Ethnobotanical database development and screening of medicinal plants in villages under the Jongilanga Traditional Council, Mpumalanga

Boikanyo Calvin Mophuting

25469551

Submitted in partial fulfilment of the requirements for the degree

Magister Scientiae (Option: Medicinal Plant Science)

Faculty of Natural and Agricultural Sciences

Department of Plant Science

University of Pretoria

Supervisor: Dr T.E. Tshikalange

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

DECLARATION OF ORIGINALITY

I Boikanyo Calvin Mophuting (u25469551) hereby declare that this study, except where properly acknowledged on and referenced in the text, is my own work and has not been previously submitted to this or any other institution. I understand what plagiarism is and I authorize the University of Pretoria to reproduce the contents for research purposes.

Signature……. Date……..

Boikanyo Calvin Mophuting

Acknowledgements

I am grateful to the following individuals and organisations who contributed towards this dissertation. First and foremost I thank God for his purposeful guidance throughout my life and studies. I express my sincere gratitude to my supervisor Dr Emmanuel Tshikalange for his patience, guidance, suggestions, encouragements, support and excellent advice throughout the course of this study, especially during the preparation of this dissertation. I would like to express my appreciation to the technical assistants Mrs Magda Nel for her help during the identification of medicinal plants, as well as Garland More and Thomas Nembudani willingness to go on fieldtrips for plant collection. I am very grateful to Smeetha Singh for carrying out the cytotoxity assay. I am grateful to Mr James Mahore for his cooperation and patience to allow me to record his ethnobotanical knowledge, and Guides & Trackers Direct for the planning and administrative support for this study. My great acknowledgement goes to the funding bodies National Research Fund (NRF) and MR Christian Courtin Clarins for funding this project. Finally I thank my family and Milcah Masemola for their moral support and encouragement throughout the study.

Table of Contents

Chapter 2: Methodology

Chapter 3: Results and discussion

Chapter 4: General discussion and conclusion

Chapter 5: Appendices

List of figures

List of tables

List of abbreviations

ATCC	American type culture collection
CFU	Colony forming units
CH	Circulatory system and Hepatic disorders
CS	Conservation status
D	Declining
DC	Dermatological disorders and cosmetics
DMSO	Dimethyl sulphoxide
DPPH	1,2-diphynyl-2-picrylhydrazyl
GI	Gastro-intestinal disorders
IC_{50}	Inhibitory concentration at 50%
INT	2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl
LC	Least Concern
MIC	Minimum inhibitory concentration
MMC	Minimum microbicidal concentration
NE	Not evaluated
RT	Respiratory diseases
SM	Skelto-muscular pain and swelling
SS	Sensory system disorders
STI`s	Sexually transmitted infections
T.I.	Therapeutic index
UG	Ureno-genital disorders
UR	Use report

UV	Use value
WHO	World Health Organisation
XTT	2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide

Abstract

Ethnobotanical database development and screening of medicinal plants in villages under the Jongilanga Traditional Council, Mpumalanga.

Boikanyo Calvin Mophuting

Degree: MSc (Option: Medicinal Plant Science)

Department of plant Sciences

Faculty of Natural and Agricultural sciences

University of Pretoria

Supervisor: Dr T.E. Tshikalange

Ethnopharmacological relevance: Medicinal plants still play an integral part in the livelihood of people in the rural areas. The aim of this study was to document information about the medicinal plants used by Shangaan people in villages under Jongilanga Tribal Council, Ehlanzeni district, Mpumalanga. This is the first documentation of medicinal plant uses in the study area, and it provides significant pharmaceutical information. **Methods**: An ethnobotanical survey of medicinal plants was conducted using a semi-structured questionnaire with 15 traditional healers as informants; one of them also served as a field guide during data collection. The results were analysed by using various quantitative indices such as information consensus factor, user report and conservation status. Biological assays (antimicrobial assay for *Prevotella intermedia* and *Candida albicans*, antioxidant assay was tested using 1.1-dipheny-2-picrylhydrazyl free radical scavenging assay and cytotoxicity assay was determined using the XTT method) of the medicinal plants were analysed.

Results: The study reported 207 medicinal plant species belonging to 54 families. The Fabaceae family was the most represented family of all the medicinal plants recorded in the study. Roots were the most frequently used plant part, accounting for 56 % of the parts reported, while decoction is the most common method of preparation to treat various ailments. The most treated ailment category is skelto-muscular pain and swelling and ureno-genital disorders, which account for 20% and 19% of plants collected, respectively. The medicinal plant use value ranged from 0.06 to 0.27. From the 207 collected medicinal plant species, ethanol extracts of 90 plants were prepared and tested for their antimicrobial properties. Eighteen plant extracts showed good activity compared to the positive control Chlorhexidine. The most significant antimicrobial activity was shown by *Combretum collinum* and *Terminalia sericea* with MIC and MMC values ranging between 0.39mg/ml and 3.13mg/ml. All 18 extracts that showed antimicrobial activity were also tested for antioxidant and cytotoxicity activities. The best antioxidant activity was shown by *Combretum collinum*, *Sphedamnocarpus pruriens, Rhoicissus tridentata* and *Kalanchoe thyrsiflora* respectively, with IC_{50} values ranging from 1.653µg/ml to 1.81µg/ml, compared to the positive control vitamin C, which is 1.62µg/ml. Six plant extracts (*Catunaregam* sp A, *Peltophorum africanum, Phyllanthus reticulatus, Sphedamnocarpus pruriens, Terminalia sericea* and *Xerophyta retinervis*) showed little toxicity or were not toxic at all against human cell line U937. **Conclusion**: This study revealed that medicinal plants are still widely used in rural areas and this documentation can serve as an ethnopharmacological basis for selecting plants with potential pharmaceutical properties. Future work will focus on identifying and elucidating the active constituents of the active plant extracts.

Chapter 1: Background and Literature Review

1.1 Background on medicinal plants

Ethnobotany refers to all the studies which concern the mutual relationship or interaction between plants and people (Davidson-Hunt, 2000). The definition itself is still being debated because through history ethnobotany has stemmed from differences in the interests of the workers involved in the study as they come from different disciplines (Cotton, 1996; Ghorbani *et al.*, 2006). In the past the field of ethnobotany has belonged to the explorers and adventurers, who were recording and taking notes of the uses of plants by the aboriginal people they came across around the world. This started with Christopher Columbus who discovered, among other plants, tobacco (*Nicotiana* species) in Cuba. The world then followed with interest in ethnobotany to find plants that can help fight illness (Veilleux & King, 1996). Since the 1990s, plants have re-emerged as a significant source of pharmaceuticals. South African National Biodiversity Institute (SANBI) has an ethnobotany unit which focuses on traditional uses of southern Africa's plants for their conservation, sustainable use and development as some plants are getting over exploited and are now becoming threatened. SANBI's contributions are made towards documenting the cultural, medicinal and economic value of these plants (SANBI, 2011).

Humans have been dependent on plants for food, shelter and medicine since prehistoric times. Plants have been brought from the wild and domesticated, aside from their use as sources of drugs, food and industrial material plants (production of soaps and toiletries, perfumes and spices, and insecticides) (McChesney *et al.*, 2007). There are many threats to

the natural resources of our planet; such threats include habitat loss, degradation, over-exploitation of resources and climate change. Biodiversity refers to the full variety of life and complexity (DeLong, 1996). Plant diversity is a major part of the total biodiversity. Plant conservation is important as there is extinction of certain species that are used for food, shelter and medicine (DeLong, 1996). There is a major correlation between indigenous knowledge and plant biodiversity conservation (Warren, 1996).

Plants are natural producers of medicinal substances. Anthropologists hypothesize that animals evolved a tendency to search for bitter plant elements in response to illness. This behaviour arose because bitterness is an indicator of presence of the secondary compounds (Palo, 1984; Williams *et al.*, 1989). The earliest written account of herbal remedies comes from China and dates back to 2800 BC. Medicinal plants are plants that one or more of their parts contain a physiologically active ingredient which can be used for therapeutic purposes or consist of compounds that can be used for drug synthesis. Medicinal plants have been used for centuries (Hoareau & DaSilva, 1999) and herbal practitioners have developed various preparation methods appropriate for releasing the healing properties of different plants. Most appropriate herbs need to be selected for the patient by the practitioner also taking note of the best way to prepare them (Hoffman, 2003).

1.2 Traditional medicine

Plants have played a major role in maintaining human health and improving the quality of human life for thousands of years in traditional medicine. Traditional medicine refers to the knowledge, skills and practices that are based on theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness (World Health, 2002). The medicines used vary from country to country (World Health, 2002). Traditional medicine has been a primary health care provider in different communities for thousands of years. Traditional medicine is researched on by ethnobotanists to find and record the medicines used by indigenous people or traditional medicine practitioners, to screen for plants that are used which can improve human health (World Health, 2002). The traditional medicines that are not based on belief can be identified and the plants that are used can be given a voucher specimen number and tested in the laboratory for activity in improving health against diseases like cancer, sexually transmitted diseases and tuberculosis. Thus traditional medicine helps scientists to target plants that are medicinally useful instead of random sampling to do trial and error (Fennell *et al.*, 2004). This requires the knowledge of ethnopharmacology which is to test the traditional medicine for pharmacodynamic and pharmacokinetic activity. By 2001 there was already a reported estimation of over 122 drugs developed from 94 plant species that have been discovered through ethnobotanical leads (Fennell *et al.*, 2004).

There is great interest of herbal products around the world, especially in the United States (Sardesai, 2002). Surveys of plant and medicinal usage by the American public have shown

an increase from 3% of the population in 1990 to over 12% in 1997 (Sardesai, 2002). An estimated $2 billion was spent in 1996 buying herbs in bulk. This includes capsules, tablets, extracts and teas. The sale of the herbal products reached over $3.5 billion in 1999 (Sardesai, 2002). Southern Africa is rich in plants and traditional knowledge of medicinal uses of plants. These plants are prepared or formulated differently to have a final medicinal product.

1.3 Traditional knowledge and formulation of medicinal plants

Formulation method is a process in which different chemical substance are processed and combined to produce the final medicinal product. There are mainly five preparation methods, namely; decoction, maceration and expression, digestion, percolation, and infusion. Other methods such as burning, poultice and bathing are used in traditional medicine.

Decoction is a water-based preparation made by gently simmering the herb in boiling water. Decoction differs from infusion in that it is more appropriate for tougher plant parts, such as roots, bark, and seeds (Hoffmann, 2003). Traditional healers commonly use this method of preparation by grinding roots, leaves, seeds to boiling water thereafter simmering in boiling water.

Maceration and expression is the process of softening plant material by soaking in cool water; facilitating the dissolution of the soluble constituents it plays important roles in many official preparations, including tinctures, extracts, syrups, wines and vinegars (Hoffman, 2003).

Digestion is a form of maceration that involves application of a gentle heat to the substrate being extracted. It is used in cases where a moderately elevated temperature will help increase the solvent powers of the menstruum. Digestion differs from decoction in that preparations made through the process of digestion are alcohol based, rather than water based (Hoffman, 2003)

Percolation is a process by which a powder contained in a suitable vessel is deprived of its soluble constituents by the descent of a solvent through the material (Hoffman, 2003).

Infusion is a water- based preparation made by steeping leaves, flowers, and other non woody plant parts in either hot or cold water. Obviously, this method of preparation is appropriate only for herbs with water-soluble constituents (Hoffman, 2003).

Poultice is a process where herbs are directly applied to the skin mainly on rashes or wounds by squeezing the herb or mashing (Iwu, 2014).

Bathing is a process where the medicinal plant is added to bathing water and the patient is soaked in it. The active constituent is then absorbed by the skin (Kelmanson *et al*., 2000).

Burning is a process where the herb is burned to minimally or to ashes to enhance or reduce the active constituent (Kelmanson *et al*., 2000).

1.4 Oral pathogens

Traditional remedies are widely used in South Africa for treatment of ailments. Antimicrobial agents refer to drugs, chemical or other substances that kill or slow down the growth of microbes (bacteria, fungi, virus or parasite). Periodontal disease is a term used to describe all disorders of the supporting structure of the teeth; this includes gingival, periodontal ligament and the underlying alveolar bone (Kinane, 2001). The infection level can range from gingivitis, which is the inflammation of the gingival to full blown periodontitis that can result in tooth loss. Unlike most illness, bacteria that are foreign to the body do not cause periodontal diseases (Williams, 1990; Kinane, 2001). Periodontal disease is caused by the microbes that inhabit the oral cavity (Zambon, 1996; Gendron *et al.*, 2000). The attack is triggered by the shift in the balance of oral microflora towards more invasive microbes (Williams, 1990; Zambon, 1996; Gendron *et al.*, 2000; Kinane, 2001; Arwa *et al.*, 2008). These microbes can cause inflammation leading to periodontal disease by either directly invading the surrounding tissues or indirectly by emitting a toxin (Kinane, 2001).

Dental professionals and Pharmaceutical companies have been waging a successful war against pathogens that cause tooth decay, gingivitis and periodontitis (Arbes Jr & Matsui, 2011). The corrective treatments for such infectious diseases required the reduction and or elimination of bacterial accumulation in the relative sites such as the teeth surfaces and between teeth by brushing daily and frequent dental cleaning or prophylaxys (Alviano *et al.*, 2008). A number of antiseptic agents including chlorhexidine, cetyl pyridinium chloride, fluorides and phenol derivatives are used frequently in dentistry to inhibit bacterial growth inhibit bacterial growth (Alviano *et al.*, 2008). The steady decrease of tooth decay and periodontal disease worldwide is evidence of the success (Arbes Jr & Matsui, 2011). The

antiseptic agents however have several adverse effects such as vomiting, diarrhoea and teeth staining (Alviano *et al.*, 2008). Thus it is important to research and develop a natural antimicrobial agent targeting specific oral pathogens while being safe for the host (Alviano *et al.*, 2008). There has been research conducted to find whether there is a correlation between oral pathogens and allergic disease. During the past half century there have been observations that led researchers to hypothesise that exposures to oral bacteria, including pathogens associated with periodontal diseases, such as gingivitis and periodontitis might play a protective role in the development of asthma and allergy (Arbes *et al.*, 2011). Polyphenols have been suggested to be responsible for the antibacterial properties upon *Streptococcus mutans* (Sampaio *et al.*, 2009). In this study the oral pathogens *Candida albicans* and *Prevotella intermedia* were selected on their role to being mainly linked to periodontal disease (Rosas-Pinon *et al.*, 2012).

1.4.1 Selected oral pathogens

Candida albicans is a diploid fungus which grows as a yeast cell and filamentous cell. It is a constituent of the normal gut flora and lives in the human mouth and gastrointestinal tract. The excessive growth of *C. albicans* causes candidiasis which is commonly restricted to the mucosal membranes in the mouth or vagina, this type of infection is called thrush. Candidiasis is curable in people who are not immune compromised (Barasch & Griffin, 2008). Thus candidiasis is often observed in immune compromised individuals that are infected with Human Immune Virus (Gimeno *et al.*, 1992; Berman & Sudbery, 2002).

Prevotella intermedia are a gram negative anaerobic pathogenic bacterium commonly inhabiting the human buccal epithelial cavity (Gursoy *et al.,* 2009). *Prevotella intermedia* have been implicated as a putative periodontal pathogen because it is isolated from lesions of patients with early periodontitis, advanced periodontitis and acute necrotizing ulcerative gingivitis (Dorn *et al.*, 1998). It has been shown that *Prevotella intermedia* are capable of invading human oral epithelial cells, coronary artery endothelial and smooth muscle cells in vitro. Through a study of subgingival plaque and saliva samples of pregnant women, it was concluded that *Prevotella intermedia* is associated with hormone induced pregnancy gingivitis (Gursoy *et al.*, 2009). Selection and testing of 90 ethanol plant extracts against the above mentioned microorganisms is reported below.

1.5 Selected plant description

Eighteen plant species that showed significant antimicrobial activity (chapter 3) are described as follows:

Acacia karroo is a multi-stemmed shrub or tree growing to 15 metres in height. *A. karroo* is widely distributed throughout the country. Medicinally the bark, roots and gum are commonly except the roots. In the Cape the bark and leaves are used as a remedy for diarrhoea and dysentery (Van Wyk *et al*., 1997). The ailments such as colds, conjunctivitis and haemorrhage are treated with the bark, gum and leaves which act as an emollient and astringent. The gum is most used to treat oral thrush. The active ingredients have been reported to be the high tannin content found in the leaves and bark (Van Wyk *et al*., 1997).

Figure 1.1: *Acacia karroo* (Adapted from http://witbos.co.za)

Antidesma venosum (Tassel berry) is a glossy bright green shrub or small tree with spreading branches, from the Euphorbiaceae family (Figure 1.2). The root extract has been proven to have physiologically effects on the heart (Watt & Breyer-Brandwijk, 1962). The leaves, fruit, and bark are used by the Zulus and Swahilli as one of the infusions for abdominal pains. The leaf contains alkaloids and the root has been reported as toxic (Watt & Breyer-Brandwijk, 1962).

Figure 1.2: *Antidesma venosum* leaves (Adapted from: www.westafricanplants.senckenberg.de)

Catunaregam spA is a flowering plant belonging to the family Rubiaceae, native to tropical Africa and tropical Asia. *Catunaregam spinosa* a plant form the same genus, stem bark was reported to have a significant sedative activity in a study evaluating the effect of the plant extracts on locomotor activity using an actophotometer and anxiolytic activity involving several models including elevated plus maze and thiopentone sodium induced sleeping time (Madhavan *et al.*, 2011).

Combretum collinum is an aromatic plant from the Combretaceae family (Figure 1.3). A decoction of the plant is drunk in Eastern Uganda for the treatment of diarrhoea, sterility and pyomyositis. An infusion from the roots is administered orally to delivering women to enhance labour (Watt and Breyer-Brandwijk, 1962).

Figure 1.3: *Combretum collinum* (adapted from: www.zimbabweflora.co.zw)

Dichrostachys cinerea subsp. *nyassana* (sickle bush) is a shrub or small tree with curly and twisted indehiscent pods, and unpaired spines from the Mimosaceae family (Figure 1.4). The powdered root bark of the plant is used in Northern Zimbabwe for wounds and is said to promote wound healing. The cold water infusion of the powdered leaf is squirted into the

nose and mouth to relieve headache. The leaf and root is smoked in Northern Zimbabwea for pulmonary tuberculosis (Watt & Breyer-Brandwijk, 1962).

Figure 1.4: *Dichrostachys cinerea* (Adapted from http://shivanidiwani64.blogspot.com)

Diospyros lycioides subsp. *lycioides* (Blue bush) is a berry-fruited small or medium sized tree, with ascending branches from the family Ebenaceae (Figure 1.5) (Van Wyk & Van Wyk, 1997; Dziba *et al.*, 2003). The plant root is frequently used as a chewing stick to clean teeth, and has been reported to have antibacterial compounds against oral pathogens (Cai *et al.*, 2000; Ndukwe *et al.*, 2005; More *et al.*, 2008).

Figure 1.5: *Diospyros lycioides* subsp. *lycioides* (Adapted from ttp://www.kyffhauser.co.za)

Faurea saligna (Transvaal beech) is a small to medium sized semi deciduous tree from the family Proteaceae, which occurs mostly in bushveld on sandy soil (Figure 1.6). The bark is often used for tanning leather and is well known to be unsusceptible to borers and termites. The bark is also medicinally used as a tonic and the roots are boiled to treat diarrhoea and indigestion. The leaves have been reported to be traditionally used for antibacterial and haemorrhoid treatment (Chitemerere & Mukanganyama, 2011).

Figure 1.6: *Faurea saligna* leaves (Adapted from http://www.zimbabweflora.co.zw)

Kalanchoe thyrsiflora is a succulent with fleshy leaves from a slightly swollen rootstock (Figure 1.7) and anatomically the xylem cells are not found in clusters. The plant usually dies after it flowers. *Kalanchoe thyrsiflora* is from the Crassulaceae family, and occurs in grassland (Van Wyk *et al.*, 1997b). *Kalanchoe thyrsiflora* has been reported to have bufadienolides that are poisonous to livestock (Fouche *et al.*, 2008).

Figure 1.7: *Kalanchoe thyrsiflora* (Adapted from http://fr.academic.ru)

Ochna natalitia is a multistemmed evergreen small shrub from the Ochnaceae family; all species of this family have attractive yellow flowers (Figure 1.8). The leaves of *Ochna natalitia* are alternate and the bark is dark and rough. The leaf hexane, acetone, dichloromethane and methanol extracts of *Ochna natalitia* were tested for antimicrobial activity against bacteria (*Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) and various fungi (*Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans*, *Microsporum canis* and *Sporothrix schenckii*) (Mokoka *et al.*, 2010; Suleiman *et al.*, 2010). *Ochna natalitia* had an inhibitory activity between 0.002 and 0.12 mg/mL for all extracts against *Cryptococcus neoformans* a fungus causing cryptococcosis in humans and animals (Mokoka *et al.*, 2010).

Figure 1.8: *Ochna natalitia* with yellow flowers (Adapted from http://www.sciag.ukzn.ac.za)

Pappea capensis (Jacket plum) is a deciduous tree (7-13 m) with spreading branches and furry green capsule fruit from the Sapindaceae family (figure 1.9). The tree is heavily browsed by game and stock. The seeds oil is edible and somewhat purgative and used topically for ringworms. The bark is medicinally used in Bechuana-land for the treatment of venereal diseases. The broth decoction of the bark is used by the Masai as an aphrodisiac for men (Watt & Breyer-Brandwijk, 1962). Alcoholic beverages or fruit can be made from the fruit. The seed oil is used for oiling guns and soap making. The plant has been reported to be used for chronic Joint pains in Kenya (Wambugu *et al.*, 2011). *Pappea capensis* dichloromethane leaves extract has been reported to have good anti-inflammatory activity. The extract showed high inhibition activity against COX-1 and moderate inhibition against COX-2 (Mulaudzi *et al.*, 2013).

Figure 1.9: *Pappea capensis* fruit (Adapted from http://www.zimbabweflora.co.zw)

Peltophorum africanum (weeping wattle) is a small to medium sized tree with a dense leafy crown occurring in the bushveld Savanna from the Fabaceae family (Figure 1.10). The plant excretes almost pure water and drips constantly to the ground, thus the common name weeping wattle (Van Wyk & Van Wyk, 1997). The bark and the roots are used medicinally. For example, an infusion of the bark is taken orally as anthelmintic and for abdominal disorders (Watt & Breyer-Brandwijk, 1962).

The powdered root and stem bark are taken by the Karanga for diarrhoea and dysenteries. Tannins have been reported to be found in the bark and the roots (Watt & Breyer-Brandwijk, 1962).

Figure 1.10: *Peltophorum africanum* (Adapted from: www.zimbabweflora.co.zw)

Phyllanthus reticulatus (Potato bush) is a multi-stemmed shrub or small tree (Figure 1.11) from the family Euphorbiaceae occurring in bushveld (Van Wyk & Van Wyk, 1997). A strong scent of potatoes is emitted by the plant mostly spring and summer evenings. The fruit is used to make black dye and the leaves are used for medicinal purpose (Van Wyk & Van Wyk, 1997). The powdered leaves are used by several tribes as well as European farmers to apply to sores, burns and venereal sores. The bark and leaves are also used as a diuretic. The roots were used to poison criminals in Zambia who were sentenced to death (Watt & Breyer-Brandwijk, 1962; Van Wyk & Van Wyk, 1997).

Figure 1.11: *Phyllanthus reticulatus* with fruit (Adapted from http://2.bp.blogspot.com)

Rhoicissus tridentata (Figure 1.12) is a deciduous climber or sprawling shrub commonly known as bushman's grape from the family Vitaceae. The plant has nearly entire leaf margins and up to four teeth on the terminal leaflet tip. It is found in the eastern parts of South Africa and in most of Zimbabwe growing in grassy slopes and rocky areas. *Rhoicissus tridentata* subsp. *tridentata* was reported to be used by the Zulu to treat abdominal pains, cuts, epilepsy, infertility, menorrhagia, to ensure a safe delivery during pregnancy, renal complaints, sprained ankles, stomach ailments, and sores (Lin *et al.*, 1999). Decoctions and infusions of *Rhoicissus tridentata* subsp. *cuneifolia* roots and lignotubers are used during pregnancy (Katsoulis *et al.*, 2000). To investigate the pharmacological action of an aqueous extract of *R. tridentata* subsp. *cuneifolia* roots, isolated rat uterus and ileum were used. The extract directly stimulated concentration dependent contractions of the uterus and ileum (Katsoulis *et al.*, 2000).

Figure 1.12: *Rhoicissus tridentata* (adapted from www.zimbabweflora.co.zw)

Senna petersiana (Monkey pod) is a shrub or small tree with large loose branched flowers from the Fabaceae family occurring in bushveld and sand forest (figure 1.13) (Van Wyk & Van Wyk, 1997). The plant has indehiscent pods, which are flattened and dark brown in colour. *Senna petersiana* is used to treat sexually transmitted diseases and was reported to

have good antimicrobial activity and cytotoxicity, luteolin was isolated from the extract and tested for antiviral activity (Tshikalange *et al.*, 2005). The extract and Luteolin showed some activity at the highest nontoxic concentration of 24 and 500 µg/ml, respectively (Tshikalange *et al.*, 2005).

Figure 1.13: *Senna petersiana* flowering (Adapted from http://upload.wikimedia.org)

Solanum tomentosum var. *tomentosum* a soft wooded shrub covered with fine bristles from the family Solanaceae, and commonly found in grassland (Figure 1.14). The fruit are yellow to bright orange in colour. The methanol extract was reported to have inhibitory activity to the growth of fungi with percentage ranging from 47.22 to 50.56 on *Aspergillus niger* and *Fusarium oxysporum*, respectively (Aliero & Afolayan, 2006).

Figure 1.14: *Solanum tomentosum* var. *tomentosum* leaves and fruit (Adapted from http://lh5.ggpht.com)

Sphedamnocarpus pruriens var. *pruriens* a deciduous climber with twining stems and leaves densely covered with greyish or silvery hair from the family Malpighiaceae (Figure 1.15). It is found in open woodlands, open grass land and forest floors of Namibia, South Africa, Zimbabwe and Botswana (Van Wyk *et al.*, 1997b). *Sphedamnocarpus pruriens* is used in Southern zimbabwe as a snake bite remedy. A mixture containing *S. pruriens* and *Securidaca longipedunculata* is used by the Chopi as medicine to treat persons possessed by evil spirits (Watt & Breyer-Brandwijk, 1962). The species has been reported to be macerated with various plants to treat infertility (Tan & Wiart, 2014).

Figure 1.15: *Sphedamnocarpus pruriens* var. *pruriens* (Adapted from http://www.zimbabweflora.co.zw)

Terminalia sericea (silver cluster leaf) is a small to medium sized tree with silvery grey foliage from the Combretaceae family (Van Wyk & Van Wyk, 1997).The roots are used by the Tswana for the treatment of stomach disorders and diarrhoea. The leaves are chewed to arrest purging. Eye lotions are made from the decoction of the root and the bark is taken against diabetes. *Terminalia* species contain several pentacyclic triterpenoids of which the main compounds of the root include sericic acid and sericoside (Van Wyk *et al.*, 1997a). Triterpenoids are known for their antimicrobial and anti-inflammatory activity (Van Wyk *et al.*, 1997a).

Figure 1.16: *Terminalia sericea* leaves [Adapted from http://www.westafricanplants.senckenberg.de]

Xerophyta retinervis (Black sticky lily or monkey`s tail) is an irregularly branched fibrous perennial from the Velloziaceae family (Figure 1.17). The dried roots of the plant are smoked for asthma relief and the whole plant is burned as the smoke is reported to stop nose bleeds (Van Wyk *et al.*, 1997a). The chemical compositions of *Xerophyta* are poorly known, the only main compound discovered was amentoflavone. Flavonoids are said to be responsible for the physiological activity as flavonoids are known to reduce capillary fragility (Van Wyk *et al.*, 1997a).

Figure 1.17: *Xerophyta retinervis* with flowers [Adapted from http://www.fao.org]

1.6 Aims and objectives

- To collect and record the ethnobotanical knowledge of a local shangaan traditional healer.
- To determine the conservation status and use value of documented medicinal plants.
- To investigate antimicrobial activity of selected plants against oral pathogens.
- To investigate antioxidant and cytotoxic activities of active plants.

1.7 Scope of dissertation

Chapter 1, outlines background medicinal plants, oral pathogen and literature review on traditional medicine. Chapter 2 focuses on methodology, which covers ethnobotany, antimicrobial assay, antioxidant assay and cytotoxicity assay. Results for ethnobotany, antimicrobial assay, antioxidant assay and cytotoxicity assay are discussed in chapter 3. The general discussion, conclusion and future work for the study are outlined in chapter 4. Chapter 5 includes appendices for semi-structured questionnaire, ethnobotanical database plant list for plants identified and cytotoxicity IC_{50}.

1.8 References

Acacia karroo. [Online]. (Available from: http://witbos.co.za/photos/acacia-karroo-77d9d.jpg) [Accessed: 12 August 2014]

Aliero AA & Afolayan AJ (2006). Antimicrobial activity of Solanum tomentosum. *African Jounal of plant science and Biotechnology* **5,** 369-372.

Alviano WS, Alviano DS, Diniz CuG, Antoniolli AR, Alviano CS, Farias LM, Carvalho MAR, Souza MMG & Bolognese AM (2008). *In vitro* antioxidant potential of medicinal plant extracts and their activities against oral bacteria based on Brazilian folk medicine. *Archives of Oral Biology* **53,** 545-552.

Antidesma venosum. [Online]. (Available from: http://www.westafricanplants.senckenberg.de/images/pictures/Antidesma_venosum_PBirnb_CAN04253 _100698.JPG) [Accessed: 12 August 2014]

Arbes Jr SJ & Matsui EC (2011). Can oral pathogens influence allergic disease? *Journal of Allergy and Clinical Immunology* **127,** 1119-1127.

Arwa PS, Onyango JC & Nyunja RO (2008). Phytochemical compounds and antimicrobial activity of extracts of *Rhoicissus* plant (*Rhoicissus revoilli*)(Planch). *Plant Sciences Research* **1,** 68-73.

Barasch A & Griffin AV (2008). Miconazole revisited: new evidence of antifungal efficacy from laboratory and clinical trials. *Future microbiology* **3,** 265-269.

Berman J & Sudbery PE (2002). Candida albicans: a molecular revolution built on lessons from budding yeast. *Nature Reviews Genetics* **3,** 918-932.

Cai L, Wei G-X, van der Bijl P & Wu CD (2000). Namibian chewing stick, *Diospyros lycioides*, contains antibacterial compounds against oral pathogens. *Journal of Agricultural and Food Chemistry* **48,** 909-914.

Chitemerere TA & Mukanganyama S (2011). In vitro antibacterial activity of selected medicinal plants from Zimbabwe. *African J Pl Sci Biotech* **5,** 1-7.

Combretum collinum. [Online]. (Available from: http://www.zimbabweflora.co.zw /speciesdata/images/14/141830-2.jpg) [Accessed: 12 August 2014]

Cotton CM (1996). *Ethnobotany: principles and applications*. John Wiley & Sons.

Davidson-Hunt I (2000). Ecological ethnobotany: Stumbling toward new practices and paradigms. *MASA Journal* **16,** 1-13.

Dichrostachys cinerea. [Online]. (Available from://sshivanidiwani64.blogspot.com) [Accessed 12 August 2014]

Diospyros lycioides. [Online]. (Available from: http://www.kyffhauser.co.za

/Plants1/Diospyros_lycioides_subsp_lycioides/Image4.jpg)
[Accessed: 12 August 2014]

Dorn BR, Leung KP & Progulske-Fox A (1998). Invasion of human oral epithelial cells by *Prevotella intermedia*. *Infection and immunity* **66,** 6054-6057.

Dziba LE, Scogings PF, Gordon IJ & Raats JG (2003). Effects of season and breed on browse species intake rates and diet selection by goats in the False Thornveld of the Eastern Cape, South Africa. *Small Ruminant Research* **47,** 17-30.

Faurea saligna. [Online]. (Available from: http://www.zimbabweflora.co.zw /speciesdata/images/12/120700-5.jpg) [Accessed: 12 August 2014]

Fennell CW, Lindsey KL, McGaw LJ, Sparg SG, Stafford GI, Elgorashi EE, Grace OM & Staden Jv (2004). Assessing African medicinal plants for efficacy and safety: Pharmacological screening and toxicity. *Journal of Ethnopharmacology* **94,** 205 - 217.

Fouche G, Cragg GM, Pillay P, Kolesnikova N, Maharaj VJ & Senabe J (2008). *In vitro* anticancer screening of South African plants. *Journal of Ethnopharmacology* **119,** 455-461.

Gendron R, Grenier D & Maheu-Robert L-F (2000). The oral cavity as a reservoir of bacterial pathogens for focal infections. *Microbes and infection* **2,** 897-906.

Ghorbani A, Naghibi F & Mosaddegh M (2006). Ethnobotany, ethnopharmacology and drug discovery. *Iran J Pharm Sci* **2,** 109-118.

Gimeno CJ, Ljungdahl PO, Styles CA & Fink GR (1992). Unipolar cell divisions in the yeast S. cerevisiae lead to filamentous growth: Regulation by starvation and< i> RAS</i>. *Cell* **68,** 1077-1090.

Gursoy M, Haraldsson G, Hyvonen M, Sorsa T, Pajukanta R & Kononen E (2009). Does the frequency of Prevotella intermedia increase during pregnancy? *Oral Microbiology and Immunology* **24,** 299-303.

Hoareau L & DaSilva EJ (1999). Medicinal plants: a re-emerging health aid. *Electronic Journal of biotechnology* **2,** 3-4.

Hoffmann D (2003). Medical herbalism. *The Science and Practice of Herbal Medicine. Rochester, VT: Inner Traditions/Bear & Co*.

Kalanchoe thyrsiflora. [Online]. (Available from: http://fr.academic.ru/pictures/frwiki/75/ Kalanchoe_thrysiflora.jpg) [Accessed: 12 August 2014]

Katsoulis LC, Veale DJH & Havlik I (2000). The pharmacological action of Rhoicissus tridentata on isolated rat uterus and ileum. *Phytotherapy Research* **14,** 460-462.

Kinane DF (2001). Causation and pathogenesis of periodontal disease. *Periodontology 2000* **25,** 8-20.

Lin J, Opoku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, K JÃeger A & Van Staden J (1999). Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. *Journal of Ethnopharmacology* **68,** 267-274.

Madhavan V, Vedavathi B, Raju A, Murali A & Yoganarasimhan S (2011). Sedative activity studies on the aqueous and alcohol extracts of the stem bark of Madanaphala-an ayurvedic drug (*Catunaregam spinosa* (Thunberg) Tiruvengadam). *Asian Journal of Traditional Medicines* **6,** 203-210.

McChesney JD, Venkataraman SK & Henri JT (2007). Plant natural products: Back to the future or into extinction? *Phytochemistry* **68,** 2015-2022.

Mokoka TA, McGaw LJ & Eloff JN (2010). Antifungal efficacy of ten selected South African plant species against *Cryptococcus neoformans*. *Pharmaceutical Biology* **48,** 397-404.

More G, Tshikalange TE, Lall N, Botha F & Meyer JJM (2008). Antimicrobial activity of medicinal plants against oral microorganisms. *Journal of Ethnopharmacology* **119,** 473-477.

Mulaudzi RB, Ndhlala AR, Kulkarni MG, Finnie JF & Staden JV (2013). Anti-inflammatory and mutagenic evaluation of medicinal plants used by Venda people against venereal and related diseases. *Journal of Ethnopharmacology* **146,** 173-179.

Ndukwe KC, Okeke IN, Lamikanra A, Adesina SK & Aboderin O (2005). Antibacterial activity of aqueous extracts of selected chewing sticks. *Journal Contemporary Dentistry Practice* **6,** 086-094.

Ochna natalitia. [Online]. (Available from: http://www.sciag.ukzn.ac.za/bcs/images/Gdnplants/Ochna%20natalitia.JPG) [Accessed: 12 August 2014]

Palo RT (1984). Distribution of birch (Betula spp.), willow (Salix spp.), and poplar (Populus spp.) secondary metabolites and their potential role as chemical defense against herbivores. *Journal of Chemical Ecology* **10,** 499-520.

Pappea capensis. [Online]. (Available from: http://www.zimbabweflora.co.zw/speciesdata/images/13/137450-1.jpg) [Accessed: 12 August 2014]

Peltophorum africanum. [Online]. (Available from: http://www.zimbabweflora.co.zw/speciesdata/images/12/127220-5.jpg) [Accessed: 12 August 2014]

Phyllanthus reticulatus. [Online]. (Available from: http://2.bp.blogspot.com/Phyllanthus+reticulatus1.jpg) [Accessed: 12 August 2014]

Rhoicissus tridentate. [Online]. (Available from: http://www.zimbabweflora.co.zw/speciesdata/images/13/137860-3.jpg) [Accessed: 12 August 2014]

Rosas-Pinon Y, Mejia A, Diaz-Ruiz G, Aguilar MI, Sanchez-Nieto S & Rivero-Cruz JF (2012). Ethnobotanical survey and antibacterial activity of plants used in the Altiplane region of Mexico for the treatment of oral cavity infections. *Journal of Ethnopharmacology* **141,** 860-865.

Sampaio FbC, Maria do Socorro VP, Dias CS, Costa VCO, Conde NCO & Buzalaf MAR (2009). *In vitro* antimicrobial activity of *Caesalpinia ferrea* Martius fruits against oral pathogens. *Journal of Ethnopharmacology* **124,** 289-294.

SANBI (2011) Annual report. [Online]. (Available from: http://www.sanbi.org/sites/default/files/documents/documents/sanbi-annual-report-2011-2012.pdf) [Accessed: 02 February 2012]

Sardesai VM (2002). Herbal medicines: Poisons or potions? *Journal of Laboratory and Clinical Medicine* **139,** 343-348.

Senna petersiana. [Online]. (Available from: http://upload.wikimedia.org/wikipedia/commons/2/2d/Senna_petersiana,_bloeiwyse,_Kloof.jpg) [Accessed: 12 August 2014]

Solanum tomentosum. [Online]. (Available from: http://lh5.ggpht.com/Solanum tomentosum fruit.jpg) [Accessed: 12 August 2014]

Sphedamnocarpus pruriens. [Online]. (Available from: http://www.zimbabweflora.co.zw/speciesdata/images/13/133640-2.jpg) [Accessed: 12 August 2014]

Suleiman MM, McGaw LI, Naidoo V & Eloff J (2010). Detection of antimicrobial compounds by bioautography of different extracts of leaves of selected South African tree species. *African Journal of Traditional, Complementary and Alternative Medicines* **7**.

Tan KK & Wiart C (2014). Botanical descriptions, Ethnomedicinal and no-medicinal uses of the genus Artabotrys R. BR. *Internatinal Journal Current Pharmceutical Research* **6,** 34-40.

Terminalia sericea. [Online] (Available from: ttp://www.westafricanplants.senckenberg.de/images/pictures/terminalia_sericea_dsc_0936_azizka_2982_bcbb0d.jpg) [Accessed: 12 August 2014]

Tshikalange TE, Meyer JJM & Hussein AA (2005). Antimicrobial activity, toxicity and the isolation of a bioactive compound from plants used to treat sexually transmitted diseases. *Journal of ethnopharmacology* **96,** 515-519.

Van Wyk B-E, Oudtshoorn Bv & Gericke N (1997a). *Medicinal Plants of South Africa*. Briza, Pretoria.

Van Wyk B, Malan S, Lowrey TK & Pienaar A (1997b). *Field Guide to the Wild Flowers of the Highveld: Also useful in adjacent grassland and bushveld*. Struik, Cape Town.

Van Wyk B & Van Wyk P (1997). *Field Guide to Trees of Southern Africa*. Struik, Cape Town.

Veilleux C & King SR (1996). *An Introduction to Ethnobotany*.

Wambugu SN, Mathiu PM, Gakuya DW, Kanui TI, Kabasa JD & Kiama SG (2011). Medicinal plants used in the management of chronic joint pains in Machakos and Makueni counties, Kenya. *Journal of Ethnopharmacology* **137,** 945-955.

Watt J & Breyer-Brandwijk M (1962). *The medicinal and poisonous plants of Southern and Eastern Africa*. E. & S. Livingstone LTD., Edinburgh and London.

Williams DH, Stone MJ, Hauck PR & Rahman SK (1989). Why are secondary metabolites (natural products) biosynthesized? *Journal of Natural Products* **52,** 1189-1208.

Williams RC (1990). Periodontal disease. *New England Journal of Medicine* **322,** 373-382.

World Health Organisation (2002). WHO traditional medicine strategy 2002-2005.

xerophyta retinervis. [Online].(Available from: http://www.fao.org/ag/agp/AGPC/doc/show/SAfrica/FIGURE37.JPG) [Accessed: 12 August

Zambon JJ (1996). Periodontal diseases: Microbial factors. *Annals of Periodontology* **1,** 879-925.

Chapter 2: Methodology

2.1 Ethnobotanical survey

2.1.1 Study area description (geography, geology, climate, population, languages and terrestrial flora)

Southern Africa has a rich and varied flora (Botha & Penrith, 2008). South Africa occupies only about 2% of the world's land area, but is home to about 10% of all plant species (Biggs *et al*., 2006). Three of the world's 34 biodiversity hotspots are the Cape Floristic Region, the Succulent Karoo and the Maputaland-Pondoland Region (Biggs *et al*., 2006). This indicates the great potential for the discovery of novel bioactive chemicals from the plants (Louw *et al.*, 2002). Mpumalanga is one of the nine South African provinces in the Maputaland-Pondoland Region harbouring the southern half of the Kruger National Park and other centres of endemism. Mpumalanga (Figure 2.1) is divided into three districts, namely; the Gert Sibande District, Nkangala district and Ehlanzeni District. The Ehlanzeni District Municipality (figure 2.1) is located in the north eastern part of Mpumalanga Province bordered by Mozambique and Swaziland. The Ehlanzeni district municipality covers an area of 27 895.47 km^2. Thus the district is divided into five local municipalities, namely; Mbombela, Nkomazi, Bushbuckridge, Umjindi, and Thaba Chweu (Figure 2.1). The Bushbuckridge Local Municipality covers an area of 2 589.59 Km^2 with Dwarsloop, Thulamahashe, Maviljan, Shatate, Mkhuhlu and Marite the main townships. The rest of the geographical area in Bushbuckridge is made up of villages (Mpumalanga Provincial Government, 2011). The dominant languages in Mpumalanga include Siswati 30% a language from the neighbouring country Swaziland, with 26% speaking isiZulu, 10.3% isiNdebele, 10.2% Northern Sotho and 11.6% Xitsonga.

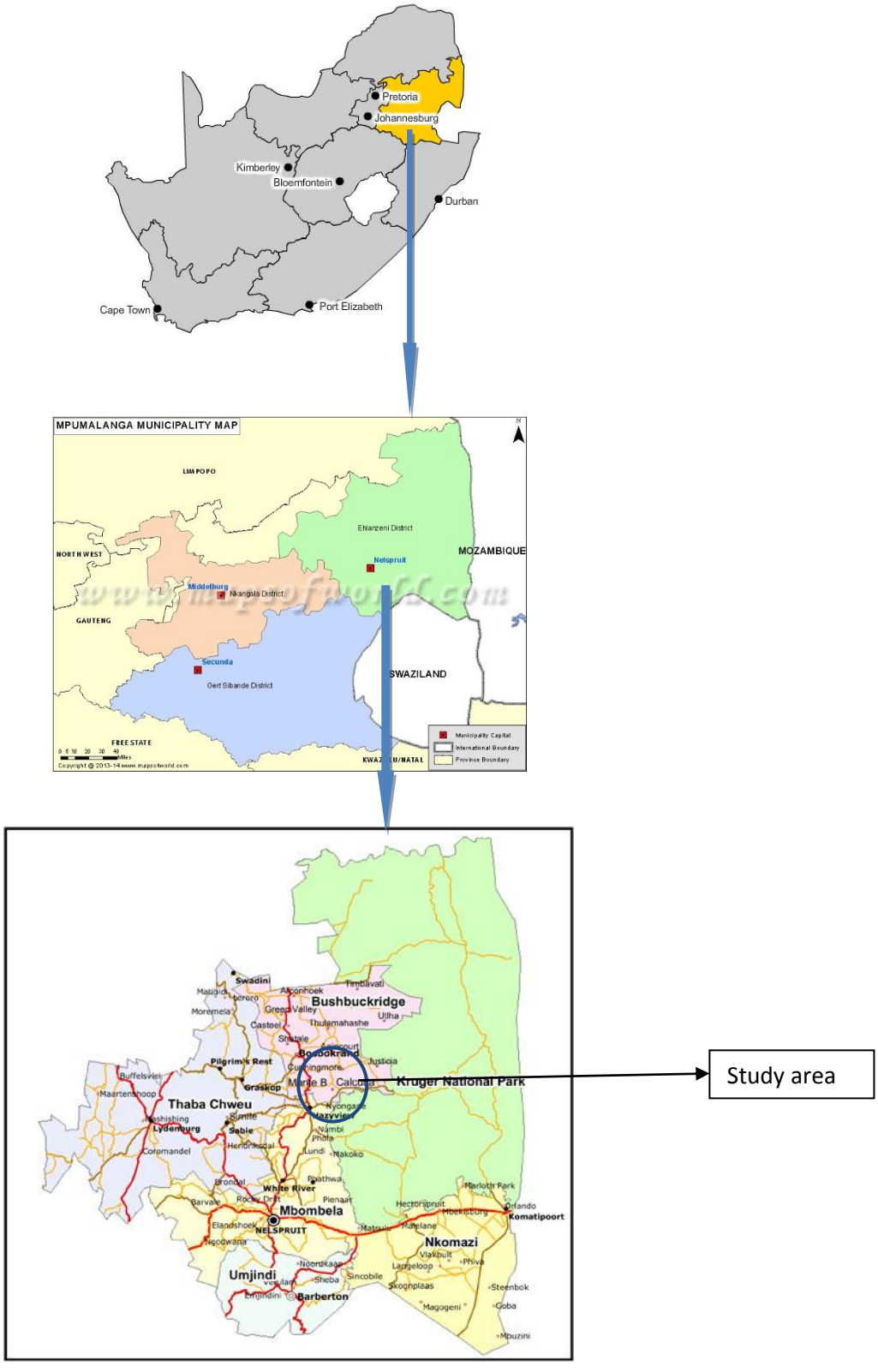

Figure 2.1: South African map showing Mpumalanga province and Ehlanzeni district municipality [adapted from www.mpumalanga.gov.za]

The Tsonga are a diverse people including the Shangaan, Tonga and smaller ethnic groups. The Shangaan tribe came to be in the 19th century when King Shaka Zulu, sent Soshangane to conquer the Tsonga people in the southern Mozambique area. Soshangane decided to settle and make the place home, inhabited by scattered communities of peace loving people during the Mfecane upheaval rather than return to Shaka (Mwakikagile, 2008). The Shangaan were a mixture of Nguni (Swazi, Zulu and Xhosa) and Tsonga speakers (Ronga, Ndzawu, Shona, Chopi tribes), which were conguered and subjugated by Soshangane (Mwakikagile, 2008). The Shangaan community structure is based on tribal relationships. The authority of the specific tribal area comes from one chief or hosi who is recognized by the people (Anthony *et al.*, 2011).The shangaan today live in areas mainly between the Kruger National Park and the Drakensburg Mountains, in Mpumalanga and the Limpopo Province of South Africa, with sister tribes in southern Mozambique (Siyabona Africa, 2012).

The knowledge about potential medicinal plants is known by the specialists within an indigenous community in most cases traditional healers (Vandebroek *et al.*, 2004). The knowledge is passed down from generation to generation. The knowledge of medicinal plant is slowly getting lost, Because of deforestation and migration thus the relationship between people and plants is also lost (Nesheim *et al.*, 2006). Traditionally Shangaan people are agriculturalists, with each family living in a family village. Because of war and famine the shangaan people were forced to leave their farming way of life to settle in townships and cities. Recording the shangaan traditional medicine is crucial for the heritage of the country. No account of shangaan traditional medicine has been found in literature. Existing villages under the Jongilanga Traditional council like Agincourt, Cunningmoore, Croecketlawn, Dumphries, Huntington, Justicia, Kildare, Lillydale and Ronaldsey (Figure 2.2) are suitable for such a study, because traditional healers are still the first option when seeking healing. An

Ethnobotanical survey is relevant to record the information and further tests such as antimicrobial assay can be done on the collected plant samples for bioprospecting.

Figure 2.2: Study area map with GPS coordinates 24°S 31°E CC and 24°S 31°E CD [adapted from www.maps.google.co.za]

The shangaan people are losing their traditional way of life losing the important knowledge of traditional medicinal plants. This makes it crucial to research the shangaan traditional medicine for the heritage of the country. As herbal medicine is a growing field each year this will also have economic benefits for the villages and the country if a stage of commercialization is reached. There are four approaches to select for pharmacological study namely ethnobotanical, taxonomic, ethno-medicinal and the random approach (Ahmad *et al.*, 2006). Sometimes there is a combination of several criteria for an ethnobotanical survey. The ethno-medicinal approach credibility is given to traditional use as this has proven use over years and the plants are collected and tested. This purpose allows for finding new plants and novel chemical compounds with economic interest.

James Mahore is a registered shangaan traditional healer living and working in Cork, a village near Hazyview in Mpumalanga. Guides and Trackers Direct use to train field guides in traditional shangaan medicinal uses of plants. It was during these training sessions that Guides and Trackers realized that Mr. J. Mahore`s plant knowledge was enormous and he did not have a daughter or son interested in learning from him the traditional uses of plants, meaning his knowledge would be lost forever. Guides and Trackers Direct managed to negotiate a sponsorship grant for recording of Mr. J. Mahore's knowledge of plants and their traditional uses to secure his knowledge for future generations. Guides and Trackers Direct approached the University of Pretoria to help to successfully record this information in the correct format and following legal and scientific protocol.

2.1.2 Legal cover of the study

To discover and develop new pharmaceutical includes sample acquisition, primary in vitro qualitative assay, bioassay directed isolation of active constituent, structural elucidation, tertiary assay and preclinical development respectively (McChesney *et al.*, 2007). Before a research study commences, parties are identified and information is disclosed. Contracts are drawn up, in case a plant with active constituent is acquired from traditional or indigenous people, to evaluate agreed upon the benefits should the product reach commercialization. Proper intellectual property and information transfer agreements are important to prevent bio-piracy lawsuits. Bio-piracy refers to an un-authorized use of traditional knowledge or biological resources (Kartal, 2007).

All legal aspects for the study were adhered to and the confidentiality agreement, material transfer agreement were signed during different phases with the stake holders. The

bioprospecting discovery phase notification form was completed by the University and sent to the minister of the Department of Environmental Affairs.

2.1.3 Ethnobotanical data collection

Six field trips were carried out to collect ethnobotanical data and medicinal plants in villages under the Jongilanga Traditional Council, from April 2011 to April 2013. Ethnobotanical data was collected with randomly selected informants (traditional healers) and field interviews with key informant (Mr James Mahore). All ethical aspects were adhered to before the data collection. The investigation was carried out using questionnaires designed to facilitate semi-structured face to face interviews with traditional healers. The objectives of the study were explained before seeking their consent to engage in these interviews. The Questionnaire (Appendix I) was mainly focused on the medicinal plant uses, local names, preparation methods and administration. A total of 15 traditional healers from 8 different villages were interviewed after getting their voluntary consent. The interviews were carried out in the homes of the traditional healers, with the exception of our key informant who was interviewed in the field during plant collection.

2.1.4 Collection of plants

Field work essentially needs a global positioning system (GPS) device, plant presser with blotting paper, camera, pruning scissors, collection bags and a spade. All plant specimens were collected from different villages within the Jongilanga community in the

presence of key informant and a translator so as to ascertain the correct identification and ensure local names are spelled correctly (Figure 2.3). Most of the plant species were collected in both flowering and fruiting conditions. Herbarium specimens were prepared following standard procedure (Fish, 1999); Victor *et al*., 2004) and deposited into the HGWJ Schweickert Herbarium of the University of Pretoria. Each plant species was provided a specimen number, local name, location and GPS coordinates were recorded in a field note book.

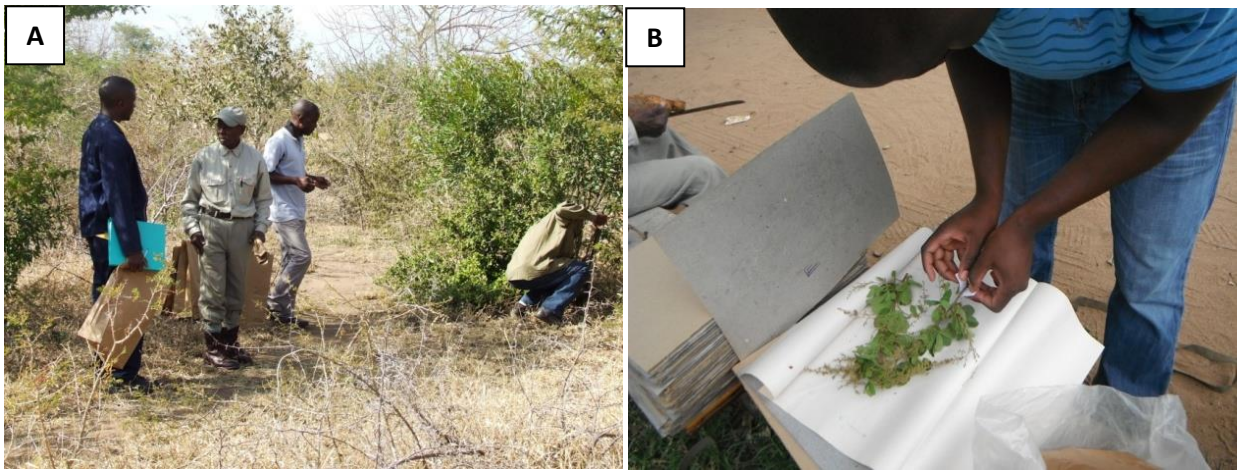

Figure 2.3: (A) Field work the collection (B) pressing of plant material

2.1.5 Taxonomic identification

The plants were identified and their scientific names were recorded from reputed flora and fauna (Van Wyk *et al.*, 1997; Van Wyk & Van Wyk, 1997). Some of the medicinal plants were identified in the field or through a literature review. Voucher specimen numbers were allocated to the herbarium specimens after taxonomic identification. The herbarium specimens that could not be taxonomically identified at the HGWJ Schweickert Herbarium of

the University of Pretoria were sent to South African National Biodiversity Institute for further analysis. Each plant species was assigned a unique voucher specimen number.

2.1.6 Quantitative analysis of ethnobotanical results

The data were entered into Microsoft Excel sheets for analysis and identifying various proportions, such as plant part used, plant families and number of plant used per ailment category. Various quantitative indices, including the informant consensus factor, use value and use report, were applied.

2.1.6.1 Informant consensus factor

The frequency of individual plants medicinal use can be used for analysing the ethnobotanical importance of the respective taxa (Ankli *et al.*, 1999). Species that are frequently cited in a group of traditional uses are regarded to be of greater ethnobotanical importance than those which are cited by few informants. Usage frequency for each plant and the informant consensus factor for each category of use, reveals a consensus among the healers on the use of plant species and diseases treated (Amiguet *et al.*, 2005). Thus a greater consensus factor can be the basis for further phytochemical and pharmacological evaluations of the traditional uses (Ankli *et al.*, 1999).

Traditional healers were selected in different villages to get a consensus factor for medicinal usage of plant species. The sampling was non-random as local traditional healers were interviewed under the assumption that local traditional healers would provide more specific and higher quality medicinal plant information (Gazzaneo *et al.*, 2005).

The data were analysed using Microsoft Excel sheet to identify various proportions such as plant part used, plant families, number of plant used per ailment category. The informant concensus factor (Fic) for different ailment categories was calculated using the expression:

$$F_{ic}=N_{ur}-T/N_{ur}-1$$

Where N_{ur}= number of use reported in a particular ailment category and T= number of plant species used to treat that particular category by informants.

2.1.6.2 Use value (UV) and use report (UR)

The use value (UV) refers to a demonstration of the relative importance of plants known locally. The UV is calculated using the formula (Bibi *et al.*, 2014).

$$UV = \sum U/n$$

Where UV is the value of the species, U is the number of use reports cited by each informant for a given plant species and n is the total number of informants interviewed for a given plant (Bibi *et al.*, 2014). If there are many use reports for a plant species, the UV will be high (near 1). If there are fewer reports related to the plant species the UV values are close to 0. The UV will not indicate whether a plant is used for a single or multiple purposes (Bibi *et al.*, 2014). To determine the plants with the highest use in the treatment of an ailment UV is applied, while use report (UR) is the use recorded for every species (Bibi *et al.*, 2014; Yaseen *et al.*, 2015).

2.1.7 Conservation status

The conservation status of a species indicates whether the species still exist or the likelihood the species will still exist in the future (Baillie *et al.*, 2004). There are a number of factors taken into account when assessing the conservation status of a species. These include the number remaining, the overall population increase or decrease over time, breeding success rates and known threats (Baillie *et al.*, 2004). The International Union for Conservation of Nature (IUCN) has a Red List of threatened species, ranking system and best known worldwide conservation status (Baillie *et al.*, 2004; Rodrigues *et al.*, 2006). South Africa is one of the few countries that fully assessed the conservation status of its entire flora. Thus the Red List of South African Plants Online was used to provide up to date information on the national conservation status of South Africa's indigenous plants (redlist.sanbi.org).

2.2 Biological assays

2.2.1 Preparation of extracts

The plant material was air dried at room temperature (25 °C), and ground to fine powder using a Janke & Kunkel (IKA Labortechnik, Germany) grinder. The powdered material was extracted by shaking with 400 ml ethanol (Merck Chemicals (Pty) Ltd Wadeville, South Africa) on a Labcon shaker (Lab Design Engineering, Maraisburg, South Africa). The samples were filtered through a Whattman No.1 (110 mm diameter) (Merck Chemicals (Pty) Ltd Wadeville, South Africa) filter paper using a vacuum filter (Merck Chemicals (Pty) Ltd Wadeville, South Africa). The process was repeated several times. The

solvent was evaporated in a BÜCHI Rotavapor (Labotec (Pty) Ltd. Halfway House, South Africa) at a temperature of 40 °C. The extracts were further dried at room temperature after which they were subjected to antimicrobial assay.

2.2.2 Antimicrobial assay

Aromatic and saturated organic compounds in plants generally possess antimicrobial activity and are initially extracted from plants with ethanol or methanol. Disc diffusion and minimum inhibitory concentration (MIC) assay are two of the most used methods to investigate the antimicrobial activity of South African medicinal plants (Van Vuuren, 2008). Disc diffusion studies were initially the preferred method due to its simplicity and capacity to analyse a large number of test samples. Extract studies to determine antimicrobial activity are reported to mainly use methods for MIC assay. The other method in plant extract studies used is the bio-autographic assay which is the one used to evaluate antimicrobial of isolated bioactive compounds (Van Vuuren, 2008). A quantitative method to determine antimicrobial activity by measuring MIC based on the principle of contact of a test organism to a series of dilutions of the test substance (Van Vuuren, 2008). Most assays involving MIC methodology are widely used and accepted criterion for measuring the susceptibility of organisms to inhibitors. Use of a more quantifiable MIC assay techniques are presently the more preferred method of antimicrobial assessment (Van Vuuren, 2008).

2.2.2.1 Microbial strains

Candida albicans (ATCC10231; 4×10^7 CFU/ml) and *Prevotella intermedia (*ATCC 25611; 4×10^8 CFU/ml) were used.

2.2.2.2 Determination of minimum inhibitory concentration and minimum microbicidal concentration

An *in vitro* micro-dilution assay on a 96-well micro plates (Eloff, 1998) was used to obtain the MIC and MMC values of the ethanol extracts against the microorganisms under study. The ethanol extract of the selected 90 plants were serially diluted in the 96 well micro-plates with 48 hours old microorganisms *Candida albicans* (ATCC10231; 4×10^7 CFU/ml) and *Prevotella intermedia (*ATCC 25611; 4×10^8 CFU/ml) grown at 37 °C in Casein peptone soymeal peptone broth. The final concentration of extracts ranged from 25.0 mg/ml to 0.195 mg/ml. Chorhexidine was used as a positive control and for the negative control broth was used. Microbial growth was indicated by adding 40 µl of (0.2 mg/ml) 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (INT) to micro-plate wells and incubated 37 °C for 48 hours. MIC was defined as the lowest concentration that inhibited the colour change of INT. The MMC was determined by adding 50 µl of the suspensions from the wells, which did not show any growth after incubation during MIC assay, to 150 µl of fresh nutrient broth. These suspensions were re-incubated at 37 °C for 48 hours. The MMC was determined as the lowest concentration of extract which inhibited 100% growth of the microorganism.

2.2.3 Anti-oxidant assay

There has been great interest for the last several decades in identifying free radical scavengers or antioxidants that can retard the oxidative damage of DNA (Abbas *et al.*, 2014). An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent (Agbor *et al.*, 2006). It has been shown that various antioxidants occur naturally in plants (Hall & Cuppett, 1997). Natural antioxidants are constituents of many fruits and

vegetables. This has attracted a great deal of public and scientific attention because of their anticarcinogenic potential and other health promoting effects (Anwar *et al.*, 2006). Diets rich in fruits and vegetables containing natural antioxidants such as plant carotenoids, polyphenols, Vitamin C, Vitamin E, and flavonoids (flavonols, flavones, isoflavonoids) are correlated with reduced incidence of cardiovascular, neurological diseases and cancer (Velioglu *et al.*, 1998; Anwar *et al.*, 2006). A number of *in vitro* and *in vivo* assays have been developed to measure the antioxidant activity of plant extracts (Gheldof *et al.*, 2002). 1,1 – diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical with an unpaired valence electrons at a nitrogen atom forming bridge (Alrahmany, 2012). DPPH can initiate a chain reaction which causes the removal of an electron from another molecule to complete its own orbital. The free valence electrons form the basis of the DPPH assay as plant extracts are tested to determine the scavenging capability of the DPPH radical (Kedare & Singh, 2011). The IC_{50} refers to a point where 50% of the DPPH is inhibited. When DPPH is reduced by an antioxidant it changes from purple to colourless thus by measuring the absorbance at 550 nm the IC_{50} of the plant extract can be determined.

2.2.3.1 Chemicals

1,1 – diphenyl-2-picrylhydrazyl (DPPH) and ascorbic acid that were used were of analytical grade and they were both supplied by Sigma Chemicals Co. (St. Louis, MO, USA).

2.2.3.2 Determination of radical scavenging capacity

Determination of the radical scavenging capacities of the plant extracts was determined by using the method of (du Toit *et al.*, 2001). Plant extracts and the positive control (Vitamin C) were used for the study. Stock solutions of Vitamin C and the plant extracts were prepared at a concentration of 2 mg/ml and 10 mg/ml. Antioxidant activities of all plant extracts were tested using DPPH free radical scavenging assay. To each well in the top row of a 96-well micro-plate, 200 µl of distilled water was added. To the rest of the wells were 110 µl of distilled water was added as a medium. Then 20 µl of plant extract was added to first top wells, in triplicate followed by serial dilution with the final concentrations ranging between 3.9 µg/ml to 500 µg/ml for the plant extracts and 0.718 µg/ml for Vitamin C. Finally 90 µl of 0.04M DPPH ethanolic solution was added to each well except the negative control where distilled water was added instead of DPPH to determine the radical scavenging capacity.

Ascorbic acid (Vitamin C) was used as a positive control, ethanol as the blank and the plant extracts without DPPH as the negative control. The plates were covered with foil and left to stand for 30 minutes at room temperature. The plates were read after the 30 minutes period passed using a BIO-TEK Power-Wave XS multi-well reader (A.D.P., Weltevreden Park, South Africa) at a wavelength of 550 nm, using KC Junior software.

This method was used to test all the plant extracts in the study to determine their radical scavenging capacity, and their IC_{50} values were calculated using GraphPad Prism 4 software together with Windows Excel 2000.

2.2.4 Cytotoxicity assay

There is an increase in the popularity of herbal and health food remedies which are recommended for the treatment of variety of diseases (Santa Maria *et al*., 1997). This resulted in hundreds of natural products available to the consumer of which some could be potentially toxic when ingested in overdose (Santa Maria *et al.*, 1997). Cytotoxic screening models provide important preliminary data to select plant extracts with potential antineoplastic properties (Popoca *et al.*, 1998).

2.2.4.1 Cell lines and culture conditions

The U937 cells are human cell lines established from a diffuse histiocytic lymphoma of a 37 year old male patient (Deininger *et al.*, 1995). This cell line serves as a model for differentiation of monocytes and macrophages *in vitro* because the cell line is one of the few cell lines that display many monocytic characteristics. Induction of the cells to mature from the committed macrophage branch to into a monocytic stage of development can be done with a variety of agents.

2.2.4.2 Determination of cytotoxicity

In this study a robust and easily adaptable *in vitro* model that makes use of human cell lines, U937 was used to determine cytotoxic effect of selected 18 ethanol extracts at different concentrations. U937 cells were maintained in suspension culture in RPMI-1640 supplemented with 10% (v/v) heat inactivated foetal bovine serum, 2mM L-glutamine and 10mM HEPES, at 37°C in a humidified atmosphere of 5% CO_2. Cytotoxicity was measured by the XTT method using the Cell Proliferation Kit II. XTT reagent resulted in a colour change. Optical densities of each sample were read using the ELISA plate reader, and the IC_{50} for each sample was determined using the Graphpad prism 4 program.

2.3 References

Abbas SR, Sabir SM, Ahmad SD, Boligon AA & Athayde ML (2014). Phenolic profile, antioxidant potential and DNA damage protecting activity of sugarcane (*Saccharum officinarum*). *Food Chemistry* **147,** 10-16.

Agbor GA, Vinson JA, Oben JE & Ngogang JY (2006). Comparative analysis of the *in vitro* antioxidant activity of white and black pepper. *Nutrition Research* **26,** 659-663.

Ahmad I, Aqil F & Owais M (2006). *Modern Phytomedicine: Turning Medicinal Plants into Drugs*. WILEY-VCH, Heppenheim.

Alrahmany R (2012). *Antioxidant and Antimicrobial Activities of Phenolic Acids Rich Extracts Released from Oat Bran with the Aid of Carbohydrases*. Carleton University.

Amiguet VT, Arnason JT, Maquin P, Cal V, Vindas PS & Poveda L (2005). A consensus ethnobotany of the Q'eqchi' Maya of southern Belize. *Economic Botany* **59,** 29-42.

Ankli A, Sticher O & Heinrich M (1999). Medical ethnobotany of the Yucatec Maya: Healers' consensus as a quantitative criterion. *Economic Botany* **53,** 144-160.

Anthony BP, Abonyi S, Terblanche P & Watt A (2011). Towards Bridging Worldviews in Biodiversity Conservation: Exploring the Tsonga Concept of Ntumbuloko in South Africa. In *Research in biodiversity - Models and Applications* ed. Pavlinov Di. InTech.

Anwar F, Jamil A, Iqbal S & Sheikh MA (2006). Antioxidant activity of various plant extracts under ambient and accelerated storage of sunflower oil. *Grasas y Aceites* **57,** 189-197.

Baillie J, Hilton-Taylor C & Stuart SN (2004). *2004 IUCN red list of threatened species: A global species assessment*. IUCN.

Bibi T, Ahmad M, Bakhsh Tareen R, Mohammad Tareen N, Jabeen R, Rehman S-U, Sultana S, Zafar M & Yaseen G (2014). Ethnobotany of medicinal plants in district Mastung of Balochistan province-Pakistan. *Journal of Ethnopharmacology* **157,** 79-89.

Biggs R, Reyers B & Scholes RJ (2006). A biodiversity intactness score for South Africa: science policy. *South African Journal of Science* **102,** 277-283.

Botha CJ & Penrith ML (2008). Poisonous plants of veterinary and human importance in southern Africa. *Journal of Ethnopharmacology* **119,** 549-558.

Deininger M, Meyermann R & Schluesener H (1995). Detection of two transforming growth factor-Î²-related morphogens, bone morphogenetic proteins-4 and-5, in RNA of multiple sclerosis and Creutzfeldt-Jakob disease lesions. *Acta Neuropathologica* **90,** 76-79.

du Toit R, Volsteedt Y & Apostolides Z (2001). Comparison of the antioxidant content of fruits, vegetables and teas measured as vitamin C equivalents. *Toxicology* **166,** 63-69.

Eloff JN (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica* **64,** 711-713.

Fish L (1999). *Preparing herbarium specimens*. National Botanical Institute. Pretoria.

Gazzaneo LRS, de Lucena RFP & de Albuquerque UP (2005). Knowledge and use of medicinal plants by local specialists in an region of Atlantic Forest in State of Pernambuco (Northeastern Brazil). *Journal of Ethnobiology and Ethnomedicine* **1,** 1-8.

Gheldof N, Wang X-H & Engeseth NJ (2002). Identification and quantification of antioxidant components of honeys from various floral sources. *Journal of agricultural and food chemistry* **50,** 5870-5877.

Hall CA & Cuppett SL (1997). Structure-activities of natural antioxidants. *Antioxidant Methodology: In vivo and in vitro Concepts. AOCS Press Champaign, IL***,** 141-172.

Kartal M (2007). Intellectual property protection in the natural product drug discovery, traditional herbal medicine and herbal medicinal products. *Phytotherapy Research* **21,** 113-119.

Kedare SB & Singh RP (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of food science and technology* **48,** 412-422.

Louw CAM, Regnier TJC & Korsten L (2002). Medicinal bulbous plants of South Africa and their traditional relevance in the control of infectious diseases. *Journal of Ethnopharmacology* **82,** 147-154.

McChesney JD, Venkataraman SK & Henri JT (2007). Plant natural products: Back to the future or into extinction? *Phytochemistry* **68,** 2015-2022.

Mpumalanga Province. [Online]. (Available from: http:// www.mpumalanga.gov.za) [Accessed: 12 August 2014]

Mpumalanga Provincial Government (2011) Municipal districts. [Online]. (Available from: htttp://www.mpumalanga.gov.za/munic.htm) [Accessed 12 July 2012]

Mwakikagile G (2008). *South Africa and Its People*. New Africa Press.

Nesheim I, Dhillion SS & StÃ¸len KA (2006). What happens to traditional knowledge and use of natural resources when people migrate? *Human Ecology* **34,** 99-131.

Popoca J, Aguilar A, Alonso D & Villarreal ML (1998). Cytotoxic activity of selected plants used as antitumorals in Mexican traditional medicine. *Journal of ethnopharmacology* **59,** 173-177.

Redlist. [Online]. (Available from: http:// Redlist.sanbi.org) [Accessed 20 February 2015]

Rodrigues ASL, Pilgrim JD, Lamoreux JF, Hoffmann M & Brooks TM (2006). The value of the IUCN Red List for conservation. *Trends in Ecology & Evolution* **21,** 71-76.

Santa Maria A, Lopez A, Diaz M, Alban J, Galan de Mera A, Vicente Orellana J & Pozuelo J (1997). Evaluation of the toxicity of *Uncaria tomentosa* by bioassays in *vitro*. *Journal of ethnopharmacology* **57,** 183-187.

Siyabona Africa (2012). [Online]. (Available from: http://www.krugerpark.co.za/ africa_shangaan_tsonga.html) [Accessed: 12 August 2014].

South African map showing Mpumalanga province and Ehlanzeni District Municipality. [Online]. (Available from: http:// www.mpumalanga.gov.za) [Accessed: 12 August 2014].

Study area map with GPS coordinates 24°S 31°E CC and 24°S 31°E CD. [Online]. (Available from www.maps.google.co.za) [Accessed: 12 August 2014].

Van Vuuren SF (2008). Antimicrobial activity of South African medicinal plants. *Journal of ethnopharmacology* **119,** 462-472.

Van Wyk B, Malan S, Lowrey TK & Pienaar A (1997). *Field Guide to the Wild Flowers of the Highveld: Also useful in adjacent grassland and bushveld*. Struik, Cape Town.

Van Wyk B & Van Wyk P (1997). *Field Guide to Trees of Southern Africa*. Struik, Cape Town.

Vandebroek I, Van Damme P, Van Puyvelde L, Arrazola S & De Kimpe N (2004). A comparison of traditional healers and medicinal plant knowledge in the Bolivian Andes and Amazon. *Social Science & Medicine* **59,** 837-849.

Velioglu YS, Mazza G, Gao L & Oomah BD (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry* **46,** 4113-4117.

Victor J, Koekemoer M, Fish L, Smithies S & Mossmer M (2004) Herbarium Essentials: The Southern African herbarium user manual. SABONET Report No. 25. SABONET, Pretoria.

Yaseen G, Ahmad M, Sultana S, Alharrasi AS, Hussain J, Zafar M & Ur-Rehman S (2015). Ethnobotany of medicinal plants in the Thar desert (Sindh) of Pakistan. *Journal of Ethnopharmacology* **163**. 43-59

Chapter 3: Results and Discussion

3.1 Ethnobotany

The Ethnobotanical survey gave us an outline of the natural remedies used by the Shangaan people living in the villages under the Jongilanga Traditional Council at Mpumalanga. A total of 238 plants collected with 207 identified plant species from 54 families, the scientific names are given alphabetically with family names and local vernacular names in Appendix II. The plant families that had more than two plant species identified were used to tabulate the percentage of plant families identified with families that have less than two plants were grouped as Other (Figure 3.1). The most commonly used plant family was Fabaceae representing 16% of all the recorded medicinal plant species in this study, followed by Malvaceae (6%) and Combretaceae (5%). Muthee *et al*., (2011) also reported Fabaceae as the most collected plant family which was 10% of the total 80 plant species documented (Muthee *et al.*, 2011).The plant part that is mostly used for medicinal purposes are the roots (78%), whole plant (13%) and other (4%), which includes tumours, latex , parasitic or symbiotic organisms found on the plant (Figure 3.2). The wide harvesting of the roots in this study has a negative impact on the survival and continuity of useful medicinal plants and affects the sustainable use of the plants. In comparison to a study conducted by Afolayan *et al*., (2014) 106 plant species distributed in 61 families were identified with the most represented family being Solanaceae and Fabaceae was the third most collected plant species (Afolayan *et al.*, 2014).

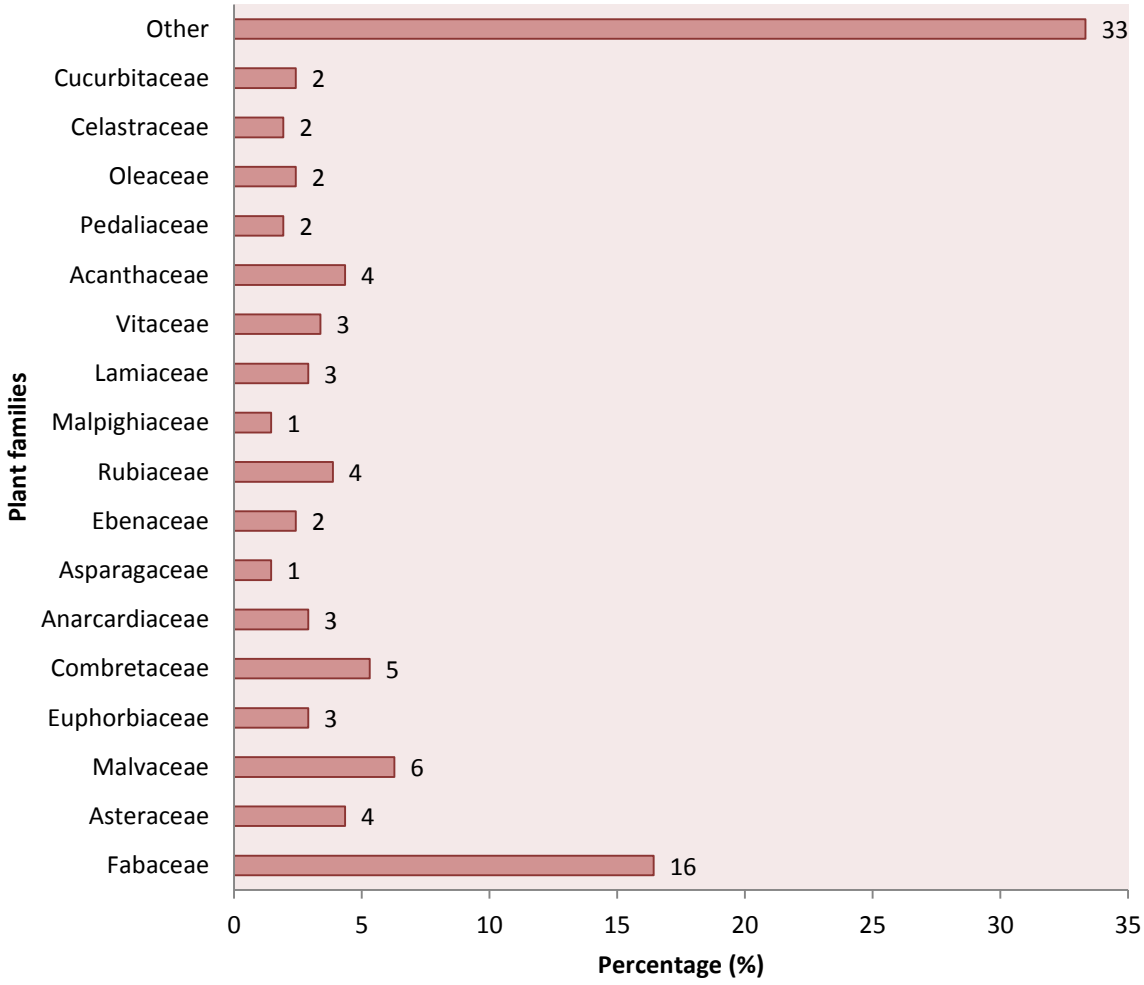

Figure 3.1: Percentage of identified plant families

The least used plant parts are the leaves (2%), stem bark (2%) and the fruit (1%). The use of the whole plant as the second most used plant part is consistent with the report by Muthu *et al.*, (2006) (Muthu *et al.*, 2006). The method of preparation used can be categorised into four, namely; Decoction, Infusion, digestion, juice extracted from the fresh plant part.

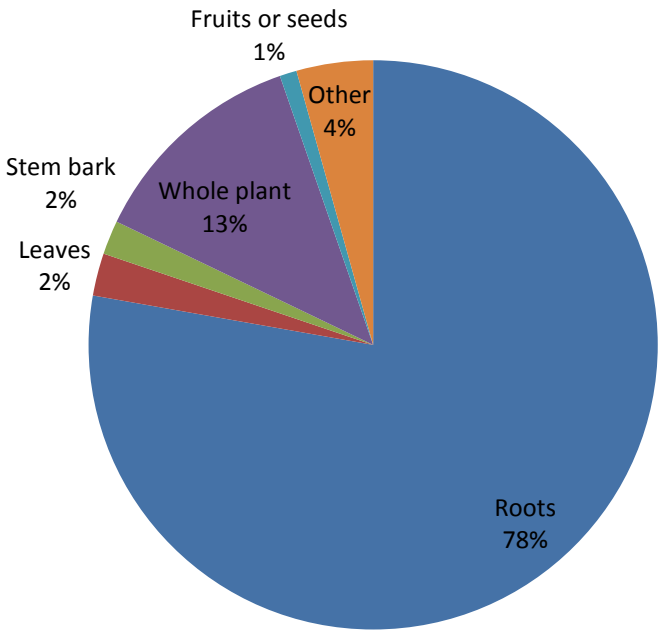

Figure 3.2: Plant part used as medicine

The administration methods were limited to external application to treat (snake bites, wounds, skin lightening, rash, and skin infections), and internal application (oral disease, ureno-genital disease, ear, eye, gastro-intestinal disorders, respiratory diseases, skeleto-muscular pain and swelling, high blood pressure, kidney disorders and hepatic disorders) (Table 3.1). The informants (15 traditional healers between ages 44-93 years) interviewed to get a consensus factor agreed to the medicinal use of major trees and common plants in the area (Figure 3.3 & 3.4).There were some differences in the mixture of plants in the concoctions based on the effectiveness or urgency of treatment of the disease. Each traditional healer had a basic use of a plant for a disease, through experience they manage to enhance or reduce the effect a plant has by combining it with other plants on the progression or severity of the disease. Some plant names are derived from the diseases they cure and the names vary from one traditional healer to the other. A single plant can be used to treat various diseases in traditional medicine. The 10 male traditional healers interviewed knew more plants which treat male related

ailments (e.g. erectile dysfunction), while the five female traditional healers that were interviewed knew more about plants used to treat female related ailments (skin lightening, bareness, yeast infection and menstrual pains). Traditional medicine knowledge varies between male and female traditional healers with a major factor being age. This has also been reported in a study conducted in Namibia (Cheikhyoussef *et al.*, 2011).

Figure 3.3: Informants interviewed for consensus of plant use (A, B, C and D)

3.1.1 Informant consensus factor

The informant consensus factor for the ailment categories ranged between 0.5 and 0.86 (Table 3.1). The usage frequency of plant species that treat respiratory infections, ureno-genital disorders, sensory system disorders, skelto-muscular pain and swelling, gastro-intestinal disorders, and circulatory and hepatic disorder was high. While the least frequently used plant species according to the consensus factor was for ailment category Other (rituals, love charm, witchcraft and mental disorders). A high consensus factor means the plant species used for the ailment category are ethno-botanically important (Kayani *et al.*, 2015). Thus a basis for further study of the frequently used plant species for their phytochemical and pharmacological activity is validated.

Table 3.1: Number of plants used for different ailment categories with factor of informants' consensus

Ailment categories	Biomedical terms	F_{ci}	Plants	%
Ureno-genital disorders (UG)	Kidneys, sexually transmitted diseases, fertility, menstrual disorders and erectile dysfunction	0.84	39	18.8
Dermatological disorders and Cosmetics (DC)	Skin problems, wounds , burns and anti-dandruff	0.75	31	15.0
Sensory system disorders	Ear, eye and oral disorders	0.84	28	13.5
Gastro-intestinal disorders (GI)	Vomiting, stomach ache, diarrhoea, laxatives and worms	0.78	23	11.1
Respiratory diseases (RT)	Chest pains and asthma,	1.00	8	3.9
Skelto-muscular pain and Swelling (SM)	Body aches, muscular pains, head ache, joint pains, swelling	0.86	42	20.3
Circulatory system and Hepatic disorders (CH)	Hypertension, hypotension and liver dysfunction	0.76	24	11.6
Other (OT)	Rituals, love charm, witchcraft and mental disorders	0.50	12	5.8

F_{ci} = Factor of informants consensus

3.1.2 Ailment Categories

According to figure 3.5 the most frequently sited species for ailment category includes skelto-muscular pain and swelling (42 plant species) followed by ureno-genital disorders (39 plant species). The moderately treated ailments include dermatological disorders and cosmetics (31 plant species), Sensory system disorders (28 plant species), Circulatory system and hepatic disorders (24 plant species), and gastro-intestinal disorders (23 plant species). The least treated ailment category is respiratory diseases (8 plant species). Skelto-muscular pain and swelling in this study commonly includes medicinal plants with anti-inflammatory and analgesic effect. In a study conducted Sewani-Rusike & Mammen (2014) the top five ailments that were treated at home were gastrointestinal problems (25 plant species), wounds (19 plant species), infections, including sexually transmitted infections (19 plant species), respiratory tract problems (19 plant species) and pain including headaches (19 plant species). Chronic diseases such as hypertension, diabetes, cancer and reproductive ailments also formed a large group of diseases self-managed at home (29 plant species) (Sewani-Rusike & Mammen, 2014).

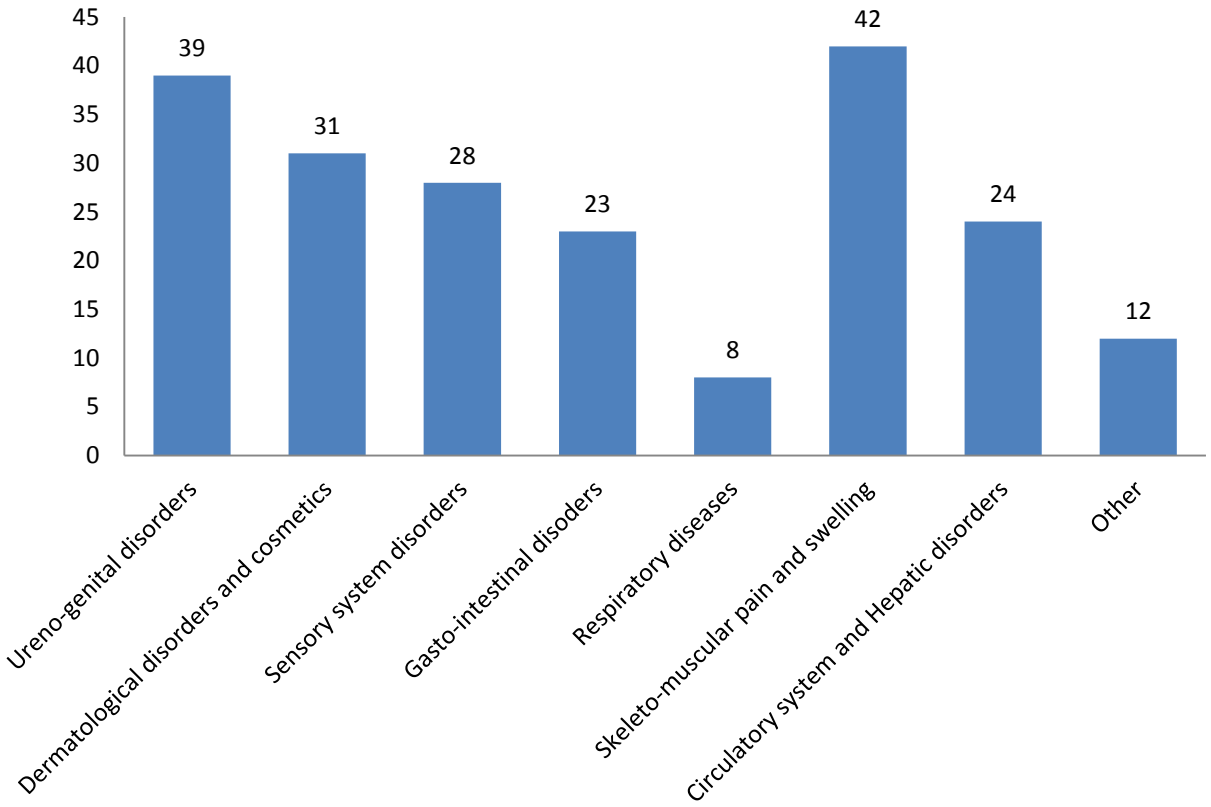

Figure 3.5: Number of plant species used per ailment category

3.1.3 Use Values (UV) and use Report (UR)

The use value for the plants collected in this study ranged from 0.06- 0.27 (Appendix II). The species with the highest use value was *Combretum collinum*. Most of the plants collected had a use value of 0.06. Compared to this study the UV of zootherapeutic resources was reported to range from 0.01 to 0.04 by Sreekeesoon & Mahomoodally (2014) in a study conducted in Mauritius (Sreekeesoon & Mahomoodally, 2014). The use report ranged between 1 and 4 uses for all the documented plants (Appendix II).

3.1.4 Conservation status

There were 166 plant species that had a Least Concern (LC) and 29 plant species with Not Evaluated (NE) conservation status (Appendix II). *Boophone disticha* and *Hypoxis hemerocallidea* were the only plant species documented with a Declining (D) conservation status. In a study conducted at the Eastern Cape Province of South Africa, *Boophone disticha* and *Hypoxis hemerocellidea* were among the medicinal plant species that were reported to be heavily traded with a high market value price even though it is protected (Dold & Cocks, 2002). Although considerable efforts have been undertaken to propagate and germinate the seeds of *Hypoxis hemerocallidea*, there has not been an acceptable method which can supply the market with the material needed at a reasonable price (Street & Prinsloo, 2013).

3.2 Biological assays

3.2.1 Antimicrobial assay

Ninety (90) ethanol plant extracts were selected and tested for antimicrobial activity against *Candida albicans* (diploid fungus) and *Prevotella intermedia* (gram negative). Eighteen plant extracts showed significant inhibitory activity against *Candida albicans* and *Prevotella intermedia* compared to the positive control Chlorhexidine (Table 3.2). The most significant antimicrobial activity was shown by *Combretum collinum* (**Fufu**) and *Terminalia sericea* (**Nkonolo**). *Combretum collinum* showed activity of MIC= 0.39 mg/ml and MMC= 3.13 mg/ml against *Candida albicans*, while it inhibited the growth of *Prevotella intermedia* with MIC= 1.56 mg/ml and MMC = 3.13 mg/ml. Ethanolic extracts of *Combretum collinum* have been reported to have larvicidal activity against *Aedes aegypti (*Odda *et al.,* 2008*).*

Terminalia sericea inhibited *Candida albicans* (MIC= 3.13 mg/ml, MMC=6.25) and *Prevotella intermedia* (MIC= 1.56 mg/ml, MMC= 3.13). The positive control Chlorhexidine showed prominent activity against *Candida albicans* and *Prevotella intermedia* with MIC at 0.390 mg/ml and the MMC at 0.390 mg/ml for both microorganisms. Although Rahalison *et al.*, (1994) suggested 0.01 mg/ml of the test compound for extract to be considered active (Rahalison *et al.*, 1994). These plant extracts possess potential to be analysed further for active constituents. The moderately active ethanol plant extracts include *Dichrostachys cinerea* (**Ndzenga**), *Rhoicissus tridentata* (**Mbhezane leyi kulu**), *Xerophyta retinervis* (**Nspwinspwela**), *Peltoforum africanum* (**Nhlanhlanu**), *Kalanchoe thyrsiflora* (**Xinyanyo**), *Acacia karoo* (**Rizaza**), *Sphedamnocarpus pruriens* (**Rexixa**), *Phyllanthus reticulatus* (**Thethenya**), *Pappea capensis* (**Xinungu**), *Ochna natalitia* (**Mahlanganisi leyi kulu**), *Diospyros lycioides* (**Xintomana**), *Faurea saligna* (**Scima mlilo**), *Solanum tomentosum* (**Nthomane**) and *Antidesma venosum* (**Ntshongwe**), with MIC and MMC ranging from 1.56 mg/ml to 25mg/ml (Table 3.2).

Table 3.2: The MIC and MMC for selected 90 ethanol plants extracts against *Candida albicans* and *Prevotella intermedia*

Plant name	**MIC (mg/ml) *Candida albicans***	**MMC (mg/ml) *Candida albicans***	**MIC (mg/ml) *Prevotella intermedia***	**MMC (mg/ml) *Prevotella intermedia***
Abrus precatorius	12.5	25.0	25.0	12.5
Acacia karoo	3.13	12.5	6.25	25.0
Acacia nilotica	12.5	>25	12.5	>25
Acacia nigrecens	25.0	>25	>25.0	25.0
Agathisanthemum bojeri	>25.0	>25.0	>25.0	>25.0
Albizia harveyi	6.25	12.5	6.25	12.5
Alectra sessiliflora	12.5	12.5	>25.0	>25.0
Antidesma venosum	12.5	>12.5	3.125	12.5
Asparagus exuvialis	12.5	25.0	>25.0	>25.0
Blephris sp.	25.0	25.0	25.0	25.0
Boophane disticha	12.5	25.0	>25.0	25.0
Canthium sp.	12.5	>25.0	25.0	>25.0
Carissa edulis	25.0	>25.0	>25.0	>25.0
Cassia abbreviate	25.0	25.0	>25.0	>25.0
Catunaregam Sp. A	3.13	12.5	6.25	12.5
Chamaecrista capensis	6.25	25.0	6.25	12.5
Clematis branchiate	6.25	25.0	12.5	12.5
Cleome angustifolia	25.0	>25	12.5	25.0
Combretum apiculatum	3.125	12.5	3.13	25.0
Combretum collinum	0.39	3.13	1.56	3.13
Combretum imberbe	6.25	12.5	6.25	12.5
Combretum mozambicans	25.0	12.5	12.5	25.0
Commelina bengalensis	>25.0	>25.0	25.0	25.0
Cordia ovalis	12.5	12.5	12.5	25.0
Crabbea hirsute	25.0	>25.0	>25.0	>25.0
Crabbea velutina	>25.0	>25.0	>25.0	>25.0
Crossandra greenstockii	6.25	12.5	>25.0	25.0
Crotalaria schinzii	6.25	12.5	12.5	12.5
Cucumis sp.	25.0	>25.0	>25.0	>25.0
Dalbergia melanoxylon	>25.0	>25.0	>25.0	>25.0
Dicerocaryum eriocarpum	12.5	12.5	25.0	25.0
Dichrostrachys cinerea	1.56	12.5	3.13	25.0
Diospyros lycioides	3.13	25.0	3.13	25.0
Diospyros mespiliformis	3.13	12.5	6.25	25.0
Elaedendron transvaalense	6.25	12.5	6.25	25.0
Faurea saligna	6.25	12.5	6.25	25.0
Ficus burkei	6.25	12.5	6.25	12.5
Gazania krebsiana	12.5	>12.5	12.5	>25.0
Gladiolis sp.	12.5	12.5	12.5	25.0
Grewia occidentalis	25.0	>25.0	6.25	25.0
Gymnosporia buxifolia	25.0	12.5	25.0	>25.0
Helichrysum pallidum	12.5	25.0	12.5	>25.0
Heliotropium steudneri	12.5	>25.0	6.25	25.0

Hypoxis hemerocollidea	6.25	>25.0	6.25	>25.0
Ipomoea crassipes	25.0	>25.0	>25.0	>25.0
Ipomoea oblongata	25.0	>25.0	25.0	>25.0
Indigofera sp.	3.13	3.13	12.5	25.0
Jasminum abyssinium	25.0	>25.0	>25.0	>25.0
Jasminum fluminense	12.5	>25.0	12.5	>25.0
Jasminum stenolobum	12.5	25.0	12.5	>25.0
Jathropa zeyheri	12.5	12.5	>25.0	>25.0
Justicia flava	6.25	>25.0	12.5	>25
Kalanchoe thyrsiflora	6.25	6.25	6.25	25.0
Ledeboria sp.	25.0	25.0	12.5	25.0
Lagerra crispata	12.5	12.5	3.125	12.5
Lannea schweinfurthii	12.5	25.0	12.5	25.0
Lippia javanica	25.0	25.0	12.5	12.5
Macrotyloma maranguense	12.5	25.0	12.5	>25.0
Mundulea sericea	25.0	>25.0	25.0	>25.0
Nuxia oppositifolia	12.5	>25.0	>25.0	>25.0
Nuxia sp.	12.5	>25.0	>25.0	>25.0
Ochna natalitia	3.13	25.0	6.25	12.5
Opuntia ficus indica	>25.0	>25.0	25.0	>25.0
Ormocarpus trichocarpum	6.25	3.13	12.5	25.0
Ornithogalum seineri	25.0	>25.0	25.0	>25.0
Ozoroa spaerocarpa	12.5	12.5	12.5	25.0
Pappea capensis	6.25	25.0	6.25	6.25
Pavetta gracilifolia	12.5	12.5	12.5	12.5
Peltoforum africanum	3.13	12.5	3.13	6.25
Philonoptera violacea	6.25	12.5	12.5	25.0
Phyllanthus reticulates	1.56	12.5	6.25	25.0
Piliostigma thoningii	3.13	25.0	12.5	25.0
Pterocarpus angolensis	>25.0	>25.0	12.5	>25.0
Pterodiscus ngamicus	25.0	>25.0	25.0	>25.0
Raphionacme procumbens	12.5	25.0	25.0	>25.0
Rhoicissus tridentata	6.25	6.25	6.25	6.25
Schontia branchypetala	25.0	>25.0	25.0	25.0
Senna italica	6.25	25.0	12.5	25.0
Senna petersiana	6.25	25.0	6.25	25.0
Sida rhombifolia	12.5	12.5	6.25	25.0
Solanum tomentosum	12.5	>12.5	6.25	>12.5
Sphedamnocarpus pruriens	3.13	12.5	3.13	6.25
Strychnos madagascariensis	12.5	>12.5	6.25	>12.5
Terminalia sericea	3.13	6.25	1.56	3.13
Trichilia emitica	12.5	25.0	>25.0	25.0
Vernonia colorata	12.5	>12.5	>12.5	>12.5
Vernonia sp.	12.5	12.5	6.25	25.0
Xerophyta retinervis	3.13	>12.5	1.56	>12.5
Ximenia americana	12.5	>12.5	12.5	12.5
Ximenia caffra	>25.0	>25.0	>25.0	>25.0
Ziziphus mucronata	>25.0	>25	>25.0	>25

3.2.2 Antioxidant assay

All the 18 ethanol plant extracts showed good antioxidant activity (Table 3.3). The best antioxidant activity was shown by *Combretum collinum, Sphedamnocarpus pruriens, Rhoicissus tridentata* and *Kalanchoe thyrsiflora* respectively, with IC_{50} values ranging from 1.653 µg/ml to 1.81 µg/ml, which is closer to that of vitamin C which is 1.62 µg/ml (Figure 3.5). This is the first time root ethanolic extract antioxidant activity of *Combretum collinum* is reported. In a study conducted by Mongalo *et al*., (2015) the roots of *Sphedamnocarpus pruriens* var pruriens reported to be mixed with a handful of the roots of *Securidaca longipedunculata* Fresen to treat someone possessed by evil spirits (Mongalo *et al.*, 2015), while Tan and Wiart (2014) reported the roots to treat mental illness (Tan & Wiart, 2014). The plants that also showed significant antioxidant activity include *Terminalia sericea, Catunaregam* spA (Figure 3.6), *Pappea capensis* and *Diospyros lycioides* with IC50 values ranging from 3.13 µg/ml to 3.87 µg/ml.

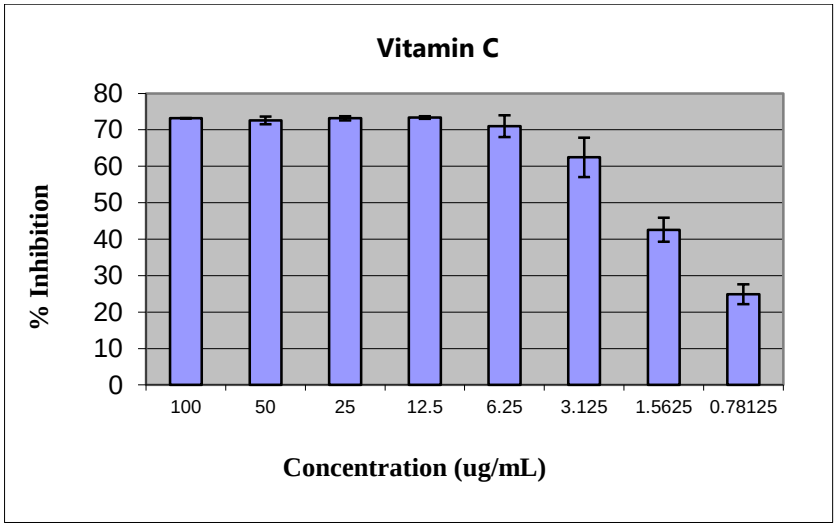

Figure 3.5: The DPPH inhibitory activity of vitamin C

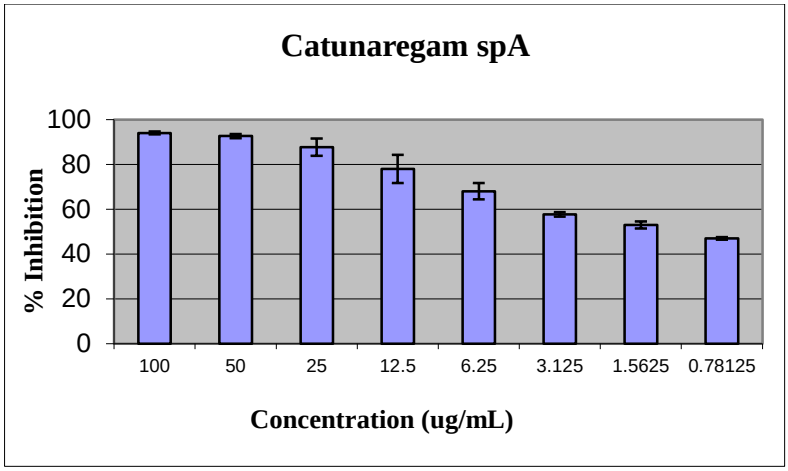

Figure 3.6: The DPPH inhibitory activity *Catunaregam* spA

Table 3.3: The IC_{50} values of 18 selected ethanol extracts and positive control vitamin C

Plant Name	**Plant part used**	**IC_{50}(µg/ml)**
Vitamin C (positive control)		1.22
Acacia karroo	Roots	4.69
Antidesma venosum	Roots	12.54
Catunaregam SpA	Roots	3.66
Combretum collinum	Roots	1.65
Dichrostachys cinerea	Roots	4.33
Diospyros lycioides	Roots	3.87
Faurea saligna	Roots	4.90
Kalanchoe thyrsiflora	Whole plant	1.81
Ochna natalitia	Roots	6.27
Pappea capensis	Roots	3.75
Peltoforum africanum	Roots	4.46
Phyllanthus reticulatus	Roots	13.68
Rhoicissus tridentata	Roots	1.72
Senna petersiana	Roots	5.41
Solanum tomentosum	Roots	12.58
Sphedamnocarpus pruriens	Roots	1.65
Terminalia sericea	Roots	3.13
Xerophyta retinervis	Whole plant	14.67

3.2.3 Cytotoxicity assay

The toxicity of plants was compared to that of actinomycin D, a known toxic antibiotic that has anti-cancer activities. The lower the IC_{50} of the plant sample the more toxic the plant. Most of the tested plants were toxic to human cell line U937, only six of the eighteen ethanol plant extracts had IC_{50} values more than 100 µg/ml (Table 3.4).

Table 3.4: The IC_{50} values and therapeutic index of 18 selected ethanol extracts and the positive control actinomycin D

Plant Name	**Plant part used**	**IC_{50}(µg/ml)**	**SD**	**TI_A**	**TI_B**
Actinomycin D (positive control)		0.002735	±1.1	-	-
Acacia karroo	Roots	39.68	±1.25	0.013	0.006
Antidesma venosum	Roots	32.02	±2.59	0.003	0.010
Combretum collinum	Roots	81.84	±1.495	0.210	0.026
Catunaregam SpA	Roots	>400	± 3.12	0.128	0.064
Dichrostachys cinerea	Roots	33.69	±1.65	0.022	0.011
Diospyros lycioides	Roots	4.156	±0.02	0.001	0.001
Faurea saligna	Roots	43.93	±1.31	0.007	0.007
Kalanchoe thyrsiflora	Whole plant	53.57	±0.70	0.009	0.009
Ochna natalitia	Roots	49.67	±0.62	0.016	0.008
Pappea capensis	Roots	68.91	±6.32	0.011	0.011
Peltoforum africanum	Roots	264.32	±3.20	0.084	0.084
Phyllanthus reticulatus	Roots	123.5	±5.87	0.079	0.020
Rhoicissus tridentata	Roots	39.89	±1.09	0.006	0.006
Senna petersiana	Roots	42.75	±0.24	0.007	0.007
Solanum tomentosum	Roots	NT	-	-	-
Sphedamnocarpus pruriens	Roots	114.3	±4.68	0.037	0.037
Terminalia sericea	Roots	242.0	±3.65	0.077	0.155
Xerophyta retinervis	Roots	>400	± 4.68	0.128	0.256

Therapeutic index of *Candida albicans*= TI_A, Therapeutic index of *Prevotella intermedia*= TI_B

Catunaregam SPA and *Xerophyta retinervis* showed good IC_{50} values greater than 400µg/ml meaning they are not toxic to human cell lines U937 (Appendix III). The average non toxic plants extracts include *Peltoforum africanum, Phyllanthus reticulatus, Sphedamnocarpus pruriens* and *Terminalia sericea.* All the six plants are not toxic to human cells. The most toxic plant is *Diospyros lycioides* which had an IC_{50} value of 4.156µg/m. To the best of our knowledge this is the first time the cytotoxic effects of the ethanolic plant extracts have been reported on this cell line. Previous studies have shown cytotoxic effects of the plants with different cell lines (Sharma & Lall, 2014). The therapeutic index (TI) for all the plants against both microorganisms ranged from 0.003 to 0.258. A high therapeutic index is preferable to have good safety profile, because of the low therapeutic index shown by all the plant extracts there is a low probability of drug development. Although there is a low therapeutic index shown by the plant extracts, it should not be ruled out that a better therapeutic index might be shown by the physiologically active constituent of the plant extracts.

3.3 References

Afolayan AJ, Grierson DS & Mbeng WO (2014). Ethnobotanical survey of medicinal plants used in the management of skin disorders among the Xhosa communities of the Amathole District, Eastern Cape, South Africa. *Journal of Ethnopharmacology* **153,** 220-232.

Cheikhyoussef A, Shapi M, Matengu K & Mu Ashekele H (2011). Ethnobotanical study of indigenous knowledge on medicinal plant use by traditional healers in Oshikoto region, Namibia. *Journal of Ethnobiology and Ethnomedicine* **7,** 10.

Dold AP & Cocks ML (2002). The trade in medicinal plants in the Eastern Cape Province, South Africa. *South African Journal of Science* **98,** 589-597.

Kayani S, Ahmad M, Sultana S, Shinwari ZK, Zafar M, Yaseen G, Hussain M & Bibi T (2015). Ethnobotany of medicinal plants among the communities of Alpine and sub-alpine regions of Pakistan. *Journal of Ethnopharmacology* **164,** 186-202.

Mongalo NI, McGaw LJ, Finnie JF & Staden JV (2015). *Securidaca longipedunculata* Fresen (Polygalaceae): A review of its ethnomedicinal uses, phytochemistry, pharmacological properties and toxicology. *Journal of Ethnopharmacology* **165,** 215-226.

Muthee JK, Gakuya DW, Mbaria JM, Kareru PG, Mulei CM & Njonge FK (2011). Ethnobotanical study of anthelmintic and other medicinal plants traditionally used in Loitoktok district of Kenya. *Journal of Ethnopharmacology* **135,** 15-21.

Muthu C, Ayyanar M, Raja N & Ignacimuthu S (2006). Journal of Ethnobiology and Ethnomedicine. *Journal of Ethnobiology and Ethnomedicine* **2,** 43.

Odda J, Kristensen S, Kabasa J & Waako P (2008). Larvicidal activity of Combretum collinum Fresen against Aedes aegypti. *Journal of Vector Borne Diseases* **45**. 321-324

Rahalison L, Hamburger M, Monod M, Frenk E & Hostettmann K (1994). Antifungal tests in phytochemical investigations: comparison of bioautographic methods using phytopathogenic and human pathogenic fungi. *Planta Medica* **60,** 41-44.

Sewani-Rusike CR & Mammen M (2014). Medicinal Plants Used as Home Remedies: A Family Survey by First Year Medical Students. *African Journal of Traditional, Complementary and Alternative Medicine* **11,** 67-72.

Sharma R & Lall N (2014). Antibacterial, antioxidant activities and cytotoxicity of plants against Propionibacterium acnes. *South African Journal of Science* **110,** 58-65.

Sreekeesoon DP & Mahomoodally MF (2014). Ethnopharmacological analysis of medicinal plants and animals used in the treatment and management of pain in Mauritius. *Journal of Ethnopharmacology* **157,** 181-200.

Street RA & Prinsloo G (2013). Commercially important medicinal plants of South Africa: A review. *Journal of Chemistry* **2013**. http://dx.doi.org/10.1155/2013/205048.

Tan KK & Wiart C (2014). Botanical descriptions, Ethnomedicinal and no-medicinal uses of the genus Artabotrys R. BR. *International Journal of Current Pharmaceutical Research* **6,** 34-40.

Chapter 4: General discussion and Conclusion

4.1 General discussion

4.1.1 Shangaan traditional medicine

Traditional medicine is still being used as primary health care. The current study revealed that Shangaan traditional healers mostly treat ureno genital disorders and skeleton muscular pain. Ureno genital disorders are normally treated with antibiotics for acute to severe cases while mild cases salts are used, which change the urinary acidity and inhibit growth of bacteria (Heinrich *et al.*, 2004). These are not suitable for all patients as blood electrolyte concentration may be affected (Heinrich *et al.*, 2004). Thus phytomedicinal treatment is the most suitable as a treatment option. The ailments are treated with a variety of plants species by the Shangaan with plant family Fabaceae being the most frequently used. The family fabaceae is characterized by a large number of traits. Most of the taxa of this family are herbaceous, sometimes shrubby and are very rarely trees. This family is also characterized by an impressive phytochemical diversity (Van Wyk & Van Wyk, 1997; Heinrich *et al.*, 2004). Flavonoids and tannins are the most common polyphenols found in the family, but for a pharmaceutical perspective the various types of alkaloids found are the most interesting and pharmaceutically relevant (Heinrich *et al.*, 2004). Observing the knowledge difference of male and female traditional healers interviewed, Male traditional healers were found to know more about plants that treat male related ailments while female traditional healers knew more about female related ailment treatments.

4.1.2 Ethnobotanical databases

Ethnobotanical surveys on the uses of medicinal plants by the indigenous people living in developing countries facing primary health care needs have been documented and reported throughout the world (Iwu, 1994). There are a number of initiatives taken in South Africa to deal with and make policies to recognise the potential role of traditional medicine in the country by developing drug and indigenous knowledge system policies (Richter, 2003). The government is supported by various institutions to record the indigenous knowledge of medicinal plant uses for pharmaceutical purposes (Sheng-Ji, 2001). A national database was then developed with the purpose to collate all scientific information on medicinal plants that are already recorded and ensure its accessibility (McGaw & Eloff, 2008). There is currently a need for studies to document all traditional medicines derived from indigenous medicinal plants to validate their safety, clinical efficacy, identify and isolate therapeutically active ingredients and establish the national database (World Health Organisation, 2002). Through these studies deductions for deforestation and depletion of the environment can also be reported. The plant species that are threatened or near extinction could be recommended for conservation management in the area (Alves & Rosa, 2007).

4.1.3 Conservation

There recently has been an upsurge of public interest to medicinal plants. This upsurge has affected the retail sales volume significantly of herbal medicine from the sale of raw unprocessed medicinal plants open markets, packaged semi-processed herbal medicines and highly refined herbal medicines processed in modern pharmaceutical industries (van Andel *et al.*, 2015). These medicinal plants are harvested from nature (wild forests, conserved areas, game reserves and national parks), with only a small fraction being from cultivated sources (Moshi & Mhame, 2013). In order to guarantee a continuous supply of medicinal plants in the future, management plans must be designed in which specified information on species occurrence and extraction localities is needed (van Andel *et al.*, 2015). The conservation of such plant species in South Africa is crucial. Conservation efforts have been taken by the government to start a Red List which exposes the status of medicinal plant. It has been reported that less than 20% of South Africa`s 322 heavily traded medicinal plant species are threatened with extinction (SANBI, 2015). The majority of medicinal plants that have been assessed on the Red List are considered Not Threatened and have a Least Concern status. Commitment to conservation measures to conserve plants that are highly threatened due to overharvesting is required.

The Jongilanga Traditional Council has conservation measures and control access to their resources. Harvesting medicinal plants in the field requires a written consent from the council or heavy penalties are enforced. There are nature watchers called “Group 10”, their duty is to patrol the fields and request permits from people harvesting medicinal plants.

4.2 Conclusion

The knowledge of a shangaan traditional healer was recorded using a semi-structured questionnaire. Confirmation of traditional uses of the plants was confirmed with other traditional healer to get a consensus factor. There were 238 plants collected with 207 plant species identified from 54 families. The most used plant family to treat ailments is Fabaceae and Malvaceae. An ethnobotanical database was started and written in a format that is useful to the traditional healer`s family and community. This was the first ethnobotanical survey study conducted in the area.

Ninety ethanol plant extracts were selected to screen for activity against oral pathogens. There were 18 plants that showed good inhibitory and microbicidal activity against selected microorganisms *Candida albicans* and *Prevotella intermedia.* The best inhibitory and microbicidal activity was shown by *Combretum collinum* and *Terminalia sericea* compared to the positive control Chlorhexidine. The eighteen plants showed good antioxidant activity and only six plant extracts (*Catunaregam* spA, *Xerophyta retinervis, Peltoforum africanum, Phyllanthus Reticulatus, Sphedamnocarpus pruriens* and *Terminalia sericea*) were not toxic to cell line U937. *Diospyros lycioides* was the most toxic plant species.

4.3 Future work

Further analysis of ethanol plant extract on *Catunaregam* spA, *Xerophyta retinervis, Peltoforum africanum, Phyllanthus Reticulatus, Sphedamnocarpus pruriens* and *Terminalia sericea Catunaregam* spA, *Xerophyta retinervis, Peltoforum africanum, Phyllanthus Reticulatus, Sphedamnocarpus pruriens* and *Terminalia sericea* needs to be done. Research on synergy of the active plant extracts. Study the metabolite content of the species to identify and elucidate the metabolite structure responsible for the effects. Acetone plant extracts of the 18 active plants need to also be tested for the same activity against oral pathogens, antioxidant activity and cytotoxicity.

4.4 References

Alves RmRN & Rosa IML (2007). Biodiversity, traditional medicine and public health: where do they meet? *Journal of Ethnobiology and Ethnomedicine* **3,** 14.

Heinrich M, Barnes J, Gibbons S & Williamson EM (2004). *Fundamentals of Pharmacognosy and Phytotherapy*. Churchill Livingstone an imprint of Elsevier Science Limited, New York.

Iwu MM (1994). African medicinal plants in the search for new drugs based on ethnobotanical leads. *Ethnobotany and Search for New Drugs* ***185,*** 116-129.

McGaw LJ & Eloff JN (2008). Ethnoveterinary use of southern African plants and scientific evaluation of their medicinal properties. *Journal of Ethnopharmacology* **119,** 559-574.

Moshi MJ & Mhame PP (2013). Legislation on Medicinal Plants in Africa. In: *Medicinal Plant Research in Africa: Pharmacology and Chemistry*. ed. Kuete V. Newnes.

Richter M (2003). Traditional medicines and traditional healers in South Africa. *Treatment Action Campaign and AIDS Law Project* **17,** 4-29.

South African National Biodiversity Institute (2015) Redlist. [Online]. (Available from: http://redlist.sanbi.org) [Accessed 02 January 2015].

Sheng-Ji P (2001). Ethnobotanical approaches of traditional medicine studies: some experiences from Asia. *Pharmaceutical Biology* **39,** 74-79.

Van Andel TR, Croft S, van Loon EE, Quiroz D, Towns AM & Raes N (2015). Prioritizing West African medicinal plants for conservation and sustainable extraction studies based on market surveys and species distribution models. *Biological Conservation* **181,** 173-181.

Van Wyk B & Van Wyk P (1997). *Field Guide to Trees of Southern Africa*. Struik. Cape Town

World Health Organisation (2002). WHO traditional medicine strategy 2002-2005. [Online]. (Available from: http://www.wpro.who.int/health_technology/book_who_ traditional_medicine_strategy_2002_2005.pdf) [Accessed 02 January 2015].

Chapter 5: Appendices

Appendix I: Semi-structured Questionnaire

1. Plant specific part

Date		Voucher specimen no.	
Collectors information			
Name	Gender	Age	Profession
Taxonomic information			
Popular names	Scientific name/s	Synonym/s	Plant family
Taxonomic indicator(endemic or invasive)			
Information of the specimen			
Short description of the plant			
Geographical/ geological information			
Province	Village name	Habitat/vegetation type	
Medicinal / pharmaceutical information			
Uses	Plant part/s used	Importance	
Preparation	Dosage	Application form	Application time
Effect/s	Observation	Side effect	Contraindication/s
Notes			

2. Information specific part

Healers (ethnological aspects)	
Which kind of healer is asked?	
How to become this kind of healer?	
Selection of plants	
What are the criteria for plant selection?	
How are the medicinal and non-medicinal plants classified?	
How are medicinal plants classified?	Are non-medicinal plants also classified?
Classification of illnesses	
Which illnesses does the healer know?	
Diagnosis	
Cause/s	Symptom/s
Concerned persons	Prevention
Who may medicate the patient and why?	Does it require a healing ceremony?

Appendix II: Medicinal plants used to treat various ailments in Villages under Jongilanga tribal council, Mpumalanga, South Africa

Scientific and Family name	Local name	Voucher number	Plant part	Preparation	Medicinal uses	CS	UR	UV
Abrus precatorius L. Fabaceae	Matihlo ya baloyi	BCM 119334	Whole plant	Decoction	Kidney problems (UG)	LC	1	0.07
***Abutilon fruiticosum* Guill. & Perr.** Malvaceae		BCM 119365	Roots	Decoction	Cramps, Muscle pulls (SM)	LC	2	0.13
***Acacia cf.* erubescens Welw. Ex Oliv** Fabaceae	Hlanganani	BCM 120535	Roots	Decoction	Swollen body (SM)	LC	1	0.07
***Acacia karoo* Hayne.** Fabaceae	Rizaza	BCM 119360	Roots	Decoction	STI's (UG)	LC	1	0.07
***Acacia nigrescens* Oliv.** Fabaceae	Nkaya	BCM 117176	Bark f the stem	Infusion	Diarrhoea (GI)	LC	1	0.07
***Acacia nilotica* Willd. Ex**	Mugamazu	BCM	Roots	Decoction	Mental illness (OT),	LC	3	0.20

Delile subsp. kraussiana (Benth) Brenan Fabaceae		117174			Headaches (SM), Wounds (DC)			
***Acacia permixta* Burtt Davy** Fabaceae	Bote	BCM 120566	Roots	Decoction	Fever	LC	1	0.07
***Agathisanthemum Bojeri* Klotzsch Subsp. bojeri** Rubiaceae	Mavunge	BCM 119330	Roots	Decoction	STI's (UG)	LC	1	0.07
Albizia harveyi Fabaceae	Ndzololwane	BCM 117161	Roots	Decoction	Rituals (OT)	LC	2	0.13
Albizia harveyi E.Fourn. Fabaceae	Nhlanhlana	BCM 120528	Roots	Decoction	Body ailments (SM), Blood cleanser (CH)	LC	2	0.13
***Albizia versicolor* Welw ex Oliv.** Fabaceae	Xivhangathana	BCM 120575	Roots	Infusion	Diarrhoea (GI)	LC	3	0.2
***Alectra sessiliflora* (Vahl) Kuntze** Scrophulariaceae	Ndluwa	BCM 119340	Roots or whole plant	Decoction	Kidney disorder (UG)	LC	1	0.07

Aloe Spp. Liliaceae	Mhangana	BCM 117180	Stem	Direct	Eyes Infections (SS), High blood pressure (CH)	-	2	0.13
***Antidesma venosum* E.Mey. ex Tul.** Euphorbiaceae	Ntshongwe	BCM 117167	Roots	Decoction	Fertility (UG)	LC	1	0.07
***Asclepias* sp.** Apocynaceae	Mahlantana	BC 103	Whole plant	Infusion	Diarrhoea, Vomiting (GI)	-	2	0.13
***Asparagus buchananii* Baker** Asparagaceae	Nkwangula-tilo lowu kulu	BCM 119329	Whole plant	Decoction	Sores, Itchy skin (DC)	LC	2	0.13
***Asparagus exuvialis* Burch** Asparagaceae	Nkwangulatilo lowu ntsongo	BCM 119347	Roots	Decoction	Back pains, Fatigue (SM)	LC	2	0.13
***Barleria affinis* C.B. Clarke** Acanthaceae	Xindzawu	BC119	Roots	Decoction	Stretch marks (DC)	LC	1	0.07
***Barleria crossandriformis* C.B. Clarke** Acanthaceae	Nyankumeni	BC113	Roots	Bath	Dandruff (DC)	LC	1	0.07
***Bauhinia galpinii* N.E.Br.**	Xikotela	BCM	Roots	Decoction	Vomiting (GI)	LC	1	0.07

Fabaceae		120563						
Blepharis subvolubilis C.B. Clarke* Subsp. *Subvolubilis Acanthaceae	Phepheyaphi	BC 129	Roots	Decoction	Kidney disorders (UG)	LC	1	0.07
***Boophone disticha* (L.f.) Herb** Amaryllidaceae	Rihemane	BC54	Bulb	Decoction	Truth serum, Good luck (OT)	D	2	0.13
***Boscia albitrunca* (Burch.) Gilg & Gilg-Ben.** Capparaceae	Gugugu/ shepherds tree	BC108	Roots	Infusion	Swollen feet, Waist pain (SM)	LC	2	0.13
***Carissa edulis* (Forssk.) Vahl** Apocynaceae	Xivambula/num-num	BCM 119351	Roots	Infusion	Internal bleeding (SM), Ear Infection (SS)	LC	2	0.13
***Cassia abbreviata* Oliv. Subsp. beareana (Holmes) Brenan** Fabaceae	Mlumanyama	BC 124	Stem bark or roots	Decoction	Body aches (SM)	LC	1	0.07
Catunaregam sp. A Poaceae	Xirhombe	BCM 119345	Fruits	Infusion	Purgative, Laxative (GI)	LC	2	0.13

Catunaregam spp. Rubiaceae	Xirhuki	BCM 117170	Fruits, Roots	Decoction	Purgative (GI), Rituals (OT)	LC	2	0.13
***Chamaecrista capensis* (Thunb.) E.Mey. var. capensis** Fabaceae	Mahlakule	BCM 119343	Roots	Decoction or chew	Witchcraft (OT)	LC	1	0.07
***Chascanum incisum* (H.Pearson) Moldenke** Verbanaceae	Ximbangana	BC 155	Roots	Infusion	Blood thinner (SM)	LC	1	0.07
***Chlorophytum galpinii* (Baker) Kativu var. galpinii** *Anthericaceae*	Xiyandana	BCM 120516	Whole plant	Infusion	All ailments (OT)	LC	1	0.07
***Cienfuegosia hildebrandtii* Garcke** Malvaceae	Ntsutsuma	BCM 120554	Whole plant	Infusion	STI's (UG)	LC	1	0.07
***Cissus cornifolia* (Baker) Planch.** Vitaceae	Mbhezana lowu tsongwe	BC107	Roots	Infusion	Rashes, Allergies (DC)	LC	2	0.13
***Clematis branchiata* Thunb.**	Mahlungu ya	BC 73	Roots	Decoction	Snake bites (SM),	LC	2	0.13

Ranunculaceae	tinyoka				Swollen scrotum (UG)			
***Cleome angustifolia* forssk. Subsp. Petersiana (Klotzsch ex sond.) kers** Capparaceae	Ngwarhele	BCM 120533	Roots	Decoction	All ailments (OT), Blood cleanser (SM)	LC	2	0.13
Coccinia sp. Cucurbutaceae	Xinanjana	BCM 120520	Leaves, stem or roots	Infusion	Ear infections, deafness (SS)	LC	2	0.13
***Combretum apiculatum* Sond. Subsp. apiculatum** Combretaceae	Xihlalavhana	BCM 119358	Whole plant	Decoction	Mouth discolouring (DC)	LC	1	0.07
Combretum collinum Combretaceae	Fufu	BCM 117156	Roots	Infusion	Shortage of blood water Painful legs, Cramps, Joint pains (SM)	LC	4	0.27
Combretum hereroense Combretaceae	-	BC 190	Roots	Decoction	STI's (UG)	NE	1	0.07
Combretum imberbe Combretaceae	Mondzo	BCM 117175	Roots or Stem	Infusion	Menstruation (UG)	LC	1	0.07

Combretum molle Combretaceae	Mbika nyama	BC194	Roots	Decoction	Tongue sores (GI)	LC	1	0.07
Combretum mossambicense combretaceae	Fuka labangafa	BC176	Roots	Decoction	Coma (SM)	LC	1	0.07
Commelina africanum Commelinaceae	Nihundzule	BC198	Roots	Decoction	Joint pains, Blood cleanser (SM)	LC	1	0.07
Commelina africana L. var lancispatha C.B. Clarke Commelinaceae	Nyamhuntana	BC156	Roots	Burn and sniff	All ailments (OT)	LC	1	0.07
Commelina bengalensis Commelinaceae	Mkhegula	BC154	Roots	Decoction	Tapeworms (GI)	LC	1	0.07
Commiphora africana Burseraceae	Xigunge	BC111	Roots	Decoction	Heartburn, Stomach cramps (GI)	LC	2	0.13
Cordia ovalis Boraginaceae	Mpungwana Xinyanyam	BCM 117159	Bark of the stem	Bath	Good luck (OT)	LC	1	0.07
Cordia sp. Boraginaceae	Xinkuwana	BCM 120522	Roots	Decoction	Strengthen teeth (GI)	LC	1	0.07
Crabbea hirsuta Acanthaceae	Xitsayitsayi	BCM 119366	Roots	Infusion	Eye Infections (SS)	LC	1	0.07

Crabbea hirsute Harv. Acanthaceae	Xirhuwela	BC228	Roots	Burn to ashes and let the smoke reach the area	Haemorrhoids (DC)	LC	1	0.07
Crabbea velutina S.Moore Acanthaceae	Xikunbyanbya	BCM 120517	Roots	Infusion	Stiff neck (SM)	LC	1	0.07
Crinum sp. Amaryllidaceae	Forodji	BCM 120526	Bulb	Decoction	Back pains (SM)	-	1	0.07
Crossandra greenstockii Acanthaceae	Dinda leri kulu	BC110	Roots	Infusion	Warts (DC)	LC	1	0.07
Crotalaria agatiflora Fabaceae	Mahlampyana	BCM 119344	Roots	Infusion	Laxative (GI)	NE	1	0.07
Crotolaria cf. burkeana Fabaceae	Phuphuma	BCM 117184	Roots	Lotion	Strong Love charm, Good luck (OT)	LC	2	0.13
Crotalaria lanceolata E.Mey. subsp. Lanceolata Fabaceae	Nyokana	BCM 120557	Roots	Infusion	Epilepsy (SM)	LC	1	0.07
Crotalaria schinzii Baker f.	Nchimbeni	BCM 120514	Roots	Infusion	Tonsillitis (GI)	LC	1	0.07
Cucumis hirsutus	Nunankulu	BC93	Roots	Decoction	STIs (UG)	LC	1	0.07

Cucurbitaceae								
***Cucumis* sp.** Cucurbitaceae	Mluma nyama	BCM 119363	Roots	Decoction	Menstrual pain (UG)	LC	1	0.07
***Cucumis* sp.** Cucurbitaceae	Xirhakarhana	BCM 120529	Roots	Infusion	Waist pain (SM)	LC	1	0.07
Cyanotis speciosa (L.f.) Hassk Commelinaceae	Xipfala nomo	BCM 120552	Tuber	Poultice	Cramps (SM)	LC	1	0.07
Cyphostemma humile (N.E.Br.) Desc. Ex Wild & R.B.Drumm.subsp. dolichopus(C.A.Sm.) wild & R.B.Drumm Commelinaceae	Xirhandzana	BCM 120567	Tuber	Infusion	Asthma (RT), High blood pressure (CH)	LC	2	0.13
Cyphostemma puberulum (C.A.Sm.) Wild & R.B. Drumm. Vitaceae	Xiyandla xa mfenhe sa nuna	BCM 120521	Whole plant	Decoction	Side stitch (SM)	LC	1	0.07
Cyphostemma sp.	Marhumbini	BCM	Tuber	Infusion	Itchy armpits (DC)	-	1	0.07

Vitacea		120548						
***Dalbergia Melanoxylon* Guill.& Perr.** Fabaceae	Xiphaladzi	BCM 117154	Roots	Infusion, Decoction	Rashes (DC)	LC	1	0.07
Dicerocaryum eriocarpum (Decne.) Abels Pedaliaceae	Dinda	BCM 119332	Whole plant	Decoction	Cow delivery inducer (UG), Headaches (SM)	LC	2	0.13
***Dichrostachys cinerea* L. Wight & Arn. subsp. nyassana (Taub.) Brenan LC Nyassana** Fabaceae	Ndzenga	BCM 117157	Roots or pods	Decoction	Snakebite (SM)	LC	1	0.07
Diospyros lycioides Desf. **subsp. Lycioides** Ebenaceae	Xintomane	BCM 119336	Roots	Decoction	Ear infection (SS)	LC	1	0.07

Diospyros mespiliformis Hochst. ex A.DC **Ebenaceae**	Ntoma	BCM 117182	Roots or leaves	Decoction	STI's (UG)	LC	1	0.07
Dombeya rotundifolia (Hochst.) Planch. Var. rotundifolia	Risavana	BCM 120553	Roots	Decoction	Back pain (SM)	LC	1	0.07
***Drimea* sp.** Hyacinthaceae	Makatsana	BC62	Bulb	Decoction	Tapeworms (GI)	-	1	0.07
Ehretia sp. Boraginaceae	Nta byela mani	BCM 120518	Roots	Decoction	Blood cleanser (SM)	-	1	0.07
***Elaeodendron transvaalense* (Burtt Davy) R.H.Archer** Celastraceae	Ngwavuma	BCM 117182	Bark of the stem	Infusion	Purgative (GI), Good luck charm (OT)	NT	2	0.07
***Euclea crispa* Gürke subsp. crispa** Ebenacea**e**	Nhlangula lowu tsongo	BC02	Roots	chew	Toothbrush (GI)	LC	1	0.07
***Euclea natalensis* A.DC.**	Nhlangula lowu kulu	BC01	Roots or stem bark	Decoction or chew	Skin care (DC), STIs	LC	3	0.20

subsp. natalensis Ebenaceae					(UG), Toothbrush (SS)			
Eugenea altisima Myrtaceae	Makatsana	BC125	tuber	Decoction	STIs (UG)	NE	1	0.07
Equisetum ramosissimum Desf. Subsp. ramosissimum Equisetaceae	Ndoda emnyama	BCM 120525	Whole plant	Decoction	Comma (OT)	LC	1	0.07
Euphorbia sp. Euphorbiaceae	Nhlayiseni	BCM 120560	Whole plant	Paste	Sores (DC)	-	1	0.07
***Faurea saligna* Harv.** Proteaceae	Scima mlilo	BCM 118700	Leaves	Decoction	Epilepsy (SM)	LC	1	0.07
Ficus burkei (Miq.) Miq. Moraceace	Nhlulabambe	BC89	Roots	Infusion	Eye infections (SS)	LC	1	0.07
***Ficus capreifolia* Delile** Moraceae	Vhoka bafhile	BC 146	Roots	Decoction	Immune boaster (OT)	LC	1	0.07
Fucospora gilva	Xivindzi/ bracket fungi	BC118	Whole fungus	Decoction	Liver dysfunction (CH)	-	1	0.07
***Gazania krebsiana* Less.**	Rhuketela	BCM	Roots	Direct	Head sores (DC)	LC	1	0.07

subsp. krebsiana Asteraceae		119369						
***Gladiollis* sp.** Iridaceae (11)	Byanyi-byanhova	BCM 119353	Bulb	Infusion	Purgative (GI)	LC	1	0.07
Gloriosa superba L. Colchicaceae	Khondywasi	BC238	Tuber	Lotion	Good luck charm (OT)	LC	1	0.07
Gnidia cf. capitata L.f. Thymelaeaceae	Tlakula	BCM 120565	Roots	Decoction	All ailments (OT)	NE	1	0.07
***Grewia occidentalis* L. var. occidentalis** Malvaceae	Nsihana	BCM 117158	stem	Place stem in your car	Good luck charm (OT)	LC	1	0.07
***Gymnosporia buxifolia* (L.) Szyszyl.** Celestraceae	Xihlangwe	BCM 117155	Leaves or Roots	Infusion, Decoction	Epilepsy (SM)	LC	1	0.07
***Gymnosporia buxifolia* (L.) Szyszyl.** Celastraceae	Rigumkela	BCM 119357	Leaves and roots	Infusion	Epilepsy (SM), Fire burns (DC)	LC	1	0.07
Gymnosporia sp. Celastraceae	Mgwili	BCM 120543	Roots	Decoction	Immune boaster (OT)	LC	1	0.07

***Harpagophytum procumbens* (Burch.) DC. ex Meisn. subsp. procumbens** Pedaliaceae	Dinda/ devils claw	BC199	Roots	Decoction	Stomach ailments (GI)	NE	1	0.07
***Helichrysum pallidum* DC.** Asteraceae	Mpetso	BCM 119348	Roots	Direct	Penal sores (UG)	LC	1	0.07
Heliotropium cf steudneri Boraginaceae	Mbavazana	BC106	Roots	Decoction	Asthma (RT)	NE	1	0.07
Hermania arrecta Starculiaceae	Mbhune	BCM 119333	Roots	Direct	Moles (DC)	NE	1	0.07
***Hibiscus calyphyllus* Cav.** Malvaceae	Rihlelo	BCM 120542	Roots	Decoction	Swollen body (SM)	LC	1	0.07
***Hibiscus pusillus* Thunb.** Malvaceae	Ghove	BCM 120534	Roots	Decoction	All ailments (OT)	LC	1	0.07
Hibiscus sp. Malvaceae	Xiveve	BC102	Roots	Decoction	Headaches (SM)	-	1	0.07
***Hilliardiella nudicaulis* (DC.) H.Rob.**	Xitsonyo	BC236	Whole plant	Direct	STI sores (DC)	LC	1	0.07

Asteraceae								
***Hypertelis bowkeriana* Sond.** Molluginaceae	Mbepana	BC167	Roots	Infusion	Aphrodisiac (UG), Flu (RT)	LC	2	0.13
***Hypoxis hemerocellidea* Fisch., C.A.Mey. & Avé-Lall.** Hypoxidaceae	Mbhumbhununu	BC42	Bulb	Decoction	High blood pressure (CH)	D	1	0.07
Indigofera arrecta Fabaceae	Khuvana	BCM 119331	Whole plant	Decoction	Sexual guard/ Anti-cheating (OT)	LC	1	0.07
***Ipomoea crassipes* Hook. var. crassipes** Convolvulaceae	Phephenya	BC162	Roots	Decoction	HIV (UG), Vomiting, Diarrhoea (GI)	LC	3	0.20
***Ipomoea oblongata* E.Mey. ex Choisy** Convolvulaceae	Dema (Black)	BCM 119362	Bulb	Decoction	Asthma (RT), High blood pressure (CH)	LC	2	0.13
Jasminum L. abyssinicum Oleaceae	Mthundangazi	BCM 119364	Roots	Decoction	Bladder cleaner (UG)	LC	1	0.07
***Jasminum fluminense* Vell.**	Maloyana	BCM	Roots	Decoction	STI's (UG)	LC	1	0.07

subsp. fluminense Oleaceae		119350						
Jasminum stenolobum Rolfe Oleaceae	Mhlaba nkonzi	BCM 120527	Roots	Decoction	Mental illness (OT)	LC	1	0.07
***Jatropha spicata* Pax** Euphorbiaceae	Xihlapfurana	BC117	Roots	Direct	Migraines (SM)	LC	1	0.07
***Jatropha zeyheri* Sond.** Euphorbiaceae	Mfelo	BCM 117173	Bulb	Chew	All ailments (OT)	LC	1	0.07
***Justicia flava* (Vahl) Vahl** Acanthaceae	Kanzabokate	BCM 120524	Roots	Decoction	Cholesterol (CH)	LC	1	0.07
***Kalanchoe thyrsiflora* Harv.** Crassulaceae	Xinyanyo	BCM 117166	Whole plant	Lotion	Love charm (OT)	LC	1	0.07
***Kyllinga alba* Nees** Cyperaceae	Xitsawana	BC230	Roots	Infusion, Bath	Haemorrhoids (DC), Painful testis (UG)	LC	2	0.13
Ledebouria cooperi (Hook.f.) Jessop Hyacinthaceae	N`wa hlokwana	BC112	Bulb	Direct	Fontanel (DC)	LC	1	0.07
Ledebouria sp.	Xinyalana	BCM	Bulb	Infusion	Erectile dysfunction	-	1	0.07

Hyacinthaceae		120558			(UG)			
***Laggera crispata* (Vahl) Hepper & J.R.I.Wood** Asteraceae	Xikhwasha	BCM 119337	Roots	Decoction	Swollen stomach (UG)	LC	1	0.07
***Laggera crispate* (Vahl) Hepper & J.R.I.Wood** Asteraceae	Rikhwekhwe	BCM 119352	Whole plant	Lotion	Swollen stomach (GI)	LC	1	0.07
***Lannea schweinfurthii* var. stuhl** Anacardiaceae	Ximbombokanyi	BCM 119341	Roots	Decoction	Body aches (SM)	LC	1	0.07
Leonotis nepetifolia (L.) R.Br. Lamiaceae	Mabala	BC95	Whole plant	Lotion	skin tone (DC)	LC	1	0.07
Leucas martinicensis (Jacq.) R.Br. Lamiaceae		BCM 120559	Roots	Decoction	STI's (UG)	LC	1	0.07
Lippia Javanica (Burm.f.) Spreng. Verbenaceae	Umsuzwane	BCM 119365	Roots	Decoction	Asthma, Chest pain (RT), Herbal tea (GI)	LC	3	0.20

Lotonotis sp. Lamiaceae	Mabala ya leyikulu	BC100	Roots	Decoction	Sores, Skin patches (DC)	-	2	0.13
***Macrotyloma maranguense* (Taub.) Verdc.** Fabaceae	Xikondlo	BCM 117171	Bulb	Chew	Swollen or painful testicles (UG)	LC	1	0.07
Maerua parvifolia Capparaceae	Nomo wako nandzika	BC163	Roots	Decoction	Throat infections (GI)	LC	1	0.07
***Melhania forbesii Planch.* Ex Mast.** Malvaceae	Mphephula	BCM 120547	Roots	Infusion	Itchy eyes (SS)	LC	1	0.07
Melhania prostrata* DC. *Malvaceae	Hlekwasi	BCM 120539	Roots	Decoction, Bath	Body pain (SM)	LC	1	0.07
***Merremia kentrocaulos* (C.B. Clarke)** Convolvulaceae	Nkaka wama blantana	BCM 120538	Roots	Decoction	High blood pressure (CH)	LC	1	0.07
Merremia palmate Hallier* f. *Convolvulaceae	Vukosimuni	BCM 120530	Roots	Infusion	Skin softner (DC)	LC	1	0.07
***Mormodica balsamia* L.** Cucurbitaceae	Nkaka	BCM 120572	Whole plant	Infusion	Asthma (RT)	LC	1	0.07

Mundulea sericea Fabaceae	vatanya	BCM 119368	Roots	Bath	Nerve tension (SM)	LC	1	0.07
Nidorella resedifolia* DC. *Subsp. resedifolia Asteraceae	Ingwe mabala	BCMm 120562	Whole plant	Infusion	Migraines (SM)	LC	1	0.07
***Nuxia oppositifolia* (Hochst) Benth.** Buddlejaceae	Mbiliya	BCM 120523	Roots	Decoction	Increases blood water (SM)	LC	1	0.07
Nuxia sp. Buddlejaceae	Ntombi yako basa	BCM 120511	Roots	Decoction	Erectile dysfunction (UG)	LC	1	0.07
***Ochna* natalitia (Meisn.) Walp.** Ochnaceae	Mahlanganisi lama kulu	BCM 118701	Roots	Decoction	Joint pain (SM)	LC	1	0.07
Ocimum americanum* L. var *americanum Lamiaceae	Manyokana	BC166	Roots	Decoction	Epilepsy (SM)	LC	1	0.07
***Ocimum filamentosum* Forssk.** Lamiaceae	Tsikisa vaxixiti	BC164	Roots	Decoction	Bed wetting (UG)	LC	1	0.07

***Opuntia ficus-indica* (l.)Mill** Cactaceae	Xitokorofiya	BCM 117178	Stem	Decoction	High blood pressure (CH)	NE	1	0.07
***Ormocarpum trichocarpum* (Talb.) Engl.** Leguminosae	Xisitane	BCM 117168	Inner bark of roots	Infusion	Erectile dysfunction (UG)	LC	1	0.07
***Ornithogalum seineri* (Engl. & Krause) Oberm** Hyacinthaceae	Xihumbana	BC183	Bulb	Decoction	STI's (UG)	NE	1	0.07
***Ozoroa sphaerocarpa* R. Fern & A. FErn** Anacardiaceae	Xinungu mafi	BCM 119359	Whole plant	Infusion, Decoction	Induces lactation, Cattle wounds (DC)	LC	1	0.07
***Pachypodium saundersii* N.E.Br** Apocynaceae	Rigumkela 2	BC116	Roots	Decoction	All ailments (OT)	LC	1	0.07
***Pappea capensis* Eckl. & Zeyh.** Sapindaceae	Xinungu	BCM 118702	Bark	Grind	Penis enlargement (UG), Male breast reduction , Immune boaster (SM)	LC	3	0.20
***Pavetta catophylla* K. schum.** Rubiaceae	Babaloyi	BCM 120507	Roots, leaves or stem bark	Direct	Ringworms (DC)	LC	1	0.07

***Pavetta* cf. *gracilifolia* Bremek.** Rubiaceae	Ncolovoti	BCM 119349	Roots	Decoction	Painful feet (SM)	LC	1	0.07
Pavetta sp. Rubiaceae	Xilapha matinyo	BCM 120573	Roots	Decoction	Swollen eyes (SS), Painful teeth (SM)	-	1	0.07
***Pechuel-Loeschea leubnitziae* (Kuntze) O. Hoffm.** Asteraceae	Mbepo	BC168	Roots	Decoction	Eye sight (SS), Side stitch (SM)	LC	2	0.13
***Pellaea calomelanas (*sw.) Link var. calomelanos** Pteridaceae	Xiome	BCM 120510	Roots	Direct	Stroke (SM)	LC	1	0.07
***Peltophorum africanum* Sond.** Rosaceae	Nhlanhlanhu	BC40	Roots	Decoction	Body pain (SM)	LC	1	0.07
***Periglossum mackenii* Harv.** Apocynaceae	Nhlakwazi	BCM 120550	Tuber	Decoction	Swollen testicles (UG)	LC	1	0.07
***Philenoptera violacea* (Klotzsch) Schrire**	Mbhandzu/Apple leaf	BCM 119335	Roots	Infusion	Purgative (GI), Good luck Charm (OT)	LC	2	0.13

Fabaceae								
***Phyllanthus maderaspatensis* L.** Euphorbiaceae	Matene	BCM 120546	Roots	Infusion	Epilepsy (SM)	LC	1	0.07
Phyllanthus reticulatus* Poir. Var. *reticulatus Euphorbiaceae	Xincimban/Potato bush	BCM 118705	Roots	Decoction	Drip (CH)	LC	1	0.07
***Piliostigma thonningii* (Schumach.) Milne-Redh.** Fabaceae	Nkholonkhotlho	BCM 117183	Roots and leaves	Infusion, Decoction	Bone aches (SM), Erectile dysfunction (UG)	LC	2	0.13
***Pterocarpus angolensis* DC.** Fabaceae	Mrhotso	BCM 117169	Roots	Decoction	Heart burn , Purgative (GI)	LC	2	0.13
Pterocarpus rotundifolius* (Sond.) Druce subsp. *rotundifolius Fabaceae	Nxelele	BCM 117164	roots	Infusion	Fertility in cows (UG)	LC	1	0.07
***Pterodiscus ngamicus* N.E.Br. ex Stapf** Pedaliaceae	Nkukute	BC136	Whole plant	Decoction	Lactation (CH)	LC	1	0.07

***Raphionacme procumbens* Schltr.** Asclepiadaceae	Dema	BCM 117172	Bulb	Infusion	Painful waist (SM), Aphrodisiac (UG)	LC	2	0.13
***Rhoicissus tridentate* (L.f.) Wild & R.B. Drumm. subsp. cuneifolia (Eckl. & Zeyh) Urton** Vitaceae	Mbhezane leyi kulu	BCM 119338	Roots	Decoction	STI's (UG)	NE	1	0.07
***Rhychosia minima* (L.) DC. var. minima** Fabaceae	Mahlantswana	BCM 120531	Roots	Decoction	Waist pain (SM)	LC	1	0.07
***Schkuhria pinnata* (Lam.) Kuntze ex Thell.** Asteraceae	Nyokana	BCM 120515	Whole plant	Infusion	Tapeworms (SM)	NE	1	0.07
***Schotia capitata* Bolle** Fabaceae	Xilopilopi	BC99	Roots	Decoction	Pulmonary Infections (RT), STI's (UG)	LC	2	0.13
***Schotia branchypetala* Sond.** Fabaceae	Mvhomvhomvho	BCM 119370	Roots and seeds	Decoction	Shoulder pains, Sternum pains (SM)	LC	2	0.13
***Schotia capitata* Bolle**	Xilopilopi	BC99	Roots	Decoction	Pulmonary infections	LC	2	0.13

Fabaceae					(RT), STI's (UG)			
***Sclerocarya birrea* (A. Rich.) Hochst. Subsp. *caffra* (Sond.) Kokwaro** Ananacardiaaceae	Bheduka	BCM 120544	Roots	Infusion	Loose sphincter (GI)	LC	1	0.07
***Searsia pyroides* (Burch.) Moffett var. *integrifolia* (Engl.) Moffett** Anacardiaceae	Khaloli	BCM 120574	Roots	Infusion	Spinal pain (SM)	LC	1	0.07
***Searsia transvaalensis* (Engl.) Moffett** Anacardiaceae	Nkhwa-Nkhwa	*BC195*	Roots	Decoction	Smelly armpits (DC)	LC	1	0.07
***Senna italica* Mill. subsp. avachoides (Burch.) Lock** Fabaceae	N`warimangana	BCM 117179	Roots	Decoction	STI's (UG)	LC	1	0.07
***Senna petersiana* (Bolle) Lock** Fabaceae	Nembenembe	BC08	Roots	Infusion	Bladder infection, Fertility (UG), High blood pressure (CH)	LC	3	0.20
***Sida cordifolia* L. subsp.**	Mbemane	BC122	Roots	Direct	Nose bleeds (SS)	LC	1	0.07

cordifolia Malvaceae								
Sida rhombifolia* L. subsp. *rhombifolia Malvaceae	Tihoveta vhalungu	BCM 119355	Whole plant	Bath	Dandruff (DC)	LC	1	0.07
***Solanum tomentosum* L. var. tomentosum** *Solanaceae*	Nthomane	BCM 117177	Roots	Infusion	Eye infections (SS)	LC	1	0.07
Sphedamnocarpus pruriens* (A.Juss.) Szyszyl. Subsp. *Pruriens Malpighiaceae	Nqhayiye	BCM 119342	Roots	Infusion	Pubic lice (DC)	LC	1	0.07
Sphedomnocarpus pruriens2 Malpighiaceae	Khodaxu	BCM 118703	Roots	Decoction	STI's (UG)	LC	1	0.07
***Striga asiatica* (L.) Kuntze** Orobanchaceae	Mlulwana	BCM 120561	Roots	Lotion	Smelly armpits, Body scent (DC)	LC	1	0.07
***Strychnos madagascariensis* Poir** Loganiaceae	Nkwakwa	BCM 117163	Roots	Decoction/ Infusion	Purgative (GI), Transference of spirits (OT)	LC	1	0.07

Stylechiton sp. Araceae	Bafana vati mbuti	BC101	Roots	Infusion	Ear infections (SS)	-	1	0.07
***Stylosanthes fruticosa* (Retz.) Alston** Fabaceae	Zulanezwe	BCM 120561	Roots	Paste	Verukas (DC)	LC	1	0.07
***Synadenium cupulare* (Boiss.) L.C. Wheeler** Euphorbiaceae	Nunankulu n`wa nuna	BC109	Bulb	Decoction	Erectile dysfunction (UG)	NE	1	0.07
***Syncolostemon ellioti* (Baker) D.F. Otieno** Lamiaceae	Hanyana	BCM 120519	Roots	Decoction	Immune boaster (SM)	LC	1	0.07
***Tephrosia Purpurea* (L.) Pers. subsp. leptostachya (DC.) Brummitt var. leptostachya** Fabaceae	Mpeto	BCM 120540	Roots	Direct	Sinus or nasal congestion (SS)	LC	1	0.07
***Terminalia sericea* Burch. Ex DC.** Combretaceae	Nkonolo	BCM 118704	Tumors of plant	Decoction	Tonsils (GI)		1	0.07

***Terminalia prunioides* M.A. Lawson** Combretaceae	Homu	BCM 120537	Roots	Decoction	Bad body scent (DC)	LC	1	0.07
***Triaspis hypericoides* (DC.) Burch** Mapighiaceae	Hluphekile	BCM 120512	Roots	Infusion	Immune boaster (SM)	LC	1	0.07
***Tricalysia junodii* (Schinz) Brenan var. *kirkii* (Hook.f.) Robbr** Rubiaceae	Rhukana	BCM 120509	Roots	Decoction	Dry coughs (RT)	LC	1	0.07
***Trichilia emetic* Vahl subsp. emetica** Meliaceae	Nkuhlu/Natal mahogany	BCM 119354	Roots and stem bark	Infusion	Painful feet, Blood cleanser (SM)	LC	1	0.07
***Triclicerus glanduliferum* (Klotzsch) R.Fern.** Turneraceae	Funani	BCM 120513	Roots	Decoction	Hiccups (RT)	LC	1	0.07
***Turraea nilotica* Kotschy & Peyr.** Meliaceae	Masunungulu	BCM 119361	Roots	Chew	Stomach disorders, Tapeworms (GI)	LC	2	0.13

***Turraea obtusifolia* Hochst.** Meliaceae	Mafehlana	BCM 120545	Roots	Infusion	Eye infections (SS)	LC	1	0.07
***Tylosema fassoglensis* (Schweinf) Torre & Hillc** Fabaceae	Mbambati	BCM 120568	Roots	Decoction	Miscarriage (UG)	LC	1	0.07
Vangueria infausta* Burch. Subsp. *infausta Rubiaceae	Xinyathelo	BCM 117162	Roots	Decoction	Snake repellent (OT), Snake bites (SM)	LC	2	0.13
***Vernonia colorata* subsp. colorata** Compositae	Mpyila	BCM 117160	Roots	Decoction	Foot ailments (SM)	LC	1	0.07
***Xerophyta retinervis* Baker** Velloziaceae	Nspwinspwela	BC126	Whole plant	Decoction	Painful feet (SM)	LC	1	0.07
***Ximenia americana* L. var. *microphylla* Welw. Ex Oliv.** Olacaceae	Nthunduluka lowu tsongo	BC114	Roots or fruits	Decoction	STI's (UG)	LC	1	0.07
Ximenia caffra* var. *caffra Olacaceae	Nthunduluka lowu kulu	BCM 119346	Roots	Decoction	Blood cleaner (SM), Erectile dysfunction (UG)	LC	1	0.07

***Ziziphus mucronata* Willd. subsp. mucronata** Rhamnaceae	Mpasamhala	BCM 117165	Leaves or Roots	Lotion	Edible fruits, even skin tone, patched skin (DC)	LC	3	0.2
To be identified	Mphovhane	BC32	Roots	Decoction	Wounds (SM)	-	1	0.07
To be identified	Mhlambululo wangati	BCM 119339	Roots	Decoction, Bath	Body pains (SM)	-	1	0.07
To be identified	Mpempenya	BC89	Roots	Decoction	STI's (UG)	-	1	0.07

CH= Circulatory system and hepatic disorders, CS =Conservation status, D=Declining, DC= Dermatological disorders and Cosmetics, LC= Least concerned, NE= Not evaluated, OT= Other, RT= Respiratory diseases, SM= Skelto-muscular pain and Swelling, Sensory system disorder= SS, UG= Ureno-genital disorders, UR=Use report, UV= Use value

Appendix III: Cytotoxicity IC_{50}