It was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated a deoxysugar characteristic of cardenolides, either a violet ring may appear below the ring, while in the acetic acid layer; also a greenish ring may form just gradually throughout the thin layer.

*4.2.1.4. Test for terpenoids (Salkowski test)*

About 0.5 g of the plant material was dissolved in distilled water and filtered. Five milliletres of the extract was mixed in 2 ml of chloroform and about 3 ml of the concentrated sulphuric acid ($H_2SO_4$) was carefully added to form a layer. A reddish brown colouration of the interface formed showed positive results for the presence of terpenoids.

*4.2.1.5. Test for steroids*

Approximately, 2 ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml of $S_2SO_4$. The colour change from violet to blue indicating the presence of steroids.

*4.2.1.6. Test for flavonoids*

One gram of the powdered plant sample was heated with 10 ml of ethyl acetate over a steam bath for 3 minutes. The mixture was then filtered and 4 ml of the filtrate was

shaken with 1 ml of dilute ammonia solution. A yellow colouration indicated a positive test for flavonoids.

*4.2.1.7. Test for tannins*

In the test for the presence of tannins, about 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were then added to the solution and brownish green or a blue-black colorationrevealed the presence of tannins.

*4.2.1.8. Test for Saponin*

About 2 g of powdered plant material was boiled in 20 ml of distilled water using a water bath and the boiled extract was filtered. Ten milliliters of the filtrate were mixed with 5 ml of distilled water and then shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken again and then observed for the formation of emulsion as indication of saponin.

**4.2.2. Antibacterial activity**

The microplate method of ELOFF (1998) was used to determine the minimal inhibitory concentration (MIC) values for plant extracts with antibacterial activity. Residues of plant extracts were dissolved at 50 mg/ml with the extracting solvents. All extracts were initially tested at 12.5 mg/ml in 96-well micro plates and serially diluted two-fold to 0.098 mg/ml, after which 100 µl bacterial cultures were added to each well. The antibiotic neomycin was included as a standard in each assay. Extract-free solution was used as a blank control. The micro plates were incubated overnight at 37°C. As an indicator of bacterial growth, 40 µl p-iodonitrotetrazoliumviolet (INT) dissolved in water was added to the wells and incubated at 37°C for 30 minutes. MIC values were recorded as the lowest concentration of the extract that completely inhibited bacterial growth, i.e., a clear

well. Two Gram-negative bacteria namely *Escherichia coli* (ATCC 8739), *Klebsilla pneumoniae* (ATCC 25922) and two Gram-positive bacteria, *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (ATCC 10702) were used.

### 4.2.3. Antifungal activity

Standard strains of *Candida albicans* and *Trichophytan mucoides* were obtained from the University of Fort Hare. The water extract residues were redissolved in water and the organic solvent extract residues were dissolved in dimethyl sulfoxide (DMSO). All extracts were dissolved at a concentration of 100 mg/ml.

A modification of the NCCLS proposed method (M27-P) broth microdilution test was performed (ESPINEL-INGROFF AND PFALLER 1995; MOTSEI *et al*., 2003). Four millilitres of sterile saline were added to approximately 400 µl of 24-h-old cultures, a 1: 1000 dilution with broth (e.g. 10 µl stock culture: 10 ml broth) was prepared.

One hundred microlitres of MH broth were added to each well of a 98-well microplate. One hundred microlitres of the water extract were added to well (A) and serially diluted from (A) by taking 100 µl into (B). This two-fold dilution was continued down the plate and 100 µl from the last well (H) were discarded. In the case of organic solvent extracts 25 µl of the extracts were added to 175 µl broth and serially diluted. Three replicates were prepared for each extract. All the wells were filled with 100 µl of stock fungal culture. Amphotericin B was used as a standard for this experiment and the following controls were prepared: wells containing broth only, fungal strains with no extract, and serial dilutions of Amphotericin B with the fungi at the recommended inhibitory concentrations. The plates were, covered with parafilm and incubated at 33ºC overnight. As an indicator for fungal growth, INT dissolved in water was added to the wells and incubated for 30 minutes.

### 4.2.4. Antimycobacterial activity

*M. tuberculosis* (ATCC 25177) was maintained in Middlebrook 7H9 broth containing 10% OADC (oleic acid + albumin + dextrose + catalase). Inoculum was prepared by transferring the stock bacterial culture to supplement 7H9 (Middlebrook 7H9 + 10% OADC) and grown for 72 hours on a shaker. Five (5 ml) supplemented 7H9 broths was inoculated by the bacteria culture and grown for 72 hours. Twenty percent sterile glycerol was added to each culture and 500 µl aliquots was made into sterile Eppendorf tubes. These stocks were named G1 stocks and were stored at -30 ºC. A single G1 stock was used to inoculate supplemented Middlebrook 7H10 agar (7H10 + 10% OADC) plates and was incubated at 37 ºC until growth was observed. From this culture a single colony was used to inoculate 5 ml supplemented 7H9 broth. This was grown on a shaker at room temperature for 72 h and was used for the experiment.

The broth microdilution method (SWENSON *et al*., 1982) was used to determine the MIC of *Mycobacterium tuberculosis* of the investigated plant extracts. The aqueous residues were re-dissolved in water and other extract residues were dissolved in dimethyl sulfoxide (DMSO). All extracts were dissolved to a concentration of 100 mg/ml. One hundred microliter of the supplemented 7H9 broth was added to all the wells of microtitre plates. All extracts were tested at a concentration of 25 mg/ml and serially diluted to 0.195 mg/ml. The optical density of the 72 hours broth culture was determined and adjusted at 550 nm. The controls included the solvent used to dissolve plant extracts, Middlebrook 7H9 broth alone, and the antibiotic streptomycin (1.56 mg/ml) as a positive control. The plates were covered and incubated at 37 ºC for 72 hours. After incubation, 40 µl of 0.4 mg/ml solution of INT was added to each well of the plate. The plates were covered and incubated for 24 h at 37 ºC. All extracts were tested in triplicates.

## 4.3. RESULTS AND DISCUSSION

### 4.3.1. Screening for the presence of secondary metabolites

The results of eight plant species screened for phytochemicals are presented in Table 7. Plants were screened for the presence of chemical constituents such as tannins, saponins, flavonoids, alkaloids, steroids, terpenoids, cardiac glycosides and anthraquinones. The phytochemical investigation carried out revealed some of the secondary metabolites being absent in some of the plants tested and present in others. *X. undulatum* and *E. automnalis* revealed the presence of saponins only (Figure 12). A study conducted by AMUSAN *et al*. (2007) also reported that X. *undulatum* contained saponins as one of the secondary metabolites. According to KOSE *et al*. (2015), *X*. *undulatum* was reported to treat a total of 38 ailments by the Lesotho traditional healers and this was due to its chemical composition. *Lentsweni* and *H. depressa* revealed the presence of tannins and saponins, with *Lenstweni* going to an externt or revealing the presence of terpenoids (Figure 13), whereas *L. lanceolata* and *S. harveianus* revealed the presence of tannins, saponins and cardiac glycosides. *H. depressa* revealed the presence of tannins, saponins, flavonoids and cardiac glycosides. According to XU *et al*., (1996); ASL *et al*., (2008) there is evidence of saponins in traditional medicine preparations where oral administrations might be expected to lead to hydrolysis of glucose from terpenoids. Saponins are well known bioactive phytochemicals and have been investigated for a multiple of activities including antimicrobial, cytotoxicity, anti-inflamatory and immunostimulatory (HOSTETTMAN AND MARSTON, 1995). Plants containing saponins are being promoted commercially as dietary supplements and nutraceuticals (WEBMD, 2015). Traditionally, saponins have been extensively used as detergents, piscicides and molluscicides in addition to their industrial applications as foaming and surface active agents (HOWES, 1930; SHI *et al*., 2004). Since prehistoric times, cultures throughout the world have used piscicidal plants, mostly those containing saponins, for fishing (HOSTETTMANN AND MARSTON, 1995; FRANCIS *et al*., 2002).

Flavonoids were only observed in *H. depressa*. Flavonoids are found present in all dietary plants, such as fruits and vegetables. Additionally, flavonoids are found in several medicinal plants and herbal remedies and have been used in folk medicine around the world (REN *et al*., 2003). The present study has revealed that *D*. *anomala*, *D*. *depressa*, *H*. *depressa*, *L*. *lanceolata*, *Lentsweni* and *S. harveianus* have tannins present in them. It is well known that plants containing tannins have astringent, hemostatic, antiseptic and toning properties (FRANCIS *et al*., 2002). Herbal preparations of plants containing tannins are generally used to stop burns, scars of the skin, wounds and pain on the skin. Tannins are considered antioxidants and they prevent the onset of degenerative diseases such as cancer and cardiovascular disease.

### 4.3.2. SCREENING PLANT EXTRACTS FOR ANTIBACTERIAL ACTIVITY

Results for antibacterial activity are presented in Table 8. A total number of four bacterial strains were used in the test for antibacterial activity using a microplate bioassay. INT was used as an indicator for the presence of bacteria. Minimal inhibitory concentration (MIC) values were recorded as the lowest concentration of the extract that completely inhibited bacterial growth, i.e. a clear well. Extracts were considered as highly active if their inhibitory concentration ranged between 0.098 and 1 mg/ml and said to have minimal activity if the MIC value was 1.5 mg/ml. Some of the medicinal plants screened gave remarkable results when tested against *Klebsiella pneumoniae, Bacillus pumilus, Escherichia coli,* and *Staphylococcus* aureus.

Plant extracts exhibiting low or no antibacterial activity were *D. anomala* (methanol and aqueous), *X. undulatum* (acetone and aqueous), *Lentsweni* (methanol), *E. automnalis* (ethanol and methanol) and *D. depressa* (acetone and methanol). Among the plant species screened for antibacterial activity, some of the extracts displayed very good activity against the bacterial strains used.

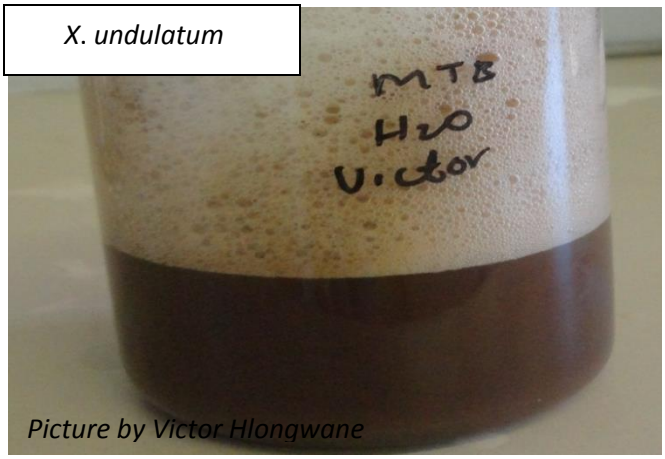


**Figure 12: The extract of *X. undulatum* revealing the presence of saponins**

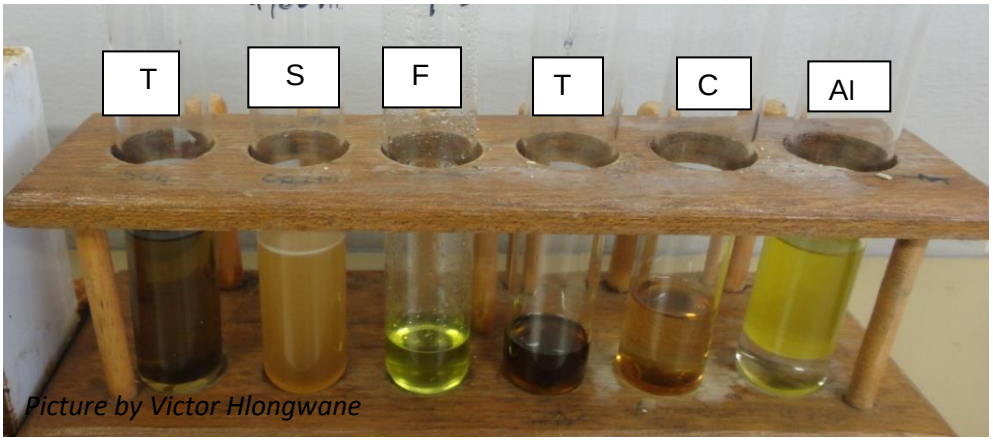


**Figure 13: *H. depressa* showing the presence of a certain group of secondary metabolites from the respectives test tubes as labeled. T** = Tannins; **S** = Saponin; **F** = Flavonoids; **Al** = Alkaloids; **St** = Steroids; **Tp** = Terpenoids; **CG** = Cardiac glycosides

**Table 7: Secondary metabolites observed from the medicinal plant species used against TB in the Free State, SA**

| | | SECONDARY METABOLITES | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Plant name** | **Part used** | **T** | **S** | **F** | **St** | **Tp** | **Cg** | **A** | **Al** |
| *D. anomala* | Leaves | + | + | - | - | + | + | - | - |
| *D. depressa* | Underground | + | + | - | - | + | - | - | - |
| *E. automnalis* | Underground | - | + | - | - | - | - | - | - |
| *H. depressa* | Underground | + | + | + | - | - | + | - | - |
| *L. lanceolate* | Underground | + | + | - | - | - | + | - | - |
| [a]*Lentsweni* | Underground | + | + | - | - | + | - | - | - |
| *S. harveianus* | Underground | + | + | - | - | - | + | - | - |
| *X. undulatum* | Underground | - | + | - | - | - | - | - | - |

**T**-tannins, **S**- saponin, **F**-flavonoids, **Al**-alkaloids, **St**- Steroids, **Tp**-terpenoids, **Cg**-Cardiac glycosides, **A**-anthraquinones

**-** = negative, + = positive

The acetone and ethanolic extracts of *D. anomala* had the highest inhibitory activity against both the Gram-positive and Gram-negative bacteria. The MIC values for acetone extracts against *K. pneumonia* and *E. coli* were 0.130 and 0.781 mg/ml, respectively, and 0.098 mg/ml against both the Gram- positive bacteria (Figure 14). GELFAND *et al*. (1985) reported on antibacterial and antiinflamatory properties of *D. anomala* roots extracts.

Minimum inhibitory activity, against all the bacterial strains, was detected with *X. undulatum*, *L*. *lanceolata* and *D. depressa* ethanolic extracts with MIC values of 1.563 mg/ml. The ethanolic extract prepared from *Lentsweni* displayed a minimum inhibitory concentration against *B. pumilus* (1.563 mg/ml) and *K. pneumoniae* (1.563 mg/ml). However, a study that was carried out by BUWA (2006) reported *X. undulatum* to exhibit poor or no antibacterial activity.

Extract prepared from *H. depressa* and *S. harveianus* showed the best MIC values against the four bacterial strains used. The MIC values of the organic extracts prepared from *H. depressa* were low (0.098 mg/ml). Surprisingly, good activity was also detected with the aqueous extracts prepared from *H*. *depressa* (Figure 15). The aqueous extracts of *S*. *harveianus* also revealed good antibacterial activity against *K*. *pneumoniae* and *B*. *pumilus* (0. 781 mg/ml), *S*. *aureus* (0.391mg/ml) and *E*. *coli* (0.098 mg/ml) (Figure 16). Currently, there is no available report or literature on the antibacterial activity of *S*. *harveianus*. These results on water extracts are interesting since the local traditional healers and herbalists use water in the preparation of their traditional remedies.

A study conducted by APPIDI *et al*. (2009) also reported on the antibacterial activity of *Hermannia* species water extracts against the Gram-positive bacteria. Various researchers have already shown that Gram-positive bacteria are more susceptible towards plant extracts as compared to the Gram-negative bacteria (LIN *et al*., 1999; PAREKH AND CHANDA, 2006). The differences between these two strains may be attributed to the fact that the cell wall for the Gram-negative strain has a multilayered structure whereas the Gram-positive bacteria are characterized by a single layer (YAO *et al*., 1995).

The present study also revealed good antibacterial activity with all extracts prepared from *H*. *depressa* against all the tested bacterial strains with MIC values ranging between 0.326–0.098 mg/ml. The methanol extract prepared from the roots of *Hermannia* species was reported to inhibit the growth of both Gram-positive and Gram-negative strains (ESSOP, 2005; APPIDI *et al*., 2009). According to REID *et al*. (2005), the acetone and ethyl acetate extracts prepared from *H. depressa* (leaves, stems and roots) were found to exhibit moderate antibacterial activity with MIC values ranging between 0.195 to 3.125 mg/ml. In addition, *H. depressa* extracts exhibited the highest overall activity, which may play a role in combating coughs, diarrhea and stomach-ache caused by bacterial infections (REID *et al*., 2005). Water extracts prepared from *L*. *lanceolata* had good antibacterial activity against *E*. *coli* and *S*. *aureus* with MIC values of 0.098 mg/ml. Appreciable antibacterial activity was detected with the methanolic and acetone extracts. No studies have revealed the pharmacological screening of this plant species.

Antibacterial activity was also observed with the acetone extracts prepared from *Lentsweni* against the test microorganisms (0.098 mg/ml). *E*. *autumnalis* aqueous extracts exhibited good activity (0.195 mg/ml) against all the test microorganisms. *E*. *autumnalis* leaves and bulbs were previously screened for the presence of pharmacological properties and were reported to relieve pain and inflammation (ZSCHOCKE *et al*., 2000). *D. depressa* aqueous extract exhibited very good activity against *K. pneumoniae*, *B. pumilus*, *E. coli* and *S. aureus* with MIC values of 0.098 mg/ml. The ethanol extract of *D*. *depressa* also exhibited minimal antibacterial activity against all the test microorganisms with MIC values of 1.563 mg/ml.

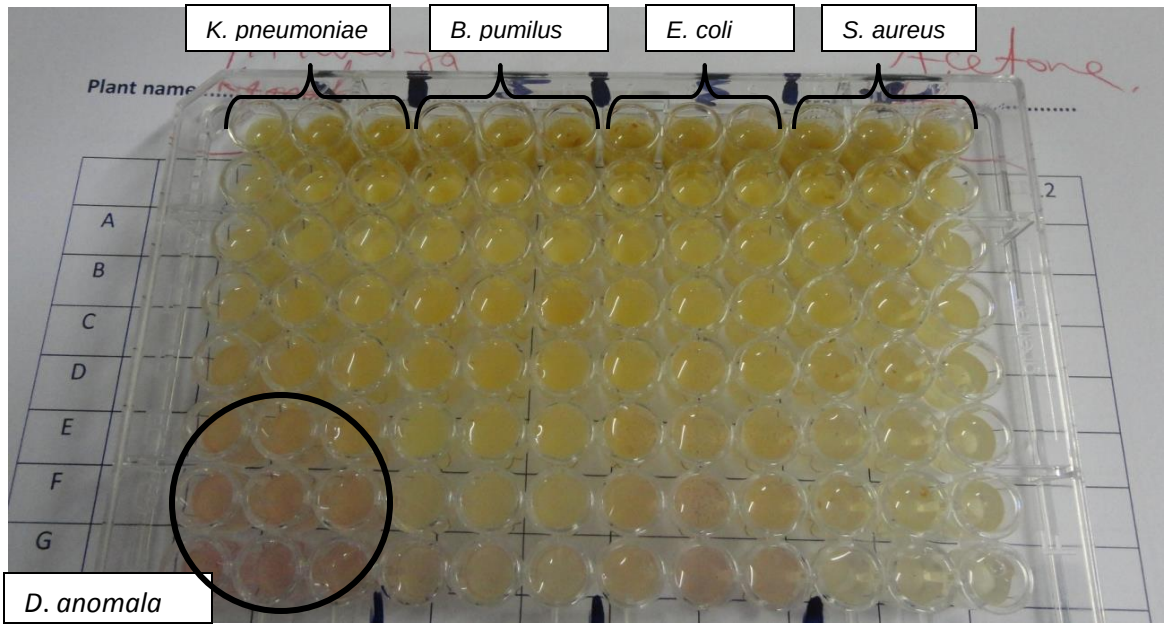


**Figure 14: The acetone extract prepared from *D. anomala* showing the lowest level of inhibition against the four bacterial straints used. Circle shows the wells with extracts that did not inhibit the growth of the bacteria.**

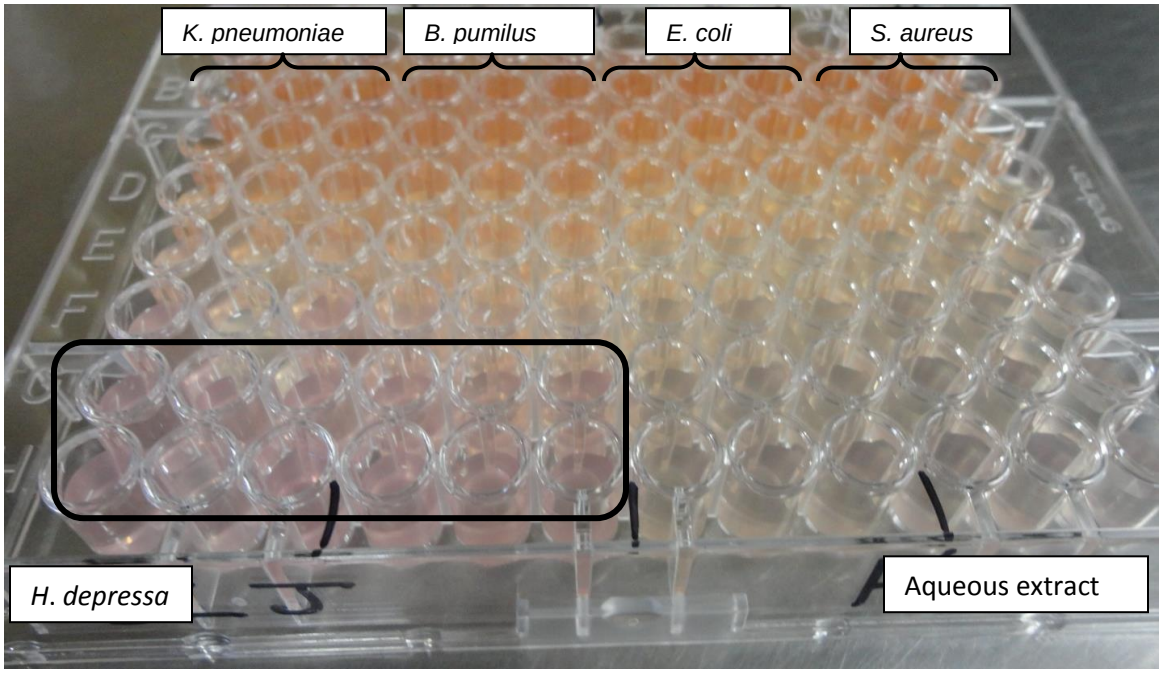


**Figure 15: The aqueous extracts of *H. depressa* having the lowest MIC value with all the bacterial strains. The rectangle shows the wells that did not inhibit the growth of the bacteria.**

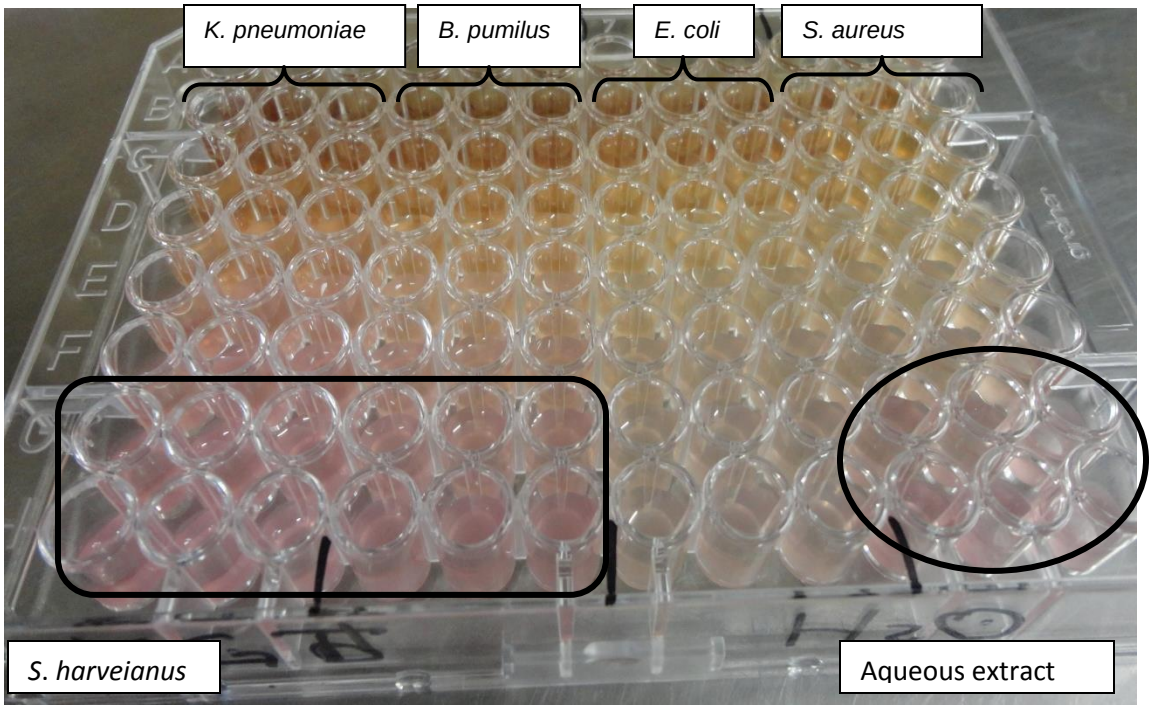


**Figure 16: The water extract of *S. harveianus* showing best activity against the test organisms. The rectangle and the circle show the wells with extracts that did not inhibit the test microorganisms.**

**Table 8: Antibacterial activity of medicinal plants used against TB in the Free State Province (MIC values in mg/ml)**

| Plant name | Family | Plant part used | Extract | Extract yield (mg) | Bacteria | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Kp | Bp | Ec | Sa |
| *Dicoma anomala* (Sond) | Asteraceae | Leaves | Acetone | 310 | 0.781 | 0.098 | 0.130 | 0.098 |
| | | | Ethanol | 337.6 | 0.781 | 0.781 | 0.098 | 0.391 |
| | | | Methanol | 1730 | 3.125 | 2.116 | 3.125 | 3.125 |
| | | | Water | 3220 | 3.125 | 3.125 | 3.125 | 3.125 |
| *Drimia depressa* (Baker) | Hyacinthaceae | Underground | Acetone | 1002.3 | 12.5 | 12.5 | 6.25 | 12.5 |
| | | | Ethanol | 234.8 | 1.563 | 1.563 | 1.563 | 1.563 |
| | | | Methanol | 2315 | 3.125 | 3.125 | 3.125 | 3.125 |
| | | | Water | 3232.4 | 0.098 | 0.098 | 0.098 | 0.098 |
| *Eucomis automnalis* (Mill.) | Hyacinthaceae | Underground | Acetone | 420.2 | 0.391 | 0.163 | 0.260 | 0.098 |
| | | | Ethanol | 1020.1 | 3.125 | 3.125 | 1.563 | 1.563 |
| | | | Methanol | 3606.9 | 3.125 | 3.125 | 3.125 | 3.125 |
| | | | Water | 5889.4 | 0.195 | 0.195 | 0.195 | 0.195 |
| *Hermannia depressa* (N.E.Br) | Sterculisceae | Underground | Acetone | 515.9 | 0.098 | 0.098 | 0.098 | 0.098 |
| | | | Ethanol | 997.9 | 0.098 | 0.098 | 0.098 | 0.098 |
| | | | Methanol | 1471.8 | 0.195 | 0.098 | 0.098 | 0.098 |
| | | | Water | 855.4 | 0.391 | 0.391 | 0.098 | 0.098 |
| *Lotononis lanceolata* (E. Mey) | Fabaceae | Underground | Acetone | 2700 | 0.195 | 0.195 | 0.326 | 0.098 |
| | | | Ethanol | 1673.3 | 1.563 | 1.563 | 1.563 | 1.563 |
| | | | Methanol | 5920 | 1.563 | 0.195 | 1.563 | 0.098 |
| | | | Water | 2850 | 5.208 | 2.604 | 0.098 | 0.098 |
| [a]*Lentsweni* | unidentified | Underground | Acetone | 118 | 0.098 | 0.098 | 0.098 | 0.098 |
| | | | Ethanol | 1083.8 | 1.563 | 1.563 | 0.098 | 1.563 |
| | | | Methanol | 3199.6 | 6.25 | 6.25 | 6.25 | 6.25 |
| | | | Water | 3789.8 | 6.25 | 3.125 | 0.78 | 0.325 |
| Neomycin (µg/ml) | | | | | 0.39 | 0.78 | 0.78 | 0.78 |

**Table 8 Continued**

| Plant name | Family | Plant part | Extract | Extract yield (mg) | Bacteria | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | ***Kp*** | ***Bp*** | ***Ec*** | ***Sa*** |
| *Senecio harveianus* (MacOwan) | Asteraceae | Whole plant | Acetone | 535.4 | 0.326 | 0.098 | 0.098 | 0.098 |
| | | | Ethanol | 675.4 | 0.31 | 0.391 | 0.391 | 0.391 |
| | | | Methanol | 1365.6 | 0.195 | 0.195 | 0.098 | 0.391 |
| | | | Water | 1410.5 | 0.781 | 0.781 | 0.098 | 0.391 |
| *Xysmalobium undulatum* (L.) | Apocynaceae | Whole plant | Acetone | 689.5 | 6.25 | 6.25 | 6.25 | 0.625 |
| | | | Ethanol | 1019 | 1.563 | 1.563 | 1.563 | 1.563 |
| | | | Methanol | 1365 | 8.333 | 8.333 | 0.098 | 8.854 |
| | | | Water | 1410.5 | 3.125 | 3.125 | 3.125 | 3.125 |
| Neomycin (µg/ml) | | | | | 0.39 | 0.78 | 0.78 | 0.78 |

[a]-Unidentified

*Kp-Klebsiella pneumoniae; Bp-Bacillus pumilus; Ec- Escherichia coli*; *Sa-Staphylococcus aureus*

### 4.3.3. SCREENING PLANT EXTRACTS FOR ANTIFUNGAL ACTIVITY

The antifungal activity of the acetone, ethanol, methanol and water extracts prepared from *D*. *anomala*, *X*. *undulatum*, *H*. *depressa*, *L*. *lanceolata*, *S*. *harveianus*, *Lentsweni*, *E*. *automnalis* and *D*. *depressa* are presented in Table 9. The MIC values were recorded as the lowest concentrations of plant extracts that completely inhibited fungal growth.

Among the plant species tested, the organic solvents prepared from *D*. *depressa*, *H*. *depressa* (Figure 17A and 17B), *E*. *autumnalis*, *D*. *anomala* (Figure 18A and 18B) and *L*. *lanceolata* displayed the best activities against *C. albicans* and *T. mucoides* with MIC values ranging between 0.098 to 0.781 mg/ml. The aqueous extracts from *D*. *anomala* leaves, *H*. *depressa* roots and the bulb of *E. automnalis* showed promising results against the *Candida* and *Trichophyton* species with MIC values of 1.563 mg/ml respectively. According to studies undertaken by SHALE *et al*. (1999) and REID *et al*. (2005), minimal or poor activity observed in some plant species might be due to that aqueous solvents do not extract all the active compounds that might be present in the plant as compared to the lipophilic solvents. Water extraction, is one of the mode of preparation used by most of the traditional healers to prepare the traditional remedies. Hence, the medication is applied at high volumes, whereas applying the same dosage from organic solvent may be dangerous (REID *et al*., 2005).

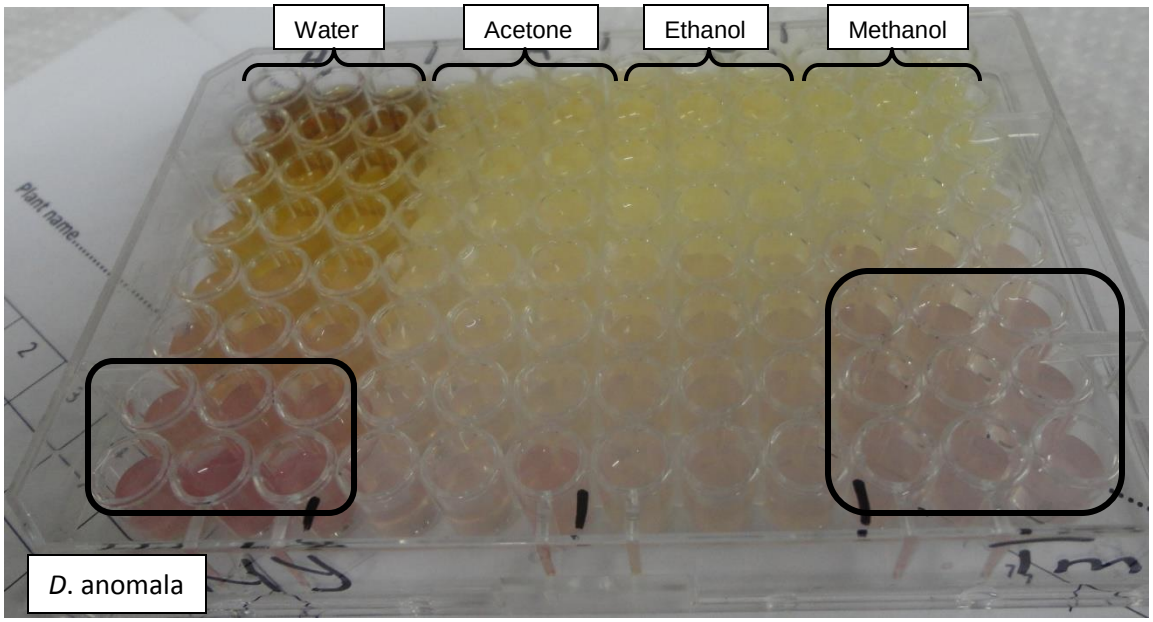


**Figure 17A: The extracts of *D. anomala* revealing good antifungal activity against *T. mucoides*. The rectangle show the wells that had fungal growth.**

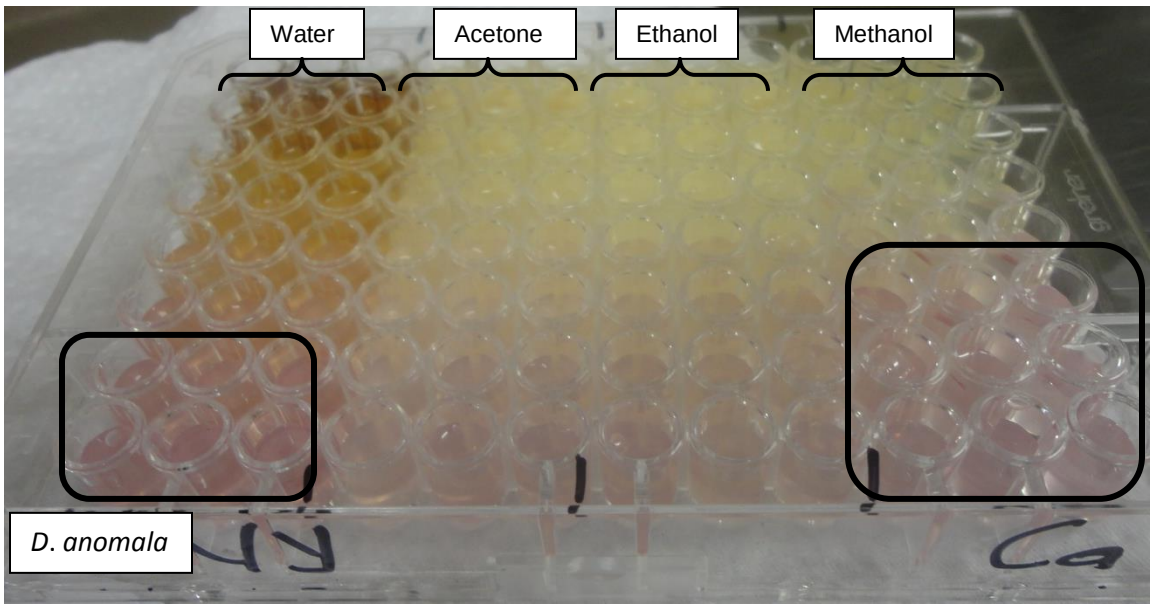


**Figure 17B: The extracts of *D. anomala* revealing good antifungal activity against *C. albicans*. The rectangles show the extracts that did not inhibit the growth of the fungi.**

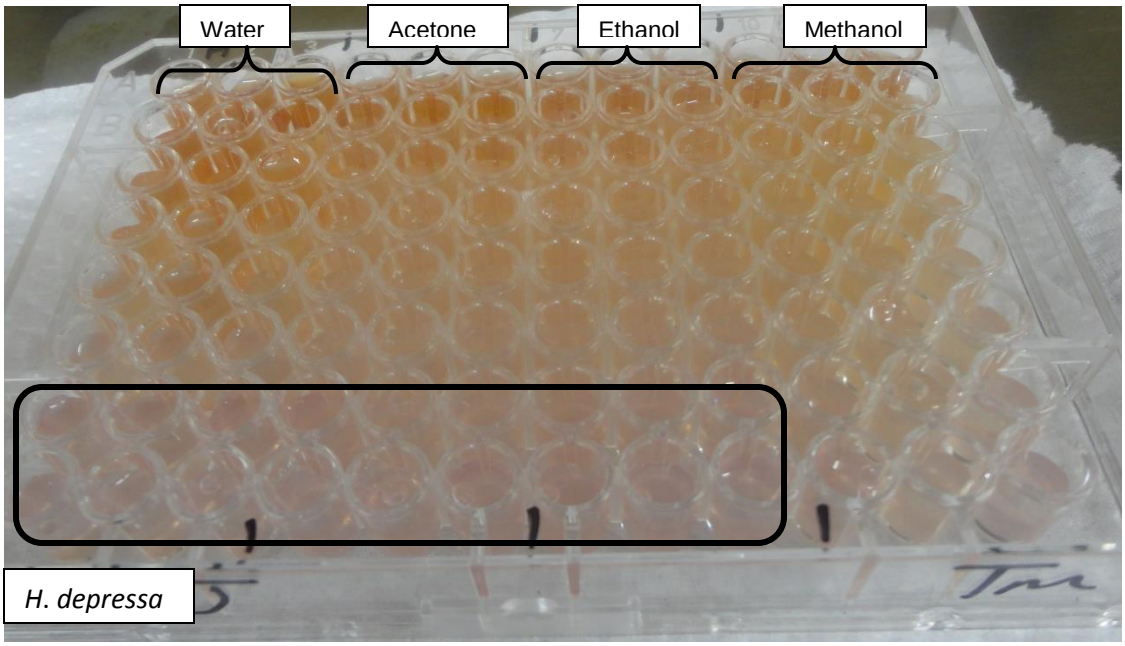


**Figure 18A: The organic extracts of *H. depressa* showing good antifungal activity with the lowest MIC value 0.391 mg/ml against *T. mucoides*. The rectangle shows the wells that had fungal growth.**

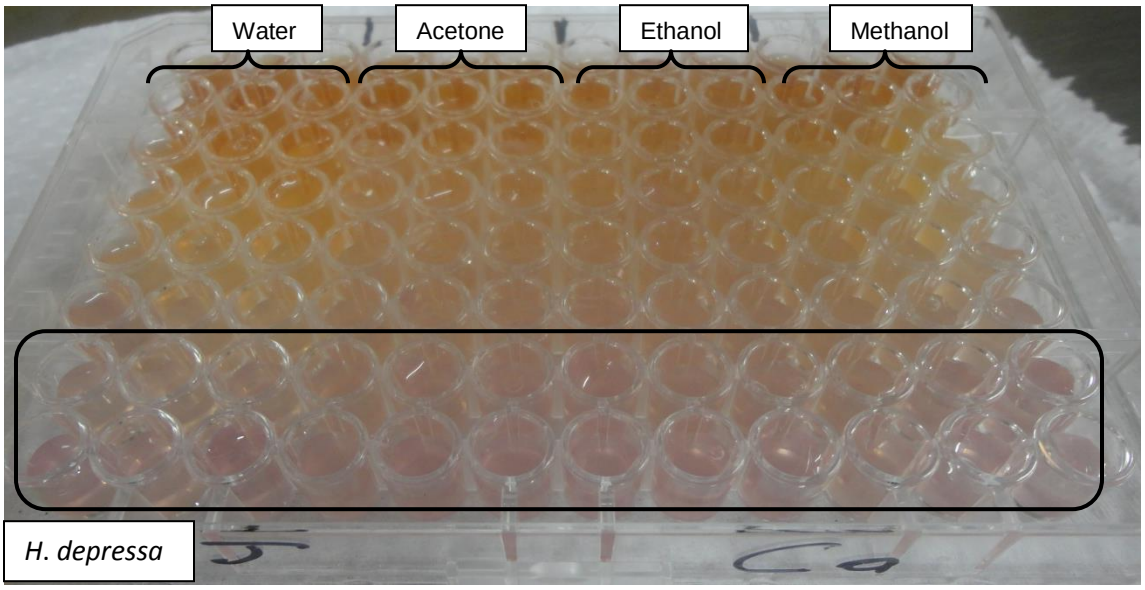


**Figure18B: The organic extracts of *H. depressa* showing good antifugal activity with the lowest MIC value 0.391 mg/ml against *C. albicans*. The rectangle shows the wells with extracts that did not inhibit the growth of the fungi.**

**Table 9: Antifungal activity of medicinal plants used against TB in Free State Province (MIC in mg/ml).**

| Plant name | Plant part | Extract | Yield (mg) | Fungi | |
|---|---|---|---|---|---|
| | | | | ***Ca*** | ***Tm*** |
| *Dicoma anomala* | Leaves | Acetone | 170.8 | 0.391 | 0.195 |
| | | Ethanol | 454.2 | 0.391 | 0.195 |
| | | Methanol | 1078.8 | 0.781 | 0.781 |
| | | Water | 1081.5 | 1.563 | 3.125 |
| *Drimia depressa* | Bulb | Acetone | 940 | 0.098 | 0.195 |
| | | Ethanol | 1110 | 0.098 | 0.195 |
| | | Methanol | 1290 | 0.049 | 0.195 |
| | | Water | 2560 | 0.391 | 0.782 |
| *Eucomis automnalis* | Bulb | Acetone | 190.4 | 0.049 | 0.049 |
| | | Ethanol | 1169.8 | 0.049 | 0.049 |
| | | Methanol | 33566.6 | 3.125 | 0.049 |
| | | Water | 1701.2 | 1.563 | 3.125 |
| *Hermannia depressa* | Roots | Acetone | 426.5 | 0.391 | 1.563 |
| | | Ethanol | 966.6 | 0.391 | 0.391 |
| | | Methanol | 1310.7 | 0.391 | 0.391 |
| | | Water | 215.2 | 1.563 | 1.563 |
| *Lotononis lanceolata* | Underground part | Acetone | 426.4 | 0.049 | 0.049 |
| | | Ethanol | 1437 | 0.391 | 0.049 |
| | | Methanol | 1492.5 | 0.391 | 0.049 |
| | | Water | 769.9 | 1.563 | 0.782 |
| [a]*Lentsweni* | Underground part | Acetone | 1021 | 0.391 | 3.125 |
| | | Ethanol | 906.7 | 0.391 | 1.563 |
| | | Methanol | 1200.5 | 0.391 | 1.563 |
| | | Water | 1774.4 | 0.782 | 0.782 |
| *Senecio harveianus* | Whole plant | Acetone | 1238.6 | 1.563 | 1.563 |
| | | Ethanol | 1000 | 1.563 | 1.563 |
| | | Methanol | 1089.1 | 3.125 | 0.049 |
| | | Water | 703.2 | 6.25 | 12.5 |
| *Xysmalobium undulatum* | Whole plant | Acetone | 290.3 | 0.781 | 0.391 |
| | | Ethanol | 667.7 | 0.781 | 0.391 |
| | | Methanol | 1062.5 | 1.563 | 3.125 |
| | | Water | 1484.4 | 3.125 | 1.563 |
| Amphotericin B (µg/ml) | | | | 0.012 | 0.012 |

[a] -Unidentified

*Ca- Candida albicans*

*Tm- Trichophyton mucoides*

### 4.3.4. Antimycobacterial activity

The results for antimycobacterial activity of plant extracts against *M*. *tuberculosis* are presented in Table 10. MIC values were recorded as the lowest concentration of the extract that completely inhibited mycobacterium growth. In the study, extracts having activities with MIC values that range between 0.195 to 1.56 mg/ml were considered to be highly active, whereas those having an MIC value of 3.125 mg/ml were considered to possess minimal activity.

Among the plant species screened against *M*. *tuberculosis,* the organic solvents of *D*. *anomala* displayed the best activity with an MIC value of 0.195 mg/ml. *H*. *depressa* extracts showed good activity against the test *Mycobacterium* with MIC values of 0.78 mg/ml. The *H*. *depressa* aqueous extract also displayed good activity against *M. tuberculosis*, with an MIC value of 1.56 mg/ml. Very good activity was detected with the organic solvent prepared from *L. lanceolate* with MIC values ranging between 0.195 and 0.65 mg/ml. Organic extracts prepared from *S*. *harveianus* also displayed good antimycobacterial activity gainst *M. tuberculosis* with the MIC value of 0.195 mg/ml.

The water extract prepared from *S*. *harveianus* and H. *depressa* displayed good activity against *M. tuberculosis* with MIC values of 0.39 mg/ml and 1.56 mg/ml, respectively. The activity observed from the aqueous extracts was considered to be the best activity because the local traditional healers and herbalist use water when preparing traditional remedies.

The acetone extract prepared from *Lentsweni* also displayed some antimycobacterial activity with an MIC value of 0.33 mg/ml. Minimal activity was observed with the aqueous extracts prepared from *D*. *anomala*, and *E*. *automnalis* (6.25 mg/ml and 3.125 mg/ml, respectively). Although water is the most common solvent used by traditional healers to extract the active compounds due to its availability, antimycobacterial sceening of *S*. *harveianus* generally resulted in higher inhibitory activity from organic extract as compared to water (SHALE *et al*., 1999). In addition, considering the fact that

the dosage prescribed by the traditional healers/herbalists is usually very high, for instance, three to four cupfuls per day for adult, water can still be considered as an appropriate extracting solvent for traditional remedies (SHALE *et al*., 1999).

Concerning *X*. *undulatum* methanol extract, poor activity was observed against *M*. *tuberculosis*. A study undertaken by BALLEL *et al*. (2000) revealed that minimal or poor activity might be due to the complex lipoglycan calyx on the cell surface that provides a significant physical barrier to intracellular acting compound. The lack of penetration is thought to be the reason why many antibiotics become resistance to the *Mycobacterium* strain (GAO *et al*., 2003). The cell wall biosynthesis is believed to be chemotherapy (ELDEEN AND VAN STADAN, 2007). Conversely, negative results do not mean absence of bioactive constituents and not that the plant extract is inactive. Plant extracts may act in other ways by stimulating the immune system of the patient, or by creating internal conditions that are unfavourable for the multiplication of the microorganisms (BUWA AND AFOLAYAN, 2009).

**Table 10: Antimycobacterial activity of traditional medicinal plants used against TB in the Free State, SA (MIC in mg/ml)**

| Botanical name | Plant part | Extraction solvent | Extract yield (mg) | *M. tuberculosis* |
|---|---|---|---|---|
| *D. anomala* (Sond) | Leaves | Acetone | 320 | 0.195 |
| | | Ethanol | 200 | 0.195 |
| | | Water | 1130 | 6.25 |
| *D. depressa* (Baker) | Bulb | Acetone | 200 | 5.21 |
| | | Ethanol | 240 | 12.5 |
| | | Methanol | 660 | 12.5 |
| | | Water | 2010 | 12.5 |
| *E. automnalis* (Mill.) | Bulb | Water | 4050 | 3.125 |
| *H. depressa* (N.E.Br) | Roots | Acetone | 350 | 0.78 |
| | | Ethanol | 170 | 0.78 |
| | | Methanol | 250 | 0.78 |
| | | Water | 790 | 1.56 |
| Streptomycin (µg/ml) | | | | 0.012 |

**Table 10 Continued**

| **Botanical name** | **Plant part** | **Extraction solvent** | **Extract yield (mg)** | ***M. tuberculosis*** |
|---|---|---|---|---|
| *L. lanceolata* (E. Mey) | Bulb | Acetone | 520 | 0.195 |
| | | Ethanol | 880 | 0.195 |
| | | Methanol | 340 | 0.65 |
| [a]*Lentsweni* | Roots | Acetone | 380 | 0.33 |
| *S. harveianus* (MacOwan) | Whole plant | Acetone | 150 | 0.195 |
| | | Ethanol | 150 | 0.195 |
| | | Methanol | 450 | 0.195 |
| | | Water | 270 | 0.39 |
| *X. undulatum* (L.) | Whole plant | Methanol | 1090 | 6.25 |
| Streptomycin (µg/ml) | | | | 0.012 |

# CHAPTER 5

## GENERAL CONCLUSION

Ever since ancient time, healing with medicinal plants has played a vital role in treating various ailments and will continue to play an important role in search and development of pharmaceuticals. The healing properties of various plants have been observed from the monkey and ape species that repeatedly consume botanical “plants” species containing chemical components that act as analgesics, antimicrobials, antiinflamatories, immunostimulants, antidiarrheals, digestive aids, and fertility regulators (AIKMAN, 1977; ANDERSON, 1977; HALBERSTEIN, 2005). There is also copious archaeological evidence indicating that medicinal plants were regularly employed by people in prehistoric times. In turn, the pharmaceutical industries reliance on natural products also has an immense impact on certain plant species and their natural habitats. Plant extracts are also processed or refined to produce therapeutic tincture, syrups, sauces, oral sprays, tablets, encapsulated powders, snuffs, and lozenges. Both traditional cultures and their biological resources become increasingly vulnerable to the pressures of market economics.

The scientific investigation of the potential antibacterial, antifungal and antimycobacterial activities of plants used by the traditional healers and herbalists in treating TB is important. The selected medicinal plants used against TB and other respiratory ailments in the Free State Province demonstrated significant antibacterial, antifungal and antimycobacterial activities which may explain and justify the usage of the plants by traditional healers and herbalists. In addition, most of the plant species also revealed the presence of phytochemicals such as tannins, saponins, steroids, cardiac glycosides and terpenes, with one of the plant species having flavonoids. In search for alternatives to production of desirable medicinal compounds from plants, secondary metabolites (phytochemicals) and plant tissue cultures are found to have potential as a supplement to traditional agricultural products in the industrial production of bioactive plant metabolites. A complex of some secondary metabolites found in botanical species (i.e. bark of the *Taxus* tree) are found to be one of the most promising

TB agents known due to its unique mode of action. Though the growing demand and popularity of medicinal plants is under threat of extinction, some of the traditional healers interviewed in this study apply sustainably use of medicinal plants by cultivating them in their gardens. Very good antimicrobial activities were observed with the medicinal plants used by the traditional healers and herbalists from the Free State Province, with some of the medicinal plant species displaying the high activity and some minimal activity when screened for antibacterial, antifungal and antimycobacterial activities against the selected pathogens. This study has validated the claims made by the traditional healers on the use of medicinal plants to treat various ailments, particularly TB. In addition, those plants that revealed good antimycobacterial activity could be targeted for future study on isolation of active compounds.

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# Appendix 1

## Example on finding the final mass:

**Using *D. anomala* Acetone extract (Table X).**

Pre-weighed Flask: Mass= 92.29 g

Re-weight Flask: Mass= 92.60 g

ΔM = Reweight – Pre-weighted

92.60 g-92.29 g

**0.31 g**

Mass converted to mg= 0.3 g*1000= 310 mg

Residues were re-dissolved a respective solvents:

Calculation:

Ratio: 50mg: 1ml

310 mg: X

Cross multiplying

50 mg.X=310 mg*1 ml

**X= 6.2 ml, the total volume of the solvent expected to re-dissolve the dried plant extract.**