

**Ecology and conservation status of six medicinal
plants commonly used to treat diabetes in the
eastern Free State, South Africa**

By

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Abstract

Diabetes mellitus is a global disease with an extreme effect on the quality of life of the patients and it is increasing among South Africans. South African population is predominantly black and medicinal plants represent an important asset to their livelihoods as it is to many people in developing countries. The communities from both rural and urban areas still rely on medicinal plants for their primary health care and income generation. However, the growing population and the general field collection practices of healers, herbalists and commercial gatherers are posing a serious extinction threat to the wild populations of medicinal plants. Six medicinal plants viz.: *Hypoxis hemerocallidea*, *Dicoma anomala*, *Morella serrata*, *Gazania krebsiana*, *Xysmalobium undulatum* and *Eriocephalus punctulatus* were identified to be the most commonly used to treat diabetes in the eastern Free State. The purpose of this investigation was to (1) conduct a survey on the herbarium collection of six medicinal plants used to treat diabetes (2) conduct a survey to determine the collecting practices of traditional healers and herbalist and the impact these practices have on the wild populations and (3) to conduct an ecological survey to determine the current population trends of the six selected plants in their natural habitats.

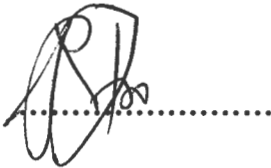
A total of six herbaria (Uniqwa Herbarium, Sterkfontein Dam Nature Reserve Herbarium, Geo-Potts Herbarium, National Museum Herbarium, Free State National Botanical Garden Herbarium and Bews Herbarium) were surveyed and there were only a few specimen of the studied plants found in four surveyed herbaria. The collection labels generally lacked information on the precise location, distribution and frequency of occurrence, and most of them were very old. The present study showed that most of the traditional medicine practitioners were women with a low education level and they had little appreciation of the

impact of their activities on wild populations of the medicinal plants. The indiscriminate collecting practices posed a serious extinction threat to the plants used in traditional medicine. The ecological survey identified two species *Eriocephalus punctulatus* and *Morella serrata* as potentially threatened within the study area.

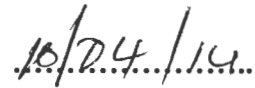
Herbaria should strive to become aligned with the “13-point strategy to meet conservation challenges” and this would make integral part of conservation strategies. Extensive study to determine the extent of the threat to the two identified species needs to be undertaken and their status on the Red Data List need to be revised. Traditional medicine practitioners (healers, herbalists and commercial gatherers) need to be informed about sustainable usage of natural resources and about environmental legislation regarding their business. Strong working relationships between government (Department of Environmental Affairs), Universities, South African National Parks and traditional medicine practitioners need to be established to protect the environment. Failure to use medicinal plants in sustainable way will have a negative impact on both the biodiversity and to the general health of the population.

Certification

This is to certify that this research work was carried out by Teboho Raymond Pitso, with the student number 1992345635, in the Department of Plant Sciences, University of the Free State – Qwaqwa Campus, under my supervision

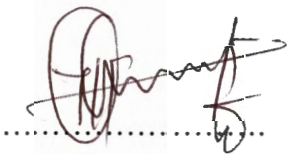


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Dedication

I dedicate this work to my wife Matshidiso Pitso, my daughter Mpho Pitso and to my parents Tefo Pitso and Kelibone Pitso.

Acknowledgements

I would like to first and foremost, thank Almighty God for his protection and for providing all those involved in this study with wisdom.

I am also grateful to my supervisor, Dr Anofi O.T. Ashafa for his patience, understanding, guidance, contribution and availability during this study. This thesis would not have seen the light of day if it was not for his dedication in seeing all his students succeed. May God continue to bless you and provide you with more strength in your endeavours to promote other students.

I would also like to sincerely thank all the herbalists and traditional healers from Maluti-a-Phofung Municipality for agreeing to share their vast knowledge of medicinal plants with me and to leave their business and accompany me to the field during the ecological survey.

I also would like to thank the Research Committee at the Qwaqwa campus for providing financial support that helped me carry out the required field work.

Lastly, I would like to thank my dear wife for her understanding, patience and for motivating me to see my studies through.

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CHAPTER 1

1. INTRODUCTION

People of all cultures around the world have always relied on plants as source of medication for treating various ailments and for their general well-being. Humans have over the years, identified a large number of medicinal plants and this knowledge has been passed from one generation to the next. Over time, people in the more developed countries started relying on more sophisticated pharmaceutical chemicals to treat illnesses. Medicinal plants remained an important and integral part of the indigenous health system of the most of the populations in the developing world. It is estimated that as much as 80% of the developing world population still relies on traditional medications (Institute of Natural Resources, 2003). Herbal medicines are an important part of the culture and traditions of African people. Most populations in urban and small rural communities are reliant on herbal medicines for their healthcare needs (Fennell et al., 2004).

In recent years, there has been a renewed interest in usage of traditional medicines treatment against different diseases. On one hand, scientists are looking towards traditional medicines for new drugs and on the other hand the expensive drugs and their adverse side effects have encouraged more people to resort to traditional medicine. Traditional medicines are generally less expensive and very effective. Thus people tend to prefer them over synthetic drugs. World Health Organisation (WHO) has realised the importance of traditional medicine and has recommended that their efficacy should be researched. WHO has also called on African countries to formally recognise traditional medicine as this will help them to achieve total healthcare coverage for their people (Kofi-Tsekpo, 2004).

Plants are the most common source of traditional medicines and the renewed interest in this practice has placed a severe pressure on the natural resource and even threatens its survival.

In the past, the collection of medicinal plants from the wild was the sole responsibility of traditional healers and their trainees. They had all the knowledge of good collection practices and these practices allowed for the sustainable cultivation of the different species.

The increased demand in traditional medicine has seen the increased involvement of commercial harvesters who are in search of an income. To maximise their income, the commercial gatherers will collect as much as they can and then sell to traders. Commercial harvesters tend to “mine” the natural resources and not manage them. Some of the plants are collected by harvesters for pharmaceutical companies thus placing further pressure on the survival of these plants. The rapid urbanization in many African countries threatens plants as the development reduces the wild habitat.

1.1 OVERVIEW OF DIABETES

Diabetes mellitus is recognised as being a syndrome, a collection of disorders that have hyperglycaemia and glucose intolerance as their hallmark, either due to insulin deficiency or to the impaired effectiveness of insulin’s action, or to a combination of the two. The body becomes unable to produce insulin and this condition is characterised by polyuria (frequent and abundant urine), glycosuria (presence of glucose in urine) and hyperglycaemia which is glucose rate on an empty stomach higher than 1.2 g/l in plasma blood and confirmed in at least two occasions (N’guessan et al., 2009).

An understanding of diabetes is preceded by an understanding of physiological processes during and after meals, as food passes through the digestive system, where nutrients including proteins and carbohydrates are absorbed through the bloodstream. The presence of sugar signals to the endocrine pancreas to secrete insulin. Insulin then causes the uptake of

sugar by almost all tissue types in the body, especially the liver, musculature and fat tissues (Anderson, 2003).

WHO (2006 a) published the classification of mellitus diabetes and other categories of glucose intolerance in 1985 (Table 1). The report provided the standard diagnostic criteria and terminology suitable for clinical and epidemiological research. The classification and diagnostic criteria has been reviewed due to the expanding knowledge base on diabetes. In 1998, WHO published a revised classification and diagnostic criteria for diabetes that followed the American Diabetes Association's extensive review of the subject.

The WHO working group has eliminated the use of the terms "insulin dependent diabetes mellitus" and "non –insulin dependent diabetes mellitus and their acronyms as they were confusing and often resulted in patients being classified based on treatment rather than on pathogenesis but retained the terms type-1 and type-2 (Mollentz and Levitt, 2012).

1.1.1 The Extent of Diabetes Globally

Diabetes, together with cardiovascular disease, cancers and chronic lung diseases are part of non-communicable diseases (NCDs) that are currently the leading cause of death worldwide and it was estimated that in 2008, they caused 57 million deaths globally. In 2008, diabetes was directly responsible for 1.3 million deaths and its prevalence was estimated at 10% of the global population (Day, 2001). Estimates showed that in 2000, 171 million people worldwide had diabetes and this number projected to grow to 366 million by 2030. The incidence of type 1 and particularly type 2 diabetes are increasing in all societies and on all continents. The type 2 was traditionally prevalent in the elderly but it is now emerging in early childhood (Day, 2001). The above prevalence showed that diabetes has reached Table

1: Classification of diabetes mellitus and other categories of hyperglycaemia (adapted from Mollentze and Levitt, 2012)

Clinical class	Type
Diabetes mellitus	Insulin-dependent diabetes mellitus (IDDM) Non-insulin dependent diabetes mellitus (NIDDM) Malnutrition-related diabetes mellitus (MRDM) Impaired glucose tolerance (IGT) Gestational diabetes mellitus (GDM)

epidemic proportion and poses a major international challenge for optimal intervention and prevention strategies (WHO, 2012).

In the United Kingdom, diabetes is reported to affect 2.4 million people of whom 1.4 million (which is prevalence of 2.5%) are diagnosed and 1 million are undiagnosed (Day, 2001). In the United States of America, the prevalence rate of diabetes is estimated at 6.3% of the population but it differs significantly in adults in different ethnic groups, for example it is 10% among adults of European origin, 20% of Hispanics and 50% of Native American Pima Indians (Day 2001). In Asia the prevalence is at 2.5% of the population but it is estimated to exceed 3.5%. In India alone to projected increase in the number of people diabetes was from 19 million in 1995 to 57 million in 2025. The projection in China showed an increase to be from 16 million people in 1995 to 38 million in 2025 (Day, 2001).

There is limited data on the epidemiology of diabetes in Africa but a recent review has shown that the prevalence of type 2 diabetes has increased over the past decade. The reported increased rates varied from low (in rural areas) to moderate (some countries in North and North Eastern Africa) and high (among urban communities in Cairo and population of mixed Egyptian ancestry in Northern Sudan) (Rheeder, 2006). In 1997, the diabetics in Africa were estimated at 7.8 million, of whom 4.5 million were in North Africa, 1.08 million in Western Africa, 1.05 million in Eastern Africa, 0.2 million in Central Africa and 0.82 million in Southern Africa (Mollentz and Levitt, 2012), In Ivory Coast, it is estimated that the prevalence rate of diabetes is between 3 and 7% (N'guessan et al., 2009). In Nigeria, the Diabetes Association of Nigeria estimated that in 2004 there were 10 million diabetic people in the country (Jegede et al., 2011).

Diabetes mellitus is associated with reduced life expectancy; significant morbidity due to specific diabetes related microvascular complications and diminished quality of life. All these

listed conditions forces government to spend a high percentage of the health budgets on the disease. The American Diabetes Association has estimated that diabetes cost the nation \$US132 billion in 2002 and the cost will increase to \$US192 billion in 2020 (WHO, 2006 b).

1.1.2 Extent of Diabetes in South Africa

In South Africa and many other African countries, black people are increasingly coming under western influence which has led to adoption of western culture including their dietary habits. The country is experiencing rapid urbanization that comes with the adoption of cosmopolitan behaviour and consumption of unhealthy food resources. The fast foods consumed by the population are characterised by high fat, sugar and salt content. This change in nutritional composition has led to an increase in the occurrence of diabetes mellitus (Deutschländer et al., 2009 a). Previously diabetes was common among the white people in urban areas but it is fast growing among the rural population due to influence of westernization on people's lives (Moodley and Rambiritch, 2007).

The data on the prevalence of diabetes mellitus in South Africa showed that it varies between 3 and 28.7% depending on ethnic group and the community (Mellutz and Levitt, 2012). The Asians had the highest prevalence (20%), followed by the Whites (10.8%) , Coloureds (9%) and the Blacks 4.6%) (Mellutz and Levitt, 2012). The communities with the highest prevalence was Indian community of Durban 13%) and Coloured community of Cape Town (28.7%). The International Diabetes Federation's Diabetic Atlas reported that the prevalence among South African aged between 20 and 79 was 3.4% and it is expected to increase to 3.9% by 2025 (Rheeder, 2006).

Hughes and Bradley (2006) reported that the prevalence of diabetes mellitus among rural people in South Africa ranged from 4.5% in the Kwazulu Natal to 6% in the Free State. The study also reported that in Cape Town, the prevalence increased from 3.6 in the 1960's to

8%. In the rural Limpopo Province, the prevalence of diabetes was reported to be 8.8% in women and 8.5% in men (Hughes and Bradley, 2006). In another study, it was reported that the prevalence of diabetes mellitus in Qwaqwa was 4.8% and in Mangaung was 6.0% (Levitt, 1996).

One of the factors that seems to be driving the increased prevalence of diabetes mellitus is the suboptimal healthcare delivery. The poorly funded healthcare results in overcrowded clinics and hospitals, inadequate number of qualified staff and poor availability of medication for diabetes.

Due to lack of knowledge about diabetes, most people are diagnosed late and this usually leads to long term complications. If people had adequate knowledge they would prevent the impending chronic and co-morbidities of the disease by seeking early treatment and thus take charge of their situation. In a study conducted at clinics in Kwazulu-Natal, it was found that only 69% of the people had adequate knowledge of diabetes (Moodley and Rambrith, 2007). The study also showed that only 31.5% of the people with the disease could identify the symptoms of hypoglycaemia. The rural people had less knowledge of the disease as compared to the urban dwellers (Moodley and Rambrith, 2007).

1.1.3 Available treatment options

Currently, there is no cure for diabetes, but it is only managed by controlling blood sugar levels through a healthy diet, exercise and medication thus decreasing the long term complications. General objectives of diabetes management include to relieve symptoms, to correct associated health problems, to reduce morbidity, mortality and economic costs of the disease, to prevent possible acute and long term complications and to improve quality of life and productivity of individual with diabetes (WHO, 1994). The progressive nature of the

disease necessitates constant re-assessment of glycaemic control in people with diabetes and appropriate adjustment of therapeutic regimes. When a single agent is no longer good enough in controlling glycaemic levels, the addition of another drug is usually more effective than switching to another single agent (Deutschländer et al., 2009 a).

The long term complications associated with diabetes are expressed through different organs and tissues and they can include:

- i. Eyes – cataracts and retinopathy (gradual damage to the eye) that may lead to blindness
- ii. Kidneys – various diseases that can lead to kidney failure
- iii. Feet – ulcers, infections and gangrene
- iv. Nerves – neuropathy (gradual damage to the nerves
- v. Cardiovascular system – hardening of arteries, heart disease and stroke
(Heart foundation, 2012)

The management of diabetes is a four step process and the steps are diagnosis (based on symptoms), classification (type of diabetes), initial assessment (a full history, a complete examination and laboratory assessment) and treatment. The treatment has three major components namely, diet, oral hypoglycaemic therapy and insulin treatment (WHO, 1994). Diet is the basic part of diabetes management and treatment cannot be effective unless an appropriate nutrition is ensured. Physical activity is also important in type 2 diabetes mellitus as it has been associated with a decrease incidence of the disease (Bastaki, 2005). A recommended diet for diabetics include low fat and low carbohydrate diet, usage of sweeteners instead of sugar, reduction of protein intake to levels recommended by WHO (0.6 g/kg/day), restriction of sodium intake and intake of a 400 µg/day of folic acid (Bastaki, 2005).

Oral therapy for diabetes includes the use of Sulfonylureas (SUs) and Biguanides (BGs). SUs act by stimulating insulin release from the beta cells and also by promoting its action through extra-pancreatic mechanism while BG decrease gluconeogenesis and by increasing the peripheral utilization of glucose (WHO, 1994). The first generation of sulfonylureas includes tolbutamide, acetohexamide, tolazamide and chlorpropamide and a second generation that has emerged includes glibenclamide, glibizide, gliclazide and glimepiride (Bastaki, 2005). Pherformin is a biguanide that was withdrawn due to increase in frequency of lactic acidosis and metformin is the only BG now being marketed in most Eastern Mediterranean Region countries (Bastaki, 2005)

In insulin therapy, adequate education of the patients cannot be overemphasised and it requires a close cooperation of the patient, his family and the healthcare team. It is aimed at achieving good metabolic control by mimicking physiological insulin secretion as much as possible and minimizing the risk of hypoglycaemia (WHO, 1994). There are many insulin preparation available and they are grouped according to their duration of action: rapid-acting formulations (e.g. Lispro, Aspart and Glulisine) are designed to cover meals, intermediate-acting (e.g. neutral protamine Hagedon and lente insulin) and longer-acting formulations (e.g. Ultralente insulin and protamine zinc insulin) are designed to provide steady basal levels between meals and overnight (Bastaki, 2005).

1.2 OVERVIEW OF TRADE IN MEDICINAL PLANTS

1.2.1 International Trade in Medicinal Plants

Medicinal plants are in demand from both the pharmaceutical companies and traditional medical practitioners. Pharmaceutical companies use medicinal plants for the isolation of single purified drugs, extraction of active compounds and as a starting material for production of other semi-synthetic pharmacologically active substances (Laird, 2005). Traditional

practitioners use medicinal plants as raw material for preparation of various medicines (e.g. infusions, decoctions, etc.).

The demand of medicinal plants has also created a vast international trading market in medicinal and aromatic plants (MAP). Plants are no longer used by local people only but they are now also exported to other countries. The international market for medicinal plants is vast and it is very difficult to quantify due to the fact that few companies keep data for the amount of plants used. Small companies are involved in the wild harvesting of the medicinal plants as they seek them for the manufacture of pharmaceutical drugs and for herbal medicine (Hamilton, 1992). Some of the large traders in natural medicinal products include Hong Kong (77 250 tonnes), Germany (42 800 tonnes) and France (15 950 tonnes) and Europe accounts for about 25% of the international trade (Institute of Natural Resources, 2003). It has also been estimated that the global sales of herbal medicines totalled \$60 000 million in 2002 (Laird, 2005 and Hassan, 2013). The study by Hassan (2013) also reported annual European imports of MAPs from Africa and Asia worth about US\$1 billion.

The market structure of pharmaceuticals has been divided into five major blocks by the International Medical Statistics Health. These blocks consist of North America with 2005 trade estimated at \$US340.15 billion, Europe with estimated sales of \$US169.5, Japan with estimated sales of \$US60.3 billion, Asia/Africa/Australia with estimated sales of \$US46.4 billion and Latin America with estimated sales \$US24.0 billion (Institute of Natural Resources, 2003). There is also a market for botanical medicines where whole plant material contains a large constituents and active ingredients are used to produce medicines. This industry consists mainly of phytomedicines, herbal remedies, and cosmeceuticals. These products are sold in various forms that include capsules, tablets, herbal teas, extracts, tinctures, dried and fresh herbs (Loundou, 2008)

1.2.2 Medicinal plants trade in South Africa

It is estimated that there are about 27 million South Africans that still rely or use traditional medicine (Street et al., 2008). Factors driving this reliance on traditional medicine are largely based on the fact that they are accessible, affordable and the extensive knowledge among the local communities. The trade in medicinal plants in South Africa is largely informal, occurring mostly on street markets, muthi markets and muthi shops (Figure 1). Large quantities of both semi-processed and unprocessed medicinal plants are sold at these markets which are found in almost all the cities and towns. It is estimated that between 35 000 and 70 000 tonnes of medicinal plant material traded by the herbal consumers (Loundou, 2008).

There are about 200 000 practicing traditional healers in the country (Institute of Natural Resources, 2003). The high number of healers coupled with high number of herbalists and rapid urbanization are the driving forces behind the trade in medicinal plants. The trade in medicinal plant, which is estimated at R62 million per annum is a part of a R500 million traditional medicine business. It is estimated that in Kwazulu Natal alone about 4500 tonnes of 450 plants are traded annually (Institute of Natural Resources, 2003). Most households spend between 4% and 8% of their annual income on traditional medicinal services (Deuschländer et al., 2009 b).

Traditional healers use different parts of the plants as medicine. Some plants are harvested for their roots, while others are harvested for their stems, bark or leaves. The amount of wild plants collected by harvesters/commercial gatherers are determined by demand of the traders, healers and the general public. With the high demand in medicinal plants and the collecting methods has led to a serious decline in wild stocks of medicinal plants. There are reports of some of the most popular medicinal plants have been harvested to extinction. The Wild ginger (*Siphonochilus aethiopicus*) and pepper bark tree (*Warburgia salutaris*) can no longer

be found in the wild. This clearly indicates that the harvesting of wild stocks is not sustainable as the current rates of harvesting exceed the ability of wild stocks to meet the demands.

Presently most of the research on medicinal plants are focused on identifying their active compounds and the possibility of establishing new drugs. There is a serious threat of harvesting medicinal plants to extinction and losing valuable plants forever. It becomes very important that more studies need to be undertaken to determine possible threats to species survival before it is too late. The health of millions of people could be at risk because of the over-exploitation of medicinal plants. The loss of medicinal plant diversity can be viewed as a quiet disaster (Edwards, 2009). Therefore documenting the trade in plant species in general and medicinal plants in particular should be seen as a first step in identifying species in need of conservation and sustainable usage of the biodiversity resources.



Figure 1: Medicinal plant trade at the Setsing Shopping Complex in Phuthaditjhaba (Pitso 2013)

1.3 TRADITIONAL MEDICINE IN THE TREATMENT OF DIABETES

1.3.1 Use of medicinal plants in treatment of diabetes

The World Health Organization advocates for a three component management plan for diabetes treatment. The components are diet modification combined with exercise, oral hypoglycaemic therapy and insulin treatment. Some of the medications for diabetes have serious side effects that include liver failure, respiratory infection, headache, nausea, diarrhoea, skin rash and itching (WHO, 1994). On top of these side effects, the medicines are not easily accessible and are too expensive for the majority of the population in the rural areas in the developing countries. People have increased their interest in traditional medicines to overcome the problems associated with diabetes mellitus.

Before the advent of insulin in 1922, starvation diets and traditional plant treatments were the only anti-diabetic remedies (Day, 1990). In occidental societies, insulin was hailed as the miracle life-saver and traditional plant treatments were forgotten, but in many deprived regions of the globe, the traditional herbal remedies continue to form the cornerstone of diabetic treatment (Day, 1990).

Plants that have hypoglycaemic properties have been used in traditional medicines for a very long time around the world (Yeh et al., 2003). There are over 1200 different plant species that have been reported to be used by traditional healers around the world to treat diabetes and only 30% of these plants have been pharmacologically investigated (Ayyanar et al., 2008). WHO has identified the scientific or medical scrutiny on traditional medicines used to treat diabetes as an area that needs serious attention (Swanston-Flatt et al., 1990).

Many plants synthesize substances that are useful in the maintenance of health in both humans and animals. Most of these substances are secondary metabolites like phenols or their

oxygenated substituted derivatives such as tannins. Plants that naturally synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannin and volatiles and contain minerals and vitamins are the ones that possess medicinal properties (Faleyimu et al., 2010).

Most of the anti-diabetic medicines that have been developed over the years are chemical or biochemical agents aimed at controlling or lowering blood glucose levels to a normal level. Despite the advances in health sciences and medical care, millions of people around the world are still using traditional plant remedies or herbal formulations to treat hypoglycaemic conditions. Insulin is a life saver for most diabetes sufferers but it is not a cure at all, as the majority of type 2 sufferers are insulin-resistant to a point where even the supra-normal insulin concentration are insufficient to control hyperglycaemia (Day, 1990).

Ethno-botanical surveys on medicinal plants used by local populations have been performed in different parts of the world and there is a considerable number of plants described as anti-diabetic. Alexiou and Demopoulos (2010) listed 147 plants used to treat diabetes from around the world. These plants belonged to different families but most of them were from Asteraceae, Fabaceae, Rutaceae, Liliaceae, Araceae and Leguminosae. Ten plant species were used by two tribal groups in South Tamil Nadu India two and were from the family Asclepiadaceae and two from Acanthaceae (Ayyanar et al., 2008). A review of herbs and dietary supplements for glycaemic control in diabetes by Yeh et al. (2003) identified 108 human trials using 12 medicinal plants and they all had glycaemic activity. A study by Swanston-Flatt et al. (1990) showed that some of the plants used to treat diabetes are not active in reducing or altering hyperglycaemia or hypoinsulinaemia. In this study, only five of the eleven plants (*Agrimonia eupatoria*, *Medicago sativa*, *Coriandrum sativum*, *Eucalyptus globulus* and *Juniperus communis*) retarded the development of streptozotocin diabetes in mice and the rest of the plants (*Rubus fruticosus*, *Chemlidonium majus*, *Allium sativum*,

Alchemi vulga ris, *Glycyrhizza glabra* and *Convallaria majalis*) failed to reduce the hypoglycaemia levels in diabetic mice.

Studies have been conducted in different African countries to identify medicinal plants used to treat diabetes. A study by Faleyimu et al. (2010) identified eleven forest plants used by traditional healers to treat diabetes in one local government in Nigeria. Traditional healers in Agbonville, Côte-d'Ivoire, used 28 different species of plants which belonged to 22 families in the treatment of diabetes (N'guessan, et al., 2009).

1.3.2 Medicinal plants used to treat diabetes in South Africa

Deuschländer et al. (2009 a) published an inventory of plant species (32 species) used in the treatment of diabetes by traditional healers in South Africa, which also included the species' phytochemistry and/or bioactivity. Further studies of four species from the inventory (*Euclea undulata*, *Schkhuria pinnata*, *Elaeodendron transvaalense* and *Pteronia divaricata*) showed hypoglycaemic activity which validated their usage in traditional medicine (Deuschländer et al. (2009 a).

A study by Afolayan and Sunmonu (2010) reported that 32 medicinal plant species have been identified for the treatment of diabetes from ethnobotanical surveys in South Africa covering Eastern and Western Cape Provinces, South-eastern Karoo, Khoi-San and Cape Dutch areas. Only nine of these plants (*Hypoxis hemerocallidea*, *Vernonia amygdalia*, *Catharanthus roseus*, *Leonotis leonurus*, *Catha edulis*, *Momordica foetida*, *Sclerocarya birrea*, *Psidium guajava* and *Sutherlandia frutescens*) have documented reports of *in vivo* anti-diabetic activities (Afolayan and Sunmonu, 2010). An earlier study conducted in the Eastern Cape, by Erasto et al. (2005) reported on the use of 14 medicinal plants used by the Xhosa people in the treatment of diabetes. The dominant family in this study was Asteraceae, represented by seven species (*Helichrysum odoratissimum*, *H. nudifolium*, *H. petiolare*, *Artemisia afra*,

Vernonia oligocephala, *V. amygdalina* and *Brachylaena discolor*) followed by families with two species, Hypoxidaceae (*Hypoxis hemerocallidea* and *H. colchifolia*) and Asphodelaceae (*Bulbine natalensis*, and *B. frutescence*). The remaining three families had one representative, Apocynaceae (*Catharanthus roseus*), Apiaceae (*Heteromorpha arborescens*) and Buddlejaceae (*Chilianthus olearus*).

The Bapedi tribe in Limpopo Province, used 24 plants species belonging to 20 families in treating diabetes (Semenya et al., 2012). The most represented family was Asteraceae with 13% of the species and 42% of these anti-diabetic plants are also sources of food. It has been reported that in the eastern Free State, 24 medicinal plants species belonging to 17 families were used by traditional healers in treating diabetes (Tshabalala, 2012). The family with highest representation was Asteraceae (29%), followed by Fabaceae (12%) and Asphodelaceae (12%). Only two of the species in Tshabalala's study (*H. hemerocallidea* and *Cannabis sativa*) have been reported in other ethno-botanical surveys and the rest were reported for the first time (Tshabalala, 2012).

1.4 ECOLOGY AND CONSERVATION OF MEDICINAL PLANTS

It is estimated that approximately 3 billion people in the developing world still rely on traditional medicines, 80% of them are plants based (Kala, 2000). About 75 – 80% of the medicinal plants are sourced from the wild collection (Hawkins, 2008). This high demand and pressure on the natural resources raise a serious concern on the survival of these species. The WHO realised the threat to the continued existence of these important natural resources and developed a number of documents that have been used to guide the international community conserving medicinal plants. The 1988 Chiang Mai Declaration "Saving lives by saving plants" formed the basis of the document titled the "Guidelines on the conservation of medicinal plants" published in 1993. These guidelines were revised in 2003 due to new

global developments in conservation of medicinal plants and their use over the intervening decade. Other important documents based on the guideline include the World Wide Fund (WWF) for Nature “People and Plants (1993 – 2004)”, International Union for Conservation of Nature’s (IUCN) Medicinal Plant Specialist Group (1994)” and WHO’s Traditional Medicine Strategy 2002 – 2005 published in 2002” (Kathe, 2006).

A meeting held between representatives of WHO, IUCN, WWF and TRAFFIC International to discuss challenges of conservation of medicinal plants recognised the need to respond to the increasing political, economic and consumer demand to product quality and to create a strong awareness that product quality is determined by both the pharmaceutical quality and by sustainability of practices of sourcing the product (Kathe, 2006).

South Africa is blessed with a very immensely rich biodiversity characterised by high levels of endemism and genetic diversity. There are over 20 300 flowering plants in South Africa and about 2000 of them are used in traditional medicine (SANBI, 2013). The conservation of the biodiversity is enshrined in the constitution of the Republic of South Africa with Chapter 2 firmly entrenching protection of the environment as a fundamental human right and Section 24 imposing a duty to the state to protect the environment for the benefit of current and future generations through legislative and other means, and to ensure ecologically sustainable usage of the natural resources while pursuing economic and social development (van der Linde, 2006). The National Environmental Management Act (NEMA) of 1998 was promulgated to provide legal framework to concretize the rights guaranteed by the section 24 of the constitution. This act is based on the White Paper on the Conservation and Sustainable Use of South Africa’s Biological Diversity published in 1997.

Like many other countries, South Africa has developed a plant Red Data List (RDL) that identify extinct, threatened and potentially threatened species. Other categories of threat to

species perpetuation have arbitrary thresholds between rare and not rare, vulnerable, and endangered (Ungricht et al., 2005). In developed countries, information in such lists is used to establish new legislation but it has yet to make impression in the developing countries (Golding, 2001). RDL provides data on the conservation status of species and this information can then be used by conservationists to prevent known species to be listed as “extinct”. RDL usually lack geo-referenced primary data and are thus inadequate in conservation of species but they can still be used together with regional checklists to guide prioritization of primary data for conservation. In the context of Convention on Biological Diversity, governments are obliged to pay particular attention to all species endemic within their borders (Schatz, 2002).

The government of South Africa has shown willingness to meet their constitutional obligation in conserving natural resources. The main challenge in this country is the lack of knowledge on social and economic values of biodiversity and thus the potential impact of biodiversity on social wellbeing is not fully recognised (Turpie, 2003). This has resulted in the under-funding of conservation projects due to their low priority at national level.

Conservation depends on the sufficient space to maintain a viable population, the size of which will vary from one species to the next depending on life histories and habitat requirements. The ideal exercise in determining conservation status is to undertake Population Viability Analysis (PVA) which can provide the exact sufficient space for each species. Undertaking PVA is time consuming and the next best thing is to use Geographical Information System (GIS) tools to estimate the species vulnerability to threat and therefore prioritize conservation of vulnerable species. There are inherent assumptions in making such estimates:

- i. The known collections and locations of a species are a valid reflection of their abundance and distribution
- ii. The widespread and common species will be at lower risk than restricted and rare ones
- iii. Species occurring within protected areas will be at lower risk than those in unprotected areas (Schatz, 2002).

1.5 THE ROLE OF HERBARIA IN PLANT CONSERVATION

Plant taxonomist have over the years used herbarium specimens to erect taxonomic frameworks within which species are defined, circumscribed and delimited from related species (Schatz, 2002). Herbarium collections have also been used as source for computing and comparing diversity indices (Ungricht et al., 2005). Well prepared specimen can provide important information such as full description of location and abundance of a particular species at a particular time. The voucher specimens thus provide permanent and variable data on a species at a particular time.

Golding and Smith (2001) proposed a 13 point flora strategy of using herbarium collections to meet conservation challenges. They pointed out that floras are not necessarily produced to assess RDL status of species but as taxonomic tools. The strategy goes a long way in adding value to herbarium collections and deconstructing the erroneous view that there is no potential of applying taxonomic science in implementing conservation strategies. The strategy recommends the approach that needs to be taken by herbarium field workers so that the gathered data becomes relevant to conservation strategies. The herbaria need to define their role in the broader conservation spectrum by aligning herbaria activities with the needs of conservation. It was recommended that specimen that are known to be on RDL should be

clearly marked by using a variety of methods such as computer coding, stickers, coloured species covers, labels that need to be relevant for both the taxonomist and the conservationist by always including data such as habitat, distribution and population.

There are limitations in using herbaria data in conservation strategies. The specimen sheets are generally regarded as qualitative rather than quantitative sources of data (Willis et al., 2003). The collections are usually concentrated near settlements or in areas of easy road access and the number of collection vary over time. The data acquired from herbaria specimen often only provide a rough approximation of species distribution. Earlier collections usually lack precise coordinates and contain imprecisely locations in relation to population centres and thus cannot be geo-referenced. Historical specimen may only contain sparse handwritten details of the plant name, collector and locality and therefore will be of limited value to the conservation analysis (Wills et al., 2003). Other limitations arise due to the fact that population reductions cannot be determined from herbarium labels data and there is no indication of a number of individuals in a population. The collections of individual specimen in particular area are sporadic and there are no follow up on collections of a particular species. Once specimen/sample is collected it becomes difficult to determine population trends. Another problem is the collection of species in areas where they are unusual and ignored where they are common so the collections become unsystematic and so some areas will be under-represented.

Data on herbarium collections can be used in conservation as a way to explore the options and demonstrate the role that such data can play in identifying sampling gaps, improving survey design, reducing collecting biases, building richness restricted areas (endemicity) and distribution maps and selecting priority biodiversity sites (Funk and Richardson, 2002).

An area of conservation that still needs to be exploited and integrated into the main stream conservation strategy, is the indigenous knowledge and conservation systems. There is an increasingly comprehensive appreciation of traditional ecological knowledge and ethnoecology as systems of local and indigenous conservation. These conservation knowledge is mostly acquired after a resource has been depleted. They can provide important indicators and early detection of threatened species (Berkes and Turner, 2006). Conservation strategies need not be blind to the fact that people and societies are capable learning from experience, modifying their knowledge and rule sets, and passing their knowledge on to others.

1.6. HYPOTHESIS AND OBJECTIVES OF THE STUDY

The hypothesis of this study was that the increased awareness of the advantages of traditional medication over modern medication, easy access and affordability of traditional medicines and increasing prevalence of diabetes among the black population will negatively impact the natural populations of the medicinal plants used by traditional healers and herbalists.

The primary objective of this study was to determine the conservation status of the six plants identified as the most commonly used to treat diabetes in the eastern Free State by :

1. Identify the sites where the healers and herbalists commonly collect the plant materials
2. Conduct a survey of herbarium specimen of the study plants, focusing on dates of collection, number of specimen and area of collection
3. Conduct ecological surveys of the study plants at the identified sites to determine the population sizes

1.6. RATIONALE

Diabetes is a growing problem among the black population in South Africa. The study area falls under an area that was identified as poverty nodal point by the government under the leadership of President Thabo Mbeki in February, 2001 (thepresidency, 2001). The poverty level was high due to a high unemployment rate (88%) and thus it becomes difficult for most people to access basic health and mainstream health products (the presidency, 2001). Most of the people in the area still rely on traditional healers for their health requirements.

The problem of drug resistance to modern medicine has necessitated the search for new efficacious agents with fewer side effects. Plants used in traditional medicine to treat diabetes offer the best option in this regard. There is a growing interest in the use of medicinal plants which are almost always collected from the field. The traditional medicines are usually cheap and easily accessible and have fewer side effects.

The increased demand in traditional medicine has placed a serious pressure on these natural resources. There is a concern that overharvesting may threaten the continued existence of the important resources. These plants have a potential to provide new more potent drugs and hence it has become imperative to conserve them.

1.8 STRUCTURE AND SCOPE OF THIS DISSERTATION

The scope of this dissertation includes a literature review on diabetes, medicinal plants used to treat diabetes (globally and in South Africa) and conservation of medicinal plants in Chapter 1. The study or research question, objectives and rationale are also outlined in Chapter 1. Chapter 2 deals with morphology, ecology and pharmacological activity of six medicinal plants used to treat diabetes in the eastern Free State, South Africa and also cover the description of the study area.

Chapter 3 deals with the questionnaire administered to the traditional healers and herbalists in the study area and also cover description of the herbarium collection and ecological survey of the six studied plants. Chapter 4 contains the results gathered from the questionnaire from both the herbarium and ecological surveys. Chapter 5 deals with the discussion of the observed results. Chapter 6 brings together all the findings of the investigation and ends by making recommendations for a more integrated approach to conservation of the wild medicinal plant populations.

CHAPTER 2

PLANTS COMMONLY USED TO TREAT DIABETES IN THE EASTER FREE STATE PROVINCE OF SOUTH AFRICA

2.1 STUDY AREA

The study was based in the Eastern Free State in Maluti-A-Phofung Municipality which falls under the Thabo Mofutsanyana District Municipality (Figure 2). The study area is in the north-eastern Free State, in the former homeland of the Basotho tribe, Qwaqwa. It is situated south of latitude 28° S and west of 28° 30' longitude with the highest peak at 2217 m . The eastern Free State lies in the summer rainfall region of South Africa and receives more than 80% of its annual precipitation during September and March, mainly in the form of thunderstorms. The annual precipitation for the lower lying plateau is approximately 700 mm increasing gradually to 1340 mm in the south (Moffett et al., 2001). Hail storms are not uncommon, occurring about three times per annum in the north-west and up to eight times in the south-east. In June and July the minimum temperature is often below -10 °C and on the summit plateau can reach -20 °C. Frost is common in the lower lying areas any time between April and October. Snow can also fall at any time of the year in the higher parts, but usually only falls in the winter months.

Geology of the area was described by Moffett et al. (2001) as an area underlain by rock formations representing the upper Karoo Sequence which is intruded by dolerite dykes and sills. Recent alluvium and scree covers the valley floors and mountain slopes . The Beaufort group is represented by red mudstones and the light brown fine grained Feldspathic sandstone of the Tarkastad Subgroup. The four geological formations that are visible in the area, from bottom top are Molteno, Elliot, Clarens and Drakensberg.

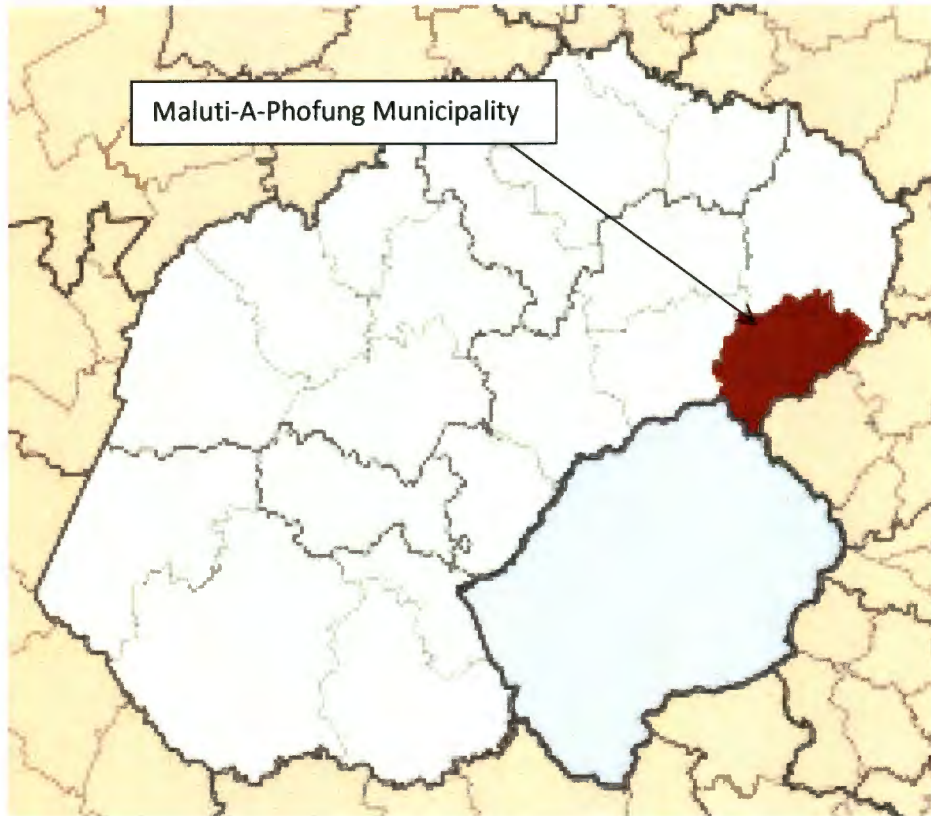


Figure 2: Map of the municipality demarcations in the Free State, with Maluti-a-Phofung municipality (study area) highlighted (wikipedia.org)

The vegetation type of the study area is generally classified as the Eastern Free State Grassland (Mucina and Rutheford 2006), generally characterised by closed grassland dominated by *Eragrostis curvula*, *Tristachya leucothrix* and *Themeda triandra*.

2.2 PLANTS COMMONLY USED TO TREAT DIABETES IN THE EASTERN FREE STATE PROVINCE, SOUTH AFRICA

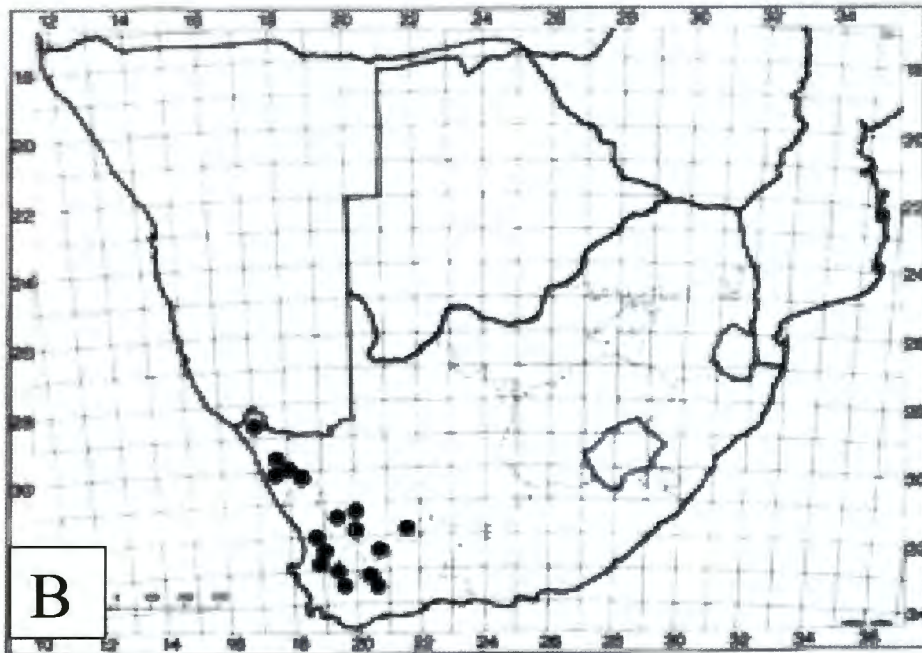
The plants being studied were identified from a previous study conducted at the Department of Plant Sciences of the Qwaqwa campus of the University of the Free State in 2012. A total of 24 species were identified as being used to treat sugar related disorders by traditional healers in the eastern Free State (Tshabalala, 2012) and the six most frequently mentioned by the healers were selected for the current study (Table 2). Traditional healers were requested to confirm the use of the selected plants in treating diabetes as part of a questionnaire.

Table 2: Plants used by traditional doctors to treat diabetes in the Eastern Free State and their frequency of usage (adopted from Tshabalala, 2012).

Scientific Name	Sesotho Name	Family	Frequency of mention by healers /20
<i>Eriocephalus punctulatus</i>	Sehalahala sa matlaka	Asteraceae	10
<i>Hypoxis hemerocallidea</i>	Tapole	Hypoxidaceae	8
<i>Dicoma anomala</i>	Hlwenya	Asteraceae	8
<i>Xysmalobium undulutum</i>	Pohotshehla	Apocynaceae	6
<i>Morella serrata</i>	Monna motsho	Myricaceae	6
<i>Gazania krebsiana</i>	Tsikitlane	Asteraceae	6



A



B

E. punctulatus

Figure 3: A - Picture of *Eriocephalus punctulatus* (google.co.za) and B - its distribution map (plantzafrica.com)

2.2.1 *Eriocephalus punctulatus*

Eriocephalus punctulatus is a member of the Asteraceae. It is a highly aromatic shrub and is easily identifiable from late spring to early summer as is covered in fluffy white heads. It is characterised by simple and quite small leaves that are covered with pitted glands (Figure 3A). *E. punctulatus* flowers profusely bearing bright white ray florets (Pooley, 1998)

Ecologically, *E. punctulatus* is mostly restricted to the winter-rainfall regions with the Greater Cape Floristic Region. It grows from Namaqualand and south to the Roggeveld and Witteberg Mountains (Plantzafrica, 2013) (Figure 3B). It also grows in the north-eastern slopes of the Drakensberg Mountains in the eastern Free State (Mierendorff et al., 2003). A 2005 assessment gave *E. punctulatus* RDL status of Least Concern (LC) (SANBI, 2013).

The genus *Eriocephalus* is an important part of traditional medicine among the ethnic groups in both South Africa and Namibia. Leaf infusions of *E. africanus* are usually used as diuretics and diaphoretics and in the treatment of gastro-intestinal complications, inflammation and other dermal complications. Oils extracted from the plant has aromatherapeutic properties that includes being analgesic, anti-allergic, antiseptic, antidepressant, and anti-inflammatory (Njenga, 2005; Njenga and Viljoen, 2006). *E. Racemosa* has also been used for treatment of respiratory ailments, gastro-intestinal disorders and various skin inflammation diseases, *E. tenuifolius* as a substitute for “buchu” due to the presence of compounds with diuretic effects and *E. karooicus* as wild “dagga” due to compounds that induce psychotropic effects (e.g. linalyl acetate and β -caryophyllene (Njenga, 2005).

E. punctulatus is used to treat stomach diseases, as anti-inflammatory, or to fumigate huts (Mierendorff et al., 2003; Njenga and Viljoen, 2006). The oil of *E. punctulatus* is blue in colour due the presence of azulene derivatives in the oils. They are formed by decomposition

of proazulenes during steam distillation (Mierendorf et al., 2003). In GC-MS analysis of the oil of the plant, 123 compounds were identified with the aliphatic esters being the dominant group as they amounted to more than 50% and the main component was 2-methylbutyl 2-methylpropanoate with 21.2% composition. The terpenoid component was 18.9 % and it consisted of 27 oxygenated monoterpenes and 15 monoterpene hydrocarbons and the dominant compounds in this case were linalyl acetate and α -pinene with 4.4% and 1.9% composition respectively (Mierendoff et al., 2003). The extracts of *E. punctulatus* showed inhibitory activity against 5-lipoxygenase enzyme which validated the use of the plant to treat inflammatory diseases (Njenga and Viljoen, 2006).

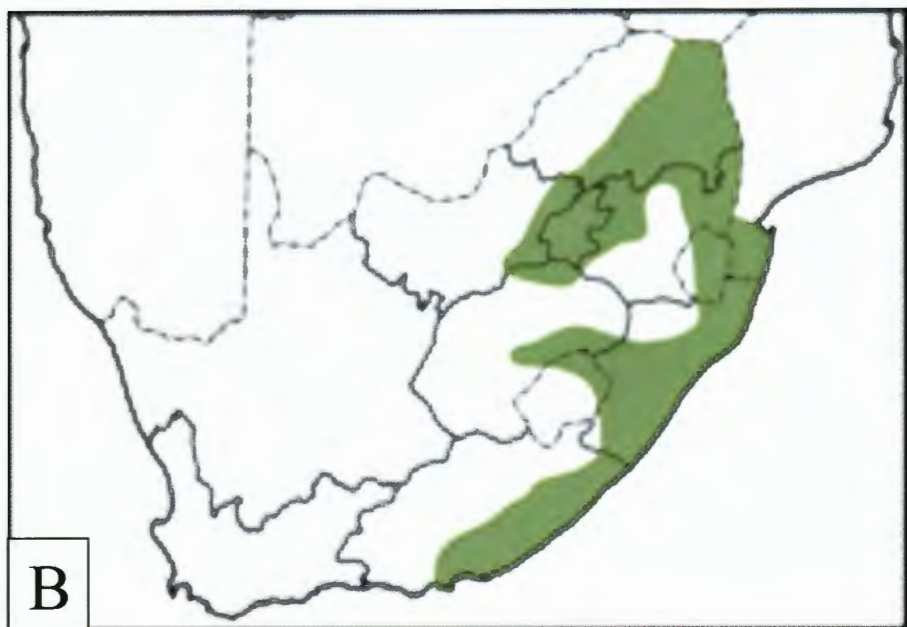


Figure 4: A - *Hypoxis hemerocallidea* (en.wikipedia.org/wiki/Hypoxis_hemerocallidea, 2013) and B - the distribution map of *H. hemerocallidea* (e-monocot.org/taxon/urn, 2013)

2.2.2 *Hypoxis hemerocallidea*

H. hemerocallidea is a tuberous perennial that can be up to 25 - 70 mm in diameter. It has strap-like leaves that can grow up to 400 mm and they are arranged in 3 ranks arching outwards and they appear above ground in spring (Figure 4A). The flowers are yellow and star shaped. The flowers are carried on 5 or 6 erect inflorescences each having between 5 and 13 flower (Pooley, 1998).

Ecologically, *H. hemerocallidea* is not endemic to South Africa. It occurs in both grasslands and woodlands across the country. The only Provinces where the plant has not been recorded are Northern Cape and Western Cape, but it is also found in Lesotho, Mozambique, Zimbabwe and Botswana (Figure 4B). It is scarce in the other countries as compared to South Africa (Pooley, 1998). The conservation status of *H. hemerocallidea* has been listed as Declining on the RDL due to extensive commercial exploitation since 1997, habitat loss and degradation (redlist.sanbi.org.za).

Through evolution, plants have developed many strategies to adapt to their environment and one of the most important tools in these strategies is the production of secondary metabolites. These secondary metabolites are particularly involved in the plant-microbe interactions as both weapons and defence mechanisms (Popovici et al., 2010). *H. hemerocallidea* and other members of the genus *Hypoxis* have been used extensively in traditional medicine to cure various conditions and disorders. *H. hemerocallidea* is used to treat all kinds of human and veterinary diseases in South Africa and hence it is referred to as 'zifozoneke' which translates into a plant that can cure all diseases. It has been hailed as a miracle 'muthi' (Zulu word that refers to herbal remedy) and wonder potato and it has become one of the best known medicinal plant in the country. It is also said to be one of the most researched medicinal plants in Africa (Ncube et al., 2013). The wide range of medicinal usage includes the

treatment of intestinal infections, urinary infections, infertility, vomiting, nausea, cough, palpitations, heart weakness, impotency, anxiety, insanity, lice, common cold, flu, ulcers, wounds, arthritis, cancers, conditions related to HIV/AIDS, hypertension, diabetes, tuberculosis, asthma, central nervous system disorders including epilepsy and childhood convulsions, depression, laxative, vermifuge, burns, prostatitis, and many more (Ncube et al., 2013).

The decoctions of *H. hemerocallidea* are used for cardiac diseases, impotency, barrenness, intestinal parasites, cancer, headaches and testicular tumours, HIV, diabetes, pimples, high blood pressure, benign and prostrate hypertrophy, dermatitis and mental disorders (Drewes et al., 2008). The plant is also used as generic tonic for good health and as a purgative. The infusions of the corms are used as emetics to treat dizziness, urinary tract infections and insanity (Ojewole et al., 2009)

The ethnomedical use of *H. hemerocallidea* has been supported by a number of pharmacological studies. Ojewole et al. (2009), showed that the corm aqueous extracts possess antidiarrheal activity. It was speculated that this activity was due to the presence of sterols, stanols, and sterols especially rooperol and β -sitosterol in the extract. The presence of phytosterols with their recognised 5, α -reductase and aromatase inhibition account for the activity of the plant extracts in amelioration of benign prostrate hypertrophy and urinary infection as well as immune modulation. The sterols have also been reported to possess immune modulating properties which may have some use in the treatment of TB (Katerere and Eloff, 2008).

The South African Health Ministry has recommended the use traditional medicine with antiretroviral treatment. The principal species recommended are *H. hemerocallidea* and *Sutherlandia* sp. The use of these plants in HIV/AIDS treatment is also supported and

recommended by 14 other SADC countries. Studies have shown that the extracts of the plant have the potential to interfere with the efflux of nevirapine across epithelial cell and potentially increase the bioactivity of this antiretroviral drug when taken concomitantly (Ncube et al., 2013). On the other hand, Mills et al. (2005) reported that *in vitro* model showed that *Hypoxis* has the potential to interact with the HIV drug metabolizing enzyme, which could lead to drug resistance, drug toxicity and/or treatment failure.

One of the most common traditional use of *H. hemerocallidea* extracts is the treatment of cancer. Steenkamp and Gouws (2006) found out that the aqueous extract of the plant significantly stimulated the prostate cancer cell growth more than a well-known anti-tumour agent, cisplatin. *In vitro* studies have shown the compound hypoxoside to be cytotoxic to cancer cells at concentrations of up to 100 µg/mL. β-3glucosidase (aglucone of rooperol) is a potential oral prodrug for cancer therapy in humans due to its first-pass metabolism into non-toxic conjugate rooperol which may be activated in tumour cells with high deconjugase activity (Ncube et.al., 2013).

Rooperol from *H. hemerocallidea* caused a moderate, transient increased cardiac output, stroke volume, and vascular pressure without increased heart rate or filling pressures which suggested the increased myocardial contractility (Ncube et al., 2013). In a study by Ojewole et al. (2006) it was shown that a significant concentration-dependent positive inotropic and chronotropic responses was shown and the extracts also led to dose related transient but significant reductions in the systemic arterial blood pressure and heart rates of hypertensive rats. These observations suggested that *H. hemerocallidea* corm extracts contain some active chemical constituents with some cardiovascular activities and validated the traditional medicinal use of the plant in treating heart related diseases.

Ojewole (2006), investigated and reported that the decoctions of *H. hemerocallidea* as useful remedies for the management of painful arthritic and inflammatory conditions. Experiments with experimental animals have shown that the rats had an increased threshold to pain when aqueous extracts of the plant were administered and that the extracts also led to significant reduction in fresh egg albumin-induced inflammation of rat hind paw. The evaluation of anti-diabetic activity of extracts of *H. hemerocallidea* on rats induced by intraperitoneal injection of streptozotocin showed hypoglycaemic activity as evidenced by a significant reduction in the blood glucose concentrations. It was concluded that the plant extract induced hyperglycaemia by stimulating insulin release thereby enhancing the cellular uptake and utilization by animals (Drewes et al., 2008). These studies supported the folkloric claims and gave credence to the traditional usage of the plant as anti-diabetic and anti-inflammatory agents.

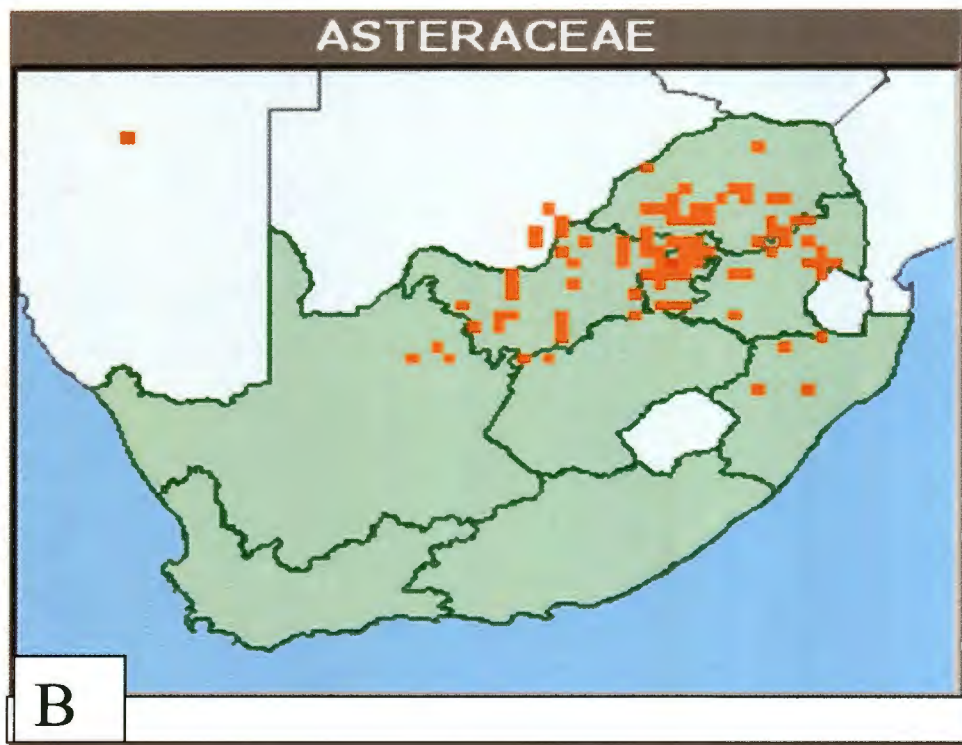


Figure 5: A - Picture of *Dicoma anomala* (Pitso 2013) and B- Distribution map of *Dicoma anomala* (redlist.sanbi.org.za)

2.2.3 *Dicoma anomala*

Dicoma anomala belongs to the Asteraceae. It is an erect, sub erect, prostrate, decumbent perennial herb bearing semi-woody tubers at the base of a woody subterranean stem. The stems are branched yellowish, trail along the ground and over surrounding plants and rock. They are thinly covered by hairs. The leaves are simple, narrow, positioned alternately on the stem 4 to 5 cm apart, stalk-less and lanceolate. The lower surface is white and hairy with a faintly uneven surface. Flower heads are terminal, cup or cone shaped, cream to pinkish-white disk florets, with a fluffy top (Figure 4). Flowering occurs from November to July, with peak flowering occurring in February (Kazembe and Chinyuku, 2012).

Ecologically, *D. anomala* is a grassland species widely distributed in sub-Saharan Africa, growing in stony or wooded grasslands in summer rainfall areas, in stormy grasslands, hillsides or flat grasslands in savannah, or dolerite or sandy soils at altitudes ranging from 165 to 2075 m (Kazembe and Chinyuku, 2012). In South Africa it occurs in all Provinces except in the Western Cape and Northern Cape (Figure 5) (redlist.sanbi.org.za).

In traditional medicine, *D. anomala* is used as a root decoction to treat blood disorders, colic, diarrhoea, dysentery, toothache, fever, malaria, coughs, haemorrhages, ulcers, dermatosis, venereal diseases, labour pains, purgative for intestinal worms and as an ingredient in the preservation of medication. Other parts of the plant are used to induce vomiting in suspected food poisoning and also to treat respiratory conditions (Kazembe and Chinyuku, 2012). Such uses can be linked to pharmacological properties of the decoction: antibacterial, antihelmintic, antiviral, anti-plasmodial, anti-spasmodic, wound healing, analgesic and anti-inflammatory (Becker et al., 2011). A previous study reported the antibacterial and anti-inflammatory properties of the root extracts of *D. anomala* and another

study demonstrated the *in vitro* anti-cancer activity of extracts of another member of the genus, *Dicoma capensis* (Steenkamp and Gouws, 2006).

Phytochemical studies have identified several classes of secondary metabolites including acetylenic compounds, phenolic acids, sesquiterpene lactones, triterpenes, phytosterols, asymmetrical sesquiterpene dimers with potent anti-plasmodial and anti-cancer properties (Becker et al., 2011, Kazembe and Chinyuku, 2012) . A compound from *D. anomala* with anti-malarial activity was isolated and identified as dehydrobrachylaenolide, a sesquiterpene lactone, and it is said that it could play a valuable role as a drug lead (Becker et.al., 2011). This new discovery could help in the fight against malaria which is becoming more resistant to standard anti-malarial agents.



Figure 6:Field photograph of *X. undulatum* (Royal Museum for Central Africa)

2.2.4 *Xymalobium undulatum*

Xymalobium undulatum is a member of the Asclepidaceae. It is a geophytic herb that can grow up to 2 m high. It usually sprout from a rootstalk in spring and die back in winter. It has large hairy leaves that are almost stalkless and bears many stalked inflorescences at the axils of the leaves. Young leaves bears densely pubescent indumentum with numerous non-glandular hair-like trichome on both surfaces. The leaves are amphistomatous, having equally abundant stomata on both surfaces. It bears large inflated fruit that are covered by re-curved soft bristles (Figure 6). All parts of the plant exudes milky sap in response to damage or injury (Pooley 1998, Naidoo et al., 2009; SANBI, 2013).

Ecologically, *X. undulatum* is widely distributed mainly in the eastern parts of southern Africa. In South Africa, it is found in all Provinces and common in Namibia, Botswana, Lesotho and Swaziland . It usually grows in areas with higher rainfall, in open or moist grasslands, particularly in wetlands and seepage areas (Pooley, 1998). The plants has been awarded the LC status on the RDL since it was not selected one of the four screening processes for highlighting potential taxa of conservation status (redlist.sanbi.org).

Literature review showed that *X. undulatum* has been used in traditional medicine to treat various ailments such as diarrhoea, menstrual cramps, skin diseases, fever, coughing, influenza, urinary tract infections, gonorrhoea, headache relief and hysteria in young women, (Pedersen et al., 2006, Naidoo et.al., 2009). The plant is also used as charms to divert storms, prevent poisoning and make dog s keen hunters (Pooley, 1998). The plant together with *Agapanthus campanulatus*, *Boophone disticha*, *Mondia whitei*, were reported to exhibited anti-depressant like activity in three *in vivo* models for depression (Stafford et.al., 2008). The powdered tuber of the plant is used to treat sores, wounds and abscess (Steenkamp et.al.,

2004). Decoction of the plant is usually taken for the treatment of syphilis (Buwa and van Staden, 2006).

Pharmacological studies have shown that *X. undulatum* does not possess antibacterial activity (Rabe and van Staden, 1997, Kelmanson et al., 2000, Steenkamp et al., 2004). The presence of serotonin receptor in the extracts of the *X. undulatum* could account for the observed clinical effects in treating central nervous system (CNS) disorders (Nielsen et al., 2004). The neuroprotective effect of the plant has been ascribed to several glycosides (Adewusi and Steenkamp, 2011). In a study by Buwa and van Staden (2006) it was shown that water extracts of the plant had antibacterial and antifungal activity.

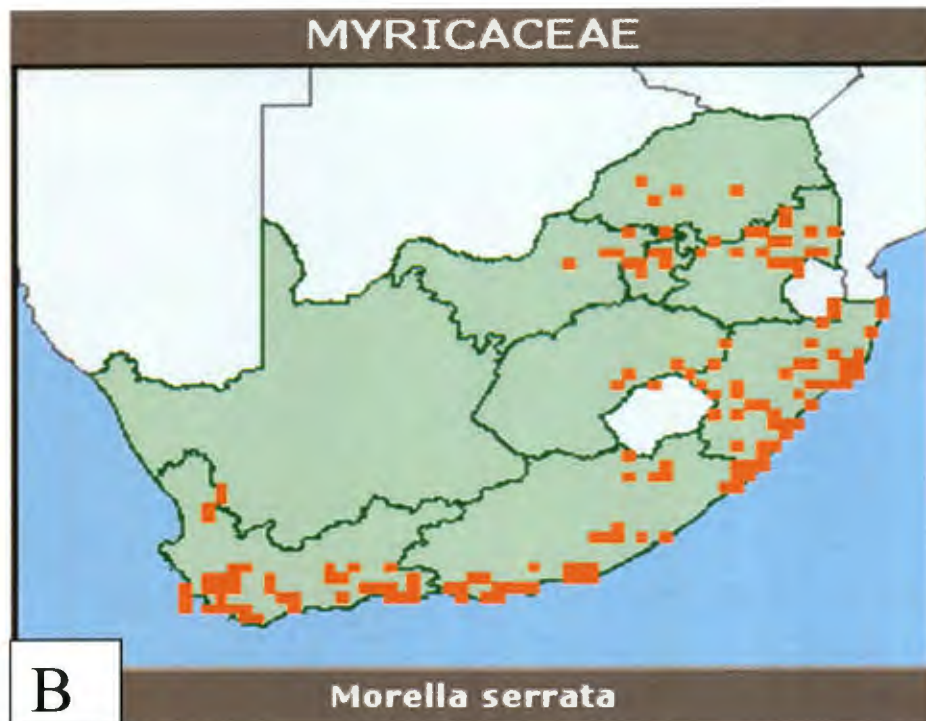


Figure 6: A - Photo of *Morella serrata* (Pitso, 2013) and B – Distribution map of *Morella serrata* (redlist.sanbi.org.za)

2.2.5 *Morella serrata*

Morella serrata belongs to Myricaceae. It is a dioecious densely leafed, multi-stemmed shrub that can grow up to 2 m tall (Figure 7A). It has narrow toothed leaves that taper at both the apex and base (Ashafa, 2013).

Ecologically, *Morella serrata* occurs in all South African provinces except in the Northern Cape (Figure 7B) (redlist.sanbi.org.za). The conservation status of this species has been listed as LC on RDL.

M. serrata is used in the Xhosa medicinal practice to treat chest related ailments like asthma, coughing and shortness of breath. The Basotho usually use the plant to treat various ailments like painful menstruation cold, coughs, and headaches. The plant is also used in enhancing male sexual performance, the management of sugar related disorders and as a laxative in treating constipation. In Zimbabwe, the plant is used to treat headaches and as an insurance against bad luck (Ashafa, 2013).

Phytochemical investigation of *M. serrata* has revealed the presence of tannins, phlobatanins, saponins, flavonoids, terpenoids and steroids. There were alkaloids and cardiac glycosides detected. The extracts from the plant were shown to be able to inhibit a number of human pathogenic bacteria and fungi at relatively low concentrations. These results validated the use of the plant in traditional medicine in treating various human diseases (Ashafa, 2013).



Figure 8: A - Picture of *Gazania krebsiana* (<http://navigate.botanicgardens.org>) and B - distribution of *Gazania krebsiana* (redlist.sanbi.org.za)

2.2.6 *Gazania krebsiana*

Gazania krebsiana is a member of daisy family (Asteraceae). The plants are semi decumbent (prostrate to ascending), perennial and herbaceous and can reach up to 150 mm high. They are referred to as tufted groundcovers because many individuals growing together give a mat-like appearance. In young plants, the leaves can vary in shape but matured ones are compound and divided into small narrow regular lobes. The flower-heads can measure up to 60 mm in diameter and the upper ray florets are dark red or orange (Figure 8) (redlist.sanbi.org.za).

Ecologically, *Gazania krebsiana* is widely distributed in South Africa, especially in the winter rainfall areas of the country (Figure 8). The plants are found mainly in the southern parts of the country in the Western and Northern Cape, Free State, Kwazulu-Natal and Lesotho (Graham, 2011). It grows mostly along roadsides, flats or lower slopes, exposed hills and rocky outcrops and showing a preference for clay and sandy soils. The species was given an automated status of LC since it was not selected in any one of four screening processes for highlighting potential taxa of conservation concern (redlist.sanbi.org).

Extensive literature search has shown that there has been no pharmacological study based on *G. krebsiana*.

CHAPTER 3

METHODOLOGY

3.1 HERBARIA

3.1.1 Selection of herbaria

Six herbaria were selected as part of the study. Three of them were Provincial herbaria focusing on species collected in the Provinces (Free State and Kwazulu-Natal), two were local herbaria and the remaining one was a regional herbarium. Though the selected herbaria specialised in collections from province, region or local area, they all have collections from other parts of the country, hence the inclusion of the Bews Herbarium located in the Kwazulu-Natal in the study.

The selected herbaria were:

1. Uniqwa Herbarium: Located at the department of Plant Sciences at the Qwaqwa campus of the University of the Free State in Phuthaditjhaba. This is a local herbarium focusing on collections from the Qwaqwa area and other parts of the eastern Free State. It is located within the study area.
2. Sterkfontein Dam Nature Reserve Herbarium: It is also a local herbarium located within the study area. It specialises in collections from within the reserve.
3. Geo-Potts Herbarium: –located at the department of Plant Science at the main campus of the University of the Free State in Bloemfontein: This is a Provincial herbarium that specialises in collections from all over the Free State Province.
4. National Museum Herbarium in Bloemfontein: This is regional herbarium specialising in collection from the central parts of the Free State.

5. Free State National Botanical Garden Herbarium in Bloemfontein: A local herbarium specializing in collection from around the Bloemfontein area
6. Bews Herbarium located at the department of Botany at the Pietermatzburg campus of the University of Kwazulu-Natal: One of the largest herbaria in South Africa. It is provincial herbarium specialising in collection from the all over the Kwazulu_Natal Province.

3.1.2 Herbarium survey

A physical search for all the collection of the plants under study was conducted at the selected herbaria. Each specimen was located and the information on the collecting labels was recorded focusing on the collection, locality and coordinates, abundance of the species and distribution of the species in the collection area. The number of specimen for each species were also counted and recorded. The collection trends of each of the herbaria were also noted to determine whether the collection can be of value to conservation practices.

3.2 TRADITIONAL HEALERS AND HERBALISTS

3.2.1 Selection of traditional healers and the determination of the collection practices

A questionnaire was prepared and handed over to the traditional healers and herbalists at their place of work. For those who were not fluent in English, the questionnaire (Appendix 1) was treated as interview and the researcher then translated the answers from Sesotho (local language) into English and filled in the questionnaire. The data collected was analysed to confirm the usage of the selected plants in treating diabetes, and to determine their collection practices and their attitude towards conservation. The healers and herbalists were also requested to identify the sites where they usually collect the selected plants.

A total of 20 traditional healers were chosen randomly from Phuthaditjhaba and surrounding villages, and 10 herbalists operating at the Setsing Shopping Complex in Phuthaditjhaba were selected to participate in the study by responding to the questionnaire. The ones that refused to answer questions or cooperate with the researcher were not considered.

3.3 Ecological survey

The sampling sites were identified by the traditional healers and herbalists that agreed to accompany the researcher to the field. The plants grow in different environments and hence they could not all be sampled in the same area. Once the collection areas were identified sampling plots were measured out and the population size of the selected plants was determined by calculating the individuals present in each plot (Elzinga et al., 2001). A total of 36 plots were sampled and each was 10 m². The data collected was then analysed to determine the population trends of the plants. This data was then used to determine the conservation status of the plants by looking at frequency of occurrence, density and total number of individual species per plot according to Roberts-Pichette and Gillespie (1999).

CHAPTER 4

RESULTS

4.1 HERBARIUM SURVEY

The results of searches of different herbaria for the six selected medicinal plants are shown in Table 3, and the results for information collected from collection labels (i.e. exact locality, distribution and abundance) that is important in determining conservation status in Figure 9. These results were obtained by physically visiting the selected herbaria and conducting physical search for the species. Three of the herbaria were fully computerised and one was partially computerised (collection data captured to computer software).

The Free State National Botanical Garden Herbarium (GOFS) and the Sterkfontein Dam Nature Reserve Herbarium (SDNR) had no any collections of the six plants. The reason for this was that all their specimens were transferred to the National Herbarium in Pretoria(PRE) and hence it was not possible to determine the number of specimen collected in the study area. The information of the relocation of specimen was discovered only once the researcher arrived at the two institutions to conduct the survey. According to Smith and Willis (1999), GOFS had a sizable collection of about 5000 specimens while SDNR had a very small collection of only 398 specimens.

Table 3: The total number of herbaria specimen collections of the six medicinal plants commonly used to treat diabetes in the eastern Free State Province, South Africa (QWA: Uniqwa Herbarium, NU: Bews Herbarium, BLFU: Geo-Potts Herbarium, NMB:National Museum Herbarium, GOFS: Free State National Botanical Garden Herbarium and SDNR: Sterkfontein Dam Nature Reserve Herbarium).

Plants Species	QWA	NU	BLFU	NMB	GOFS	SDNR
<i>Hypoxis hemerocallidea</i>	1	0	2	0	0	0
<i>Xysmalobium undulatum</i>	1	0	0	6	0	0
<i>Morella serrata</i>	3	0	4	6	0	0
<i>Gazania krebsiana</i>	9	2	4	29	0	0
<i>Dicoma anomala</i>	4	2	8	6	0	0
<i>Eriocephallalus punctulatus</i>	1	0	6	8	0	0

4.1.1 UNIQWA HERBARIUM

Uniqwa Herbarium (QWA) is the only one still active and located within the study area. In the report by Smith and Willis (1999), it had over 4800 specimens. Among the selected medicinal plants, *Gazania krebsiana* was the one with the most collection (9), followed by *Dicoma anomala* (4) and *Morella serrata* (3). The remaining three species, *Hypoxis hemerocallidea*, *Eriocephalus punctulatus* and *Xysmalobium undulatum* had one collection each.

The labels had adequate information on the locality of the species but information on abundance and distribution was inadequate. The labels of the more recent collections (1998 and beyond) had space for this information but the collectors did not fill it up. Only three collections had information on distribution and one with information on the abundance of the species. Seven collection labels (three of *G. krebsiana*, two of *D. anomala* and one each of *H. hemerocallidea* and *M. serrata*), had information on the exact locality, but none of them had the coordinates of the collection area.

The other observation made was that there were no specimens of the selected medicinal plants collected beyond 2002. Most of the collections were collected from the mid 1980's to the late 1990's. There was no system in the herbarium to clearly mark the medicinal plants from the rest of the collections.

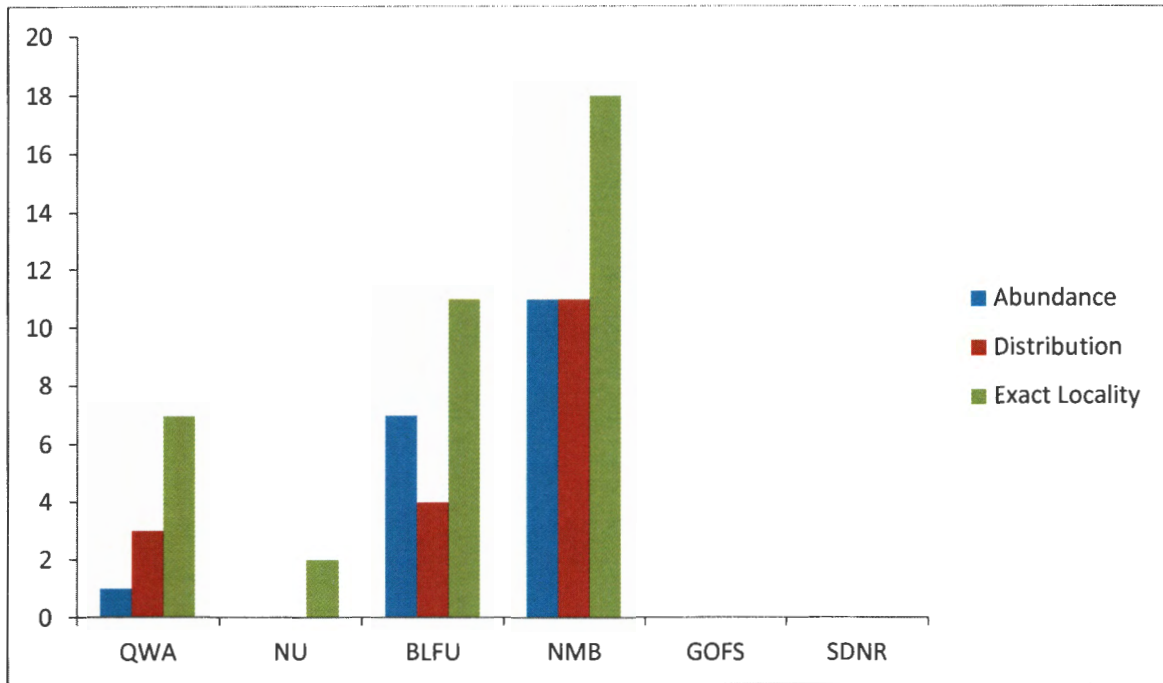


Figure 9: The number of collection labels with information on abundance, distribution and exact locality of the selected species.

4.1.2 GEO-POTTS HERBARIUM (BLFU)

BLFU is a medium sized herbarium with well over 20 000 specimens and it is located in the central part of the Free State, with collection from all over the Province. A total of 24 specimens of the study plants were located in this herbarium. The species with most collections from the study sites was *D. anomala* (8), followed by *E. punctulatus* (6), *M. serrata* and *G. krebsiana* (both with 4). There were only 2 collections of *H. hemerocallidea* and none for *X. undulatum*. The collection labels lacked information on abundance and distribution of the species and only 2 labels had information on the exact locality. All the labels lacked information on the coordinates of the collecting sites.

This herbarium also did not have separate or distinct medicinal plant collection. The collections of the selected plants in this herbarium were very old with earliest being a 1928 collection and the latest being a 1974 collection. Most of these collections were in the 1960's and early 1970's. The reasons for the non-collection of the study plants in BLFU need to be identified and rectified. Like all the herbaria, BLFU needs to strive for a more regular and complete collection of all the species in the Province.

4.1.3 BEWS HERBARIUM (NU)

NU is located in Pietermaritzburg, KwaZulu-Natal. It is a big herbarium that has well over 120 000 specimens. It mostly has collections from Kwazulu-Natal but it is in the process of building up collections from all over the country and other southern African countries. A total of only 4 collections of the study plants in the study area were located, 2 were *G. krebsiana* and the other 2 were *D. anomala*. The rest of the species were not represented. To achieve its goal of building country wide collection of different species, the herbaria can establish cooperation and collaboration with other herbaria in different provinces and exchange donated specimens.

The labels lacked information on abundance and distribution of the two species in the area and two labels had information on exact locality but there were no coordinates. All of the specimens were old with the earliest one being collected in 1947 and the most recent one being a 1986 collection. Two of the collection labels had information on the exact locality but none had coordinates recorded.

In this herbarium the medicinal plant collections are distinguished from the rest of the collections by different coloured specimen covers.

4.1.4 NATIONAL MUSEUM HERBARIUM (NMB)

NMB is a medium sized herbarium with over 25 000 specimens specializing in collection from the whole of the Free State and Northern Cape. Its collections include medicinal plants. This was the herbarium with the most collections of the selected medicinal plants from the study area with a total of 55 specimens. There were no collections for *H. hemerocallidea*, but there were 29 collections of *G. krebsiana*, 6 collections of *X. undulatum*, *M. serrata* and *D. anomala* each and 8 collections for *E. punctulatus*.

Only 18 of the labels had information on the exact locality and the coordinates of the collection site were recorded. It was found out that 11 of the labels had information on abundance and distribution of the species. The earliest collection was in 1932 and the latest was a 2002 collection. Most the collections (36 out of 55) were between 1991 and 1996. There was no way of distinguishing medicinal plant collections from the rest of the collections in this herbarium. NMB need to identify all the medicinal plants among their specimens and then distinguish them by using different coloured specimen covers.

4.2 TRADITIONAL HEALERS AND HERBALISTS

The traditional healers and herbalists usually collect their plants for medicine from the field. They harvest the raw materials and then prepare them before prescribing and administering them to their patients. Field collections directly impact on the wild populations of the medicinal plants and hence it becomes important to study their field practices.

4.2.1 Profiles of the traditional healers and herbalists

The results from profile survey of the healers and herbalists involved in the study are listed in Table 4. The dominant gender of the participants was female with 63% while male made up only 37%. The age profile ranged from 20 to above 45, years most of the participants with age above 36 years making up 90%. Three percent of the participants were in the age group 20-25, and 7% were in the age group 31- 35 years.

The results showed that the education levels of the participants ranged from pre matric to post matric levels. Most of the participants (76%) had an education level below matric, 17 % having matric and 7% having post matric qualifications. Those with post matric level were practicing traditional healers who were also school teachers. The results also showed that a large number of the participants (67%) were traditional healers, followed by herbalists (23%). Some of them (10) were practicing as both healers and herbalists.

Table 4: The profile of traditional healers and herbalist who participated in the study

Category	Description	Percentage (%)
Gender	Male	37
	Female	63
Age (in years)	20 – 25	3
	26 – 30	0
	31 – 35	7
	36 – 40	23
	41 – 45	37
	45+	30
	Highest level of education	Pre-matric
Matric		17
Post-matric		7
The type of practice	Traditional healer	67
	Herbalist	23
	Both traditional and herbalist	10

4.2.2 Collecting practices of the participants

There is a direct correlation between medicinal plant collection practices and the impact of harvesting on the wild populations of the species. The result of the field practices of the participants are shown in table 5.

The results showed that 67% of the participants received the formal healer's training, while 11% learned about traditional medicine from working with a relative or a parent who was a healer and 22% learned from working with herbalist. It was determined that only 12% of the participants had the collecting permits from the Provincial Department of Environmental Affairs and 88 percent did not have such permits.

The kind of training received informed the collecting methods. Traditional healers are usually trained to collect only what they need and not to over harvest. The amount collected by herbalists is generally guided by the amount of money they need to make to survive.

The survey showed that 24% of the participants undertook less than 3 collecting trips per week, 12% took more than 3 collecting trips per week, 56 percent collected only when their stock were running low and 8% did not undertake collection trips, but bought their medicinal plants from herbalists and commercial gatherers. The results also showed that 35% of the participants collected as much as they could on each trip, whereas 12% collect only a little, 25% collect only as much as they need and 28% collect enough to be able to sell to other healers, herbalists and directly to the public.

The results showed that 45% of the participants collected the same medicinal plants more than three times from the same area, 22% collected less than 3 times, 12 % collected once in the same area and then moved to another area. The remaining 21% collected from the same area until the species is depleted and only then do they move to another area.

Table 5 Collecting practices of the traditional healers and herbalists participating in the study

Category	Description	Percentage
Type of training in traditional medicine	Formal healer's training	67
	Learned from a parent/relative who was a healer	11
	Learned from another herbalist	22
Possession of a collector's permit	Yes	12
	No	88
Frequency of medicinal plant collecting trips	< 3 times/week	24
	> 3 times/week	12
	Only when the stock is low	56
	Never, I buy from commercial gathers	8
Quantity of a particular medicinal plant collected per trip	As much as I can collect	35
	As little as possible	12
	Only as much as needed	25
	Enough to sell to other herbalists, healers & public	28
Frequency of collecting the same plant from the same area	More than 3 times per year	45
	Less than 3 times per year	22
	Once and the move to another area	12
	Until the plant is depleted in the area	21

4.3 ECOLOGICAL SURVEY

The summary of the results for ecological survey of the six medicinal plants used to treat diabetes is presented in table 6. Both the frequency of occurrence and density of the *H. hemerocallidea* were high (50 and 1.0 respectively). These results are indicated by the high number of individual plants counted from all the sampling sites (total of 867) and hence it was easy to locate the species in the field as it still occurred in large numbers. Other species with high frequency were *D. anomala* and *G. krebsiana* (47 and 57 respectively) with an average density (0.3 and 0.4 respectively) across all the sampling sites. The frequency of *M seratta* and *X. undulatum* was very low (8 and 22 respectively) and similarly, their density was also very low (0.1 and 0.3 respectively).

The results of the individual species count in each of the sampling plots are represented in Figure 14. The three species with the highest frequencies, *H. hemerocallidea*, *G. krebsiana* and *D. anomala* occurred together in only 5 (14%), and 10 (27%) plots had two different species and the rest of the plots had only one species (68%).

The results indicated *H. hemerocallidea* as the species with the highest number of individuals, 867 which is 49.79% of all species counted. The sampling site with highest number of *H. hemerocallidea* individuals was site S29 with 78 individuals and the site with lowest individual was site S1 with only 17 individuals and it did not occur in 18 plots.

According to South African National Biodiversity Institute (SANBI, 2013), the existence of *H. hemerocallidea* is under threat due to commercial exploitation since 1997 and by loss of habitat loss even though the threat is countered by the fact the species is naturally abundant

and it is widespread. In the Eastern Cape, the plant is heavily traded, unsustainably harvested and sold at high prices (Dold and Cocks, 2002). On the Red Data List it has the status declining due to this kind of practices.

The species with second highest count was *G. krebsiana* with a total of 324 individuals (18.61%) and the plot with the highest number of individuals was site S21 (with 25) and the one with the lowest individuals was site S2 (with 7) and the species was not present in 15 in 15 sites.

D. anomala was the third species highest individual counts adding up to 311 individuals (17.86%), and the plot with highest count was site S25 with 36, the one with lowest count was site S6 with 7 individuals and *D. anomala* was present in 19 sampling plots. *X.*

undulatum with 227 individuals (13.04%), was the species with the fourth highest individual count. The sampling plots with the highest individuals was plot S36 with 45, the one with lowest count was plot S11 with 10 individuals and. *M. serrata* was the species with the least count with only 12 individuals (0.7%) and it was recorded in only three sampling plots and the one with the highest individuals count was S32 with 6.

E. punctulatus was not recorded in any of the 36 sampling plots. The traditional healers and herbalists had identified 5 of the sampling plots (S13, S15, S28, S30 and S35) as areas where

they had previously collected the plant. They pointed out that the species is becoming very scarce and the situation is getting worse as they had to rely on commercial gatherers to supply them.

The absence of *E. punctulatus* in all the sampling sites is of great concern to the survival of the species within the study area. Previous report by Mierendorff et al. (2003) confirmed that the species growing at the slopes of the Drakensberg mountains in the Free State. The absence of this species is most probably due to over-harvesting as it is also used to treat various stomach diseases and fumigation of huts. In the Red Data List, the plant has been give the status of Least Concern (LC) because it has not yet received a detailed assessment for conservation (Sanbi, 2013). The plants used for the commercial production of oil from *E.punctulatus* in the Eastern Cape depends on the cultivars and the possibility of cultivating it in the study area should therefore be investigated.

Table 6: Summary of data collected from all the sites in the ecological survey of the six medicinal plants used to treat diabetes in the eastern Free State (F – frequency, D – density and TI – total individual)

Species	F	D	TI	Remark
<i>H. hemerocallidea</i>	50	1.0	867	High frequency
<i>D. anomala</i>	47	0.3	311	High frequency
<i>G. krebsiana</i>	56	0.4	324	High frequency - Equal frequency across all plots
<i>M. seratta</i>	8	0.1	117	Equal frequency in all plots
<i>X. undulatum</i>	22	0.3	227	Relative high frequency
<i>E. punctulatus</i>	0	00	0	Not recorded in all plots

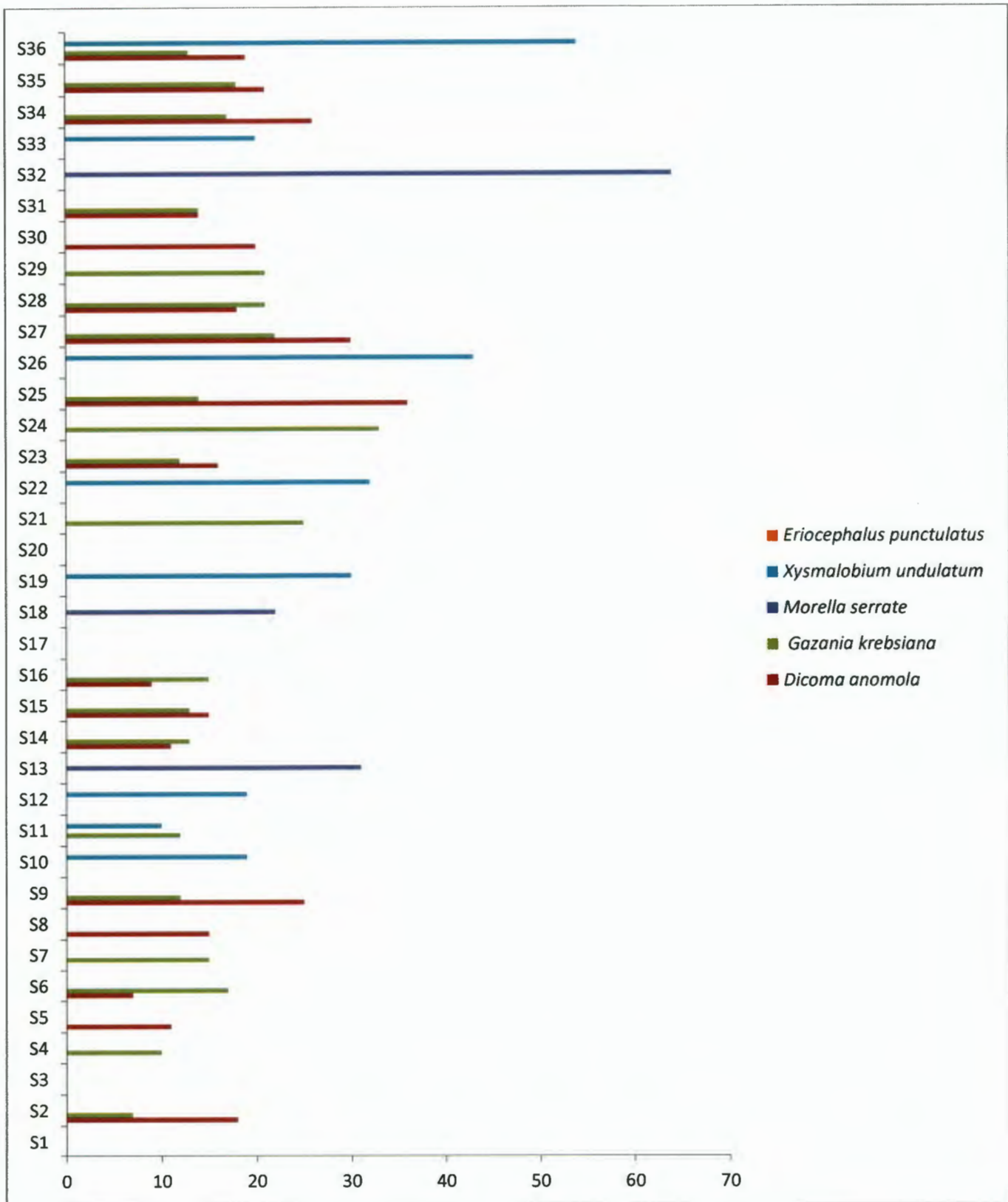


Figure 14: The number of individual species counted per sampling plot (S – sampling site)

CHAPTER 5

DICUSSION

Traditional healing has always been a component of healthcare(Puckree et al., 2002).Traditional medicines are an integral part of both physical and mental welfare of poor communities and hence traditional healers herbalists are established healthcareproviders within their communities (Semenya and Potgieter, 2014). It has been estimated that in South Africa, the ratio of medical doctors to the total population is 1:17400 and for the traditional doctors the ratio is 1:700-1200 (Cunningham, 1993).

The gender profile ratio of participants in the current study (37% and 63% male and female respectively) which differs from previous study in the Cape Peninsula where the ratio was 56% and 44% (male and female respectively) (Loundou, 2008). The gender profile obtained in the current study agrees with the profile (80%-90% women to 20%-10% men) reported by Institute of Natural Resources that more women are involved in trade of medicinal plants. The high number of women to men (69% to 31% respectively) in the medicinal trade was also reported by Cunningham (1993). Semenya and Potgieter (2014) reported that in the Vhenda area, men comprised two thirds of those involved in traditional medicine which is in contrast to the findings of the current study.

Previous studies did not report on the age profile of the traders and whether they were healers or herbalists. The educational profile (93% with secondary and lower education level and 7% with tertiary education) obtained in the current study mirrors the one reported by Loundou (2008) that 97% of the traders had a below matric and only 3% had tertiary education. Similar educational profile was reported by Mander et al. (2006) where 65.3 % of respondents in their study had education levels lower than matric, 26% had matric qualification and 8.7% had post matric qualification. Semenya and Potgieter (2014) reported

that the majority of males in traditional medicine (76%) had no formal education as compared to healers, 67% of 46% of females.

In the current study, the majority of the participants (89%) received healing knowledge from fellow healers or herbalists and only 11% received it from parents/relative. This is in contrast to the report by Semenya and Potgieter (2014) who reported that the majority (52% of males and 70% females) received their knowledge from parents rather than from fellow healers (48% males and 30% females).

The majority of the participants (88%) did not have a collector's permit from the Provincial Department of Environmental Affairs. The implication is that they did not attend training sessions and workshops arranged by the department and hence, they were not aware of biodiversity conservation legislation. According to Semenya and Potgieter (2014), the traditional healers viewed the permit system as an obstacle to their practice and they claimed that environmental statutes had no bearing on their profession and they also viewed plants as a common property. According to Thisikhawe (2012), the harvesting of medicinal is being dominated by commercial gathers who are only concerned with earning as much as possible as they do not have any other source of income. The author further suggests that harvesting of medicinal plants for trade in order to meet the urban trade is an environmentally destructive activity.

The results from the present study have confirmed that the assignment of conservation status for some plants species on the RDL was based on poor taxonomy as stated by Callmander et al. (2005). Most of the herbaria collections surveyed were from material gathered a long time with no information that can be used to assess the current conservation status of the species. Most of the specimens in the surveyed herbaria were old collections whose collection labels lacked information that is useful to conservationists. Generally there was no systematic

collecting approach and hence there was no follow up collections. It became impossible for one to determine population trends by studying the specimen. The results confirm the observation by Willis et al. (2003) that specimen sheets are a qualitative rather than quantitative data source and this places limitations in using herbaria data. The study of Willis et al. (2003) also stated that locality co-ordinates data acquired from herbarium specimen data will often only provide a rough approximation of species distribution and the same was observed in the current study. Specimens sheets need to include information on parameters used for generating Red List assessments to become useful to broader, conservation orientated user group (Willis et al., 2003). These parameters are extent of occurrence (EEO), area of occupancy (AOO), severe fragmentation and continuing decline of species (Golding and Smith, 2001). The first step in enhancing and fast-tracking conservation related initiative such as RDLs is “specimen stock taking” by herbaria staff. The focus of such activity need to be on identification of species with only a few specimens or those known from only a single locality, making specimen labels relevant to both taxonomists and conservationists (Golding, 2001).

There was a serious lack of coordinates and description of exact location that can be used by current conservationists to assess population trends in a particular area. Herbaria have always been the domain of plant taxonomist and most of the collections are only known to the few practicing taxonomists (Callmander et al., 2005). It has become imperative that taxonomists and conservationists work closely together to vast information resources in herbaria to conserve natural resources and to integrate conservation assessment with taxonomic re-evaluation and revision. According to ter Steege et al, (2000), botanical collectors usually collect for museums (herbaria) and they only collect taxa selectively and mostly from areas they can access easily. These practices have produce overestimates or underestimates of species in an area.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

The herbaria survey did have a systematic approach to the collection of plants in their specific areas. Most of the collections of the studied plants were very old and their collection labels generally lacked adequate information that can be used by conservationists in evaluating the conservation status of species. The collecting practices of the different herbaria are not systematic and hence cannot provide information that can be used to conduct comprehensive assessments of the population trends. QWA is located within the study area, and one would expect to find adequate number of specimen of the study plants, but this was not the case. There was only one collection of the most common species, *H. hemerocallidea* and this confirmed the trend from other herbaria that plants not deemed rare or those that the collectors are not interested are usually excluded during the collecting trips.

It is important to note that though herbarium specimens are in general more reliably determined than mere field observations, they are more biased towards rare or unusual species. The collectors are also biased to more favourable groups and often exclude species difficult to reach or to preserve (Schmidt et al., 2005). The survey of the herbarium collection has shown that even the species that are common and well distributed in an area are usually excluded during collecting trips.

Only one of the herbaria surveyed, Bews Herbarium, followed some of the points from the “13-point flora strategy for conservation challenges” as outlined by Golding and Smith (2001) using different coloured species cover for medicinal plants. According to points of the strategy, information that needs to be mentioned on collection labels include whether a taxon is type specimen, taxon is known from more than one location (second location should be stated), a taxon is known from fewer than five collection, a taxon is endemic to an area,

frequency of occurrence to be included with habitat data, citing of the most recent collections, relevant information on distribution, a note on recorded uses of the species, collection from National Parks, conduction of stock taking by herbarium staff to identify and prioritise collection of taxa represented by a few specimen or from single locality, identify specimen on Red List by using computer coding, stickers, coloured species cover etc., specimen labels relevant to both taxonomists and conservationists and Red List assessment when publishing monographs, revisions etc.

It is recommended that all the herbaria should re-evaluate their collecting strategies so that they can be aligned to the 13-point flora strategy to meet conservation challenges” by Golding and Smith (2001). This will assist to make herbaria important tools for conservationists in conducting fast and accurate assessments of the population that can then be used for RDLs. QWA herbarium is small and it will be a perfect pilot study. All the medicinal plants in the study area need to be identified and collected (with all the relevant data on the collection labels), then marked with different coloured specimen covered.

The traditional healers and herbalists in the current study were mostly women who survived and raised their families on the proceeds from their medicinal plant businesses. The participants were mostly middle aged and they had very low education level. The level of education has the potential of limiting their appreciation of the conservation laws and strategies. Most of the participants did have collecting permits and did not attend workshops organised by the Provincial Department of Environmental Affairs. Most of the healers and herbalists have the tendency of collecting the same plant from the same area and that practice has a potential threat to the survival of medicinal species in the wild.

It is therefore recommended that efforts should be taken to forge proper links between the traditional healers and herbalists, the Department of Environmental Affairs and the Golden Gate Highlands National Park. The cooperation will help to build trust between the scientists, government officials and traditional medicine practitioners and hence there can be exchange of information between all parties. The healers and the herbalists should be provided with training that will emphasise sustainable usage of natural resources and the importance of conserving the biodiversity. The Department of Environmental Affairs should be encouraged to continue handing out collecting permits but this service needs to be brought closer to the people by establishing regional offices. The number of people actively collecting the plants from the wild can only be reduced if the government succeed in creating more job opportunities.

The ecological survey identified only two plants that are potentially threatened within the study area, *E. punctulatus* and *M. serrata*. The latter was available in only a few locations and it was generally one or few individuals per site. At some of the sites there was evidence where the young plant were uprooted instead of just harvesting the roots. *E. punctulatus* could not be located in all the sites that were identified by the participants. The plant has been over-harvested to the point that healers and herbalists can now only source it from commercial gatherers from other parts of the country. The rest of the study plants are still well distributed all over the study area and they are easily accessible.

It is also recommended that easier and more rapid field assessment method for the determination of population trends should be developed as a matter of urgency. It is very important that the impact that the collection of medicinal plants by the healers, herbalists and commercial gatherers should be quantified throughout the country.

REFERENCES

- Adewusi, E.A. and Steenkamp, V. 2011. *In vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from southern Africa. *Asian Pacific Journal of Tropical Medicine* 4(10): 829-835.
- Afolayan, A.J. and Sunmonu, T.O. 2010. *In vivo* studies on antidiabetic plants used in South African herbal medicine. *Journal of Clinical Biochemistry and Nutrition* 47: 98-106.
- Alexiou, P. and Demopoulos, V.J. in Medicinal plants used for the treatment of diabetes and its long-term complications. Editor Kokkalou, E. 2010. *Plants in Traditional and Modern Medicine: Chemistry and Activity*. pp 69-175. ISBN: 978-81-7895-432-5.
- Ashafa, A.O.T. 2013. Medicinal potential of *Morella seratta* Lam. Killick (Myrecaceae) root extracts: biological and pharmacological activities. *BMC Complementary and Alternative Medicine* 13: 163.
- Ayyanar, M., Sankarasivaraman, K. and Ignacimuthu, S. 2008. Traditional herbal medicines used for the treatment of diabetes among two major tribal groups in South Tamil Nadu, India. *Ethnobotanical Leaflets* 12: 276-280.
- Bastaki, S. 2005. Review: Diabetes mellitus and its treatment. *International Journal of Diabetes and Metabolism* 13: 111-114
- Becker, J.W., van der Merwe, M.M., Brummelen, A.C., Pillay, P., Crampton, B.G., Mmutlane, E.M., Parkison, C., van Heerden, F.R., Crouch, N.R., Smith, P.J and Matucama, D.T. 2011. *In vitro* plasmodial activity of *Dicoma anomala* subsp. *gerradii* (Asteraceae): identification of its main active constituent, structure activity relationship studies and gene expression profiling. *Malaria Journal* 10: 295.

- Berkes, F. and Turner, N.J. 2006. Knowledge, learning and the evolution of conservation practices for social-ecological system resilience. *Human Ecology* 34(4) 479-494.
- Buwa, L.V. and van Staden, J. 2006. Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. *Journal of Ethnopharmacology* 103: 139-142.
- Callmander, M.W., Schatz, G.E. and Lowry, P.P. 2005. IUCN Red List assessment and the Global Strategy for Plant Conservation: taxonomists must act now. *Taxon* 54(4): 1047-1050
- Day, C. 1990. Traditional plant treatments for diabetes mellitus: pharmaceutical foods. *British Journal of Nutrition* 80: 5-6.
- Day, C. 2001. The rising tide of type 2 diabetes. *The British Journal of Diabetes and Vascular Disease* 1: 37-43.
- Deuschländer, M.S., Lall, N. and van der Venter, M. 2009(a). Plant species in the treatment of diabetes by South African traditional healers: An inventory. *Pharmaceutical Biology* 47(4): 348-365.
- Deuschländer, M.S., van der Venter, M., Roux, S. Louw, J. and Lall, N. 2009 (b). Hypoglycaemic activity of four plant extracts traditionally used in South Africa for diabetes. *Journal of Ethnopharmacology* 124 (3): 619 – 624.
- Dold, A.P. and Cocks, M.L. 2002. The trade in medicinal plants in the Eastern Cape Province, South Africa. *South African Journal of Science* 98: 589-597
- Drewes, S.E., Elliot, E., Khan, F. Dhlamini, J.T.B. and Gcumisa, M.S.S. 2008. *Hypoxis hemerocallidea* – Not merely a cure for benign prostate hyperplasia. *Journal of Ethnopharmacology* 119: 593-598.

- Edwards, R. 2009(January). Medicinal plants on the verge of extinction. *New Scientist*: 1-4
- Elzinga, C.L., Salzer, D.W., Willoughby, J.W. and Gibbs, J.P. 2001. Monitoring plants and animal populatios. Library of Congress Cataloguing-in-Publication Data. Blackwell Sciences, Inc.
- Erasto, P., Adebola, P.O., Griesom, D.S. and Afolayan, A.J. 2005. An ethnobotanical study of plants used for the treatment of diabetes in the Eastern Cape Province, South Africa. *African Journal of Biotechnology* 4(12): 1458-1460.
- Faleyimu, O.J., Mohammed, A.A. and Akinyemi, O. 2010. Efficacy of forest plants in the treatment of diabetes in Birnin Gwari local government area, Kaduna State, Nigeria. *International Journal of Biomedical and Health Sciences* 6(1): 83-90.
- Fennell, C.W., Lindsey, K. L., McGaw, L. J., Sparg, L. G., Stafford, G. I. Elgorashi, E. E., Grace, O. M. and van Staden, J (2004). Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. *Journal of Ethnopharmacology* 94: 205-217.
- Food and Agricultural Organization of the United Nation. Trade in medicinal plants. Raw Materials, Tropical and Horticultural Products Service, Commodities and Trade Division, Economic and Social Department, Rome (<ftp://ftp.fao.org/docrep/fao/008/af285e/af285e00.pdf>) (Accessed 2012)
- Funk, V.A. and Richardson, K.S. 2002. Systematic data in biodiversity studies: use it or lose it. *Systematic Biology* 51(2): 303-316
- Golding, J.S. 2001. Southern African herbaria and Red Data Lists. *Taxon* 50: 593-602

- Golging, J.S. and Smith, P.P. 2001. A 13-point flora strategy to meet conservation challenges. *Taxon* 50:475-477
- Graham, D. 2011. *Gazania krebsiana* subsp. *arctotiodes* (Compositae). *Curti's Botanical Magazine* 28(4): 333-340
- Hamilton, A. 1992. International trade in medicinal plants: conservation issues and potential roles for botanical gardens. WWF International, Panda House, Weyside Park, Godalming, Surrey GU7 1QJ, UK.
- Hassan, S. 2005. Collection and marketing of high value medicinal and aromatic plants from District Swat, Pakistan. Pakistan Strategy Support Program: Working Paper No. 011:pp 23
- Hawkins B. 2008. Plants for life: Medicinal plant conservation and botanic gardens. Botanic Gardens Conservation International, Richmond, UK.
- Heart Foundation. 2012. [www.heartfoundation.co.za] (2012/06/13)
- Hughes, G.D. and Bradley, H. 2006. Ability to manage diabetes – community health worker's knowledge, attitudes and beliefs. *Journal of Endocrinology, Metabolism and Diabetes of South Africa* 11(1): 10-14
- Institute of Natural Resources. 2003. Indigenous medicinal plant trade – sector analysis. Investigative Report number 248.
- Jegade, A., Aladosu, P., Ameh, S., Kolo, I., Izebe, K., Builders, P., Yahaya, T., Okhale, S., Mustapha, B., Busu., S., Shitu, H. and Gamaniel, K. 2011. Status of management of diabetes mellitus by traditional medicine practitioners in Nigeria. *Journal of Medicinal Plants Research* 5(27): 6309-6315

- Kala, C.P. 2000. Status and conservation of rare and endangered medicinal plants in the Indian trans-Himalaya. *Biological conservation*, 93:371-379
- Katerere, D.R. and Eloff, J.N. 2008. Anti-bacterial and anti-oxidant activity of *Hypoxis hemerocallidea* (Hypoxidaceae): Can leaves be substituted for corms as a conservation strategy? *South African Journal of Botany* 74(4): 613-616
- Kathe, W. 2006. Revision of the “guidelines on the conservation of medicinal plants” by WHO, IUCN, WWF and TRAFFIC: process and scope. In Bogers, R.J., Cracker, L.E. and Lange, D. (eds). *Medicinal and Aromatic Plants*. Springer, Netherlands, 109-120
- Kazembe, T. and Chinyuku, J. 2012. *In vitro* Babesiosis Assaying using *Acacia karoo* and *Dicoma Anomala* plant extracts and extract fortified antimalarial drugs. *Bulletin of Environment, Pharmacology and Life Sciences* 1(9): 26-31.
- Kelmanson, J.E., Jager, A.K. and van Staden, J. 2000. Zulu medicinal plants with antibacterial activity. *Journal of Ethnopharmacology* 85: 241-246.
- Kofi-Tsekpo, M. 2004. Institutionalization of African traditional medicine in healthcaresystems in Africa. *African Journal of Health Sciences* 11(1-2): i-ii.
- Laird, S.A. 2005. Medicinal Plants in International Trade: Conservation and Equity Issues. In: *Ethnopharmacology, Encyclopedia of Life Support Systems (EOLSS)*, developed under the auspices of the UNESCO, EOLSS Publishers, Oxford, UK. www.eolss.net. 13 pp
- Levitt, N.S. 1996. Diabetes mellitus in black South Africans. *International Journal of Diabetes in Developing Countries* 16:41-44.
- Loundou, P-M. 2008. Medicinal plant trade and opportunities for sustainable management in the Cape Peninsula, South Africa. Masters Thesis, University of Stellenbosch.

- Mander, M., Ntuli, L., Diederichs, K. and Mavundla, K. 2007. Economics of the traditional medicine trade in South Africa: healthcaredelivery. South African Health Review. 189-196
- Mierrendorff, H., Stahl-Biskup, E., Posthumus, M.A. and van Beek, T.A. 2003. Composition of commercial Cape chamomile oil (*Eriocephalus punctulatus*). Flavour and Fragrance Journal 18: 510-514
- Mills, E., Coper, C., Seely, D. and Kanfer, I. 2005. African herbal medicine in the treatment of HIV: *Hypoxis* and *Sutherlandia*. AN overview of evidence and pharmacology. Nutritional Journal 4: 1-6
- Moffett, R.O., DaemanM.E., Pitso, T.R., Lentsoane, R. and Taona, T.R.N. 2001. Checklist of the vascular plants of Qwaqwa and notes on the flora and vegetation of the area. Uniqwa Research Chronicle 3(2): 32-83.
- Mollentze WF and Levitt NS. 2012. Diabetes mellitus and impaired glucose tolerance in South Africa. In: Chronic Diseases of Lifestyle in South Africa: 1995–2005. Chapter 10. <http://www.mrc.ac.za/chronic/cdlchapter10.pdf> (Accessed November 2012).
- Moodley, L.M. and Rambiritch, V. 2007. As assessment of the level of knowledge about diabetes mellitus among diabetic patients in a primary healthcare setting. South African Family Practice 49(10):16
- Naidoo, Y., Samuels, J.N., Nicholas, A. and Rampersad, E. 2009. Leaf micromorphology of the African medicinal plant *Xysmalobium undulatum*. Microscopy and Microanalysis 15(Suppl 2)
- Ncube, B., Ndhlala, A.R., Okem, A. and van Staden, J. 2013. *Hypoxis* (Hypoxidaceae) in African traditional medicine. Journal of Ethnopharmacology 150(3): 818-827

- N'guessan, K., Kouassi, K.E. and Kouadio, K. 2009. Ethnobotanical study of plants used to treat diabetes, in traditional medicine, by Abbey and Krobou people of Agboville (Côte-d'Ivoire). *American Journal of Scientific Research*. Issue 4: 45-58
- Nielsen, N.D., Sandager, M., Stafford, G.I., van Staden, J. and Jäger, A.K. 2004. Screening of indigenous plants from South Africa for affinity to the serotonin reuptake transport protein. *Journal of Ethnopharmacology* 94: 159-163
- Njenga, E.W. 2005. The chemotaxonomy, phylogeny, and biological activity of genus *Eriocephalus* L. (Asteraceae). Ph.D. thesis, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg.
- Njenga, E.W. and Viljoen, A.M. 2006. *In vitro* 5-lipoxygenase inhibition and anti-oxidant activity of *Eriocephalus* L. (Asteraceae) species. *South African Journal of Botany* 72: 637-641.
- Ojewole, J.A.O., Awe, E.O. and Nyinawumuntu, A. 2009. Antidiarrheal activity of *Hypoxis hemerocallidea* Fisch. & C.A. Mey. (Hypoxidaceae) corm ('African potato') aqueous extract in rodents. *Phytotherapy Research* 23: 965-971.
- Ojewole, J.A.O. 2006. Antinoceptive, anti-inflammatory and anti-diabetic properties of *Hypoxis hemerocallidea* (Hypoxidaceae) corm ['African Potato] aqueous extract in mice and rats. *Journal of Ethnopharmacology* 103: 126-134
- Ojewole, J., Kamadyaapa, D. and Musabayane, C. 2006. Some *in vitro* and *in vivo* cardiovascular effects of *Hypoxis hemerocallidea* Fisch. & CA Mey (Hypoxidaceae) corm ('African Potato') aqueous extract in experimental animal models, *Cardiovascular Journal of South Africa: Off. J. S. Afr. Card. Soc. Card. Practitioners* 17: 166-171

Pedersen, M.E., Weng, A., Sert, A., Stafford, G.I., van Staden, J. and Jäger, A.K. 2006. Pharmacological studies on *Xysmalobium undulatum* and *Mondia whitei* – two South African plants with *in vitro* SSRI activity. *Planta Medica* 72:123

Pooley, E. 1998. A field guide to wild flowers of Kwazulu-Natal and the Eastern Region. Natal Flora Publications Trust, Durban

Popovici, J., Comte, G., Bagnarol, É., Alloisio, N., Fournier, P., Bellvert, F., Bertrand, C. and Fernandez, M.P. 2010. Differential effects of rare specific flavonoids on compatible and incompatible strains in the *Myrica gale*-*Frankia* actinorhizal symbiosis. *Applied and environmental Microbiology* 76(8): 2451-2460

Rabe, T. and van Staden, J. 1997. Antibacterial activity of South African plants used for medicinal purposes. *Journal of Ethnopharmacology* 56: 81-87.

Rheeder, P. 2006. Type 2 diabetes: the emerging epidemic. *South African Family Practice* 48(10):20.

Roberts-Pichette, P. and Gillespie, L. 1999. Ecological monitoring and assessment network: terrestrial vegetation monitoring protocols. EMAN Occasional Paper Series Report No9. Canada Centre for Inland Waters, Burlington, Ontario, Canada.

Royal Museum for Central Africa. 2014. Prelude medicinal plants database.

http://www.africamuseum.be/collections/external/pre;ude/view_plant?pi=13190

SANBI. 2013. Life: the state of South Africa's biodiversity 2012. South African National Biodiversity Institute, Pretoria

- Schatz, G.E. 2002. Taxonomy and herbaria in service of plant conservation: Lessons from Madagascar's endemic families. *Annals of Missouri Botanical Garden* 89: 145-152
- Schmidt, M., Kreft, H., Thiombiano, A. and Zizka, G. 2005. Herbarium collections and field data-based plant diversity maps for Burkina Faso. *Diversity and Distributions* 11: 509-516
- Semenya, S., Potgieter, M. and Erasmus, L. 2012. Ethnobotanical survey of medicinal plants used by Bapedi traditional healers to treat diabetes mellitus in the Limpopo Province, South Africa. *Journal of Ethnopharmacology*, 141:440-445
- Smith, G. and Willis, C. 1999. *Index herbariorum: southern Africa supplement*. 2nd edition. South African Botanical Diversity Network Report No 8. SABONET, Pretoria
- Stafford, G.I., Pedersen, M.E., van Staden J., and Jäger A.K.. 2008. Review on plants with CNS-effects used in traditional South African medicine against mental diseases. *Journal of Ethnopharmacology* 119: 513-537
- Steenkamp, V. and Gouws, M.C. 2006. Cytotoxicity of six South African medicinal plant extracts used in the treatment of cancer, *South African Journal of Botany*, 72: 630-633.
- Steenkamp, V., Mathiva. E., Gouws, M.C. and van Rensburg, C.E.J. 2004. Studies on antibacterial, antioxidant and fibroblast growth stimulation on wound healing remedies from South Africa. *Journal of Ethnopharmacology* 95: 353-357.
- Street R.A.; Stik W.A. and Van Staden J. 2008. South African traditional medicinal plant trade – challenges in regulating quality, safety and efficacy. *Journal of Ethnopharmacology* 119. 705 – 710.
- Swanston-Flatt, S.K., Day, C., Bailey, C.J. and Flatt, P.R. 1990. Traditional plant treatments for diabetes. Studies in normal streptozotocin diabetic mice. *Diabetologia* 33: 462-464

ter Steege, H., Jansen-Jacobs, M.J and Datadin, V.K. 2000. Can botanical collections assist in a National Protected Strategy in Guyana? *Biodiversity and Conservation* 9: 215-240

The Presidency. 2001. www.thepresidency.gov.za

Tshabalala, N.T. 2012. Ethnobotanical survey of Sotho medicinal plants used in the management of diabetes in the Free State. Honours thesis, Qwaqwa Campus, University of the Free State,

Turpie, J.K. 2003. The existence value of biodiversity in South Africa: how interest, experience, knowledge, income and perceived level of threat influence local willingness to pay. *Ecological Economics* 46: 199-216.

Ungricht, S., Rasplus, J.-Y., and Kjellberg, F. 2005. Extinction threat evaluation of endemic fig trees of New Caledonia: priority assessment for taxonomy and conservation with herbarium collections. *Biodiversity and Conservation* 14: 205-232.

WHO. 1994. Management of diabetes mellitus: Standards of care and clinical practice guidelines. WHO Regional Office for the Eastern Mediterranean, Alexandria, Egypt.

WHO. 2006 a. Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia. Report of WHO/IDF Consultation. [www.who.int].

WHO. 2006 b. Traditional medicine. Fact sheet 134. www.who.int/mediacentre/factsheets/fs

WHO. 2012. A comprehensive global monitoring framework, including indicators, and a set of voluntary global targets for the prevention and control of non-communicable diseases. Revised WHO Discussion Paper [www.who.int]

Willis, F., Moat, J. and Paton, A. 2003. Defining a role of herbarium data in Red List assessment: a case study of *Plectranthus* from eastern and southern tropical Africa. *Biodiversity and Conservation* 12: 1537-1552

Yeh, G.Y., Eisenberg, D.M., Kaptchuk, T.J. and Phillips, R.S. 2003. Systematic Review of herbs and dietary supplements for glycemic control in diabetes. *Diabetes Care* 26(4): 1277-1294

Zimudzi, C., Rupende, K., Kativu, S., Jere, J. and Kunonga, N.I. 2013. Phytochemical and analgesic activity of root crude extracts of *Dicoma niccolifera* (Asteraceae) . *Journal of Applied Pharmaceutical Science* 3(04): 165-168

APPENDIX A

QUESTIONNAIRE ON TRADITIONAL HEALERS AND HERBALISTS COLLECTING PRACTICES AND CONSERVATION AWARENESS

Dear Participant	
The purpose of this questionnaire is to determine your medicinal plant collection practices. The information gathered herein will be used to help in determining the conservation of the selected plants. Please make sure that you answer all questions truthfully as you can and note that there are no wrong or wright answers (place X in the empty box next to your answer).	
SECTION A: BIOGRAPHICAL INFORMATION	
1. Gender	
a) Male	
b) Female	
2 Age category (years)	
a) 20 – 25	
b) 26 – 30	
c) 31 – 35	
d) 36 – 40	
e) 41 – 45	
f) 46+	
3. Highest level of education	
a) Pre matric	
b) Matric	
c) Post matric	
4. What is your practice?	
a) Traditional healer	
b) Herbalist	
c) Both traditional and a herbalist	
5. Have you consulted with researcher/researchers before?	
a) Yes	
b) No	
SECTION B: COLLECTION PRACTICES	
1 Training in traditional medicine	
a) Formal healer's training	
b) Learned from accompanying a parent who was a traditional healer	
c) Learned from accompanying a herbalist	
2. Do you have a collector's permit	
a) Yes	
b) No	
3. How often do you go out to collect medicinal plants?	
a) Less than 3 times a week	
b) More than 3 times a week	
c) Only when I am running low on stock	
d) As often as I can	
4. How much of a particular medicinal plant do you collect	
a) As much as I can collect on the day	

b) As little as possible	
c) Only as much as I need	
d) Enough to be able to sell to other healers/herbalists	
5. How often will you collect the same plant from the same area	
a) More than three times a year	
b) Less than three times a year	
c) Once a year and move to another area	
d) Until the plant is depleted in the area	
SECTION C: THE EASE OF LOCATING THE SELECTED PLANTS IN THE FIELD	
1. On a scale of 1-5 (1 being very difficult and 5 being very easy) indicate how difficult it is to locate the following medicinal plants in the field	
a) <i>Hypoxis hemerocallidea</i>	
b) <i>Eriocephalus punctulatus</i>	
c) <i>Morella serrata</i>	
d) <i>Dicoma anomala</i>	
e) <i>Xysmalobium undulatum</i>	
f) <i>Gazania krebsiana</i>	
2. Which of the following medicinal plants do you buy from commercial gatherers?	
a) <i>Hypoxis hemerocallidea</i>	
b) <i>Eriocephalus punctulatus</i>	
c) <i>Morella serrata</i>	
d) <i>Dicoma anomala</i>	
e) <i>Xysmalobium undulatum</i>	
f) <i>Gazania krebsiana</i>	
3. Are you willing to show the researcher the areas where you usually collect the selected medicinal plants?	
a) Yes	
b) No	

