ANTIMYCOBACTERIAL ACTIVITIES OF SELECTED PLANTS USED IN THE MANAGEMENT OF TUBERCULOSIS IN SEKHUKHUNE (LIMPOPO PROVINCE), SOUTH AFRICA.

By

Jacobus Kori Madisha

(2014116681)

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University of the Free State

Supervisor: Dr. A.O.T. Ashafa

CO-Supervisor: Dr. A.O. Aiyegoro

DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation supervised by Dr AOT Ashafa and Dr OA Aiyegoro is my original work and that I have not previously in its entirety or in part submitted at any university for a degree. I furthermore cede copyright of the dissertation in favour of the University of the Free State.

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Dr OA Aiyegoro's Signature……..

Date:…….

DEDICATION

To my late brother Moitswadi Elias Madisha who passed on this world on 09 October 2014 when I was busy with the studies, may his soul rest in peace. He will always be in our memories.

Acknowledgement

To Dr AOT Ashafa for his encouragement, patience, and most of all, his belief in me. My fellow students who assisted me in one way or the other during the program. The traditional healers and friends who helped with the collection of plant species. My sister and my brother in law, Mrs RM Mametja and Mr RH Mametja for their loving support.

RESEARCH OUTPUTS

Conference Poster

J.K.Madisha, A.O.Aiyegoro, A.O.T.Ashafa

Antimycobacterial activities of selected plants used in the management of Tuberculosis in Limpopo Province, South Africa.

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ABBREVIATIONS

AIDS Acquired Immunodeficiency Syndromes

CDC - Centres for Disease Control and Prevention

CFU - Colony Forming Units

CLSI - Clinical and Laboratory Standards Institute

COPD - Chronic Obstructive Pulmonary Disease

DMSO**-** Dimethyl Sulfoxide

HIV - Human Immuno Virus

INT- p-iodonitrotetrazolium violet

M. tuberculosis- *Mycobacterium tuberculosis*

MBC - Minimum Bactericidal Concentration

MDR - Multi Drug Resistant

MHA - Mueller-Hinton Agar

MHB - Mueller-Hilton broth

MIC - Minimal Inhibitory Concentration

STD - Sexually Transmitted Diseases

STI - Sexually Transmitted Infection

TB -Tuberculosis

WHO- World Health Organisation

XDR -Extensively Drug Resistant TB

SYMBOLS

- Negative

+ Positive

°C - Degrees Celsius

g - Gram

h - Hour(s)

L - Litre

mg - Milligram

mL - Millilitres

mm - Millimetres

µg/ml- Microgram per millilitre

µl -Microlitre

µm **-**Micrometer

A B S T R A C T

Tuberculosis (TB) continues to be a devastating disease of the world affecting more than two million people annually with one-third of the world's populations suffering from the menace. The management of TB was in the use of orthodox medicines which are not only expensive but presents severe side effects. Thus, efforts are recently geared towards the use of alternative therapy from natural sources which could offer a lasting solution to the treatment of the diseases with little or no side effects. The study investigated the antimicrobial potentials of four medicinal plants used by Bapedi tribe of Sekhukhune area, Limpopo Province of South Africa.

The antimycobaceterial efficacy of *Aloe marlothii*, *Maerua angolensis*, *Drimia elata* and *Elephantina elephantorrhiza* which were selected based on ethnobotanical study carried-out in the study was tested in four solvents such as ethanol, methanol, hydroethanol and dichlrormethane against four mycobacterium species such as *M. tuberculosis*, *M. smegmatis*, *M. peregrinum*, *M. haemophilus* and other gram positive and gram negative bacteria isolates using agar well dilution method and streak plate disc diffusion assay as a way of validating the anti-tuberculosis potentials of the plants.

The results revealed the anti TB activity of the four plants particularly *M. angolensis*, *D. elata* and *E. elephantorrhiza* which were reported for the first time in this study. Similarly, the results revealed varied degrees of antimycobacterial activities of most of the screened extracts (particularly ethanolic and methanol) going by the zone of inhibition values (10 – 32 mm) as well as minimum inhibitory concentration (MIC) values that fell within the range of 0.098 - 1.56 µg/mL and as such, could be adjudged to possess anti TB potentials.

Conclusively, the anti TB activity witnessed by the four plants could be attributed to the presence of the secondary metabolites which are responsible for the elicited effect. The study also validates the use of these plants in the management of tuberculosis by the Sekhukhune people of Limpopo Province, South Africa.

CHAPTER ONE

Background

1.1 Introduction

Tuberculosis (TB) is one of the leading causes of morbidity and mortality globally (WHO, 2008a). The global mortality rate as at ten years ago stands at two million per year with one third of the world's population infected with the bacilli (Sanjay, 2004; Centre for Disease Control 2005; WHO, 2007). It is estimated that 9.2 million new cases are diagnosed every year. According to the World Health Organisation (WHO), the incidence of tuberculosis in African countries has increased to more than twice its occurrence between 1990 and 2005 and is taking an upward trend yearly (WHO, 2008a). According to Chaisson & Martinson (2008), Africa carries 29% of the world's disease burden and 34% of the world's total death rate. South Africa is ranked 13th among the world's 22 countries with a high tuberculosis burden with an estimated incidence of 285,000 people per year and mortality of 84 deaths per 100,000 people per year (WHO, 2008a).

TB is a leading cause of death among people with human immunodeficiency virus, HIV (WHO, 2011a; WHO, 2015). Individuals infected with HIV are very susceptible to TB and often develop this disease before other manifestations of AIDS become apparent (Grange & Davey, 1990; Lall & Meyer, 1999; WHO, 2008b). The strains of TB that are resistant to all major anti-TB drugs have emerged (Buwa & Afolayan, 2009).

Multidrug resistance (MDR) is the antimicrobial resistance of microorganisms to multiple antimicrobial drugs. The emergence of drug resistant strains of *Mycobacterium tuberculosis*, is one of the major reasons contributing to the rise in

global incidence of tuberculosis since 1980 (WHO, 2015). Multi Drug Resistant (MDR) tuberculosis forms are defined as *M. tuberculosis* strains resistant to at least Rifampicin and Isoniazid which are the first line drugs used in treatment of tuberculosis (Lawn & Wilkison, 2006; WHO, 2011b). Moreover, Extensive Drug Resistant TB (XDR) is tuberculosis caused by strains resistant to first line drugs, fluoroquinolones and at least one of three injectable second-line drugs such as capreomycin, kanamycin, and amikacin (Lawn & Wilkison, 2006; WHO, 2011b).

Extensively, TB continues to be an enormous global concern as it infects millions of people annually and with the emergence of multidrug-resistant strains of *Mycobacterium tuberculosis,* hence, the need to search for new anti-TB drugs has become urgent. The emergence of TB resistance to antimicrobials, though a natural biological occurrence, has become an important public health issue in many developing countries as the treatment of TB requires the use of more expensive drugs for a longer treatment period. There is therefore, an urgent need for new, inexpensive TB drugs which are more effective and with lesser side effects. Although, a number of antimicrobial agents already exist for various purposes but the search for new antimicrobial agents should be a continuous one since the target microorganisms often evolve into new genetic variants which subsequently become resistant to existing agents.

Plants have been proven to be an important repository of future drugs and a suitable candidate in drug prospecting to manage and treat infectious diseases (Elujoba, 2005). Medicinal plants are an integral part of African culture (Buwa & Afolayan, 2009) and many higher plants are known to produce antimicrobial agents (Sakuma & Tomiyana, 1967). Indeed, extracts of plants from different parts of the world have shown to possess antimicrobial properties (Malcom & Sofowora, 1969; Bhakuni &

Bittner, 1974; Boakye-Yiadom, 1977). However, the local herbalists that use plants for medicinal purposes have no scientific knowledge of the systemic functions of the chemicals in the herbs before administering on patients, so a laboratory screening of these herbs need to be carried out, to validate the medicinal uses of plants and to assess the toxicity level and components of such plants, hence the need for studies on medicinal plants.

1.2 Herbal medicine

Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain active ingredients from plants, or materials, (WHO, 2001). Herbal medicine has a long history in the treatment of several kinds of disease (Holm *et al*., 2001). Their uses for the treatment of disease have been practised by man for many years and are still being widely practised even today (Kokwaro, 1993). For many years, peoples have developed a store of empirical information concerning the therapeutic values of local plants before orthodox medical practice appeared (Lawal, 2004). Through periods of trial, error and success, these herbalists and their apprentices have accumulated a large body of knowledge about medicinal plants (Holm *et al*., 2001).

According to Iwu *et al.* (1999), the first generation of plant drugs were usually simple botanical employed in more or less their crude form. Several effective medicines used in their natural state were selected as therapeutic agents based on empirical study of their application by traditional societies from different parts of the world. Following the industrial revolution, a second generation of plant drugs emerged based on scientific processing of the plant extracts to isolate their active constituents. Plant materials remain an important medicinal source in combating serious diseases in the world; for the therapeutic approach to several pathologies, as

well as extremely useful tools for the theoretical study of physiology and pharmacology (Dohadwalla, 1985).

Interest in medicinal plants has been overwhelming in the recent times especially as an important source of medication/health care. The annual estimate of the global market for herbal medicine was US $83 billion and expected to exponentially increase in the coming years (Robinson & Zhang, 2011). It has been globally recognized that medicinal plants play a significant role in providing health benefits to human beings. The (WHO) has estimated that 80% of the inhabitants of the world rely chiefly on traditional medicines for their primary health care needs, and it may be presumed that a major part of traditional herbal practice involves the use of plant extracts or their active principles (Fabricant & Farnsworth, 2001; WHO, 2008b).

1.3 Traditional African medicine

Africa has its own healing system of traditional medicine commonly referred to as “Traditional African Medicine” (ATM). This system is deeply rooted, and has played a key role in African culture for many centuries. The diverse way of life and culture in each separate region of Africa has led to a diverse local health care system. This medicine depends on the knowledge and practical experience of each individual healer with regard to diagnosing and treating ailments using naturally available materials. ATM is not yet supported by the government because it has not yet been incorporated into national health policy for reducing the use of western or orthodox medicine which is very expensive and sometimes unsafe.

Plants and humans are engaged in a dynamic relationship, where plants evolve creating biodiversity and humans develop strategies and solutions (Fabricant, 2001). In this relationship, plants evolve secondary metabolites to protect themselves from

human being and people find ways to use these metabolites to their advantage. Several aspects of this relationship have puzzled researchers over the past decades, especially those regarding the reasons behind plant selection criteria used by different communities around the world. The World Health Organization (WHO) estimates that up to 80% of the population in Africa makes use of traditional medicine as well as about 65% of the world's population. The used of plants in traditional medicine, is referred to as phytomedicine (Fabricant, 2001). These are plant-derived medicines that contain chemicals, more usually, mixtures of chemical compounds that act individually or in combination on the human body to prevent disorders and to restore or maintain health (van Wyk *et al.,* 2004).

Medicinal plants offer a great deal of hope to meet these needs and have been used for curing diseases for many centuries. These have been used extensively as combination or single plant. Only a few plant species have been thoroughly investigated for their medicinal properties (Heinrich, 2001). South Africa is one of the few countries of the world which has unique wealth of medicinal plants and vast traditional knowledge of use of herbal medicine for curing various diseases (Sharma, 1998). Interestingly, few plants have been tested against mycobacteria and a few plants which showed anti-TB activity were *Salvia hypargeia, Euclea natalensis*, *etc*. (Lall, 2001).

Medicinal plants are used in many parts of southern Africa to treat TB-related symptoms including chest complaints and coughing. Several recent reviews emphasize the potential of plant species and natural products as sources of antimycobacterial extracts and chemicals (Newton, 2000). The structural diversity of plant-derived antimycobacterial compounds is highlighted by the fact that the classes

to which these compounds belong include but not limited to alkaloids, terpenoids, coumarins/chromones, peptides and phenolics (Okunade, 2004).

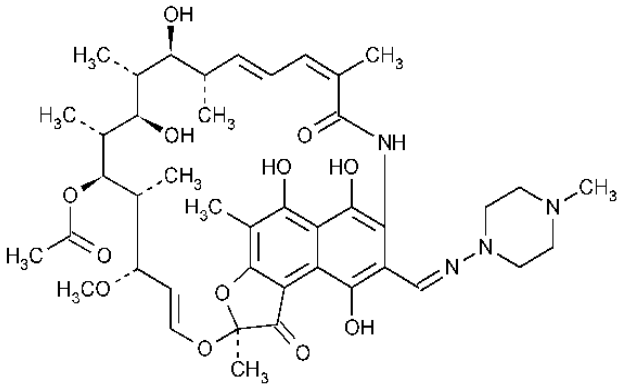

Figure 1.1 Structural formula of Rifampicin

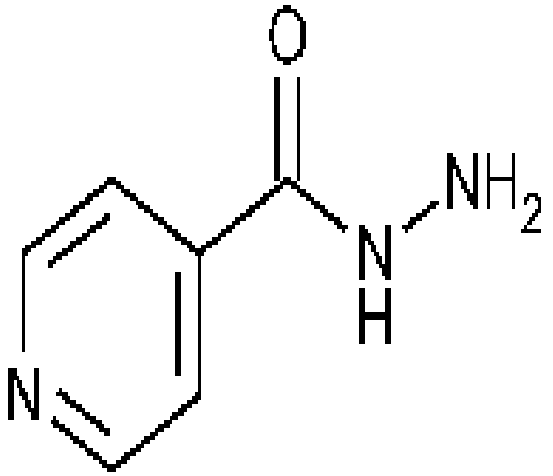

Figure 1.2 Structural formula of Isoniazid

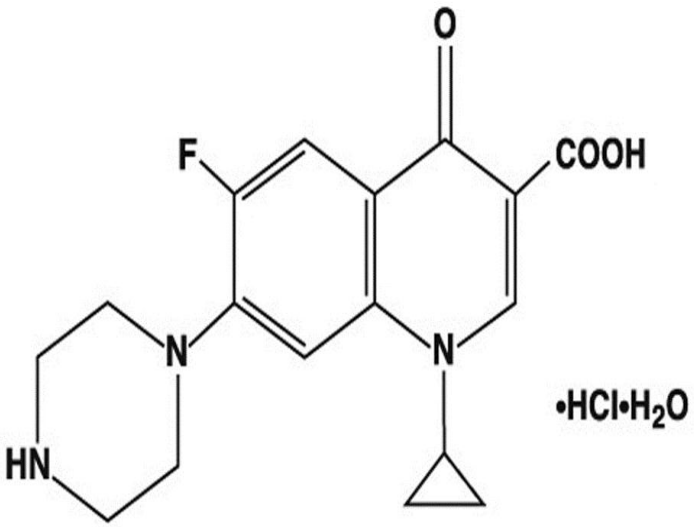

Figure 1.3 Structural formula of Fluoroquinolones

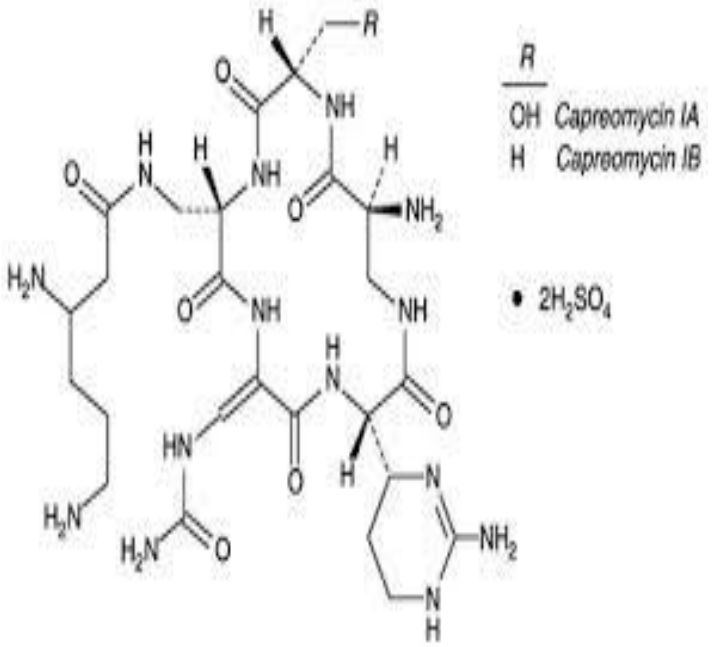

Figure 1.4 Structural formula of *Capreomycin*

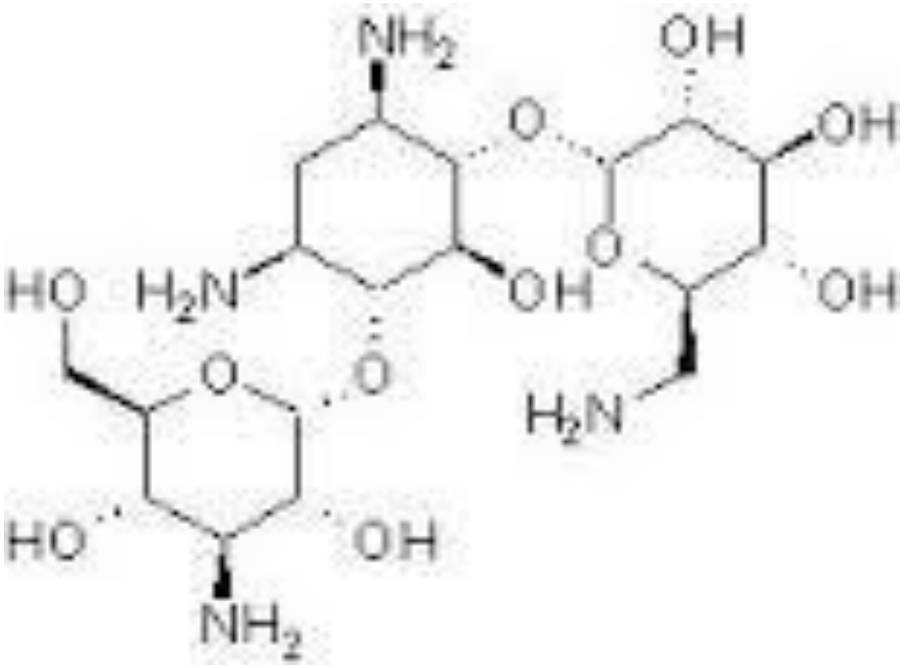

Figure 1.5 Structural formula of Kanamycin

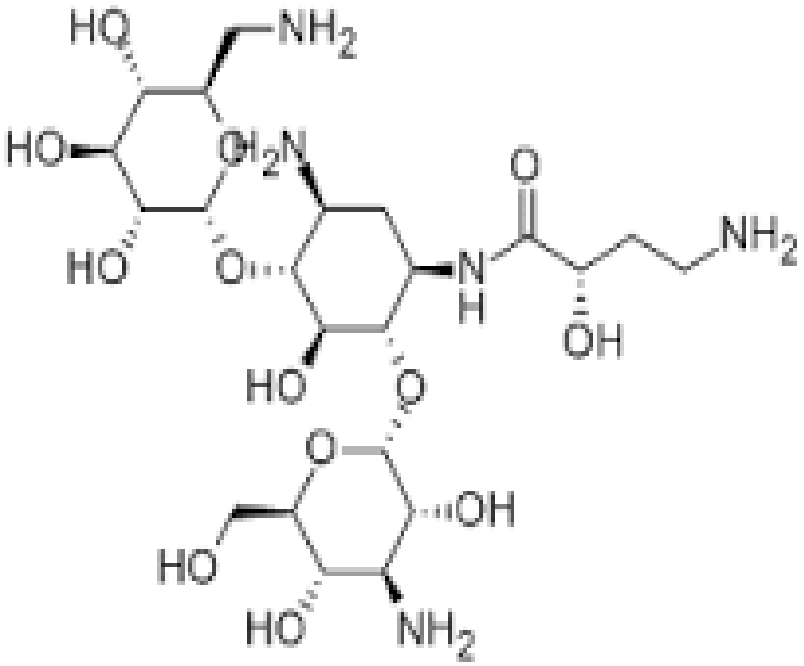

Figure 1.6 Structural formula of Amikacin

1.4 Traditional medicine in South Africa

Traditional medicine is the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness (WHO, 2001).

Traditional medicine, such as in any other third-world country still play an important role in the primary healthcare of people living in rural areas. Although, the government sometimes only supply and grant western medicine freely at no cost to the populace, most of the people still insist in consulting traditional healers. There are several reasons why traditional medicine is still so popular. These reasons include the inaccessibility of western medicine in rural areas, the high costs of these medicines as well as the cultural importance of traditional medicine.

Prescriptions and the use of traditional medicine are presently not regulated in South Africa; thus without regulation, the use of traditional medicine will always have the risk of incorrect dosage and administration by the user, which can be fatal when toxic materials are involved (Fennell *et al.*, 2004). Thus traditional medicine should be investigated to ensure that it is in fact effective for the disease it is prescribed for. If the “medicine” is effective in most cases, the effective dose, lethal dose and half-life of the extract or compounds should be determined scientifically.

South Africans have a long history of the use of medicinal plants in treating a variety of illnesses and ailments. Medicinal plants have always played a significant role within the traditional health care system of South Africa. Moeng (2010) estimated that in 1994, about 12 to 15 million or 60% of the people of South Africa used medicinal plant remedies from as many as 700 indigenous plant species. The

average South African consumer of traditional herbal medicine uses 750 g of plant material on annual basis **(**Masoko & Nxumalo, 2013**).** Since South Africa has such diverse vegetation and cultures, plants are commonly used for medicinal uses. Of the 3,000 species being used, approximately 500 species are traded in large quantities **(**Masoko & Nxumalo, 2013**)** though most of the trading occurs in informal medicinal trading markets (Light *et al.,* 2005).

1.5 The value of medicinal plants in drug discovery

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

During the last few decades, there has been a resurgence of interests in plants as sources of medicines and of novel molecules for use in the elucidation of physiological and biochemical phenomena**.** Aside this, there is an ongoing worldwide green revolution, which is reflected in the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs. Furthermore, underlying this

upsurge of interest in plants is the fact that many important drugs in use today were derived from plants or from starting molecules of plant origin: digoxin/digitoxin, the vinca alkaloids, reserpine and tubocurarine are some important examples (Iwu *et al.*, 1999). Therefore, laboratories around the world are engaged in the screening of plants for biological activity with therapeutic potentials. The potentials of higher plants as sources for new drugs are mostly unexplored (Hostettman *et al*. 2006). Among the more than 250 000 species of higher plants, only about 5-10% has been investigated chemically for the presence of biologically active compounds (Balandrin *et al*., 1993; Nahrsted, 1996)

1.6 Plants derived Antimicrobial compounds

Medicinal plants have been traditionally used for different kinds of ailments including infectious diseases. It is known that many plants especially those used by traditional healers produce pharmaceutically active compounds that have antimicrobial, antihelminthic, antifungal, antiviral, anti-inflammatory and antioxidant activity (McGaw *et al*., 2000). These plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* testing to have antimicrobial properties. Notably among these plants are wild mushroom, *Morella serrata*, *Gazania krebsina*, *Dicoma anomala* etc. (Heleno *et al.,* 2015; Tshabalala *et al.,* 2016). Furthermore, plants have provided a good source of anti-infective agents. The isoquinoline, alkaloid, emetine, obtained from the underground part of *Cephaelisi pecuanha*, and related species, has been used for many years as an amoebicidal drug for the treatment of abscesses due to spread of *Escherichia histolytica* infections (van Wyk *et al*., 2009). The higher plants have made important contributions in areas beyond infectious disease, such as

cancer therapies. Laboratories of the world now literally found thousands of phytochemicals which have inhibitory effects on all types of microorganisms (van Wyk *et al*., 2009).

1.7 Tuberculosis and burden of Disease

Pulmonary TB features (cough, fever, sweats, weight loss and haemoptysis) and extra-pulmonary lymph node swelling (lymphadenitis) are leads that is used in identifying diseases symptomatically (Fitzgerald & Haas, 2005). Apart from lung and lymph node, the disease can occur in any part of the body, including the meninges, bone and kidneys that land marks disseminated/military TB (Fitzgerald & Haas, 2005).

The use or often the misuse of drugs over the years has led to flourishing drug resistant strains (Nachega & Chaisson, 2003). The emerging and re-emerging global deadly drug resistant strains, multidrug resistance (MDRTB) and extensive drug resistance (XDR-TB) coupled with significant drug hepatotoxicity and lengthy therapy paved the irony road toward global TB therapeutic crisis (Dye *et al*., 1999; Amin *et al*., 2009; WHO, 2010).

The amount of damage caused by TB may be quite extensive, yet the symptoms may be minimal. The usual symptoms of TB are fever, coughs, weight loss, loss of appetite, weakness, night sweats, dyspnoea, shortness of breath, chest pain, signs of chest disease etc. (SATA, 1998).

1.8 Tuberculosis in South Africa

According to the South African statistics report on the causes of death in 2011, TB was acclaimed the leading cause of death in South Africa among both men and

women with 30,807 (11.8%) and 23,112 (9.5%) deaths respectively. With respect to age, there were 1,426 deaths due to TB (3.1%) among those aged between 0 -14, 36,728 (18.1) among those of 15-49, 10,983 (10.6%) among those 50-64 and 4,771 among those above 65+ years (STSA, 2014). Similarly 2013, deaths recorded from TB in South Africa were the leading cause of death with over 40,542 deaths. This was however a decrease from 2011 where an estimated 55,102 deaths were recorded, and 2012 with 48,409 deaths (STSA, 2014). These figures exclude deaths from TB and HIV co-infection which are internationally regarded as HIV deaths.

Table 1.1 South African records (2008 - 2013) of deaths from TB

Year	2013	2012	2011	2010	2009	2008
Number	40,542	48,409	55,102	63,281	69,791	75,281
% deaths	8.8	9.9	10.7	11.6	12	12.6

1.9 TB Treatment

Tuberculosis therapy has been revolutionised and the present treatment regimens for TB are based on multidrug therapy with usually 3 or 4 antituberculosis drugs. However, the problem of multi drug-resistant tubercle bacilli is emerging for various drugs for example; isoniazid, ethambutol, rifampicin, streptomycin etc. (Girling 1989; Grange & Davey 1990). Risk factors for the spread of multidrug-resistant tuberculosis (MDR TB) include poor compliance, convergence of immune suppressed patients, delayed diagnosis or treatment and poor or inadequate ventilation facilities. MDR TB is very difficult to treat and requires a longer period of treatment. Sometimes, surgery is needed to remove areas of destroyed lungs that

are heavily infected by mycobacterium and inaccessibility to drugs (WHO, 2015). A recent WHO report states that, globally, 2% of all cases of tuberculosis are multidrug resistant-by definition, resistance to rifampicin plus isoniazid [plus/minus other resistances (WHO, 2015)] .Notwithstanding, these cases can be treated in the USA and other high resource regions but at a great cost and using very long courses of rather toxic drugs, thereby raising serious problems of compliance (WHO 1997).

South Africa is witnessing an explosion in the number of cases of drug-resistant tuberculosis. In some parts of South Africa, 1 in 10 cases of TB is resistant to treatment. A research group funded by the British Pharmaceuticals Company Glaxo Wellcome reported that the number of cases of MDR TB in the KwaZulu-Natal region has risen by 300 percent in just one year. (New Scientist, 1997): An estimated 2000 South Africans contact multi drug resistant TB each year and more than half of these patients die within a period of two years (WHO/TB/98.258). It is therefore essential to have new antituberculosis agents due to the increasing resistance of mycobacterium to these classic antituberculosis drugs, preferably those that can readily and simply be produced from some local source.

1.10 MDR TB and XDR TB

Multi drug resistant (MDR) TB is the name given to TB when the bacteria that are causing it are resistant to at least isoniazid and rifampicin or two of the most effective anti TB drugs. Extensively drug resistant (XDR) TB is defined as strains resistant to at least rifampicin and isoniazid in addition to being resistant to one of the fluoroquinolones, as well as resistant to at least one of the second line injectable TB drugs like amikacin, kanamycin or capreomycin. MDR-TB and XDR-TB do not respond to the standard six months of treatment with "first line" anti TB drugs and

treatment for them can take two years or more and requires treatment with other drugs that are less active, more toxic and much more expensive. Globally, only a few thousand patients with MDR TB and XDR TB are treated each year. In areas of minimal or no multi drug resistant TB, TB cure rates of up to 95 per cent can be achieved. Cure rates for multi drug resistant TB are lower, typically ranging from around 50% to 70%.

1.11 Alternative treatment of TB

Medicinal plants are used in many parts of Southern Africa to treat TB-related symptoms including chest complaints and coughing. Several recent reviews emphasize the potential of plant species and natural products as sources of antimycobacterial extracts and chemicals (Newton 2000) .The structural diversity of plant-derived antimycobacterial compounds is highlighted by the fact that the classes to which these compounds belong include alkaloids, terpenoids, coumarins/chromones, peptides and phenolics (Okunade, 2004).

Herbal remedies play a fundamental role in health care services within rural areas of South Africa. More and more people utilize traditional medicine for their major primary health care needs (Elujoba, 2005). TB and Infectious diseases seem to be a major public health problem, which is confirmed by data from different health related bodies. This data is not accurate because traditional healers treat many cases of TB and they do not keep records. In many countries, it is fairly established that respiratory diseases, especially those that are untreated, play a role in the increase of burden of HIV (WHO, 2011b, 2015). Widespread misuse of antibiotics has led to

the emergence of new strains of TB that are resistant to rifampicin as well as isoniazid, and therefore difficult and expensive to treat.

Low costs and privacy are not the only enticements of traditional healers; there is a strong belief in the efficacy of traditional medicines. Traditional medicine is believed to be more effective because it cures the root cause of infection. Orthodox medicine addresses merely the signs and symptoms. It is also believed that the traditional medicines cleanse all the dirt left after the modern treatment; therefore some patients from hospitals go back to traditional healers (Moss *et al*, 1999; Halsey, 1999).

Traditional healers view themselves as knowledgeable and more competent to treat many illnesses that could be classified as respiratory. Recent studies have focused on medicinal plants that are rarely used traditionally to treat TB and respiratory diseases. Like western medicine, traditional medicine for TB may have very serious consequences, e.g. overdose and toxicity. All over South Africa, traditional medicine is being used to treat TB and respiratory diseases, but this study focuses on plants that are used by the Bapedi in Sekhukhune in the Limpopo province.

1.12 Diagnosis use by Traditional healer for TB

Semenya & Maroyi (2013) maintained in their reports that Bapedi traditional healers diagnosed TB based on patient's signs and symptoms. Before starting the treatment, patients were observed carefully and asked about the signs and symptoms of TB, the Bapedi traditional healers assess the treatment outcomes in patients, mainly by patient`s feedback and disappearance of TB signs and symptoms. Patient with one or combination of the signs and symptoms of TB was considered by the Bapedi traditional healers as TB "suspect". Blood in the sputum was the most commonly

cited diagnostic criterion, followed by a prolonged cough. Upon diagnosis, the healers prescribed and prepared the medication.

1.13 Conservation of Bapedi medicinal plants

Due to increased demand for plant products, over exploitation of these natural resources may occur. The medicinal application of the roots, bulbs and bark of many medicinal plants are considered to be the main factor contributing to the unsustainable use of these vascular plant species (Jain *et al*., 2005). Therefore, the non-destructive use of plant parts such as leaves or related species by traditional herbalists to improve plant conservation should be encouraged.

In Sekhukhune, medicinal plants are conserved via traditional systems which is enforce by taboo especially for those that treat cough or winter related symptom as they are only harvested during winter. Harvesting them in other season or for wood purpose is a taboo. The main aim of the system is to protect and preserve the medicinal plants and food source. Some taboos are also associated with this; example is a Bapedi tradition which dictates that if the remaining root system of *E. elephantina* is not covered after a portion has been removed; the patients treated with it will not get better. In a very wide sense this may be interpreted as a means to prevent the over-harvesting of plant material, thus aiding conservation efforts, albeit in a somewhat unconventional way.

1.14 Bapedi medicinal plants for Tuberculosis

The vegetation of the Sekhukhune district was classified by Acocks (1988) as semi-arid savannas. It is characterized by a mixture of trees, shrubs and grasses (Mucina & Rutherford, 2006). This type of vegetation has provided a diverse flora with rich medicinal plants that the people of the Sekhukhune have always used to treat many

illnesses. The ethnic group use herbal medication either alone or in combination with orthodox or western medicines for the treatment of several diseases (Semenya, 2012). Most of people living in the rural area of Sekhukhune are traditional people, hence the use of plants for common treatment of diseases such as sexually transmitted diseases (STDs), fever, coughs, weight loss, loss of appetite, weakness, night sweats, dyspnoea, shortness of breath, chest pain, signs of chest disease are predominant (Semenya , 2012).

Some of the medicinal plants used for management of ailments and infections particularly TB include but not limited to *Aloe malothii* (Kgokgopa-ya-go-ema), *Maerua angolensis* (Mogogwane), *Drimia elata* (Sekanama), *Elephantorrhiza Elephantina* (Moshitsane). Similarly, all the above mentioned plants are also used for similar health problems in other parts of the country. The fact that some of the reported plants are having similar uses elsewhere can be taken as indication of their pharmacological effectiveness having been tested in different areas by different cultures.

1.15 Literature review on studied Bapedi anti TB plants

1.15.1 *Aloe marlothii* (A.Berger)

Family: *Asphodelaceae*

Common names: Mountain aloe (Eng.); bergalwyn (Afr.); inhlaba or umhlaba (Zulu)

Botanical description

Aloe marlothii is a large, perennial, succulent leaves, with single-stemmed (Figure 7). The unbranched stem is thickly covered with old and dried up leaves. Both surfaces of the dull grey-green leaves are usually covered in numerous sharp and hard

spines. The leaf margin contains many rust-coloured spikes. The orange flowers have purple stamens, and are carried on relatively horizontal flower spikes. The fruit is a capsule (van Wyk & van Wyk, 1997).

Figure 1.7 Habitat/Vegetative morphology of *Aloe marlothii* (A.Berger)

Distribution

Aloe marlothii occurs from the North-West Province, Gauteng, Limpopo, Mpumalanga, Swaziland, Zimbabwe, Botswana, Mozambique to kwaZulu-Natal north of Durban (Pooley, 2003).

Recorded medicinal uses

Decoctions made from the leaves and roots are taken orally or as enemas to treat roundworm (Watt & Breyer-Brandwijk, 1962). Leaf decoctions are used in treating

horse sickness. Sap from the leaves is applied to the mother's breast to hasten weaning (Hutchings *et al*., 1996). Decoctions are made from the shoots and taken for stomach complaints (Watt & Breyer-Brandwijk, 1962). York (2012) described the use of *Aloe marlothii* for treatment of respiratory infections.

1.15.2 *Drimia elata* (Jacq.)

Family: *Asparagaceae*

Common names: *isiKlenama*, *Mahlokoloza*, *sekanama*, *skanama*, *uMahlogolosi*, *umHlabelo*, White *skenama* Lukhovo (Swati), *UmHlabelo*, umahlokolozi, umgulube, inguduza, *isiKlenama*,*uMahlogolosi* (Zulu)

Botanical description

Drimia elata is geophytes with large underground bulbs, strap-shaped leaves and long, slender flowering stalks. The flowers are tubular, with the tips of the petal characteristically reflexed and the stamens fused into a narrow tube (van Wyk *et al*., 2009).

Figure 1.8 Habitat/vegetative morphology of *Drimia elata* (Jacq.)

Distribution

It widely distributed over the north-eastern part of South Africa (van Wyk *et al*., 2009)

Recorded medicinal uses

The root/bulbs is use emetic and expectorant, leaves are diuretic and used for treatment of uterus and as blood purifier (van Wyk *et al.,* 2009).

1.15.3 *Elephantorrhiza elephantine* (Burch.)

Family: *Fabaceae or Leguminosae*

Common names: eland's bean, eland's wattle, elephant's root (Eng.); baswortel, elandsboontjie, leerbossie, looiersboontjie, olifantswortel (Afr.); mupangara (Shona); mositsane (Sotho, Tswana); intolwane (Xhosa, Zulu).

Description

Elephantorrhiza elephantina (Burch.) is perennial suffrutex, producing unbranched, unarmed, aerial stems up to 1 m high. The plant grows from an enormous underground rhizome of up to eight metres long. The finely divided leaves have numerous small and narrow leaflets. A cluster of small, cream-coloured flowers are produce along the lower half of the aerial stem. (van Wyk *et al*., 2009).

Figure 1.9 Habitat/vegetative morphology of *Elephantorrhiza elephantina* (Burch.)

Distribution

Elephantorrhiza elephantine (Burch.) is widespread and commonly fount from the southern parts of Angola, Namibia, Botswana, Zimbabwe, Mozambique and the South African provinces of Limpopo, Northwest, Gauteng, Mpumalanga, Free State, KwaZulu-Natal, Northern Cape and Eastern Cape as well as Swaziland and Lesotho. (Van Wyk *et al*., 2009).

Recorded medicinal uses

Elephantorrhiza elephantina root is used as remedy for dysentery and diarrhoea, stoppage of bleeding, stomach disorders, haemorrhoids, heart ailments and syphilis. It is popular for the treatment of skin diseases and acne (van Wyk *et al.,* 2009).

1.15.4 *Maerua angolensis* (PROTA)

Family: *Cappardaceae*

Common names: Kgotshane, mogogwane, bean-bean tree, moreketli.

Botanical Description

It is a shrub or small tree up to 10 m tall; branches usually pendulous, leaves are alternate, simple and entire, while flowers are bisexual with a cylindrical capsule fruit (Hutching *et al*., 1996)

Figure 1.10 Habitat/vegetative morphology of *Maerua angolensis* (PROTA)

Distribution

It is a tropical plant that is widely spread in the savannah area of tropical Africa to South Africa, Swaziland, south-east of DR Congo, Tanzania, Malawi, Zambia, Namibia, Zimbabwe and Mozambique (van Wyk *et al*., 2009).

Recorded medicinal uses

To induce vomiting after overeating the stems are chewed and the sap swallowed. The treat ulcers dressings with pounded leaves are applied (Hutching *et al*., 1996).

Table 1.2 Medicinal plants with investigated antimycobacterial activities

Botanical Name	Family	Sepedi names	Parts used	Traditional uses
A. marlothii (A.Berger)	Asphodelaceae	kgotshane,	Leaves	Treatment of cough, Flu, TB &cough (York, 2012)
M. angolensis (PROTA)	Capparaceae	moreketli, moretete	Leaves	Sore throat (Semenya, 2012)
D. elata (Jacq.)	Hyacinthaceae	Sekanama	Bulb/ Roots	To clean bladder and to treat diseases of the uterus (van Wyk & Gericke, 2009) Treatment of diarrhoea, skin problem (van Wyk & Gericke, 2009)
E. elephantina (Burch)	Fabaceae	Moshitsane	Roots	venereal disease (Semenya,2012)

1.16 Microbial Isolates

1.16.1 *Mycobacterium smegmatis*

Mycobacterium smegmatis is a rapid-growing gram-positive bacteria and non-pathogenic *Mycobacterium* species. Since it shares many biosynthetic pathways with

M. tuberculosis, (Schroeder *et al*., 2002) and is sensitive to the effect of many conventional antitubercular anti-infectives, it may serve as a good model system in investigating sensitivity patterns against *M. tuberculosis* (Glover *et al.*, 2007). The latent bacilli are opportunistic and can reactivate themselves as soon as the host becomes immune compromised (McLachlan *et al*., 2007).

1.16.2 *Mycobacterium haemophilum*

Mycobacterium haemophilum is a fastidious gram-positive bacterium, which belongs to non-tuberculous mycobacterial species, typically affects immune compromised persons. It also produces subcutaneous nodules, papules, and pustules. A times, it produces septic arthritis, osteomyelitis, pneumonitis, and disseminated infection (Megehee *et al*., 2007). *M. haemophilium* predominantly exhibit rapid growing species, have been associated with wound infections, cosmetic surgery, body piercing, and tattooing (McLachla *et al*., 2009). *M. haemophilum* infection rarely has been reported as a complication of tattooing (Giulieri *et al*., 2009; Hamsch *et al*., 2009).

1.16.3 *Mycobacterium peregrinum*

Mycobacterium peregrinum appears as straight or slightly curved rods, it is a gram positive, non-motile non-spore forming obligate aerobes belonging to genus of *Actinobacteria*, family of the *Mycobacteriaceae* (Park *et al*., 2001). *Mycobacterium peregrinum* includes pathogens known to cause serious diseases in mammals, including tuberculosis and pulmonary infection, Skin and soft tissue are the most frequent locations. Other infections include keratitis, endophthalmitis, arthritis, osteomyelitis, endocarditis, meningitis, peritonitis, urinary tract infection, infections of lung, and chronic otitis media after tympanostomy tube implantation and catheter

related bacteremia (Weber *et al*., 2000). They are mostly due to accidental inoculation from trauma, surgery, injection or aspiration (Schroeder *et al*., 2002).

1.16.4 *Mycobacterium tuberculosis*

Mycobacterium tuberculosis is a gram-positive bacterium which causes tuberculosis, the leading cause of infectious disease mortality. It produces unusually waxy walls, slow in growing and it is among the most recalcitrant bacteria to treatment (Park *et al*., 2001). *M. tuberculosis* is unusually resistant to drying and chemicals which contribute to the ease at which it is transmitted (Schroeder *et al*., 2002). The classic clinical features of pulmonary tuberculosis include chronic cough, sputum production, appetite loss, weight loss, fever, night sweats, and haemoptysis (Weber *et al*., 2000).

1.17 Combination Study

South African traditional healers combine different plant parts or plant species to achieve the most favourable outcome and they rarely use only one plant in treating an ailment (Viljoen *et al*., 2011). Synergy assessment has become important in the quest toward finding a scientific rationale for the traditional use of multidrug combinations - especially since these combined remedies are often preferred over single constituents (Wagner, 2011). Many plant compounds have proven, *in vitro*, to reduce minimum inhibitory concentration (MIC) values of antibiotics against resistant organisms (Aiyegoro *et al.*, 2010). Many *in vitro* antimicrobial studies have been used to determine synergy when combining traditionally used plant species with conventional antimicrobials (Lachowicz *et al*., 1998; Aiyegoro *et al*., 2009).

Thus in the study we have combined the four medicinal plants rarely use for respiratory disease and also combined the different extracts of different solvent, to

determine the MIC against all bacterial and to compare the MIC of different plants extract. Although the use of combined species has been mentioned in previous studies, validation of such combined uses is seldom explored. Thus in this study we validate only few of the combinations of the plants not popular because plants selected are only used as additive or in small amount in the traditional medicine against the respiratory, but mostly use for venereal disease.

1.18 Statement of research problem

Mycobacterium tuberculosis infections pose a risk to human health, particularly in developing countries. This is exacerbated by insufficient monitoring of the TB status of rural areas, coupled with the high incidence of HIV and AIDS. Many plant-based remedies are used in traditional medicine to treat TB-related symptoms. Following the discovery of plant extracts and compounds isolated from plants with promising activity against *Mycobacterium tuberculosis*, screening of plants may also yield good leads for new anti-TB drugs.

South Africa has one of the world highest tuberculosis burden; nearly half of the almost 50 million of South Africans are infected with TB of which 10% would progress to active tuberculosis infection; coupled with the incidence of resistance to the existing TB drugs by variants of *M. tuberculosis*; thus there is an urgent need for the development of new anti-TB drugs for the treatment of emerging TB diseases in South Africa.

1.19 Rationale

Tuberculosis (TB) is the world's longest running catastrophe; it has accounted for more human suffering, loss of earnings and failure of economic and social

development than any other disease (WHO, 2001). Reports showed that at every minute, one person dies of TB in South Africa and *Mycobacterium bovis* is believed to account for up to 10% of cases of human TB worldwide. The zoonotic pathogen, *Mycobacterium bovis* can spread to humans by inhalation of infectious droplet nuclei and by ingestion of infected products such as milk which has not been pasteurized or boiled properly or poorly heat-treated meat. It is reported that more than 94% of the world's population occurs in countries with no strategies in place to control *M. bovis* infections (Cousins, 2001).

The breakdown in health services, especially in developing countries such as South Africa, due to the spread of HIV/AIDS, the emergence of multi drug-resistant tuberculosis (MDR-TB) and the recent outbreak in South Africa of extreme drug resistant TB (XDR-TB) which is not only resistant to both the first line drugs (such as rifampicin and isoniazid) but also one of the three new second-line fluoroquinolones ,calls on the need of a specific drug that can treat TB in a shorter time than the usual 6-9 months (4 drug cocktail) regime. Although MDR strains of *M. bovis* have been identified, case reports show that anti-TB drugs routinely used to treat *M. tuberculosis*-infected patients are effective when properly administered (Thoen, 2006).

However, it is possible that resistance to currently used drugs will develop if the incidence of *M. bovis* infections and subsequent treatment in humans persists. In developing countries with no active bovine TB control programmes, a serious threat to human health is posed (Hope, 2007). In terms of public health, as well as economics, bovine TB control or eradication programmes should be a major target of affected countries (Hope, 2007). Natural products are proven template for the development of new scaffolds of drugs (WHO, 2015) and they have received

considerable attention as potential anti-TB agents. The discovery of novel drugs from indigenous plants would be of advantage in the developing countries. Hence, the need for investigation of medicinal plants with anti-TB activity in the search for novel drugs against TB.

1.20 Aims and objectives

1.20.1 Aim

The current study is designed to evaluate and validate the antibacterial effectiveness of some selected medicinal plants used in the management of TB and its related symptoms by the traditional healers from Limpopo Province. This study is also aimed to provide valuable information on the medicinal value of the plants while also possibly determine the bioactive components of some of the medicinal plants

1.20.2 Objectives

1) To qualitatively and quantitatively determine the presence of secondary metabolites in all the medicinal plants
2) To evaluate the plants extracts for inhibitory activity and sensitivity against susceptible bacterial strains.
3) To determine the antimicrobial activity using the minimal inhibitory concentration (MIC) of plant extracts
4) To investigate the antimicrobial potentials of the plants singly and in synergy

CHAPTER TWO

Materials and Methods

Ethnobotanical survey study

2.0 Methods

2.1 Study area and population

The study was conducted in the Sekhukhune district, Ephraim Mogale Municipality and Makhuduthamaga Municipalities of Limpopo Province in South Africa (Figure 12). The surveyed district is inhabited by black people, mostly from Bapedi ethnic group, as well as few Ndebele and White people. The Bapedi ethnic group supposedly constitutes the largest cultural group in the Limpopo Province (South Africa), comprising 57% of the total provincial population (LPG, 2012). The study was however restricted to the area around Sekhukhune in order to ensure that healer interviewed were Sepedi speaking who uses mountain and river as their closest sources of medicinal plants.

The vegetation of the district was classified by Acocks (1988) as semi-arid savannas. It is characterized by a mixture of trees, shrubs and grasses (Mucina & Rutherford, 2006). This type of vegetation has provided a diverse flora with rich medicinal plants that the people of the study areas have always used to treat many illnesses. The ethnic group use herbal medication either alone or in combination with orthodox medicines for the treatment of several diseases (Semenya, 2012). Most of the population in the Sekhukhune are sedentary to the rural lives, hence use of plants for common treatment of TB.

Fourteen villages were selected from around Sekhukhune district, however, other or distant villages were not considered due to financial constraint. Face-to-face

meetings were held with various local groups of traditional healers. The reasons for the meetings were to introduce the project, determine how active the traditional healers were in the area and to enlist them for the study. Ultimately, 35 traditional healers from 14 villages volunteered to participate. The traditional healers interviewed were of the Bapedi tribe as they are the dominant cultural group in the Limpopo Province of South Africa.

Figure 2.1 Map of South Africa

Figure 2.2 Map of Sekhukhune District Municipality

2.2 Sampling procedure

Using the random sampling technique, the study population consist of 32 young and 38 old community members who were drawn from the same villages as well as those of the 35 healers. They were interviewed to determine the attitudes of the general population to the different choices available for healing and in particular the importance of traditional healers in maintaining their good health. Participants volunteered to participate and gave their informed consent for the publication of all results and any accompanying images before commencing with the interview as required by the University of the Free State`s ethics committee. Random sampling could therefore be undertaken as a requirement that participants volunteer to be included in the study. It is worthy of note that this was a follow-up study to Semenya & Manyori (2013), a similar study conducted in the Sekhukhune district. The focus of

the present research was on depth to gain more insight into the situation of TB and in future with availability of financial resources, larger sample sizes could be selected in order to be able to generalize more fully.

2.3 Data collection

The data were collected from August 2014 to December 2014. At least five months of the rigorous fieldwork was conducted during the study year. The methods adopted included literature research, participatory investigation and key informant interviews. As earlier stated, one hundred and five informants (35 traditional healers, 32 young and 38 old) were interviewed. Informant ages ranged between 19 to 85 years old, where those aged above 65 were key informants. The interviews were carried out in standard Sepedi language because most of the populace are monolingual and have only attended a Primary school. The local Sepedi language names as well scientific or botanic names were also taken into consideration.

The study began with a literature search, which did not only help in proper identification of the study sites, but in understanding the flora of Sekhukhune for easy collection of the ethnobotanical data. The fieldwork was participatory investigation, and the main task was to search for medicinal plants with the key informants to perform a quick inventory, collect specimens, record habitats, and take photos. The preparation and consumption procedures were witnessed and recorded in the homes of the villagers. The plant specimens collected in the participatory investigation were used for reference during the key informant interviews. In the key informant interviews, detailed information about each plant, such as the local Sepedi names, habitat, medicinal parts, preparation, consumption, and medicinal function were documented.

2.4 Ethnobotanical information obtained on some of the plants

2.4.1 *Aloe marlothii* (A.Berger)

- Cut one leaf and mix it with chopped underground parts of *Euphorbia tirucalli* and a handful of crushed *E. Caffra* root. Add 1 L of boiling water and drink half a cup of the decoction once a day to treat chest pain, fever and a blocked nose. For Children, take one tablespoon of the decoction once daily.

- Crush a handful of leaves and add one cup of warm water. Take 1 syringeful (30 ml) once a day as an enema and take one teaspoon three times a day to treat cough and a runny or blocked nose. For children, take the same amount when an enema is used, but only drink one teaspoon twice daily.

2.4.2 *Drimia elata* (Jacq.)

- Pluck the bulb and mix it well with chopped underground parts of *Artemissia afra* and *Siphonochilus aethiopicus* and handful of crushed *E. caffra* root. Add 1 L of boiling water and drink half a cup of the decoction once a day to treat chest pain, fever and a blocked nose. Children should take one tablespoon of the decoction once daily.

- Mix a handful of bulb/roots with a handful of *L. javanica* leaves and bring to boil with 3 L of water. Steam the mixture for four minutes once a day. The interviewee stated that the plant is very strong, and one shouldn’t steam for longer than four minutes, while caution should be taken as regards steaming when intended to use for children. The decoction is used to treat coughs, chest pain and a runny or blocked nose.

2.4.3 *Elephantorrhiza elephantina*

- It is used in combination with *Cannabis sativa* and *L. javanica,* as described previously, to treat cough, fever and a runny or blocked nose. Mix two handfuls of leaves with one handful of *leaves* and bring to boil with 2 L of water. Drink a quarter cup three times a day to treat chest pain, cough, headaches and a runny nose. Children should take only one tablespoon of the decoction three times a day.

2.4.4 *Maerua angolensis*

Mix a handful of leaves with a handful of leaves from *Zanthaxylum capense* and bring to boil with 2 L of water. Steam until the decoction cools down, once a day, to treat chest pain, cough, fever and a blocked or runny nose. Children can steam only for five minutes per day with parental supervision.

Antimicrobial evaluation study

2.5.1 Collection of plant materials

Selection of plants with possible anti-TB activity is mostly based on their traditional use in the treatment of respiratory tract infections (RTIs), TB, pulmonary disease or related symptoms of these diseases guided by the information from traditional healers and based on no known prior record of anti-TB activity in published literature. The plants were collected over 4 months between August to December 2014 in Sekhukhune region of Limpopo Provinces, Northern South Africa. The four plant species used in the study belong to four families and the parts collected were the leaves, stem, roots and twigs. The specimen were collected with the help of the traditional healers and authentication of the plants were done by Dr A.O.T Ashafa of Phytomedicine and Phytopharmacology Research Group, Department of Plant

Sciences, University of the Free State, QwaQwa Campus, South Africa while the voucher specimen deposited at the departmental herbarium.

2.5.2 Extraction of plant materials

Plant materials were washed with sterile distilled water, cut into small pieces using a sharp knife and then air dried at room temperature for 4 days. The dried materials (approximately 500 g of each specimen) were ground into a coarse powder with aid of a hammer mill. The ground plants material was finally reduced to fine powder using an electric blender (Waring instrument, USA) and divided in to four equal parts of 125 g each which were then extracted separately with methanol (1250 mL), ethanol (1250 mL), hydro-alcohol (1250 mL at ratio 1:1) and dichloromethane (DCM; 1250 mL) for 24 hours with intermittent shakings at 2 hours intervals at room temperature. The mixtures were filtered through Whatman No.1 filter paper and the organic solvents evaporated to small fractions under reduced pressure using a rotary evaporator (Cole Palmer, Shanghai, Tokyo) while the remaining volume from the hydroethanol mixture was lyophilized using SP Scientific lyophilizer (USA). Total dryness of the filtrates was ensured by oven-drying at 37ºC until a constant dry weight of each extract of each specimen was obtained. The residues were stored at 10°C until used. A stock solution of 0.2 g/mL in dimethyl sulfoxide (DMSO) was made for each extract. All the extracts were kept at 4°C in the dark until they were further used.

2.6 Antibacterial Assay (sensitivity test)

The antibacterial assay was carried out using Agar disc diffusion method. The extracts were 125 mg dissolved in DMSO (Dimethyl Sulfoxide) to a final concentration of 25 mg/mL. The Muller Hinton Agar plates were inoculated with

(overnight 12 hours containing 10^{-5} CFU/mL) bacterial cell suspension by spread plate method. Sterile filter disks of 6 mm diameter were impregnated with 30 µL extracts and allowed to dry. The plates were incubated at 37°C for 24 hours. At the end of the incubation period, the antibacterial activity was evaluated by measuring the inhibition zone.

2.7 Streak plate disc diffusion (SPDD)

Streak plate disc diffusion assay was carried out according to the method described by Samy et al. (2006) with some modifications. Several colonies that grown on nutrient agar plates were picked and cultured in MHB until they reached their specific Optical Density (OD) at 600 nm to give a starting inoculum of 1×10^{7} bacteria/mL. Mueller-Hinton Agar plates were each divided into quadrants and labelled accordingly. One hundred microliters of inoculum, equivalent to 10 cfu was then pipetted to the MHA plate. A sterile cotton swab was used to inoculate the MHA plate by streaking over the surface with rotation to ensure even distribution of the inoculum. Impregnated discs were placed on the top layer of the MHA plates.

2.8.1 Preparation of *Mycobacterium tuberculosis* stocks

Mycobacterium Tuberculosis was maintained in Middlebrook 7H9 broth containing 10% OADC (oleic acid + albumin + dextrose + catalase). Inoculum was prepared by transferring the stock bacterial culture to supplemented 7H9 broth (Middlebrook 7H9 + 10% OADC) and grown for 72 hours on a shaker. Two (5 ml) supplemented 7H9 broths were inoculated by the bacterial culture and grown for 72 hours. Twenty percent sterile glycerol was added to each culture and 500 µL aliquots were made into sterile Eppendorf tubes. These stocks were named G1 stocks and were stored at -30°C. A single G1 stock was used to inoculate supplemented Middlebrook 7H10

agar (7H10 + 10% OADC) plates and incubated at 37°C for four days or 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 hours and used for the experiment.

2.8.2 Bacterial strains and inoculum preparation

Mycobacterium smegmatis, *Mycobacterium* peregrinum, *Mycobacterium haemophilum, Mycobacterium tuberculosis* were referenced isolates obtained from the Department of Botany, University of the Free State, Qwaqwa campus, South Africa. Bacteria were maintained on nutrient agar plates and invigorated for bioassay by culturing a single colony in 2 ml nutrient broth for 24 h. The bacterial culture was then diluted with Mueller-Hilton (MH) broth (1 ml bacteria: 9 ml broth), to make certain that the bacteria were at the start of the log phase when the test commenced.

2.8.3 Storage and Maintenance of Bacterial Strains

Gram–positive and gram–negative bacteria were used for the study. From the bacteria stocks kept at -70 ºC, bacteria cultures were prepared in 5 mL Mueller-Hilton (MH) broth (Oxoid) at 37 ºC overnight. After 18 hours the suspension cultures were streaked on Mueller-Hilton (MH) agar and then incubated overnight at 37 ºC in an incubator. The bacterial cultures were removed from the incubator and kept at 4 ºC in the fridge. This process was repeated in order to maintain the strength of the bacteria (Wistreich, 1997; Thiel, 1999).

2.9 Minimum Inhibitory concentration (MIC) test

A micro-dilution technique using 96 well micro-plates, as described by Eloff (1998) was used to obtain the MIC values of the crude extracts against the microorganisms under study. The plant extracts were diluted in 10% DMSO, to avoid the inhibiting

factor of solvents from affecting the results. A stock solution 100 mg/ml was prepared by weighing a mass of about 200 mg of the extract and dissolving it in 10% DMSO and the extracts were transferred and further dissolved inside Eppendorf tubes using the sonicator. One hundred µL of the nutrient broth was added into whole plate and followed by the addition of 100µL of distilled. One hundred µl of the plant extract (100 mg/mL) was added into the plate, 10% DMSO was use a control .

In all columns the same procedure is done but with a different extract, also control was done. The microplates were covered with parafilm and incubated at 37◦C for 24 hours after dilution and 40µL p-iodonitrotetrazolium violet (INT) reagent was added in all the wells excluding the last two column and the plates were re-incubated for 4 hours. The INT is reduced to a coloured (red) product by biologically active organisms ,bacterial growth in well will be indicated by a pink/red colour, and a total clear well indicates inhibition of growth by the plant extract. Minimal inhibitory concentration (MIC) values will be read as those concentrations where a marked reduction in colour formation is noted. Aliquots (100 µL) from the MIC wells and those of increasing concentrations were spread onto agar plates to detect minimum bactericidal concentration (MBC) values for the active substances. The MBC is defined as the concentration resulting in a sharp reduction (>99%) in the growth of mycobacteria after incubation.

2.10 Combination assays

The five different plant combinations (1:1) (Table 3.9 and Table 3.10) were prepared, respectively, from their plant extract stock solutions (64 mg/mL) to a total volume of 100 µL. For the 1:1 test combinations, equal aliquots of 50 µL each of the two extracts (tables) were mixed to make up to a volume of 100 µL while 1:1

combinations made each extract contributing 33.3 µL and 25 µL respectively to make up 100 µL in the wells of a 96-well microtitre plate. Using MIC assays, the antimicrobial activity was determined for these combinations to establish whether any interaction is evident.

2.11 Data analysis

Data obtained in the study were analysed using Graph pad Prism statistical software (San Diego, USA). Data were subjected to t-test, descriptive statistics and analysis of variance (ANOVA) while being expressed as means ± standard error of mean (SEM) of three replicate determinations. Statistical significance was considered at $p < 0.05$.

CHAPTER THREE

Results and Discussion

Ethnobotanical survey study

3.1 Results

3.1.1 Gender, age and years in practice of traditional healers

The information on the gender, age and years of practise of traditional healers during the study is shown in Table 3.1. It was observed that majority (71%, n = 25) of participating traditional healers were females, with males constituting the rest (29%, n=10). Only (30%, n=3) of the male participants were younger than 40 years, (20 %, n=2) were between 41 and 50 years, (20%, n=2) between 51 and 60 years, while (30%, n=3) were older than 60 years. The largest proportion (72%, n=18) of females was between 51 and 60 years, with none older than 60 years or younger than 30 years. Twenty percent (20%, n=5) was between 41 and 50 years, and (8%, n=2) fell within the 30 to 40 age category. Fifty percent (50%, n=5) of male healers have been in practice between 5 to 10 years, (40%, n=4) between 11 to 30 years, and only (10%, n=1) have more than 40 years' experience. Forty eight (48%, n=12) percent of female participants have been in practice for 5 to 10 years, and (52%, n=13) for between 11 and 30 years. This dominance is not unique to the Bapedi as similar findings were reported for Bapedi in Blouberg by Mathibela the Zulus in South Africa by de Wet et al (2012), for the Baka in Cameroon, the Masai in Kenya and further afield in Brazil. However, for the Bapedi in general, this female dominance is unusual. Both Moeng (2010) and Potgieter & Semenya (2012) noted the dominance of Bapedi males in traditional healing in the Limpopo Province of South Africa. Seventy percent of interviewed community members visited traditional healers.

Female traditional healers dominated the profession. Fifty six percent of the healers have no formal education, with only 10% having secondary school education. Seventy nine percent of healers see between 15 and 20 patients per month. Clinics and hospital in the vicinity have resulted in a shift by the community from using tradition-based healing to that of allopathic health care.

Table 3.1 Gender, age and years in practice traditional healers

Parameters	Age range	Males		Females	
		No	%	No	%
Age (years)	<40	3	30	2	8
	41-50	2	20	5	20
	51-60	2	20	18	72
	>60	3	30	0	0
Years of Practice	5-10	5	50	12	48
	11-30	4	40	13	52
	>40	1	10	0	0

3.1.2 Level of education

The majority of males (76 %) and less than half of the females (46%) in this study, had no formal education. A larger proportion of females had primary school education (31% vs 19%) and secondary school education (23% vs 5%) compare to their male counterpart. Various sources of traditional healing knowledge exist among the Bapedi; such as fellow healers and family members. Most males (48%) acquired their healing knowledge from fellow traditional healers, 38% from their parents and 14% from grandparents. In contrast to this, 62% of the females obtained theirs from their parents, 30% from fellow traditional healers and 8% from grandparents.

3.1.3 Plant parts used

Among the different parts of medicinal plants used by traditional healers, the leaves were most frequently used to make the prescriptions for healing treatments, while the bark and roots were second and third respectively. The most sustainable use of the plants to ensure viability is to use the leaves to avoid the threat of extinction of most of the medicinal plants. Tabuti (2010) mentioned that the use of root and tuber parts can threaten medicinal plant populations or species viability. This observation was in agreement with this study, most traditional healers submitted that some species such as *Maerua angolensis* (Mogogwane), *Drimia elata* (Sekanama), *Elephantorrhiza elephantina* (Moshitsane) are becoming rare because of over exploitation without sustainability. The interview result on different plant parts utilized revealed that leaves accounted for 34.2% of the total, followed by roots (30.9%), barks (8.2%) and other parts (19.8%).

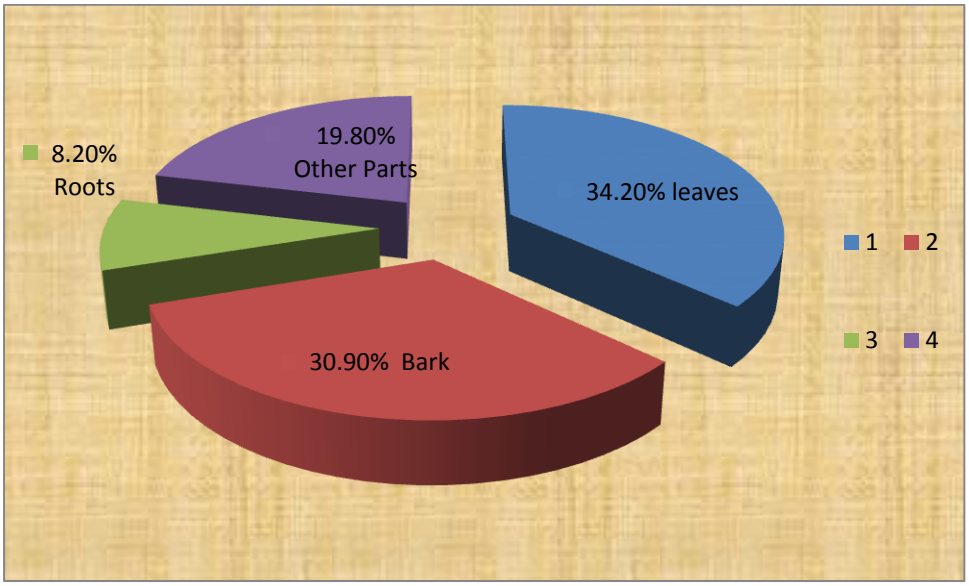

Figure 3.1 Pictorial representation of the mostly used plant's part

3.1.4 Preparation of Traditional medicine

In traditional herbal medicine, herbal remedies are prepared in several formulations which usually vary based on the plant utilized, and sometimes upon the respiratory condition being treated. Some of these methods include infusions (hot teas), decoctions (boiled teas), tinctures (alcohol and water extracts), and macerations (cold-soaking).

3.2 Discussion

Informant consensus values give good indication about particular species that serve for TB symptom's and about specific medicinal plants used for several health problems. Such information underlines the pharmacological significance of the medicinal plants in the Sekhukhune area. Medicinal plants with higher informant consensus need to be seriously considered for further ethnopharmacological studies, since they are species widely applied by many people and they have been utilized for a long time.

Interestingly, a number of plants that were reported by the informants in the study area includes *Siphonochilus aethiopicus* (Isiphephetho), *Eriocephalus africanus (kapokbos), Sutherlandia frutescens (*Lerumo-lamadi*), Citrus lemo (*Suru*), Hoodia gordonii (*bokhoring*), Helichrysum odoratissimum (*iphepho*), Tulbaghia violacea (*Isihaqa*), Artemissia Africa (*lengana, Mhloyane*), Pelargonium sidoides (*Ikhubalo*), Acacia karoo (*mookana*), Erythrophleum lasianthum (*Umkhwagu*), Cannabis sativa (*motekwane*), Lippia javanica (*musukudu*), Aloe malothii (*Kgokgopa ya go ema*), Maerua angolensis (*Mogogwane*), Drimia elata (*Sekanama*), Elephantorrhiza elephantine (*Moshitsane*), Zanthaxylum capense (*Monokwane) *Euphorbia tirucalli* (Mohloko) *as well as Zanthoxylum davyi* (Senokomaropa) and many of them are

also used to ameliorate similar health problems in some parts of the country and elsewhere. The fact that some of the reported plants are having similar uses elsewhere can be taken as indication of their pharmacological effectiveness of these plants having been tested in different areas by different cultures.

It is worth mentioning that after an exhaustive literature search and consultations with the relevant local government officials, it was revealed that currently there is no official documentation that indicates the total number of healers in either the studied municipalities or districts. Fortunately for this study, few literatures already existed on ethnobotanical knowledge in the Sekhukhune area, since this facilitated an analysis of the spatial distribution of knowledge. However, some features of the data obtained from these studies make them less than optimal for inclusion into the analysis.

Unfortunately however, the study revealed some setbacks which are attributed to the low number of questioned traditional healers due to financial resources and a clearly non-exhaustive sampling of plants, thus, results that were skewed toward one use type.

Antimicrobial evaluation study

3.3.1 Sensitivity results and discussion

The sensitivity or zones of inhibition of the four extracts of the four plants were shown in Tables 3.2-3.5. The extracts exhibit various degrees of antibacterial and antifungal potentials. The zones of inhibition was highest for *Mycobacterium tuberculosis* with mean zone diameter of inhibition value of 32 mm, while the lowest zone of inhibition was measured against *Mycobacterium haemophilum* with value of 7 mm. The highest minimum inhibitory concentration (MIC) value was recorded for *Mycobacterium haemophilum* value of 0.098 mg/ml, while the lowest zone of

inhibition was measured against *Mycobacterium smegmatis* with value of 6.25 mg/mL. The test organism that was least sensitive to most of the plant species tested was *Mycobacterium smegmatis* even though it is often used as a test organism in the initial screening of plants with possible activity against TB. Similarly, this test organism is much safer to work with and grows more rapidly as compared to *Mycobacterium tuberculosis*. There has been a reported correlation or similarity in the antimicrobial results against *Mycobacterium tuberculosis* and *Mycobacterium smegmatis* (McGaw *et al*., 2008), however this is not always the case for this study. Even though many of the once mentioned plant species extracts display some of the highest MIC values, frequency of use does not necessarily correlate with antimicrobial efficacy.

The ethanolic extract of *Aloe marlothii* is very effective against *M. tuberculosis* as evidenced from large zone of inhibition (32 mm) as observed from the study in comparison to other extracts (Table 3.2). However, the results of all the extracts against *M. tuberculosis* and *M. smegmatis* among the four mycobacterium is worthy of note because they are above 10 mm which is an acceptable zone of diameter. For *M. angolensis*, most of the four extracts showed a wider zone of inhibition with the diameter ranges between 10 – 20 mm in all the four species of mycobacterium except the ethanolic extract of *Mycobacterium haemophilum* (8 mm). Similar results were obtained for *E. elephantina* and *D. elata* except the dichlromethane extract of the latter plant (9 mm). Thus, suggesting that all the extracts are effective against all studied strains of TB.

Table 3.2 Zone of inhibition (mm) *Aloe marlothii* extract against tested isolates

Microorganisms	**Extracts (mg/mL)**			
	Ethanol	**Methanol**	**Hydroethanol**	**DCM**
1. M. tubercolosis	32	20	21	19
2.M. smegmatis	15	16	10	10
3.M. peregrinum	12	7	10	9
4.M. haemophilum	8	6	10	8
5.P.aeruginosa	21	6	7	22
6. E. faecalis	10	19	7	9
7. S. flexneri	10	7	19	8
8.E. coli	19	7	8	15
9.P. vulgaris	21	11	6	16
10.V. parahaem	20	25	25	12
11.S. aureus	19	8	8	14
12. Bacillus cereus	18	12	7	13
13.S. epiderm	8	9	14	10
14.N. gonorrhoea	9	17	18	14

Key: DCM; dichlromethane

Table 3.3 Zone of inhibition (mm) *Maerua angolensis* extract on isolates tested

Microorganisms	**Extracts (mg/mL)**			
	Ethanol	**Methanol**	**Hydroethanol**	**DCM**
1. M. tubercolosis	16	12	20	17
2.M. smegmatis	10	16	17	10
3.M. peregrinum	10	14	17	10
4.M. haemophilum	8	18	10	10
5.P.aeruginosa	6	14	22	21
6. E. faecalis	10	14	10	8
7. S. flexneri	7	20	26	20
8.E. coli	7	11	32	19
9.P. vulgaris	16	14	32	20
10.V. parahaem	9	21	32	24
11.S. aureus	7	12	21	14
12. Bacillus cereus	7	20	20	20
13.S. epiderm	8	12	10	7
14.N. gonorrhoea	18	11	10	10

Key: DCM; dichloromethane

Table 3.4 Zone of inhibition (mm) *Drimia elata* extract against tested isolates

Microorganisms	**Extracts mg/mL**			
	Ethanol	**Methanol**	**Hydroethanol**	**DCM**
1. M. tubercolosis	19	17	19	21
2.M. smegmatis	10	21	10	10
3.M. peregrinum	10	11	10	10
4.M. haemophilum	10	19	10	9
5.P.aeruginosa	11	18	19	10
6. E. faecalis	10	10	10	10
7. S. flexneri	10	16	12	10
8.E. coli	10	9	10	10
9.P. vulgaris	10	16	10	7
10.V. parahaem	9	16	14	10
11.S. aureus	9	10	9	11
12. Bacillus cereus	10	17	10	8
13.S. epiderm	10	16	16	9
14.N. gonorrhoea	10	10	10	16

Key: DCM; dichloromethane

Table 3.5 Zone of inhibition (mm) *Elephantorrhiza elephantina* extract on the tested isolates

Microorganisms	**Extracts mg/mL**			
	Ethanol	**Methanol**	**Hydroethanol**	**DCM**
1. M. tubercolosis	32	25	20	19
2.M. smegmatis	11	15	10	10
3.M. peregrinum	14	15	10	10
4.M. haemophilum	11	15	10	10
5.P.aeruginosa	10	10	10	10
6. E. faecalis	10	14	10	9
7. S. flexneri	14	16	10	17
8.E. coli	26	21	24	15
9.P. vulgaris	18	16	9	16
10.V. parahaem	7	20	9	18
11.S. aureus	18	15	10	19
12. Bacillus cereus	13	12	9	16
13.S. epiderm	16	14	10	11
14.N. gonorrhoea	11	15	10	15

Key: DCM; dichloromethane

3.3.2 Minimum Inhibitory Concentration (MIC) of the plant extracts

The interpretations of the MIC results of the plant extracts were shown in Table 3. 6-9. MIC values ranging between 0.098 to 1.56 mg/mL were considered. The ethanol extracts of *A. marlothii* showed the best MIC values against all bacteria except *P.aeruginosa* (Table 3.6). The MIC values of the aqueous extracts were high, especially against *P.vulgarisa and E.faecalis* a gram-negative bacterium, followed by *B.cerus, S.flexineri and S. aureus. A. marlothii* showed good MIC value for methanolic extract against *M. peregrinum*. Hydroethanol extract showed only weak activity against *B. cerus* and *S. epidermis*. This is interesting because drugs used by traditional healers are mostly prepared with water. It is worth noting that only in the ethanolic and hydrethanol extracts that very good activity was witnessed against *M. tuberculosis, M. smegmatis, M. pergrinum and M. haemophilum*.

The antimycobacterial activity for methanol and dichloromethane was weak as compared to that of ethanol and Hydroethanol. It has been reported for its use in traditional medicine to treat fever, colds, chest pain, various respiratory ailments, pneumonia and TB but use in small quantity in a mixture. The ethanol and hydro-Alcohol extracts exhibited very good activity against all the tested microorganisms, with concentrations ranging from 1.56 to 0.098 mg/mL. Concerning *M. tuberculosis*, the methanolic and dichloromethane extracts displayed weak activity with concentration ranging from 3.125 to 6.25 mg/ml. *Aloe marlothii* is reported to be use for respiratory infections at Maputaland (York, 2012), this corroborate the results of the study for the management of TB in Sekhukhune, thus validates the folkloric use of the plant as a good anti TB. Although, *Aloe marlothii* is no longer used

Table 3.6 MIC results for *Aloe marlothii* against susceptible bacteria isolates

Microorganisms	**Extracts (mg/mL)**				
	Gram	**Ethanol**	**Methanol**	**H-Alcohol**	**DCM**
1. M. tubercolosis	+	0.39	3.125	0.78	3.125
2.M. smegmatis	+	0.78	6.25	0.78	6.25
3.M. peregrinum	+	1.56	1.56	1.56	3.125
4.M. haemophilum	+	1.56	3.125	1.56	1.56
5.P.aeruginosa	-	3.125	0.098	0.39	0.39
6. E. faecalis	+	0.098	3.125	0.78	3.125
7. S. flexneri	-	0.195	0.78	0.39	1.56
8.E. coli	-	0.78	0.39	0.39	0.78
9.P. vulgaris	-	0.098	3.125	0.78	0.78
10.V. parahaem	-	0.39	6.25	0.78	3.125
11.S. aureus	+	0.195	3.125	0.78	1.56
12. Bacillus cereus	+	0.195	0.78	6.25	3.125
13.S. epiderm	+	0.78	3.125	6.25	3.125

Key: DCM; dichloromethane

Table 3.7 MIC results for *Maerua angolensis* (Mogogwane) against susceptible isolates

Microorganisms	**Extracts (mg/mL)**				
	Gram	**Ethanol**	**Methanol**	**H-Alcohol**	**DCM**
1. M. tubercolosis	+	3.125	1.56	1.56	3.125
2.M. smegmatis	+	6.25	0.39	3.125	3.125
3.M. peregrinum	+	3.125	0.39	0.098	3.125
4.M. haemophilum	+	3.125	0.78	6.25	1.56
5.P.aeruginosa	-	6.25	0.78	6.25	3.125
6. E. faecalis	+	3.125	0.78	6.25	0.196
7. S. flexneri	-	3.125	0.78	3.125	0.098
8.E. coli	-	3.125	0.78	0.098	0.39
9.P. vulgaris	-	3.125	0.098	3.125	1.56
10.V. parahaem	-	3.125	0.39	1.56	3.125
11.S. aureus	+	3.125	0.195	3.125	1.56
12. Bacillus cereus	+	1.56	0.39	6.25	3.125
13.S. epiderm	+	0.78	0.39	6.25	3.125

commercially in the production of laxatives; it still plays an important role in traditional medicines. It is used to treat roundworm infections, for stomach troubles such as constipation and for hastening the weaning of children (Van der Bank *et al*., 1995). Methanolic extract from the leaves of *M. angolensis* showed the best MIC values for extracts for all bacterium, with concentration ranging from 0.098 to 1.56 mg/mL (Table 3.7). Thus, revealed its strong activity against gram-positive and gram-negative bacteria. The ethanolic extract displayed the lowest antimycobacterial activity as compared to both hydro-alcohol and dichloromethane extracts. Concerning *M. tuberculosis*, methanol and hydro-alcohol extracts exhibited very good activity with concentrations of 1.56 mg/mL, the ethanolic and dichloromethane extracts had weak activity with concentration of 3.125. Bapedi use the small concentration for mixture in the treatment of various respiratory ailments. Various parts of the plant notably the leaves, roots and stem barks are claimed to reduce pain and are used to manage psychosis, epilepsy, diabetes, peptic ulcer, diarrhoea and arthritis in the traditional medicine. (Mahammed *et al.,* 2008; Magaji *et al*., 2009) Phytochemical screening of the methanolic stem bark extract revealed the major constituents as saponins, tannins, flavonoids, alkaloids and glycosides (Magaji *et al*., 2008).

Methanolic extract from the bulb/root of *Drimia elata* showed the best MIC values against all bacterium, excluding *M. smegmatis* (Table 3.8). The MIC values in aqueous extracts were high, especially against *B. cereus, S. flexnerii, E. faecalis* and *E. coli, followed by B. cerus, S. flexineri* and *S. aureus.* Ethanol and Hydro-Alcohol extracts showed only weak activity against *S. epidermidis, S. aureus, P. aeruginosa,*

Table 3.8 MIC results for *Drimia elata* against susceptible bacteria isolates

Microorganisms	**Extracts (mg/mL)**				
	Gram	**Ethanol**	**Methanol**	**Hydroethanol**	**DCM**
1. M. tubercolosis	+	1.56	1.56	1.56	0.78
2.M. smegmatis	+	12.5	3.125	6.25	0.098
3.M. peregrinum	+	6.25	0.78	6.25	0.39
4.M. haemophilum	+	6.25	0.39	3.125	0.78
5.P.aeruginosa	-	6.25	0.39	1.56	0.78
6. E. faecalis	+	0.195	0.098	1.56	0.195
7. S. flexneri	-	0.098	0.098	3.125	0.098
8.E. coli	-	0.098	0.098	6.25	0.195
9.P. vulgaris	-	0.78	0.195	3.125	0.78
10.V. parahaem	-	0.78	0.195	1.56	0.78
11.S. aureus	+	0.78	0.195	3.125	0.78
12. Bacillus cereus	+	1.56	0.098	1.56	0.78
13.S. epiderm	+	3.125	0.78	0.195	0.78

M. smegmatis, M. peregrinum, and *M. haemophilus.* It is worth noting that all extracts show very good activity against *M. tuberculosis, B. cereus and E. faecalis*. The antimycobacterial activity for ethanol *and* Hydroethanol was weak as compared to that of methanol and dichloromethane. It has been reported as traditional medicine for treating fever and various respiratory ailment but use in small quantity as a mixture. *Drimia elata* have been used as expectorants and emetics. (Watt & Breyer-Brandwijk, 1962) Leaves are said to be diuretic and are used to clean the bladder and to treat diseases of uterus (Pujol, 1990). It is used as blood purifier and as treatment for several other ailments. It is interesting to note that the closely related squil is use in several countries as a heart tonic, diuretic and expectorant. (van Wyk & Gericke, 2009)

The extracts (bulb/root) of *Elephantorrhiza elephantina* showed the best MIC values against all bacterium, excluding *V. parahaemolyticus, S. aureus* whole ethanol extract was active against *E.coli, P. vulgaris, V. parahaem, S. aureus* and *S. epiderm* (Table 3.9). The MIC values in aqueous extracts were high, especially against *M. tubercolosis*. This is interesting as drugs used by traditional healers are mostly prepared with water, with *Elephantorrhiza elephantina* use as basic medicine for venereal disease and in small quantity for respiratory disease. It is worth noting that all extract show very good activity against *M. tubercolosis, M. smegmatis, M. pergrinum, S. Aureus, V. parahaemolyticus and M. haemophilum*. This is a tradition remedy for a wide range of ailments, including diarrhoea, dysentery, stomach disorders, haemorrhoids and perforated peptic ulcers it can also as emetic. It is popular for the treatment of skin diseases and acne. (van Wyk & Gericke, 2009)

Table 3.9 MIC results for *Elephantorrhiza elephantina* against susceptible isolates

Microorganisms	**Extracts (mg/mL)**			
	Ethanol	**Methanol**	**Hydroethanol**	**DCM**
1. M. tubercolosis	0.098	0.195	0.195	0.78
2.M. smegmatis	0.39	0.39	1.56	0.195
3.M. peregrinum	0.39	0.39	1.56	0.195
4.M. haemophilum	0.78	0.098	0.78	0.39
5.P.aeruginosa	0.78	0.39	0.098	0.78
6. E. faecalis	0.195	0.78	1.56	0.78
7. S. flexneri	0.098	0.195	0.39	0.78
8.E. coli	0.78	0.098	0.098	3.125
9.P. vulgaris	0.78	0.195	1.56	6.25
10.V. parahaem	3.125	0.39	0.39	6.25
11.S. aureus	3.125	0.39	0.195	6.25
12. Bacillus cereus	0.78	0.39	0.39	0.78
13.S. epiderm	0.78	0.39	0.39	3.125

3.4 Results and discussion for combination study of the plants

Aloe marlothii, Maerua angolensis, Drimia elata and Elephantorrhiza elephantina are rarely mentioned even though they are used in small quantity for TB and respiratory infections. The antimicrobial activity of *Aloe marlothii* extract has been conducted (York, 2012). The extracts of all combinations showed noteworthy MIC values against all test microorganisms. *Maerua angolensis, Drimia elata and Elephantorrhiza elephantina* was reported for the first time for TB and respiratory infections in this study.

The MIC results for the combined plants extracts were recorded in triplicate, against each of the thirteen test microorganisms and the average MIC result for each combination was tabulated. Hundred percent of the plant extract combinations displayed noteworthy activity against all the thirteen tested microorganisms respectively. van Vuuren & Viljoen (2011) highlighted the importance of antagonistic effects due to the fact that such combinations suggest that the plant samples forming part of that mixture have opposing effects. However this is not a case in this study, it supports the traditional way of preparing the plant mixture by using combination of plants, although the combination used in the study are combination of rarely used plants which are only additive according to traditional medicine but show a significant activities against TB and respiratory infections .The MIC between of the various combinations ranges between 0.098 to 1.56 µg/mL.

Table 3.10 MIC results for combination of *Aloe marlothii*, *Maerua angolensis*, *Drimia elata*, *Elephantorrhiza elephantina*

Microorganisms	**All Plants (Ethanol)**	***A marlothii* + *E. elephantina*** **Ethanol**	**All plants (Hydroethanol)**	***E. elephantine*** **Hydroethanol + Ethanol**
1. M. tubercolosis	0.78	0.78	1.56	0.195
2.M. smegmatis	0.78	1.56	1.56	0.195
3.M. peregrinum	0.098	0.78	1.56	0.195
4.M. haemophilum	0.78	0.78	1.56	0.39
5.P.aeruginosa	0.39	0.195	0.78	0.39
6. E. faecalis	0.78	0.098	0.78	0.098
7. S. flexneri	0.39	0.39	1.56	0.39
8.E. coli	0.78	0.78	0.78	0.098
9.P. vulgaris	0.39	0.39	0.39	0.195
10.V. parahaem	0.78	0.39	0.39	0.195
11.S. aureus	1.56	0.78	0.39	0.39
12. Bacillus cereus	1.56	0.39	0.78	0.098
13.S. epiderm	0.78	0.39	0.78	0.195

Table 3.11 MIC results *for A. marlothii (*Ethanol) and *E. elephantina* (Hydroethanol)

Microorganisms	***A. Marlothii* (Ethanol) + *E. Elephantina* (Hydroethanol)**
1. M. tubercolosis	0.39
2.M. smegmatis	0.78
3.M. peregrinum	0.195
4.M. haemophilum	0.78
5.P.aeruginosa	0.78
6. E. faecalis	0.78
7. S. flexneri	0.39
8.E. coli	0.098
9.P. vulgaris	1.56
10.V. parahaem	0.39
11.S. aureus	0.78
12. Bacillus cereus	0.78
13.S. epiderm	1.56

CHAPTER FOUR

Conclusion and recommendations

4.1 Significance of the ethnobotanical survey and antibacterial

During this dissertation, plants were selected based on traditional use by Bapedi people of Sekhukhune in the management of TB. The objective of this study was to perform an ethnobotanical survey, focusing on plants used in management of Tuberculosis and related symptoms in the study area of Sekhukhune in Limpopo province in South Africa. Tuberculosis (TB) is one of the leading causes of the global mortality, and the rate stands at two million deaths per year with one third of the world's population infected with the bacilli according World Health Organisation (WHO, 2007). Tuberculosis is a leading cause of death among people with human immunodeficiency virus (HIV), as they are very susceptible to TB and often develop this disease before other manifestations of AIDS become apparent (Lall & Meyer, 1999).

The current study focused on knowledge, which makes the information recorded by the participants during the current survey valuable. During the ethnobotanical survey, 20 plant species were documented for their use in the treatment of TB by the interviewees. Three plant species *(D. elata, M. angolensis* and *E. elephantina)* were recorded for the first time globally, *A. marlothii* recorded for the second time as medicinal plants used in the treatment of TB and related symptoms.

Herbal medicine has been shown to have genuine usefulness about 80% of rural populations depend on it as their primary health care (WHO, 2010; WHO, 2011a). There has been an increased focus in recent years on ethnobotanical research and

the study of medicinal plants. Part of the increased attention to this field in South Africa has been as a result of an understanding that there is a lack of detailed documentation on the use of medicinal plants, there is an increasing threat that much of this information have disappeared over the year while the disease increase resistant on antibiotic and the increased pressure for conservation (van Wyk et al., 2009). Microbial resistance to common antibiotics is becoming a norm and an enormous threat to general health care facilities, especially in poorer countries with little or improper medical facilities and resources (Chovanová *et al*., 2013; Vigneshwari *et al.*, 2014; Marasini *et al*., 2015).

In drug discovery, ethnobotanical approach is the most productive of the plant surveying methods. The search for new active chemical compounds in high biological diversity regions has become a challenge to the modern pharmaceutical industry. The loss of both plants and knowledge has led to vital need to compile and study the information provided by the shamans and healers of these traditional cultures (Desmarchelier *et al.,* 1996). Currently over 50% of drugs used in clinical trials for anti-tuberculosis and anti-HIV activities were isolated from natural sources. The search for natural products to be used in TB and HIV/Aids represents an area of great interest in which the plant kingdom has been the most important source (Mena-Rejon *et al.,* 2008).

4.2 Correlation between traditional use and antimicrobial screening

Antimicrobial results (single and combined) could demonstrate that frequency of use do not necessarily always correlate with high antimicrobial efficacy. In this study, but less frequently used plant species show the strongest antimicrobial activities. This could indicate that, the focus is not so much on plants with the ability to heal an

infection, but on plants that are able to relieve the symptoms associated with such infections, as shown in the case of the plants use for the treatment of TB.

4.3 Conclusions

This study shows that local communities in the Sekhukhune Limpopo Province are still depend on traditional medicines to treat and manage TB. The documented medicinal plants used by the Bapedi traditional healers reflect a rich ethno medicinal knowledge in the Limpopo province. These results strengthen the firm belief that traditional medicines are readily accessible and still play an important role in meeting the basic health care of many people in developing countries and in big cities like Polokwane, Groblersdal, Pretoria, Durban, Nelspruit, Witbank, Cape Town and Johannesburg. It could be concluded some of the medicinal plants documented in this study would provide a treatment option that is readily accessible and affordable to TB patients in the Sekhukhune in Limpopo Province, the rest of South Africa and also beyond the national boundaries. The literature search has also shown that a large proportion of medicinal plants prescribed to TB patients by the Bapedi traditional healers are effective against several infectious pathogens. This is an indication of the potential value of the documented medicinal plants as sources of compounds needed for the development of plant derived anti-tuberculosis drugs.

Similarly, the results documented in this study relating to antimicrobial potentials of the studied plants correspond to the report of other literatures (van Wyk & Gericke, 2000; van Wyk *et al*., 2009) where medicinal plants were reported to be active against TB and related diseases. In general, the microorganisms investigated showed susceptibity in post-treatment with the extract. However, the CFU counts showed an increase from the initial CFU/mL of 0.3 X 10. The microorganisms

showed a decline in colonization on the agar medium. The action of microbial inhibition by tested plants in this study makes it evident that medicinal plants are effective against pathogens. Therefore these plants have a potential of being developed to new products. One of the advantages is that all tested plants showed low MIC value. The therapeutic index is the concentration at which the drug can be administered in to physiological systems. The extracts were tested directly against, *Mycobacterium smegmatis, Mycobacterium tuberculosis, M. peregrinum, and M. haemophilum.* The compounds therefore have reduced the growth and kill the bacterium in most cases. The results confirm that all the four medicinal plants have anti TB potentials. This also validates the use of the four medicinal plants on other disease and revel the possible use on other diseases which are caused by some of bacteria use in the study.

4.4 Limitations and future recommendations

This study only provided evidence of the antimicrobial potential of traditionally used plants and their combinations. Before recommending the use of these effective remedies in the treatment of TB, toxicity tests should also be carried out on plant species with unknown toxicity. Additional studies on plant remedies displaying strong antimicrobia*l* properties should involve the investigation of such remedies in clinical studies. Studies involving the analysis of plant-drug interactions should also be conducted. While results obtained during the current study is a valuable contribution toward the primary health care of the inhabitants of Sekhukhune. Therefore, plant remedies used in the current study for management of TB should also be screened for anti-HIV as the most important health problems results from the immune compromised.

These antimicrobial screenings should be followed by the identification and isolation of the active compounds. In the case of the mixed remedies, further combination studies should involve combining these plants' active compounds with each other and analysing the antimicrobial interaction between such compounds. Thus, even if most of the plant species used against TB display strong antimicrobial activity against pathogens associated with respiratory infections, it is important that further studies should also involve toxicity tests. Plants with weak antimicrobial activities could also be used because of their physiological relief of TB symptoms due to their anti-inflammatory, antispasmodic or analgesic properties. The other plant can work in the combination to reduce the combined level of toxicity, thus in turn use traditionally without any report of been toxic. This could imply that these remedies are possibly active against other TB pathogens (including *M.bovis* and *M*. *avium*) tha are not reported presently. These pathogens could not be analysed due to the availability of limited resources in the laboratory. Although many of the pathogens tested during the current study are not common causes of TB, most of them are known to cause respiratory infections specifically in people with compromised immune systems.

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4.5.APPENDIX A

QUESTIONNAIRE

Date:_____

Name of the interviewee:_____

Particulars of area:

Name of area:_____

Name Sub-area:_____

Name of Village: _____

Socio demographic data:

Age:

Gender:

Educational

background:

Plant information:

Sepedi name:_____

English name:_____

Botanical name:_____

Source:

Collected from wild	
Cultivated at home	
Both	

Part(s) used:

Other:_____

Preparation method:

a.) Method(s) of administration:

b.) Amount of plant material harvested for 1x usage:

c.) Quantify this amount (e.g.: 1 Handful=1 Cup):

d.) Is material used dry or fresh?

e.) If dry material is used, is everything used, or only some? (Specify exact amount):

f.) Preparation before taken (e.g.: Add 1cup of boiling water to handful of plant material)

Parts

Underground/root	Leaves	Bark	Whole plant	Stem

Dosage:

How much of the medicine will be taken every time?

_____

How many times days will this amount be taken?

How many weeks or months

_____

If/when this medicine is given to babies or small children, is there a difference in dosage? If yes, describe the difference(s)?

Signs and symptoms		**Extra explanation**
Fever		
Coughs		
Weight loss		
Loss of appetite		
Weakness		
Night sweats		
Dyspnoea		

Shortness of breath/ Breathlessness		
Chest pain		
signs of chest disease		
Swollen face		
Blood in the sputum		

Any extra information about the disease and/or its symptoms:_____

_____

Extra information:

Are there any other uses for the plant?

_____

_____

Where do you get your knowledge from about this plant?

_____

Why do you choose to use traditional medicine, rather than going to a clinic or a hospital?

_____

_____

Is this plant used in combination with any other plant(s)?

_____

Name the plant(s), and describe how this combination is prepared:_____

_____

_____

_____

Any other information:_____

_____

_____

_____

_____

_____

4.6.APPENDIX B

FORM OF CONSENT FOR USING ETHNOBOTANICAL INFORMATION

Researchers

JK Madisha[1], OA Aiyegoro[1, 2], AOT Ashafa[1*]

Institution: University of Free State qwaqwa campus - Phytomedicine and Phytopharmacology Research Group, Department of Plant Sciences, University of the Free State,QwaQwa Campus, Private Bag X13, Phuthaditjaba, 9866, Free State, South Africa

Research Project:

This project aims to document ethnobotanical knowledge on plants which are used to treat TB in Sekhukhune in Limpopo. The data will be conducted using structured

questionnaires. We will also collect plant material for identification and antimicrobial screening .This project is for academic purposes only and is of no commercial value. Results from this study will be presented at conferences and published in academic journals. The data will also be used towards the completion of Msc Botany by the above mentioned student.

Please take note of the following:

You are under no obligation to share any personal information which you do not feel comfortable in sharing with us.

Follow up visit:

We undertake to reveal the main results of this study to every homestead visited on completion of this project.

Signature of interviewee:

_____