Effects of pruning and fertilizer on growth, phytochemistry and biological activity of *Sutherlandia frutescens* (L.) R.Br.

By

Maanda Benjamin Raselabe

(B.Sc. Hons. University of Limpopo, South Africa)

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COLLEGE OF AGRICULTURE, ENGINEERING AND SCIENCE

DECLARATION 1 - PLAGIARISM

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DECLARATION BY SUPERVISORS

We hereby declare that we acted as Supervisor's for this MSc:

Student's Full Name: **Maanda Benjamin Raselabe**

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Regular consultation took place between the student and us throughout the investigation. We advised the student to the best of our ability and approved the final document for submission to the College of Agriculture, Engineering and Science, Higher Degrees Office for examination by the University appointed Examiners.

SUPERVISOR:	_____ PROFESSOR J. VAN STADEN
CO-SUPERVISOR:	_____ PROFESSOR J.F. FINNIE
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CO-SUPERVISOR:	_____ DR A.R. NDHLALA

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Raselabe M.B., Ndhlala A.R., Abdelgadir H.A., Finnie J.F., du Plooy C.P., Van Staden J., 2014. Effect of different pruning and spacing practices on the antibacterial activity of *Sutherlandia frutescens* (L.) R.Br. (Fabaceae). Indigenous Plant Use Forum (IPUF) 17th Annual Conference, 30 June-3 July 2014, University of the Free State, QwaQwa Campus, Phuthaditjhaba, South Africa.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
ANOVA	Analysis of variance
ARC	Agricultural Research Council
BBB	Blood-brain barrier
BCL	Bicuculline
Caco-2	Human colonic adenocarcinoma cells
CD4	Cluster of differentiation 4
CHO	Chinese Hamster Ovary Cells
COX	Cyclooxygenase
CTE	Catechin equivalents
DM	Diabetes mellitus
DNA	Deoxyribonucleic acid
FKHR	Fork hear transcription factor
Folin-C	Folin-Ciocalteu
GABA	Gamma-aminobutyric acid
GAE	Gallic acid equivalents
GC/MS	Gas chromatography/mass spectrometry
HCl	Hydrochloric acid
HIV	Human immunodeficiency virus
IC	Inhibitory concentration
ICUN	International Union for the Conservation of Nature
IL-1β	Interleukin-1 beta
LAI	Leaf area index
LADA	Latent autoimmune diabetes
LC-MS	Liquid chromatography/mass spectrometry
LD	Lethal dose
LSD	Least significant difference
MCF-7	Michigan Cancer Foundation 7
MIC	Minimum inhibitory concentration
PCT	Picrotoxin
PTZ	Pentylenetetrazole
RCBD	Randomized complete block design
SANBI	South African National Biodiversity Institute

SD	Standard Deviation
STZ	Streptozotocin
SU	Sutherlandiosides
TB	Tuberculosis
TNF	Tumor necrosis factor
TPA	2-O-tetradecanoylphorbol 13-acetate
WHO	World Health Organisation

ABSTRACT

Sutherlandia frutescens (L.) R.Br. (Fabaceae), commonly known as cancer bush, is a herb with a long history of traditional use by a variety of cultures. The plant mainly grows in the dry parts of southern Africa, mostly in the Western and Eastern Cape as well as the neighbouring countries like Lesotho, Botswana and Namibia. Cancer bush is traditionally used for the treatment of external wounds, internally for fevers, stomach problems, cancer, diabetes, influenza, HIV, depression, eye problems, TB, colds and asthma. The plant is famously known for the treatment of Type 2 diabetes, cancer and HIV. However, these claims remain inconclusive. Recent studies have shown *S. frutescens* to have antidiabetic, anti-HIV, anti-inflammatory, antibacterial, analgesic, anti-stress, anticonvulsant, antiproliferative and antithrombotic activities. Phytochemical investigations of *S. frutescens* leaves detected the presence of high levels of free amino acids and non-protein amino acids namely: canavanine, gamma aminobutyric acid (GABA) and pinitol. The presence of these compounds has been reported to be responsible for its reputed effectiveness in a wide range of illnesses. In view of its importance as a multipurpose medicinal crop, it is important to bring this plant under cultivation and determine agronomic requirements for its successful cultivation. Several factors can be investigated to enhance the growth and increase the level of active ingredients.

The current study was aimed at evaluating the effect of pruning and fertilizer levels on the growth, phytochemistry and biological activity of *Sutherlandia frutescens*. Seeds were sown in seedling trays to produce seedlings. One-month-old seedlings were then transplanted on a prepared field. The study trial was carried out at the Agricultural Research Council-Vegetable and Ornamental Plants Research Station (ARC-VOP). The experiment was conducted in a randomized complete block design (RCBD) with three pruning levels, three fertilizer levels and four replicates. There were 9 treatments, namely; no pruning (P0), tip-pruning (P1) and heading back (P2) in combination with levels of fertilizers as follows: 200 kg/ha NPK (F1), 100 kg/ha NPK (F2) and 0 kg/ha NPK (F0). Plants without treatments were considered as controls. Growth parameters taken were plant height, stem diameter, chlorophyll and Leaf Area Index (LAI). Dried leaf samples were analysed for the presence of secondary metabolites and antidiabetic activities.

There was no significant interaction effect between pruning and fertilizer levels amongst all parameters measured in this study. Pruning treatments had a significant effect on the LAI at week one and week two but did not affect the plant height, stem diameter and chlorophyll content. Different levels of fertilizers had a significant effect on the LAI, where 100 kg/ha NPK significantly increased LAI at week one and week two.

In an investigation which was conducted from January to May 2015, plants showed yellowing, stunting, and high levels of infestation expressed as extensive galling on the roots which led to the nematode infestation study. Nematodes were extracted from the roots of a healthy living, a wilted and a dead plant, as well as from the rhizosphere soil. A small population of *Scutellonema, Pratylenchus, Helicotylenchus* and *Tylenchorhynchus* were identified. Examination of the root of an infected plant revealed the presence of root-knot nematodes (*Meloidogyne javanica*) in large numbers. Juveniles, eggs and females were isolated, and the species were identified on the basis of morphological characteristics. Symptoms usually associated with root-knot nematodes were observed on the roots of the wilted plant and the soil in which the plant was growing.

Extracts from all nine treatments showed stronger activity against *α*-glucosidase than the positive control acarbose. The highest *α*-glucosidase inhibitory activity was demonstrated by the treatment with no pruning (P0) while the heading back (P2) treatment exhibited the lowest inhibitory activity. Fertilizer levels at 200 kg/ha (F1) NPK resulted in a significantly higher *α*-glucosidase inhibitory activity compared to other fertilizer treatments.

The presence of secondary metabolites (including total phenolics and flavonoids) was determined qualitatively. The total phenolic content was determined using the Folin-Ciocalteu method and flavonoids were determined using the vanillin HCL assay. The study showed that pruning and fertilizer increased the production of secondary metabolites in *S. frutescens* as compared to the control. Fertilizer at 200 kg/ha NPK (F1) did seem to improve phenolics and flavonoids with pruning but phenolic levels were actually quite low when treatment was P0F1. Total phenolics and flavonoids were significantly increased by the heading back (P2) treatment and decreased in the treatment with no pruning (P0). The application of fertilizer at 200

kg/ha NPK (F1) improved the production of secondary metabolites, and reduced production of secondary metabolites was recorded in plants that received no fertilizer. There was no direct correlation between the level of phytochemicals and the antidiabetic activity recorded.

This study examined the effects of different levels of pruning and fertilizers on the growth of *S. frutescens*. The results showed that there was no significant difference. At this stage, no positive recommendations can be made for cultivating *S. frutescens*. Plant extracts showed good antidiabetic activities in response to different pruning and fertilizer treatments. This was further seen as an increase in the production for secondary metabolites. However, further investigation of plant cultivation practices and further screening for bioactivities is required. *S. frutescens* may offer a new source of drugs for diabetes mellitus and other related diseases.

1.1. Medicinal plants

Medicinal plants are used in treating a wide range of illnesses, maintaining health and to prevent specific diseases **(FARNSWORTH and SOEJARTO, 1991; RIOS and RECIO, 2005)**. They form an important part of the culture and traditions of African people. Medicinal plants are widely used by traditional people all over the world and have frequently become more popular in communities as natural alternatives to synthetic chemicals **(VAN WYK and WINK, 2004)**. About 25% of modern medicines contain compounds extracted from plants as their main active ingredients. Frequently, isolated active compounds are channelled into drug development **(SIMPSON and OGORZALY, 2001)**. Studies to determine the chemical profile and composition of medicinal plants reveal the complexity and variety of compounds all contributing to various uses of plants in treating different diseases.

South Africa is very rich in plant biodiversity, with many of the plants having a variety of medicinal uses. Medicinal plants (locally called *muthi*) are widely used by numerous cultures for their primary health care needs in South Africa. Reports have indicated that South Africa has approximately 3000 plant species that are medicinally useful **(MANDER *et al*., 2008; VAN WYK and GERICKE, 2000)**. It is estimated that up to 70 000 tonnes of plant material are consumed annually as medicines and are sold through informal markets **(MANDER and LE BRETON, 2006)**. Medicinal use and trade is no longer limited to traditional healers, but has entered both the informal or formal business sectors of the South African economy, which has given rise to the large number of herbal gatherers and traders **(COCKS *et al*., 2004)**. **CUNNINGHAM (1991)** described it as a multi-million Rand hidden economy, with the South African trade in traditional medicines valued at R2.9 billion per annum in 2008 **(MANDER *et al*., 2008)**. Herbal medicines are also widely used for therapeutic purposes in urban areas by well-educated and modernized South Africans **(DOLD and COCKS, 2002; MANDER *et al*., 2008; NDHLALA *et al*., 2011)**.

1.2. Cultivation of medicinal plants and its importance

Only 10% of medicinal plant species are cultivated and this implies that most of medicinal plant materials are still being collected from wild resources **(JULSING *et al*., 2007)**. From the species documented, 93% are being unsustainably collected and 34% of the species have been prioritised for conservation management **(MANDER, 1998)**. Intensive collection of wild medicinal plants is a serious threat to biodiversity, with over 700 plant species actively traded in South Africa **(MANDER, 1998)**. This has resulted in overexploitation, mis-identification, and damage to the population of many species in their natural habitats eventually causing species extinction. Cultivation at a large scale remains as the only option for the conservation of many species. This will achieve sustainable development and biodiversity protection and will also generate an income through farming **(SPARG, 2003)**. Wild plants can be saved if more investments are made in the cultivation of highly wanted plants **(JÄGER and VAN STADEN, 2000)**. Cultivation also opens channels for conducting scientific research as it provides a source of plant material which can be used to select and breed high-yielding cultivars. **WHO (2003)** suggested that if there is non-availability of scientific publications or documents on cultivation data of these medicinal plants, then traditional methods of cultivation should be followed, where practicable. Otherwise a method should be developed through research **(WHO, 2003)**.

1.3. Commercialization of medicinal plants

Currently only 38 medicinal plant species have been commercialized in southern Africa. They are sold in various forms such as teas, tinctures, tablets, capsules and ointments **(VAN WYK, 2008)**. Some of the commercially cultivated plants include: buchu (*Agathosma betulina*), rooibos (*Aspalathus linearis*) and devil's claw (*Harpagophytum procumbens*). However, these products are mostly sold to overseas markets **(JÄGER and VAN STADEN, 2000)**.

1.4. Aims and objectives

There is limited understanding of the effect of cultivation practices as well as pre-harvest conditions on the biological activity of medicinal plants. Therefore, a research study on all agronomical aspects on medicinal plants needs to be investigated to ensure that the activity is not compromised. Little is known about *Sutherlandia frutescens* as a cultivated crop and there has been an increase of interest in its pharmacological activities. Scientific reports have indicated that leaf extracts of *S. frutescens* play an important role in the treatment of HIV/AIDS, cancer and diabetes. In addition, the plant also has anti-inflammatory, antioxidant and anticonvulsant effects **(VAN WYK and ALBRECHT, 2008)**. There is currently a higher demand for *Sutherlandia* and *Sutherlandia*-based products than previously. Therefore, a better understanding of its optimal cultivation and processing practices is needed to assist with successful commercialization. The overall aim of this study was to determine whether different pruning levels and application of fertilizers would have significant effects on the growth, phytochemistry and biological activity of *Sutherlandia frutescens*. However, an objective to determine the presence, distribution, and abundance of plant-parasitic nematodes in *S. frutescens* was added due the fact that a number of plants were observed with stunted growth and severe wilting. The objectives of the study were to:

- Investigate the effect of pruning and fertilizers on the growth of *S. frutescens*;
- Determine the antidiabetic activity of leaf extracts of *S. frutescens* using the *α*-glucosidase assay;
- Evaluate the total phenolics and total flavonoid content of leaf extracts using colorimetric assays; and
- Determine nematode infestation on *S. frutescens*.

1.5. Organisation of the dissertation

Chapter 1 provides a general introduction to medicinal plants, cultivation of medicinal plants and its importance, as well as recommendations on commercialization of some important medicinal plants in southern Africa. The literature review of *S. frutescens*, including a detailed overview on the medicinal uses, pharmacological uses and phytochemistry, is presented in **Chapter 2**. An

investigation on pruning and fertilizer levels on the growth of *S. frutescen*s is presented in **Chapter 3**. **Chapter 4** presents a detailed report of nematode screening on *S. frutescens*. The investigation, including materials and methods of the techniques used to determine the antidiabetic properties of the plant extracts of *S. frutescens*, are presented in **Chapter 5**. **Chapter 6** contains extraction procedures of the plant material, determination of total phenolics and flavonoid levels. **Chapter 7** summarises and concludes the thesis. This chapter also provides some recommendations for future research.

2.1. Introduction

Sutherlandia frutescens (L.) R.Br (*Lessertia*) is a well-known multipurpose medicinal plant in southern Africa **(MOSHE, 1998; VAN WYK and ALBRECHT, 2008)**. It is commonly known as the cancer bush because of its reported use against cancer. Other common names include balloon-pea (English), *umnwele* (Xhosa and Zulu), *kankerbos* (Afrikaans) and *lerumo-lamadi* in Sotho **(XABA, 2007)**. The plant is traditionally used as a medicinal remedy by different cultural groups including the Zulu, Xhosa, Sotho, Khoi-san and Cape Dutch, for treatment of stomach ailments, fever, diabetes, internal cancers, stress, asthma, wounds and arthritis **(STANDER *et al*., 2007; VAN WYK and ALBRECHT, 2008; STREET and PRINSLOO, 2012)**. It is reported that the cancer bush plays an important role in the treatment of HIV/AIDS-related illness, cancer and diabetes. It has anti-inflammatory, antioxidant and antimutagenic effects **(HARTNETT *et al*., 2005; CHADWICK *et al*., 2007; STANDER *et al*., 2007)**.

Sutherlandia frutescens belongs to the family Fabaceae, commonly known as the legume, pea or bean family. Their roots have nitrogen-fixing nodules **(ROBERTS, 1990; VAN WYK *et al*., 1997; CHADWICK *et al*., 2007)**. The Fabaceae embraces more than 600 genera and 12000 species worldwide and is represented by 134 genera and more than 1300 species in southern Africa **(JACKSON, 1990).** There are five closely related species that are often confused with *S. frutescens*, these are: *S. humilis, S. microphylla* (commonly known as bitterblaar)*, S. montana* (the mountain cancer bush)*, S. speciosa* and *S. tomentosa* (known as *eedjies*) **(PHILLIPS and DYERS, 1934; XABA, 2007; VAN WYK and ALBRECHT, 2008)**. Using enzyme electrophoresis, **MOSHE (1998)** had shown that some of these species are actually subspecies and that only two main species should be recognized, namely: *S. frutescens* and *S. tomentosa*. The genus *Sutherlandia* has been sunk into the genus *Lessertia* DC by **GOLDBLATT and MANNING (2000)** due to the assumption that *Sutherlandia* represents an adaptation to bird pollination. This remains to be validated by means of morphological or genetic analysis **(VAN WYK and ALBRECHT, 2008)**. The transfer of *Sutherlandia* to *Lessertia* required a name

change for the two species (*S*. *frutescens* and *S*. *tomentosa*) to *Lessertia frutescens* and *Lessertia canescens*, respectively **(GOLDBLATT and MANNING, 2000)**.

2.2. Taxonomic and botanical description

Cancer bush is a perennial short-lived shrub with the height reaching up to 2 m. The stems are glabrous or sparsely pubescent, with numerous leaves which are pinnately compound borne mainly towards the tip. The leaflets are 4-10 mm long **(Figure 2.1. A-C)**, grey green in colour and have a bitter taste **(XABA, 2007)**. The plant produces orange-red flowers (up to 35 mm long) from July to December **(Figure 2.1. A-C)**. The fruits are large, bladdery and papery pods are about 1.3-2 mm long and as wide **(PHILLIPS and DYERS, 1934)**. The attractive, butterfly-like red flowers are pollinated by sunbirds. The lightweight, papery, inflated pods enable the seed to be easily dispersed by wind **(LEISTNER, 2000)**.

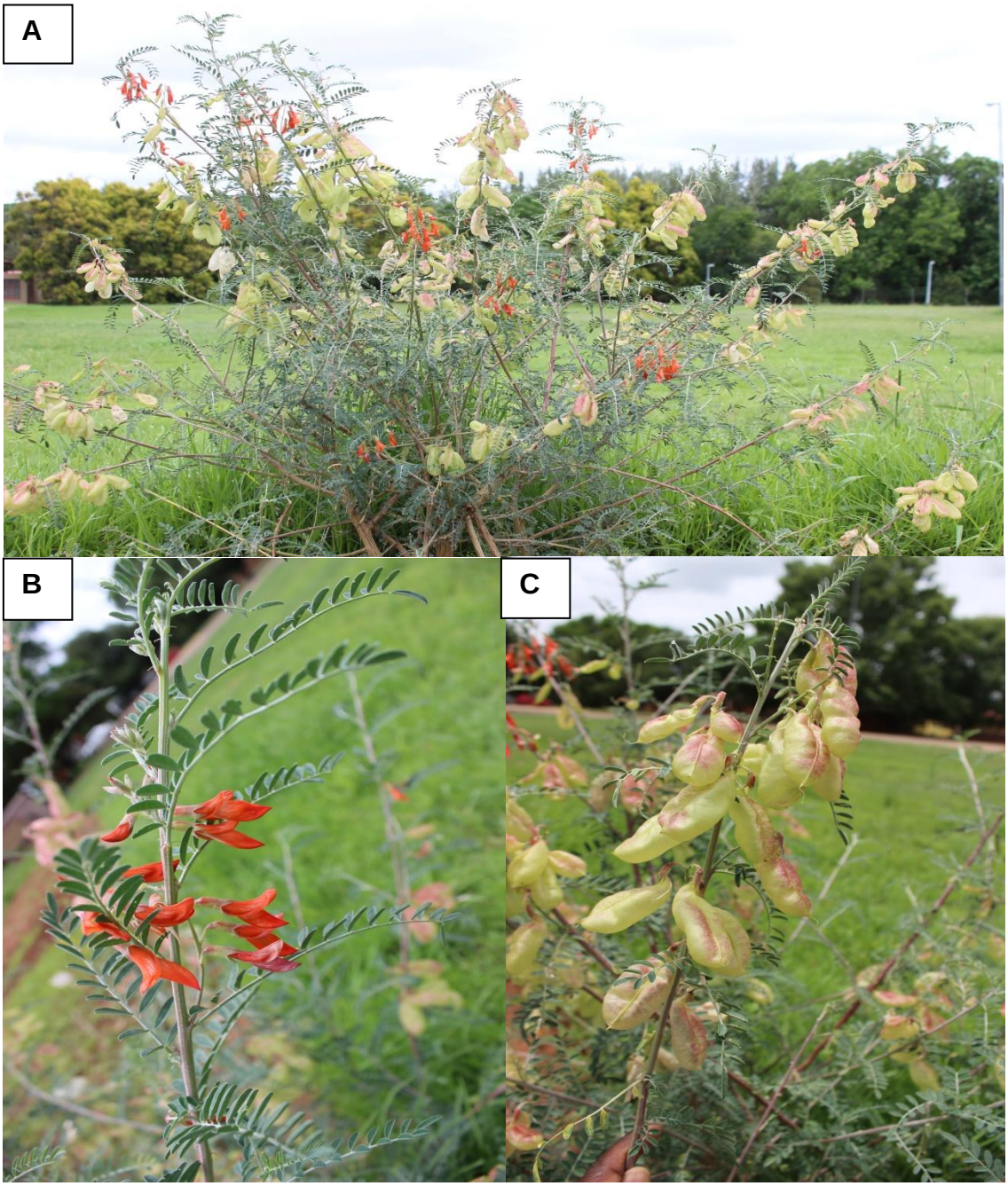

Figure 2.1: (A) *Sutherlandia frutescens* (L.) R.Br. planted at the Agricultural Research Council-Vegetable and Ornamental Plants Research Station, Roodeplaat, Gauteng, GPS coordinates: 25°59"S28°35"E (ARC-VOP), (B) Flowers and, (C) Pods of *Sutherlandia frutescens*.

2.3. Distribution

The plant is widely distributed throughout the dry areas of southern Africa, being abundant in the south Western and Northern Cape Provinces **(Figure 2.2)**, where it grows in the savannah and on hillsides near streams in drier areas **(VAN WYK and ALBRECHT, 2008)**.

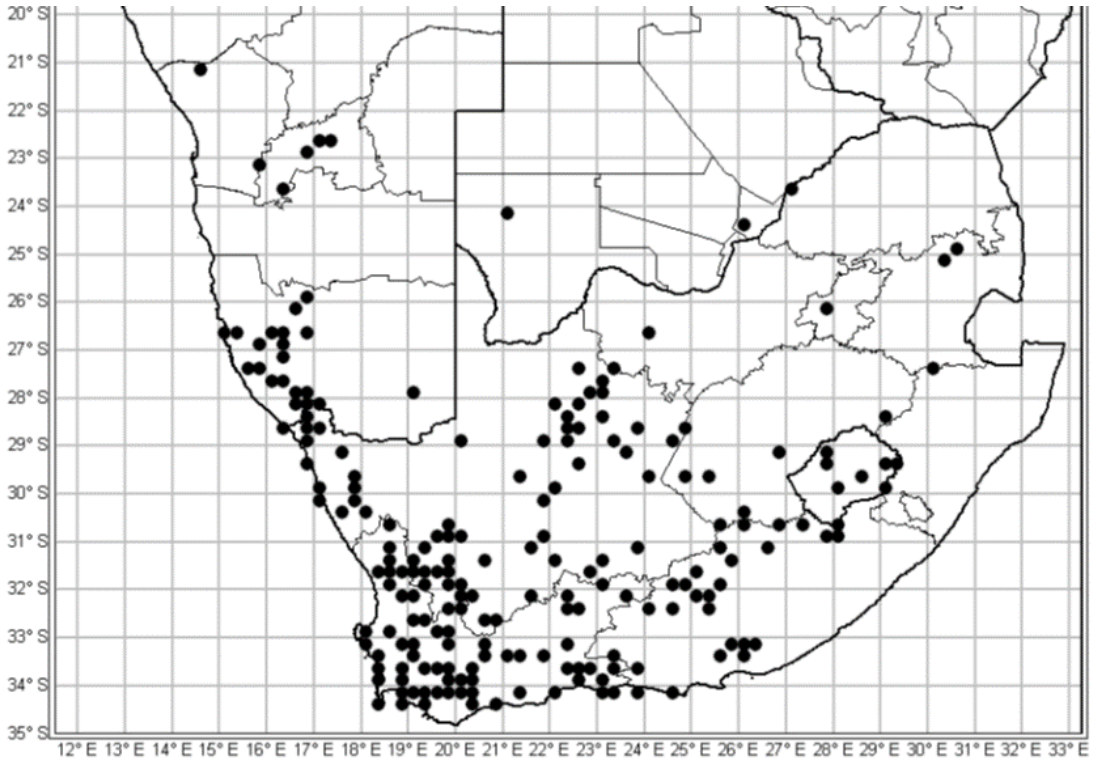

Figure 2.2: Distribution map of *Sutherlandia frutescens* (L.) R.Br. in southern Africa. Source: South African National Biodiversity Institute - SANBI distribution data (2014).

2.4. Ecology

Sutherlandia frutescens is a fast-growing plant which tolerates all soil types. The plant can grow in arid areas that have been disturbed **(XABA and NOTTEN, 2003)**. Ecologically, legumes are known for fixing nitrogen in the soil through a symbiotic relationship with bacteria. The bacteria infect the roots, forming nodules. Inside the nodules atmospheric nitrogen, which plants cannot use, is converted to ammonia **(XABA, 2007)**. The plant supplies sugars for the bacteria, while bacteria provide the biological useful nitrogen that the plant absorbs. According to the evaluation by The Red List of South African plants (2005), *S. frutescens* is not threatened.

2.5. Medicinal uses

The medicinal use of cancer bush is reported to have originated from the Khoi-san and the Nama people. *Sutherlandia frutescens* has been widely used by local traditional cultures for the treatment or relief of diseases such as cancers, HIV/AIDS, diabetes, stress and anxiety, inflammation, pain, and wounds **(HARNETT *et al*., 2005; VAN WYK and ALBRECHT, 2008)**. These uses make *S. frutescens* an ideal plant to study in order to reveal if and how the extracts are responsible for the treatment of these diseases. Branches and roots can be used to make strong decoctions. **VAN WYK and ALBRECHT (2008)** stated that the plant can be used as fodder for cattle because of the high levels of its free and protein-bound amino acids.

There have been reports on *S. frutescens* indicating that it can be used to improve the overall health in HIV/AIDS patients by improving appetite, improving CD4 counts and reducing the viral load **(CHAFFY and STOKES, 2002; WHO, 2002; HARNETT *et al*., 2005; BESSONG *et al*., 2006)**. The plant can assist with reducing muscle wasting in cancer patients, treating anxiety and depression **(TAI *et al*., 2004)**. *S. frutescens* is still one of the most commonly used medicinal plants in the Western Cape. It is also sold in *muthi* markets and in *amayeza* (medicine) shops around the country.

2.6. Phytochemistry

The chemistry of *Sutherlandia frutescens* has been studied by Professor Ben-Erik van Wyk and Dr Carl Albrecht. They identified bioactive chemicals in *S. frutescens* extracts including novel triterpenoid glycosides (currently known as SU1), GABA (gamma-aminobutyric acid), L-canavanine, pinitol, several flavonoids and non-protein amino acids **(VAN WYK and ALBRECHT, 2008)**. Hot water appeared to be the best extraction solvent for polyphenols with the total phenolic content and total flavonoid content with hot water extracts being 12.9 ± 0.2 µg gallic acid equivalents/mg and 28.7 ± 0.3 µg quercetin equivalents/mg of dried *S. frutescens*, respectively **(TOBWALA *et al*., 2014)**. **TAI *et al*. (2004)** conducted research which confirmed the presence of canavanine, gamma aminobutyric acid (GABA) and arginine in commercial *S. frutescens* tablets using gas chromatography/mass

spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS). Laboratory experiments by **SHAIK *et al.* (2010)** have shown that the yields of medicinal phytocompounds are higher in *in vitro*-grown leaf extracts than in field-grown leaf and wild leaf extracts. The five known compounds are: Gamma aminobutyric acid, pinitol, L-canavanine, L-arginine and a triterpenoid glycoside **(Figure 2.3)**.

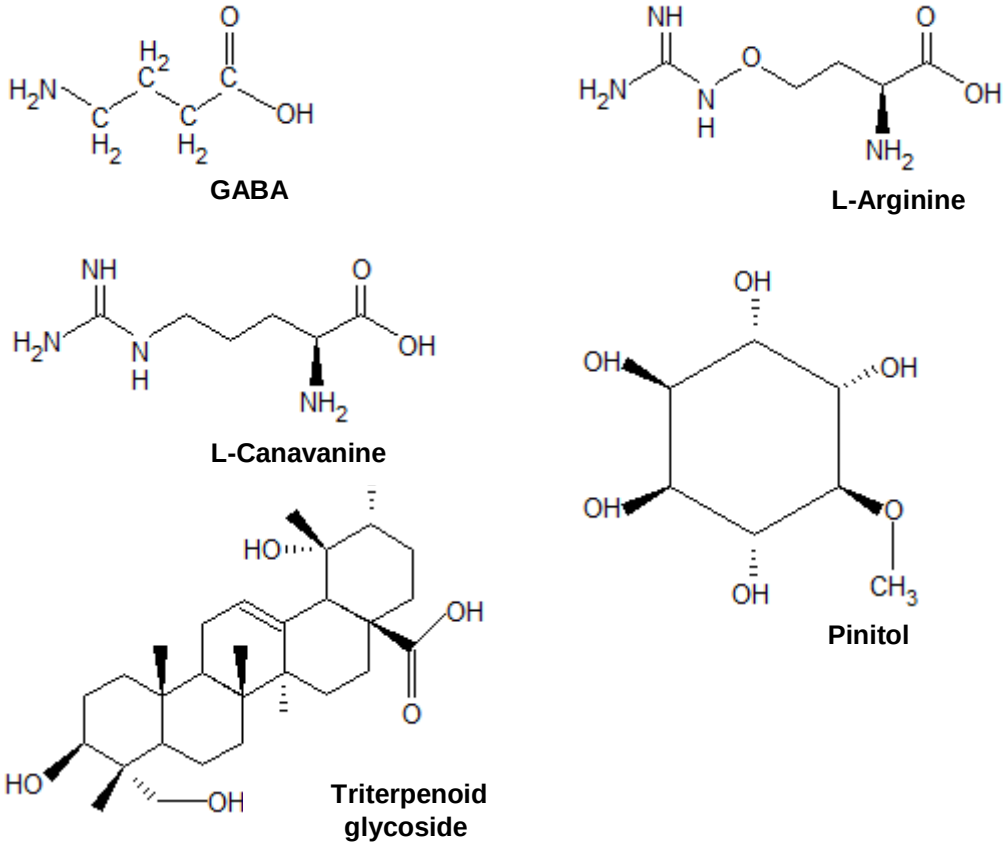

Figure 2.3: Chemical structures of compounds commonly found in *Sutherlandia frutescens*.

2.6.1. Gamma aminobutyric acid (GABA)

Gamma aminobutyric acid (GABA) is a four-carbon **(Figure 2.3)** amino acid and an important efficient neurotransmitter inhibitor which is principally located in the nervous system of some animals. It is known as the "*brain's natural calming agent*" and inhibits over-stimulation of the brain. GABA may help promote relaxation and ease nervous tension **(ZHANG *et al*., 2006)**. It is important for brain metabolism, where it also decreases neuron activity preventing them from over-firing. It has been used as a drug to reduce stress, depression, anxiety, panic and insomnia **(MAYER *et al*., 1990; BOLARÍN *et al*., 1995; MOTEETEE and VAN WYK, 2007)**.

Commercial samples of *S. frutescens* were found to contain 0.23-0.85 mg/g of GABA **(VAN WYK and ALBRECHT, 2008)**. The compound forms during the decarboxylation of glutamate **(EBADI, 2007)**. The presence of GABA also justifies the use of *S. frutescens* in treating anxiety and stress. **ABDOU *et al*. (2006)** reported that it may play a role in the general improvement of the mood and well-being which could be observed after consuming *S. frutescens.* Previous studies have indicated GABA to be used in the treatment of depression, lowering high blood pressure, bipolar disorder, seizures, as well as preventing weight loss in HIV/AIDS and cancer patients **(PETTY *et al.,* 1993; TAI *et al*., 2004)**. GABA also inhibits tumour cell migration **(ORTEGA, 2003)**.

2.6.2. Pinitol

Pinitol **(Figure 2.3)** is a type of sugar found in many legumes and it is classified as a D-chiro-inositol which acts as a second messenger in different metabolic processes including blood sugar metabolism **(LARNER, 2002; MILLS *et al*., 2005)**. It has been shown that pinitol has an insulin-like effect and could be of great use as a dietary supplement in the treatment of hyperglycemia. It is a known antidiabetic agent and is also said to be responsible for preventing weight loss and inflammation in HIV/AIDS patients **(NARAYANAN *et al.,* 1987; OSTLUND and SHERMAN, 1996)**. Using HPLC analysis, **MOSHE (1998)** found that *S. frutescens* contains up to 14 mg/g pinitol dry weight in leaves. **BATES *et al.* (2000)** tested the effects of pinitol in an animal model, where overweight diabetic mice (Type 2 diabetes) and STZ mice (Type 1 diabetes) were given 100 mg pinitol/kg body weight. This resulted in the

reduction of hyperglycemia. Pinitol assists with diabetes by lowering blood sugar levels, making glucose more available for cell metabolism **(VAN WYK and ALBRECHT, 2008)**.

2.6.3. L-Canavanine

L-Canavanine is a toxic, non-protein amino acid produced by more than 500 leguminous plants **(LI *et al*., 2001)**. It is mostly found in seeds and has a structure analogous to that of L-arginine **(VAN WYK and ALBRECHT, 2008)**. This compound has antimicrobial, anticancer, anti-viral activities, and inhibition properties for influenza virus and retroviruses **(GREEN, 1988; CROOKS and ROSENTHAL, 1994; SWAFFAR, 1995; VAN WYK *et al.,* 1997)**. L-Canavanine **(Figure 2.3)** is also a selective inhibitor of inducible nitric oxide synthase and is used in the treatment of septic shock (a syndrome associated with advanced stages of HIV/AIDS) and chronic inflammation **(ANFOSSI *et al.,* 1999; VAN WYK and GERICKE, 2000; DALVI, 2003)**. Studies found an average of 2.2 mg of L-canavanine per dry weight of *S. frutescens* leaf material. Analysis of *S. frutescens* confirmed the presence of canavanine in commercial material and recorded a level of 3 mg/g dry weight **(TAI *et al*., 2004)**.

2.6.4. L-Arginine

S. frutescens leaf extracts were analyzed and found to contain high levels of the amino acids; asparagine, proline and arginine **(VAN WYK and ALBRECHT, 2008)**. The presence of L-arginine **(Figure 2.3)** is important because it is an antagonist of L-canavanine that decreases the anti-proliferative activity of canavanine. There is approximately 3 mg/g of L-arginine in dried leaves of *S. frutescens* **(TAI *et al.,* 2004)**.

2.6.5. Triterpenoid glycosides

These compounds were first detected in *Sutherlandia microphylla* leaves by **BRÜMMERHOFF (1969)** and **VILJOEN (1969)** but the chemical structures were not determined. A pure compound known as SU1 was isolated from *S. frutescens* leaf extracts **(GABRIELSE, 1996; VAN WYK and ALBRECHT, 2008)**.

Commercially produced *Sutherlandia frutescens* material was found to contain a cycloartane-type triterpene glycoside **(Figure 2.3)** known as sutherlandioside (SU) **(FU *et al*., 2008)**. Four cycloartane-type triterpenoids, which stimulate appetite and are known for contributing to the adaptogenic and immune-boosting effects of *S. frutescens,* were isolated by **FU *et al*. (2008)**. These four compounds are known as sutherlandioside A (SU2), B (SU1), C and D. About 56 different triterpene glycosides have been detected and the mixture of cycloartane-type triterpenoid glycosides varies geographically in South Africa **(VAN WYK and ALBRECHT, 2008). ALBRECHT *et al*. (2012)** found that plants that differ due to geographic positioning can be identified based on chemical profiling.

2.7. Pharmacological activities

Previous studies have indicated *S. frutescens* extracts to have a wide diversity of pharmacological activities. The antidiabetic activities may be due to the presence of pinitol. It was indicated that the presence of canavanine may also contribute to the anticancer activity **(VAN WYK and ALBRECHT, 2008)**. Some of the most important pharmacological activities are: cancer, diabetes and HIV/AIDS (**VAN WYK and ALBRECHT, 2008**).

2.7.1. Cancer

A number of compounds were identified from isolated *S. frutescens* extracts that are known to have a direct anticancer effect and that act as an immune stimulant. L-Canavanine, considered as a leaf metabolite, was initially thought to be responsible for antiproliferative effects **(SEIER *et al.,* 2002)**. Currently it is also known that triterpenoids of *S. frutescens* (structurally related to cycloartane-type triterpenoids)

are responsible for antiproliferative effects and have been shown to have cancer chemopreventive activity **(KIKUCHI *et al*., 2007; VAN WYK and ALBRECHT, 2008). CHINKWO (2005)** stated that aqueous extracts could induce apoptosis in cultured carcinoma cells, in neoplastic cells and in Chinese Hamster Ovary Cells.

A study by **STANDER *et al*. (2007)** indicated that breast adenocarcinoma cells died (significant decrease of up to 50%) as a result of apoptosis and autophagy after their exposure to *S. frutescens* extracts (1.5 mg/ml). Methanol extracts of *S. frutescens* inhibited the DNA binding of NF-kB activated by 12-*O*-tetradecanoylphorbol-acetate (TPA) in MFC10A human breast epithelial cells in a dose dependent manner **(NA *et al*., 2004)**. It was concluded that the inhibition of TPA-induced COX-2 expression through suppression of DNA binding NF-kB may contribute to the chemo-protective activity of *S. frutescens*. Dichloromethane extracts of *S. frutescens* have been shown to exhibit antimutagenic activity **(REID *et al.,* 2006)**.

2.7.2. Diabetes

The use of *S. frutescens* as an anti-diabetic agent is due to the presence of high levels of pinitol **(MOSHE, 1998)**. The presence of pinitol, L-canavanine, and other amino acids in *S. frutescens* were also found to have an anti-diabetic activity, directly or via anti-inflammatory and NO-inhibitory activity. RAWS 264.7 cells incubated with *S. frutescens* extracts were only insignificantly affected in their expression levels of the pro-inflammatory cytokines, TNF-α and IL-1β **(SIA, 2004; TAI *et al*., 2004)**. The anti-inflammatory and NO-inhibitory activity increases insulin sensitivity at cellular levels and protects *β*-cells against free oxygen radicals **(SIA, 2004; VAN WYK and ALBRECHT, 2008)**.

There was an increase in glucose uptake in peripheral tissues, intestinal glucose uptake reduction and no weight gain in pre-diabetic rats receiving *S. frutescens* leaf extracts **(CHADWICK *et al*., 2007)**. Extracts were shown to lower the glucose concentration in the blood system. The greatest effect was observed with a dose of 100 mg/kg D-pinitol, which significantly decreased plasma glucose by 12% ($P<0.05$) at 2 h and 22% ($P<0.02$) at 6 h **(BATES *et al*., 2000)**. *S. frutescens* shoot aqueous extracts (50-800 mg/kg) were shown to reduce glucose uptake in STZ-treated mice. The plant extract (50-800 mg/kg) had a significant ($p < 0.05$-0.001) effect against

fresh egg albumin-induced acute inflammation and was also hypoglycemia significant ($p < 0.05$-0.001) in rats **(OJEWOLE, 2004)**. Studies have concluded that *S. frutescens* extracts show promise as a medication for Type 2 diabetes, but there is a need for clinical evidence to confirm these claims.

2.7.3. HIV/AIDS

Sutherlandia frutescens tablets improved the mood and appetite of patients with AIDS **(CHAFFY and STOKES, 2002; JOHNSON *et al*., 2007; VAN WYK and ALBRECHT, 2008)**. *Sutherlandia frutescens* has been reported to have antiviral activity due to the presence of L-canavanine **(VAN WYK and ALBRECHT, 2008)**. Organic and aqueous extracts from leaves and flowers were shown to have inhibitory effects on HIV reverse transcriptase and HIV target enzyme activity which makes the HIV virion less infective **(HARTNETT *et al*., 2005)**. Using the absolute CD4 count **DAVIDSON (2006)** showed a significant decrease with a p-value of 0.041 after exposure to *S. frutescens* and according to HIV guidelines the significant change in the absolute CD4 cell count in the setting of a stable CD4 percentage implies that there is immunological stability.

In a recent study by **AFRICA and SMITH (2015)** *S. frutescens* aqueous extracts decreased IL-1β secretion significantly ($P < 0.0001$), but exacerbated both monocyte chemoattractant protein-1 ($P < 0001$) - a major role player in HIV-associated neuroinflammation - and CD14+ monocyte infiltration across the BBB ($P < 0.01$).

2.7.4. Other activities

Reports indicated that *S. frutescens* extracts have anti-inflammatory, anti-analgesic and antibacterial activities. **OJEWOLE (2004)** reported statistical records of anti-inflammatory activity and *in vivo* analgesic activity. **FERNANDES *et al.* (2004)** found antioxidants to have superoxide and hydrogen peroxide scavenging activities at concentrations as low as 10 mg/ml, which contribute to the anti-inflammatory effects of *S. frutescens* hot water extracts. **TOBWALA *et al*. (2014)** also found *S. frutescens* extracts to have significant antioxidant potential using different solvent extracts where hot water extract possessed the highest radical scavenging ability (39%)

among all *S. frutescens* extracts. *S. frutescens* ethanolic extracts showed a concentration dependent antiproliferative effect on several human tumor cell lines but did not show significant antioxidant effects **(TAI *et al.,* 2004)**. A first report on antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli* was done by **KATERERE and ELOFF (2005a)** where a hexane extract was active against these bacterial strains (*S. aureus*, *E. faecalis* and *E. coli*) with MIC values of 0.31, 1.25 and 2.50 mg/ml, respectively. *In vivo* studies by **OJEWOLE (2007)** found *S. frutescens* shoot aqueous extracts to have anticonvulsant properties of the plant against pentylenetetrazole (PTZ), picrotoxin (PCT) and bicuculline (BCL) induced seizures in mice. In this study *S. frutescens* shoot aqueous extract (SFE, 50–400 mg/kg) antagonized pentylenetetrazole (PTZ)-induced seizures, antagonized picrotoxin (PCT)-induced seizures, but only weakly antagonized bicuculline (BCL)-induced seizures. *Sutherlandia frutescens* leaf extracts also showed antithrombotic activity with an IC_{50} value of 2.17 mg/ml **(KEE *et al.,* 2008)**.

2.8. Toxicology

There is a long traditional use of *Sutherlandia frutescens* with no reports of any side effects **(VAN WYK and ALBRECHT, 2008)**. The only side effects to have been reported are dryness of the mouth, occasional mild diarrhoea, and dizziness in cachectic (wasting syndrome) patients **(MILLS *et al*., 2005)**. **SEIER *et al.* (2002)** conducted the first toxicity study of *S. frutescens* herbal products, where vervet monkeys were given 0, 1, 3 and 9 times the recommended daily dose of 9.0 mg/kg, which was administered for a period of 3 months. There were no side effects found from a detailed evaluation of haematological, clinical-biochemical and other behavioural variables. The toxicity of *S. frutescens* extracts was studied by **OJEWOLE (2004)** where *Balb C* albino mice were intraperitoneally administered with aqueous extracts of *S. frutescens*. The lethal dose (LD_{50}) calculated was 1280 ± 71 mg/kg which led to the conclusion that crude extracts of *Sutherlandia* are probably safe to mammals. The consumption of *S. frutescens* products may interact with anti-retroviral or with insulin or with other diabetic medication **(SIA, 2004; MILLS *et al*., 2005; BROWN *et al.*, 2008)**. The presence of L-canavanine content may be associated with *S. frutescens* toxicities. This non-protein amino acid may, after long term usage, affect the B-cell function resulting in auto-immunity **(MILLS *et al*., 2005)**.

2.9. Commercialization and cultivation status

The first small scale cultivation and commercialization in South Africa started 20 years ago in the Eastern Cape where dried leaves were supplied to local health shops **(VAN WYK and ALBRECHT, 2008)**. Cancer bush is commercially available in various forms, such as capsules, tinctures, creams, tablets and tea bags **(Figure 2.4)**. They can be found in pharmacies and herbal shops. Phyto Nova (Pty) has large scale cultivation of *Sutherlandia* and is the distributor of both the powered encapsulated forms (Novacalm) of this herb, and has attempted to evaluate the purported benefits of this remedy in HIV/AIDS treatment.

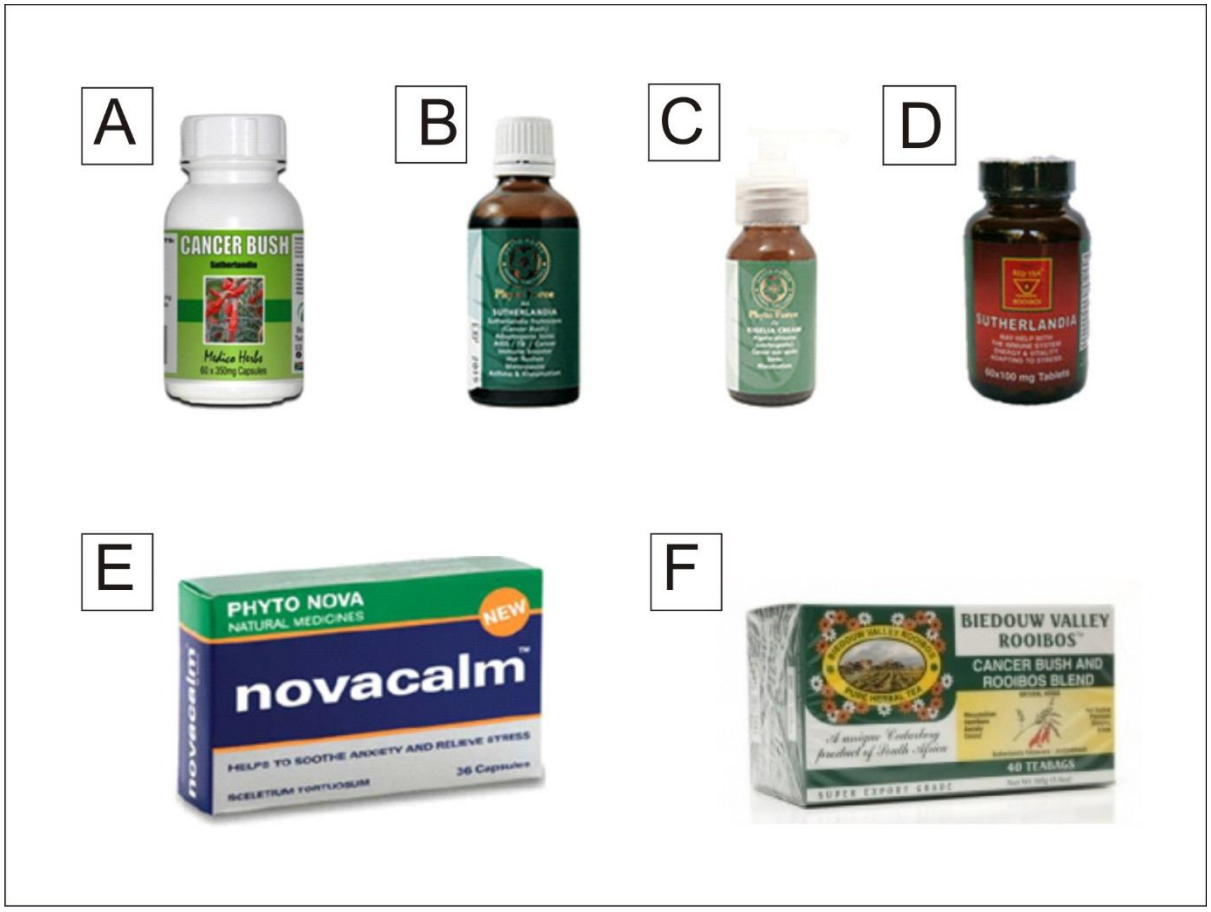

Figure 2.4: *Sutherlandia frutescens* products (A) Capsules (www.medicoherbs.co.za), (B) Tincture (www.africanbotanicals.com), (C) Cream (www.africanbotanicals.com), (D) Tablets (www.organicafricanredtea.com), (E) Novacalm Capsule (www.phytonova.co.za), (F) Teabags (www.medicoherbs.co.za).

CHAPTER 3: Effects of pruning and fertilizer on growth of *Sutherlandia frutescens*

3.1. Introduction

3.1.1. Medicinal plants and cultural practices

Medicinal plants have been considered as resources for a healthy lifestyle, however, these plants have for a long time been neglected in terms of domestication and conservation **(OFORI *et al*., 2012)**. There is growing concern about diminishing populations, loss of genetic diversity, extinctions and habitat degradation of traditional medicinal plants. A more viable long-term alternative is to increase the cultivation of medicinal plants. This offers the opportunity to optimize yield and achieve a uniform, high quality product **(OFORI *et al*., 2012)**. Cultivation methods for a number of medicinal plants have been developed but large scale cultivation of medicinal plants on farm lands is needed. Little is known about the cultivation of *Sutherlandia frutescens*, although the plant can grow in most soil types. To maximize the yield and quality of plants, agronomic practices should be optimized **(OWUOR *et al.,* 2009)**. Fertilizer, pruning, application of growth regulators and harvesting are cultural or agronomic practices that affect yield and quality of plants. Other factors that affect yield and quality parameters are seasonal variation as well as cultivar type **(SUD and BARU, 2000; OWUOR *et al*., 2009)**.

3.1.2. Pruning

Pruning is the selective removal of parts of a plant for several purposes. The technique is beneficial for a variety of plants under natural exposed sunlight or greenhouse conditions **(SARKKA and ERIKSON, 2003)**, as it improves the overall performance of plants. This method is done to control plant growth, manipulate branching, to compensate for transplant injury, to encourage flowering and fruit production and to promote plant health. Non-pruning of plants can result in reduced growth of shoots which may be due to a short-term reduction of cell activity of older shoots, causing a decline in yield **(ZIESLIN and MOR, 1981)**. Pruning should take place during the late spring or early summer where pruning wounds can heal quickly

and reduce the incidence of disease infection **(VAN OOSTEN, 2006)**. Pruning also helps the plant to produce more secondary metabolites **(ASREY *et al*., 2013)**. **MEHDI *et al*. (2006)** reported that all the pigment contents of black tea, except chlorophyll, were higher in pruned tea leaves than unpruned tea, thus enhancing the quality. Intense pruning has been reported to decrease total photosynthesis **(BALANDIER *et al*., 2000)** and carbon fixation **(GYENGE *et al*., 2010)** of trees. In addition, heavy pruning can interrupt the normal translocation of water and nutrients, exacerbating the effects of water stress **(JACKSON *et al*., 2000; GYENGE *et al.,* 2009)**.

3.1.3. Fertilization

Fertilizers play a vital role in the production of certain crops and their application is one of the quickest and easiest ways of increasing yield per unit area. To successfully manage plant nutrition, it is important to understand the relationship between plant nutrient requirements and maximum growth rate **(KELLY and ERICSSON, 2003)**.

Fertilization has been a component of improved cultural practices for most crops under frequent cultivation. Chemical fertilizers can lead to high crop yield but can also lead to ground water pollution after harvest and can affect plant quality. In a good soil fertility management programme, proper timing and good fertilization are necessary to maintain high levels of crop production (i.e. yield) **(HARPER, 1983)**. The major nutrients required for crops include: Nitrogen (N), Phosphorus (P) and Potassium (K). A sufficient supply of these nutrients is known to have a positive impact on the growth and yield of plants **(SOLUBO, 1972; NAFIU *et al*., 2011)**.

a) Nitrogen

Nitrogen (N) is important in yield production and helps foliage to grow. More importantly, plants use nitrogen for photosynthesis. It contributes primarily towards development of vegetative components of the plant **(PAPADOPOULOS, 1998)**. Nitrogen is a major constituent of amino acids and proteins, with about 85% of the plant's N present as proteins and only 5% as nucleic acids **(BENTON-JONES, 2005; BARKER and BRYSON, 2007)**. Excess application of N results in overly vegetative

plants, makes them susceptible to diseases and insect attack, and impairs blossom and fruit development **(BENTON-JONES, 2005)**.

b) Phosphorus

Phosphorus (P) enables a plant to store and transfer energy as a linkage binding site in ADP/ATP **(SANCHEZ, 2007)**. Phosphorus forms an important linkage group in membrane phospholipids, nucleotides and nucleic acids **(SANCHEZ, 2007)**. It promotes root, flower and fruit development and increases disease resistance. It is only required in small quantities for normal plant growth as it can be toxic if applied at high levels **(BENTON-JONES, 2005)**.

c) Potassium

Potassium (K) taken up by the plant as K^+ cation is an essential nutrient for plant growth. Potassium is essential for many metabolic functions. It is involved in many metabolic processes including osmotic control, enzyme activation, carbohydrate production and partitioning, and anion/cation balance **(BALLIU and IBRO, 2002)**. Plants lacking in K^+ show retarded growth, leaf edges become flaccid and chlorotic stripes starting at leaf tips develop on the margins of older leaves **(MENGEL, 2007)**.

There has been an increase in demand for *S. frutescens* for traditional use and as well as by pharmaceutical companies. Therefore, cultivation has been considered as a viable means of decreasing the pressure on natural populations of *S. frutescens.* Commercial cultivation of *S. frutescens* is hindered by lack of agronomic information for the cultivation of the crop. Data that describe the effect of pruning and fertilizer on growth of *S. frutescens* is lacking. Therefore, this study was undertaken to determine the effect of pruning and fertilizers on the growth of *S. frutescens*.

3.2. Materials and methods

3.2.1. Study site

This trial was conducted at the experimental fields of the Agricultural Research Council-Vegetable and Ornamental Plants (ARC-VOP), Roodeplaat, Pretoria, South

Africa (25°59"S, 28°35"E). Prior to planting, soil samples from the site were collected for routine analysis. A summary of the results is presented in **Table 3.1**.

Table 3.1: Soil nutrient composition of the study site at the Agricultural Research Council-VOP, Roodeplaat, Pretoria (25°59"S, 28°35"E).

Soil properties	Values (mg/kg)
Phosphorus	21.05
Potassium	173.5
Calcium	3402.5
Magnesium	783
Sodium	46.3
Nitrogen	0.0275

Soil analysis was done at the ARC-ISCW, Pretoria.

3.2.2. Experimental design and sampling

3.2.2.1. Seedling production

Seeds of *Sutherlandia frutescens* were obtained from the medicinal plant gene bank ARC-VOP, Roodeplaat. The seeds were washed with water and sown in seed trays filled with a commercial growth medium, Hygromix® (Hygrotech, South Africa) in September 2014. They were covered with a thin layer of vermiculite after seeding. They were allowed to grow under a greenhouse facility. Watering was carried out every day with a watering can, depending on the climatic conditions. To reduce loss of water from the transplants, uniform and healthy seedlings (2 months old) were transplanted early in the morning in a spacing of 0.8 m x 1 m in November 2014.

3.2.2.2. Land preparation

The field was ploughed and harrowed. The field was demarcated, lined and pegged one week before transplanting. The area was divided into four blocks. Each block was further divided into nine plots, each plot measuring 5 m x 5 m. A distance of 1 m was left between blocks and plots (6 data plants and 14 border plants).

3.2.2.3. Experimental design and treatments

The experiment was laid out as a randomized complete block design (RCBD) with four replications. The treatments were: 1) three levels of pruning, no pruning (P0), tip-pruning (P1) and heading back (P2); and 2) three fertilizer (NPK; 2:3:2 -Vita Grow) levels F0=0 g/plant (control), F1=25 g/plant, and F2=12.5 g/plant (200, 100 and 0 kg/ha application rates) giving a total of 9 treatments. Fertilizers were applied using the bend placing method. Tip-pruning (P1) was done by cutting back the growing tip of the main shoot. Heading back (P2) was performed by cutting back the terminal portion of branches to the level of a bud. No pruning (P0) involved leaving plants intact with no pruning during the experiment. These treatments were applied in February 2015.

3.2.2.4. Cultural practices

Weeds were controlled in the beds mainly by hand-hoeing. Uprooting of weeds around the plants was occasionally done. Irrigation was done once a week for two hours, using sprinklers.

3.2.3. Data collection

Growth parameters were taken from 5-month-old plants at weekly intervals. All growth parameters were recorded from six replicates. Plant height was recorded from each plot using a measuring tape. The measurements were taken from the soil level to the highest point of the stem apex and the mean was calculated. Chlorophyll content was measured by using a digital chlorophyll meter (Minolta chlorophyll meter

SPAD-502). Stem diameter was measured at 30 cm from the ground, using a digital Vernier calliper (KTV150-Major Tech). LAI was determined using an LAI 2200 plant canopy analyser (Li-cor Bioscience, USA). The instrument uses measurements made from above and below the canopy to calculate the light interception at five zenith angles, from which LAI was computed using a model of radiative transfer in vegetative canopies.

3.2.4. Statistical analysis

Statistical analyses were performed by means of the Statistix 10.0 (Analytical software, USA) programme. Data collected was subjected to 2-way analysis of variance (ANOVA) and treatment mean differences were compared by using the least significant difference (LSD) test at 0.05 as suggested by **PANSE and SUKHATME (1978)**.

3.3. Results and discussion

The influence of all treatments on plant growth was observed throughout the experiment. The results obtained for the field trial on plant height, stem diameter, chlorophyll content and Leaf Area Index (LAI) following different pruning levels and fertilizer levels are presented in **Tables 3.2, 3.3, 3.4** and **3.5**.

Treatments had no significant ($p>0.05$) effect on *S. frutescens* plant height. Although the treatments interactions were not significantly different, pruning by heading back (P2) slightly improved the plant height. Heading back shortens the existing branches to a more desired length and stimulates shoot growth below the cut **(EVANS, 2003)**. It is assumed that because of this, the plant height was enhanced. The results obtained are also supported by those of **WADE and WESTERFIELD (1999)** who report that a pruned plant re-grows to restore a balance between the shoot and root systems.

High levels of fertilizer at 200 kg/ha NPK were not as effective as 100 kg/ha NPK **(Table 3.2)**. According to **OKONWU and MENSAH (2012)** there are species and community specific responses and adaptations that enable plants to cope with specific nutrient limitations. In this case the application of 100 kg/ha NPK fertilizers

could have improved soil conditions along with better uptake of nutrients, which provided better conditions to the crop, resulting in improved plant height. **ZHILIN *et al*. (1997)** reported that plant height is increased significantly due to nitrogen application. This increase might be due to the positive effect of nitrogen on plant growth that leads to progressive increase in internode length and consequently plant height.

Table 3.2: Effect of different levels of pruning and fertilizer on plant height (m) of *Sutherlandia frutescens* cultivated at the Agricultural Research Council-VOP, Roodeplaat, Pretoria.

Treatments	Week 1	Week 2	Week 3	Week 4
Fertilizer levels (kg/ha)				
F0	1.10a	1.03a	1.07a	1.08a
F1	1.14a	1.17a	1.20a	1.21a
F2	1.15a	1.24a	1.26a	1.27a
LSD at $_{0.05}$	0.20	0.26	0.28	0.28
Pruning levels				
P0	1.10a	1.10a	1.14a	1.14a
P1	1.17a	1.14a	1.15a	1.15a
P2	1.13a	1.19a	1.24a	1.26a
LSD at $_{0.05}$	0.20	0.26	0.28	0.28
Interactions				
P0F0	0.90a	0.93a	1.01a	1.00a
P0F1	1.18a	1.23a	1.25a	1.25a
P0F2	1.12a	1.15a	1.16a	1.16a
P1F0	1.22a	0.98a	0.98a	0.97a
P1F1	1.10a	1.21a	1.20a	1.21a
P1F2	1.20a	1.25a	1.26a	1.27a
P2F0	1.20a	1.18a	1.21a	1.26a
P2F1	1.08a	1.24a	1.27a	1.28a
P2F2	1.10a	1.14a	1.24a	1.25a
LSD at $_{0.05}$	0.34	0.46	0.49	0.49

Means having the same letter along the columns indicate no significant difference ($P< 0.05$), P0= No pruning, P1= Tip-pruning, P2= Heading back, F0=0 kg/ha NPK, F1=200 kg/ha NPK and F2=100 kg/ha NPK

The results **(Table 3.3)** indicated that the application of different levels of fertilizer, pruning levels as well as their interaction insignificantly affected the stem thickness. The results showed that growth of pruned plants was not significantly different from unpruned (control) plants, indicating that removal of branches did not affect the photosynthetic capacity of these plants **(GYENGE *et al*., 2010)**. Previous studies on *Pinus ponderosa* **(GYENGE *et al*., 2010)** and *Acacia nilotica* **(SIDDIQUI *et al*., 2010)** indicated that pruning had a negative effect on stem diameter. Pruning might have decreased the resources that would be used for stem growth.

Applications of fertilizer levels did not significantly affect stem diameter. This response may be due to factors in the soil affecting phosphorus availability. The fertility of the soil can be enhanced by application of organic and inorganic fertilizers but use of any fertilizer type depends on several factors such as soil type, crop and socio-economic conditions of the area **(HAMDEN and FADNI, 2010)**. The role of phosphorus is meant to help the plant with establishment and formation of a strong root system. The strong root system absorbs more nutrients including micronutrients from the soil, which contributes towards rapid plant growth **(HARI *et al.,* 2006)**. However, in this case, the availability of phosphorus might have been affected by phosphorus fixation by the type of soil on which the experiment was conducted. The alkaline pH of the soil could also be responsible for the solubility and thus availability of phosphorus. According to **CUBERA and MONERO (2007)**, fertilization can also be detrimental, because it decreases water availability.

Table 3.3: Effect of different levels of pruning and fertilizer on stem diameter (mm) of *Sutherlandia frutescens* cultivated at the Agricultural Research Council-VOP, Roodeplaat, Pretoria.

Treatments	Week 1	Week 2	Week 3	Week 4
Fertilizer levels (kg/ha)				
F0	13.96a	20.24a	21.71a	22.23a
F1	15.10a	23.45a	25.48a	22.67a
F2	14.04a	22.63a	26.87a	27.56a
LSD at $_{0.05}$	2.85	6.18	5.75	5.57
Pruning levels				
P0	13.228a	22.66a	24.68a	26.96a
P1	15.545a	19.96a	23.25a	23.67a
P2	14.348a	23.71a	23.71a	26.96a
LSD at $_{0.05}$	2.85	6.18	5.75	5.57
Interactions				
P0F0	9.96a	17.48a	19.03a	19.04a
P0F1	16.11a	24.44a	26.65a	25.99a
P0F2	13.60a	26.05a	28.08a	29.08a
P1F0	16.92a	19.29a	18.46a	19.50a
P1F1	14.82a	24.11a	26.48a	26.84a
P1F2	14.88a	16.47a	24.82a	25.12a
P2F0	15.01a	23.96a	27.39a	28.24a
P2F1	14.37a	21.79a	23.31a	24.18a
P2F2	13.65a	25.38a	27.71a	28.48a
LSD at $_{0.05}$	4.94	10.71	9.97	9.64

Means having the same letter along the columns indicate no significant difference ($P< 0.05$), P0= No pruning, P1= Tip-pruning, P2= Heading back, F0=0 kg/ha NPK, F1=200 kg/ha NPK/ha and F2=100 kg/ha NPK

Chlorophyll content in the leaves has always been regarded as a measure of the health status of the plant **(CORTAZAR *et al*., 2015)**. **Table 3.4** shows the chlorophyll content in the leaves of *S. frutescens* in response to different pruning and fertilizer treatments. Pruning leads to enhancement of branching and hence a greater number of tender leaves. In this study pruning did not have any significant effect on the chlorophyll content of *S. frutescens.* **DAGIT and DOWNER (2002)** reported that pruning could interrupt the normal translocation of water and nutrients, increasing effects of water stress. It is reported that pruning can also decrease total photosynthesis **(BALANDIER *et al.,* 2000)** and carbon fixation **(GYENGE *et al*., 2010)**. Pruning can also imbalance the hydraulic system at the level of the whole plant.

Studies have shown that the addition of fertilizers can enhance the growth and productivity of crops, as sufficient macro- and micro-elements will be made available to the plant for normal growth **(MUCHERU-MUNA *et al*., 2013)**. The addition of fertilizers did not have a significant difference on the chlorophyll content of *S. frutescens* **(Table 3.4)**. This is probably due to the availability of the necessary macro- and micro-nutrients for chlorophyll synthesis already being sufficient in the soil. **NI *et al*. (2001)** reported that biotic and abiotic factors such as water stress, heat stress, insect feeding and aging of the plant can also cause chlorophyll degradation. A study by **MATHOWA *et al.* (2012)** reported a significant decrease in leaf chlorophyll content, which is related to plant growth due to leaching of essential plant nutrients after irrigation treatments. Treatment interactions did not have a significant overall effect on chlorophyll content of *S. frutescens*.

Table 3.4: Effect of different levels of pruning and fertilizer on chlorophyll content of *Sutherlandia frutescens* cultivated at the Agricultural Research Council-VOP, Roodeplaat, Pretoria.

Treatments	Week 1	Week 2	Week 3	Week 4
Fertilizer levels (kg/ha)				
F0	20.25a	16.0a	21.29a	19.60a
F1	27.78a	18.06a	17.71a	18.21a
F2	28.17a	19.29a	19.96a	18.63a
LSD at $_{0.05}$	7.68	6.14	8.29	6.46
Pruning levels				
P0	23.14a	17.20a	19.46a	20.68a
P1	26.57a	16.22a	19.94a	15.87a
P2	23.14a	20.01a	19.56a	19.89a
LSD at $_{0.05}$	7.68	6.14	8.29	6.46
Interactions				
P0F0	19.28a	15.08a	23.71a	21.13a
P0F1	26.17a	19.22a	19.26a	18.84a
P0F2	23.99a	17.31a	15.43a	22.08a
P1F0	18.80a	14.09a	21.63a	13.75a
P1F1	30.08a	18.54a	19.14a	18.34a
P1F2	30.84a	16.04a	19.07a	15.51a
P2F0	22.68a	19.08a	18.55a	23.91a
P2F1	27.10a	16.42a	14.75a	17.44a
P2F2	29.70a	24.53a	25.39a	18.32a
LSD at $_{0.05}$	13.30	10.64	14.36	11.19

Means having the same letter along the columns indicate no significant difference ($P< 0.05$), P1= No pruning, P1= Tip-removal, P2=head backing, F0=0 kg/ha NPK, F1=200 kg/ha NPK and F2=100 kg/ha NPK

According to **WATSON (1947)** LAI drives both the within and the below canopy microclimate, determines and controls canopy water interception, radiation extinction, water and carbon gas exchange. **Table 3.5** shows the weekly readings (means across treatments) for LAI. There was some significant difference in terms of treatment interactions in week one and week two. P2F1 (heading back and 200 kg/ha NPK interaction) had a significantly higher LAI in week one compared to other treatments, with P0F0 (control) having the lowest. This could be attributed to fertilizer that was dissolved and released nutrients, which were absorbed by the plants and enhanced better shoot growth. Significant increases in the LAI has been reported to be enhanced by increasing nitrogen rates **(VALADABADI and HOSSEIN, 2010)**. **CALATAYUD *et al.* (2008)** reported that pruned plants have a higher capacity to promote the photosynthetic light reaction, a larger number of metabolic sinks and a higher turgor pressure compared with non-pruned plants. In week two P0FI had a higher LAI amongst other treatments.

Since LAI depends on growth in leaf area **(BROWN, 1984)**, the LAI in unpruned plants would, at any time during growth of the plants, be higher than the plants that were pruned. Because of this, pruning did not have any significant difference on the plant LAI.

The application of fertilizers did not have any effect on the leaf area index of *S. frutescens* from week three to week four. These results contradict those of **TAYLOR *et al*. (1993)** and **GASTAL and LEMAIRE (2002)** who reported that nitrogen has an effect on cell division and cell expansion, which can lead to an increase in leaf area. Nitrogen deficiency in plants decreases the growth rate. Again the results are consistent with those of **FREDEEN *et al*. (1989)** who stated that the reduction of leaf expansion, number of leaves and leaf surface area are common symptoms of phosphorus deficiency. **HUBER (1985)** reported that the symptoms of insufficient potassium levels often resulted in reduced leaf expansion leading to reduced leaf size. **BREDA (2003)** also reported that the decrease in the LAI can be affected by frost, storms, defoliation, drought and/or management practices.

Table 3.5: Effect of different levels of pruning and fertilizer on the Leaf Area Index (LAI) of *Sutherlandia frutescens* cultivated at the Agricultural Research Council-VOP, Roodeplaat, Pretoria.

Treatments	Week 1	Week 2	Week 3	Week 4
Fertilizer levels (kg/ha)				
F0	1.10a	0.89b	0.99a	1.00a
F1	1.29a	1.49a	1.39a	1.49a
F2	1.50a	1.04ab	1.05a	1.25a
LSD at $_{0.05}$	0.47	0.51	0.54	0.53
Pruning levels				
P0	1.09a	1.29a	1.23a	1.14a
P1	1.35a	0.97a	0.91a	1.37a
P2	1.46a	1.15a	1.29a	1.23a
LSD at $_{0.05}$	0.47	0.51	0.54	0.53
Interactions				
P0F0	0.70c	1.13a	1.09a	0.95a
P0F1	1.02bc	1.67ab	1.47a	1.34a
P0F2	1.55ab	1.08ab	1.13a	1.13a
P1F0	1.69ab	0.85ab	1.06a	1.05a
P1F1	0.98bc	1.41ab	1.54a	1.49a
P1F2	1.24abc	0.87ab	0.75a	1.47a
P2F0	0.92bc	0.67b	0.84a	0.99a
P2F1	1.87a	1.38ab	1.15a	1.63a
P2F2	1.71ab	1.18ab	1.28a	1.14a
LSD at $_{0.05}$	0.8230	0.89	0.95	0.92

Means having the same letter along the columns indicate no significant difference ($P< 0.05$), P0= No pruning, P1= Tip-pruning, P2=Heading back, F0=0 kg/ha NPK, F1=200 kg/ha NPK and F2=100 kg/ha NPK

3.4. Conclusions

There was no significant interaction effects between pruning and fertilizer levels for all parameters measured in this study. There is no evidence that pruning and NPK had beneficial effects on the growth of *S. frutescens*. This is shown on the data collected on plant height, stem diameter, chlorophyll content and LAI. However, there were some significant differences in terms of treatment interactions for the LAI at week one and week two. For future research, the long-lasting effect of fertilizer and pruning on growth of *S. frutescens* requires follow-up research.

4.1. Introduction

Medicinal plants serve as important cash crops world-wide **(SIMON, 1986)**. Due to the extensive use of medicinal plants and natural compounds used as pharmaceuticals, nutraceuticals and in cosmetics, applied research in the field of plant pests and diseases are important steps in the process of increasing their quantity and quality. For plants to maintain the quality and amount of active ingredients, specific conditions should be considered. Among plant pathogens, nematodes are of high pathogenicity and the damage due to them is estimated at about one hundred million US$ dollars annually **(PARK *et al.,* 2004)**.

The phylum Nematoda (round worms) have been in existence for over a billion years, making them one of the most ancient and diverse types of organisms on earth **(WANG *et al*., 1999)**. Nematodes are multicellular organisms in the group Ecdysozoa and are characterized by their ability to shed their cuticle. Most of them are free-living and feed on bacteria, fungi and protozoans. Others are parasites of animals (invertebrates and vertebrates) and plants **(LAMBERT and BEKAL, 2002)**. Plant parasitic nematodes **(Figure 4.1)** are one of the main factors limiting plant growth **(NICOL, 2002)**. They can feed as ectoparasites or endoparasites **(COYNE and ROSS, 2014)**. They directly limit plant growth by the physiological responses to their feeding which expose their hosts to an array of pathogenic fungi and bacteria **(COYNE *et al*., 2014)**. The root-knot (*Meloidogyne* spp.), cyst (*Heterodera* spp.), reniform (*Rotylenchulus* spp.), and citrus (*Tylenchulus semipenetrans*) nematodes are the most commonly known plant-parasitic nematodes. These nematodes can produce large numbers of eggs, which remain in their bodies or accumulate in masses attached to their bodies.

4.1.1. Nematode biology and identification

Plant-parasitic nematodes are fairly easy to detect using a standard compound microscope. The identification of nematodes requires a detailed morphological analysis, growth of the nematode on different host plants, and or DNA or isozyme analysis **(LAMBERT and BEKAL, 2002)**. The most common morphological features used in the identification of nematodes includes: the mouth cavity, the shape and overlap of the pharyngeal glands with the intestine, size and shape of the nematode body at the adult stage, size of the head, tail, and number and position of ovaries in the female **(MAI *et al*., 1996)**.

Plant-parasitic nematodes come in a variety of shapes and sizes. The typical nematode shape is a long and slender worm-like organism, but often adults are swollen and may not resemble worms **(Figure 4.1)**. Their length ranges from 0.25 mm to >1.0 mm long, with some up to 4.0 mm. Nematodes have a body cavity that is not totally surrounded by mesoderm, so they are pseudocoelomic **(LAMBERT and BEKAL, 2002)**. In females the reproductive organs are characters used for identification because the number of ovaries and the position of the vulva in the female nematode body are easily seen under the light microscope, while male nematodes are easily identified by the presence of spicules **(Figure 4.1)**.

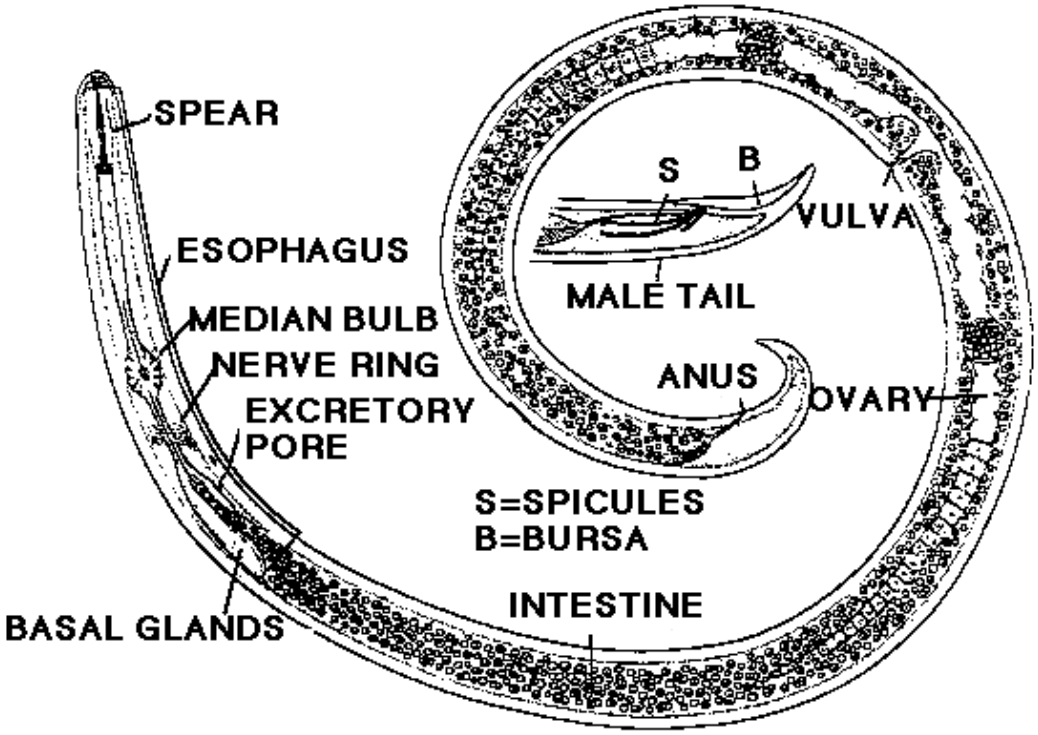

Figure 4.1: Typical plant-parasitic nematode structure **(https://smartsite.ucdavis.edu).**

4.1.2. Distribution

The plant-parasitic nematodes are distributed world-wide, mostly in warm and tropical areas. *Meloidogyne javanica*, together with *Meloidogyne incognita*, are the most common parasites of plants in South Africa, causing greater economic damage than other plant-parasitic nematodes **(KLEYNHANS *et al*., 1996; VAN DER WAL, 1999)**. Nematodes have also been recorded in protected agricultural areas and around many temperate research stations and other areas of intensive horticulture **(YANG *et al*., 1991)**.

4.1.3. Life cycle

The life cycle of plant-parasitic nematodes has 6 stages: eggs, four juvenile stages and the adult **(Figure 4.2)**. Male and female nematodes occur in most species, but reproduction can also occur without males since some species are hermaphrodites **(COYNE *et al*., 2014)**. Egg production by the individual completes the cycle. A range of between 50 and 500 eggs per female are produced, depending on the nematode species and their environment, but some can produce more than 1,000 eggs. The

length of the life cycle varies considerably, depending on nematode species, host plant, and the temperature of the habitat. Most plant nematodes complete their life cycles in about four weeks. In females, the reproductive organs are characters used for identification because the number of ovaries and the position of the vulva in the female nematode body are easily seen under the light microscope, while male nematodes are easily identified by the presence of spicules **(Figure 4.3)**.

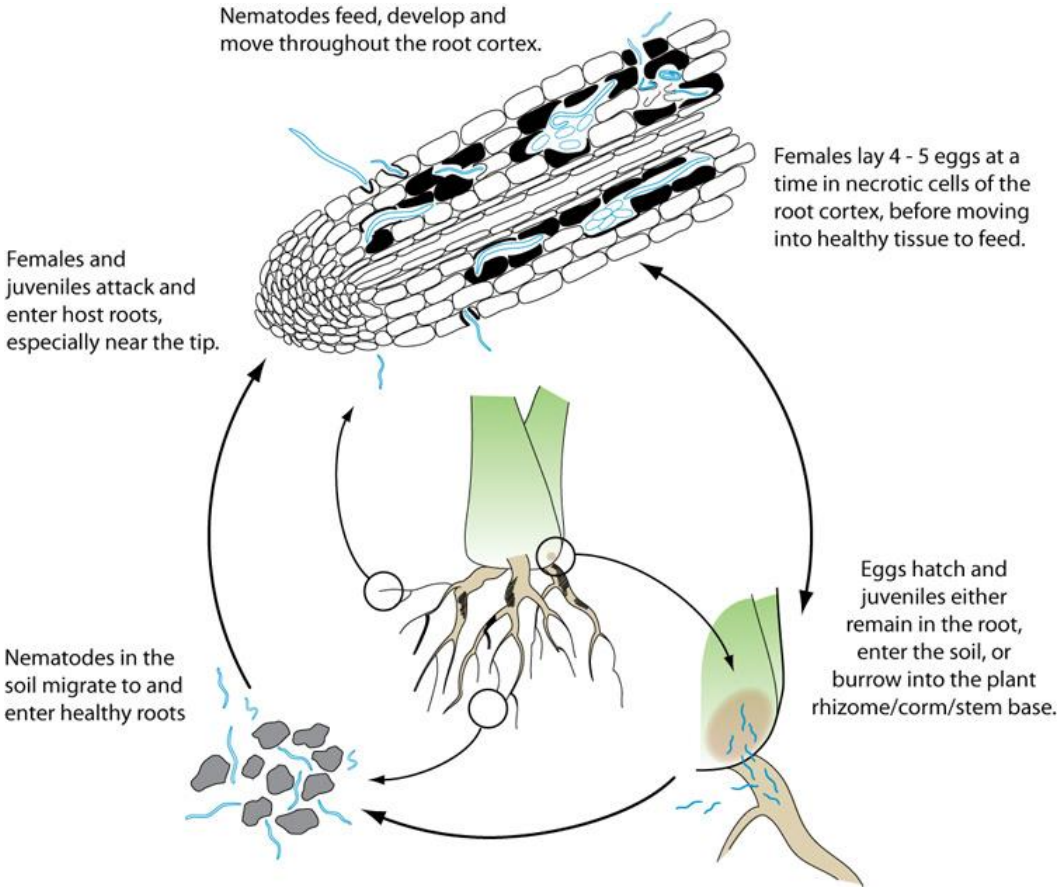

Figure 4.2: Nematode lifecycle **(http://www.apsnet.org).**

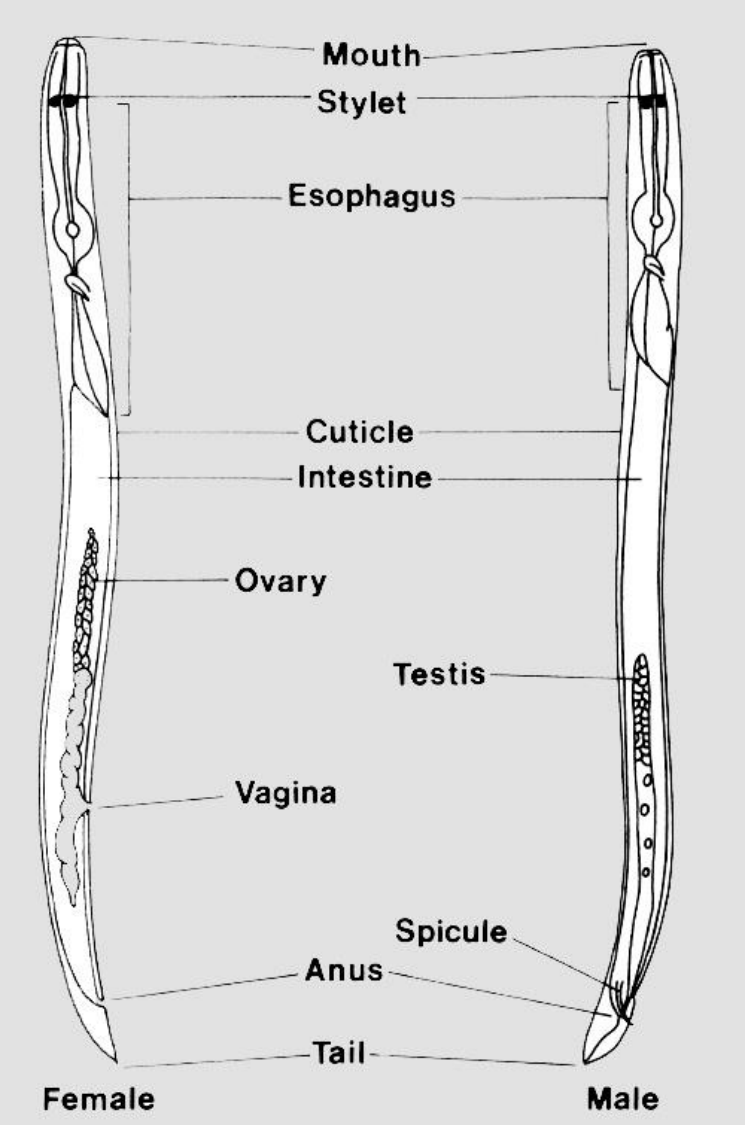

Figure 4.3: Morphological features of male and female plant parasitic nematodes. **(Schumann and D 'Arcy, 2012)**.

4.1.4. Plant nematode-interactions

Nematodes feed on roots, stems, leaves, flowers and seeds. They feed from plants in a variety of ways, but all use a specialized spear called a stylet. The size and shape of the stylet is used to classify nematodes and can also be used to infer their mode of feeding **(LAMBERT and BEKAL, 2002)**. When nematodes feed on plant cells, large lesions are formed in the plant tissue. Some nematodes do not kill the plant cells, they feed upon but "trick" the plant cells to enlarge and grow, and as a result producing one or more nutrient-rich feeding cells for the nematode **(LAMBERT and BEKAL, 2002)**.

Many plant-parasitic nematodes feed on the roots of plants. This feeding process damages the plant root system and reduces the ability of the plant to absorb water and nutrients **(LAMBERT and BEKAL, 2002)**. The damage caused by nematodes in plants also provides an opportunity for other plant pathogens to invade the root and thus further weakens the plant. Nematodes can be grouped by their feeding behaviour and motility into three main groups: migratory endoparasites that feed inside the plant root tissue; sedentary endoparasites, that, once they have reached a feeding site inside the plant, cease to be mobile and feed from a fixed location; and ectoparasites that feed on the plant from the outside **(COYNE *et al*., 2014)**.

4.1.5. Symptoms caused by nematode damage

The major challenges in identifying nematodes as the causal damaging agent of crops is the fact that most nematodes do not produce highly diagnostic symptoms, which are specific and easy to identify **(COYNE *et al*., 2014)**. Symptoms caused by nematodes are often non-specific and easily confused with symptoms caused by other abiotic or biotic stresses. For instance, chlorosis which may be due to nitrogen deficiency, poor soil fertility, moisture stress, or may be due to nematodes **(COYNE *et al*., 2014)**. Symptoms of nematode damage are found both above and below ground. Above ground nematode symptoms include; chlorosis, inflorescence necrosis, stunted growth and wilting or leaf rolling, while below ground symptoms include; galling, stubby roots, root lesions, root or tuber necrosis, rotting or death, root or tuber cracking and deformed roots.

As outlined in **Chapter 3, Section 3.2.2** above-ground symptoms (mentioned above) were noticed in 3-month-old *S. frutescens* plants grown at ARC-VOP, Roodeplaat, Pretoria. Information with regards to association of plant parasitic nematodes with the crop is often not available. Therefore, this study was conducted to determine the presence, distribution, and abundance of plant-parasitic nematodes in *S. frutescens.*

4.2. Materials and methods

4.2.1. Sampling

The field trial for *S. frutescens* was conducted at the Agricultural Research Council-Vegetable and Ornamental Plants Research Station, Roodeplaat, Pretoria (25°59"S 28°35"E) as outlined in **Chapter 3, Section 3.2.2**. During the investigation from January to May 2015, plants were observed with stunted growth **(Figure 4.4)** and severe wilting. Roots of a dead plant, a wilted plant and a healthy plant were collected for nematode examination. In all cases, soil samples from the specific spots where plants were growing were collected. Screening for nematodes was done on a dead plant, a wilted plant and a healthy living plant. Screening for nematodes was also done using soil samples.

Figure 4.4: *Sutherlandia frutescens* exhibiting chlorosis, wilting and stunted growth due to infestation by nematodes at the Agricultural Research Council-VOP, Roodeplaat, Pretoria.

4.2.2. Extraction of nematodes from the roots

Nematodes were extracted from 20 g root material using the maceration and blending method **(HUSSEY and BARKER, 1973)**. The root systems in the samples were gently washed free from soil. Infected roots were cut into small pieces and blended for 30 seconds in 1% sodium hypochlorite (NaOCl) and passed through a nest of sieves of 68 µm and 25 µm pore diameter using a strong stream of tap water. Nematodes from the 25 µm sieve were counted using a light microscope and examined for morphological identification.

4.2.3. Examination of nematodes from the soil

Nematode extraction of soil samples was achieved using the modified sugar-floatation and centrifugation method **(KLEYNHANS, 1997)**. The soil samples were poured into five litres of tap water and stirred to suspend the nematodes from the soil. When the swirl had stopped, an aliquot was quickly passed through a 250 µm, 0.63 µm and 0.25 µm nest of sieves. Nematodes on the bottom sieve were washed into 100 ml centrifuge tubes. A teaspoon of kaolin was then added in each tube to separate nematodes from the soil and centrifuged at 1800 rpm for 5 min. The water was discarded and nematodes allowed to settle at the bottom of the tubes with kaolin and soil particles. A 490 g sugar/L mixed with water was poured into the centrifuge tubes and stirred once prior to centrifuging for 1 min at 1800 rpm to suspend nematodes in the sugar solution. The aliquot was then decanted onto 0.25 µm mesh sieves. The sugar was rinsed off with tap water to free nematodes and the nematodes, which were collected from 0.25 µm mesh sieves, were poured into a 100 ml container for counting under a stereomicroscope and examined for morphological identification.

4.3. Results and discussion

Population counts identified five genera of plant nematode species namely: *Meloidogyne*, *Scutellonema*, *Pratylenchu*s, *Helicotylenchus* and *Tylenchorhynchus* associated with *S. frutescens* **(Table 4.1)**. The data represents the number of nematodes detected.

Table 4.1: Plant-parasitic nematode population found in *Sutherlandia frutescens* roots cultivated at the Agricultural Research Council-VOP, Roodeplaat, Pretoria.

	Dead plant		Wilted plant		Healthy living plant	
	Soil	Roots	Soil	Roots	Soil	Roots
Helicotylenchus spp	15	0	40	0	0	0
Meloidogyne javanica	0	0	65 jj	92 jj,58♀♀, 2800 eggs	0	0
Pratylenchus zeae	40	0	0	17	5	0
Scutellonema brachyrus	870	0	1305	33	485	15
Tylenchorhynchus brevilineatus	20	0	20	0	15	0

Key: jj=second stage juveniles; ♀♀=females

The major above-ground symptoms of nematode damage were stunted growth, wilting and chlorosis (yellowing) **(Figure 4.4)**, while the below-ground symptoms were root rotting, necrosis and small black lesions. The observations on the roots **(Figure 4.5)** revealed the presence of rotting roots similar to damage caused by root-knot nematodes (*Meloidogyne javanica*). Symptoms usually associated with root-knot nematodes were observed on the roots of the wilted plant. To confirm the species identification second stage juveniles (92), females (58) and eggs (2800) were identified using morphological characteristics **(Table 4.1)**. *Pratylenchus* spp. is the second most abundant plant pathogenic nematode after the root-knot nematode. They are distributed throughout the world and feed on a wide range of important crops **(YU *et al*., 2012)**. *Pratylenchus zeae* (lesion nematodes) were found in the soil of dead and wilted plants and in the roots of the wilted plant. As confirmation, small black lesions, which are characteristic of lesion nematodes were observed.

Scutellonema brachyurus (spiral nematodes) were found in large numbers (1305) in the soil where the wilted plant occurred, followed by 870 found in the soil where the dead plant was dug up. *Scutellonema brachyurus* is found in all nine provinces of South Africa and has also been reported numerous times at Roodeplaat **(MARAIS, 2006)**. However, this appears to be the first report of *S. brachyurus* associated with *S. frutescens.*

Helicotylenchus spp. (spiral nematodes) are ectoparasites that feed on the exterior of the root. They were found in low numbers in soils where both dead and wilted plants were obtained. *Tylenchorhynchus brevilineatus* was also detected in small numbers in both dead and wilted plants **(Table 4.1)**. Generally, where low nematode numbers result from a biological cause, the soils are referred to as suppressive **(STIRLING, 1991; ALABOUVETTE, 1993)**. Where they result from abiotic factors, the soils can be referred to as resistant **(AMIR and ALABOUVETTE, 1993)**. The observed variations in nematode densities can be caused by climate and plant host. Nematode densities can be influenced by biological, chemical and physical components of the soil **(UPADHYAY *et al*., 1972; CUC and PROT, 1992)**. Most of the observed nematodes are of limited or unknown pathogenicity **(SIPES *et al*., 2005)**. Of the nematodes observed in this study, *M. javanica* is considered to be economically damaging to most crops.

Figure 4.5: Root samples of *Sutherlandia frutescens* having root-knot nematode symptoms.

The possible reason for high infestation of nematodes on the *S. frutescens* trial site is monoculture. The crop was planted in the same field for three consecutive years and this may have encouraged the build-up of nematodes. *S. frutescens* was susceptible to nematodes and it did not survive or withstand the infestation. Most nematodes were found in the roots of a wilted plant which indicates that *S. frutescens* is a good host **(Table 4.1)**. The conditions for nematode growth were favourable and enhanced nematode reproduction. This was visually observed by the increased number of eggs on the roots. No nematodes were identified in the dead plant, possibly because it could no longer support plant parasites.

4.4. Conclusions

Five genera of plant parasitic nematodes were identified in root and soil samples where the plants were cultivated. A number of ectoparasite and endoparasite parasitic nematodes were found associated with *S. frutescens*. A wilted plant was found to contain a large number of nematodes. Both *S. brachyurus* and *M. javanica* were found in the roots and soils of the wilted plant. Reports on the association of *Meloidogyne* species with *S. frutescens* and their damage has not previously been reported in South Africa. However, it appears that plants were infested by nematode communities already existing in the soil of the field. This is also the first report of *S. brachyurus* associated with *S. frutescens*.

5.1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder caused by a lack of insulin. It is characterized by hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both **(WHO, 1999; DE FRONZO, 2004)**. DM is one of the world's most serious health concerns, developing increasingly with the increase of obesity and advancing age in the general global population **(RENGASAMY *et al*., 2013)**. In 2000 it was approximated that 171 million people in the world have DM and the number is estimated to reach 366 million by 2030 **(WHO, 2004)**. The disease is classified into 3 major categories: Type 1 DM, Type 2 DM and gestational DM.

Diabetes mellitus is very common and a prevalent disease affecting citizens in both developed and developing countries. The global prevalence of diabetes varies from 10.2% in the western pacific to 3.8% in the African region. According to the **AMERICAN DIABETES ASSOCIATION (1997)**, Asia and Africa are regions with the greatest potential increase in DM. In South Africa the number of people with diabetes has risen over the past two decades, with 1.28 million recorded in 2010. This number is expected to reach 1.64 million by 2030 **(SHAW *et al*., 2010)**. Type 2 DM is rapidly emerging as a major public health problem in South Africa **(RHEEDER, 2006)**. The prevalence of diabetes is reported to be higher in men than in women **(WILD *et al*., 2004)**. Population growth, urbanization, increasing obesity and physical inactivity are thought to be the main factors responsible for the increasing prevalence of Type 2 DM **(WILD *et al*., 2004)**. Reports have stressed high mortality from diabetes, especially among black populations **(JOFFE and SEFTEL, 1994; LEVITT, 1996)**.

5.1.1. Type 1 diabetes mellitus

Type 1 DM is insulin dependent. It was previously termed as juvenile-onset diabetes (JOD) due to its occurrence in children and young adults **(PIETROPALO, 2001)**. It is characterized by auto-immune destruction of insulin-producing beta cells which leads to insulin deficiency **(KUKREJA and MACLAREN, 1999)**. The lack of insulin leads to augmented blood and urine glucose. Typical symptoms are polyuria, polydipsia, polyphagia and weight loss. The pathogenesis of Type 1 is summarized in **Figure 5.1.**

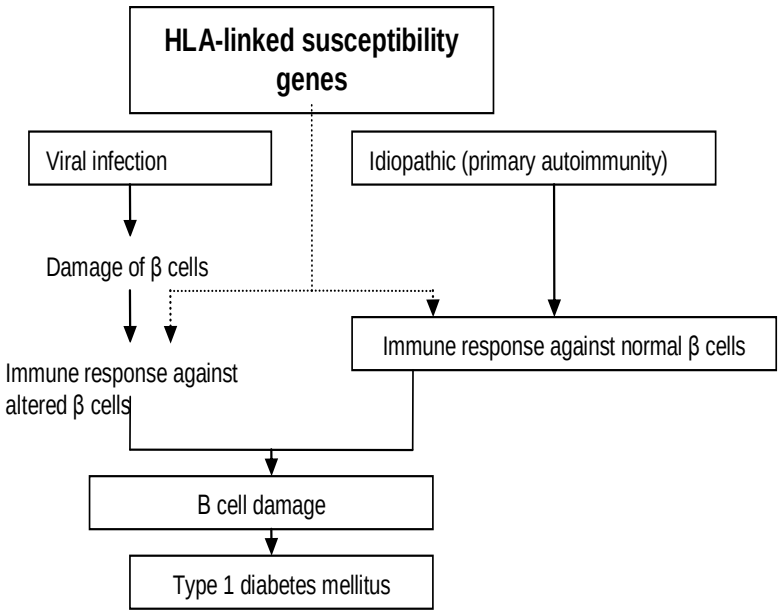

Figure 5.1: Pathogenesis of Type 1 diabetes mellitus **(KUMAR *et al*., 1992)**.

5.1.2. Type 2 diabetes mellitus

Type 2 DM (commonly known as non-insulin DM) is a metabolic disorder that is characterized by excessive hepatic glucose production, decreased insulin secretion from pancreatic beta cells, and insulin resistance in peripheral tissue such as muscle adipose and the liver **(AHMED, 2006; COOKE and PLOTNICK, 2008)**. It was previously known as maturity-onset diabetes. Type 2 DM predominantly affects patients aged 40 years and above and recently, it was shown to occur in adolescent children. It is a polygenic disorder with obesity-related insulin resistance playing a major role in its progression **(MOGALE *et al*., 2011)**. **KUMAR *et al*. (1992)** produced

convincing data to indicate a genetic component associated with insulin resistance (Insulin resistance is a feature of the off-springs of parents with Type 2 diabetes). Factors such as obesity, a sedentary life style, pregnancy and hormone excesses are also causes of insulin resistance. The pathogenesis of insulin resistance and Type 2 DM is summarized in **Figure 5.2**. Deterioration into impaired glucose tolerance occurs where either insulin resistance increases or the insulin secretory responses decrease, or both **(DE FRONZO, 2004)**. It is likely that 10-20% of individuals with Type 2 DM were initially diagnosed with Type 1, or a slowly progressing Type 1, called latent autoimmune diabetes (LADA). Postprandial hyperglycemia plays an important role in the development of Type 2 DM and chronic complications associated with the disease **(WELLS *et al*., 2009)**.

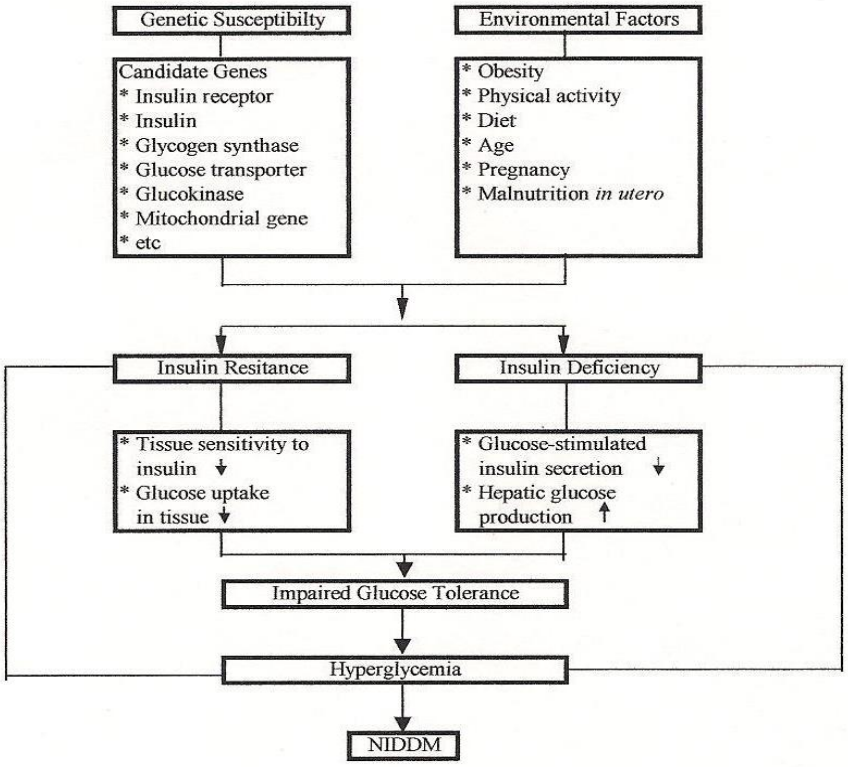

Figure 5.2: Progressive pathogenesis of Type 2 diabetes mellitus **(DE FRONZO, 2004)**.

5.1.3. Gestational diabetes mellitus

Gestational diabetes mellitus is a type of diabetes which develops during pregnancy and may improve or disappear after delivery. About 4% of pregnant women develop DM due to metabolic changes during pregnancy. Although they revert back to normal glycemia after delivery, these women are prone to develop DM later in their lives **(MAYFIELD, 1988)**.

5.1.4. Medicinal plants for diabetes

Medicinal plants have been used since ancient times for treatment and management of DM among different cultures throughout the world **(BAILEY and DAY, 1989; HEINRICH *et al*., 2004; GURIB-FAKIM, 2006)**. They continue to play an important role in the management of DM, especially in developing countries, where many people do not have access to antidiabetic therapies (**GROVER *et al*., 2002; KATERERE and ELOFF, 2005b)**. In developing countries, the use of antidiabetic herbal remedies is reported to have been declining due to the introduction of insulin and synthetic oral hypoglycaemic agents. However, in recent years there has been a renaissance of interest in medicinal plants with hypoglycaemic potential **(HAQ, 2004)**. The renewed interest in herbal antidiabetic remedies in developed countries is believed to have been motivated by several factors, including side effects, high secondary failure rates and the cost of synthetic antidiabetic remedies **(GURIB-FAKIM, 2006)**.

Ethnopharmacological surveys indicated that more than 1200 plants are used in traditional medical systems for their alleged hypoglycaemic activity **(MARLES and FARNSWORTH, 1995)**. The hypoglycaemic activity of plants has been evaluated and confirmed in animal models **(GUPTA *et al*., 2005; KESARI *et al*., 2006)**. However, the mechanism of action whereby most of these plants lower the blood glucose level remain speculative. Very little has been reported on the antidiabetic activity of *S. frutescens*. **OJEWOLE (2004)** reported the hypoglycaemic effects of *S. frutescens* extracts in streptozotocin-induced (STZ) diabetic rats. **CHADWICK *et al.* (2007)** reported similar findings in rats fed with a diabetogenic diet. The extracts demonstrated the ability to normalize insulin levels and glucose uptake in peripheral

tissues and suppress intestinal glucose uptake. There is no information on antidiabetic activities of *S. frutescens* in response to pruning and fertilizer treatments cultivation. Therefore this study was aimed at evaluating different pruning and fertilizer levels on the antidiabetic activity of *S. frutescens*.

5.2. Materials and methods

5.2.1. Plant collection

Sutherlandia frutescens leaves were harvested from plants in all of the treatments. Leaves were taken from similar positions on all plants and they were of the same approximate size and age. They were oven dried at 50 ºC. Dried plant materials were ground into powders using a mill and stored at room temperature.

5.2.2. Extraction

The powdered leaf material (2 g) of *S. frutescens* was extracted in 40 ml of 50% (v/v) methanol in a sonication bath for 1 h at 15 ºC. Temperature was maintained by addition of ice to the water bath. The extracts were filtered under vacuum through Whatman No. 1 filter paper at 30 ºC and dried at room temperature until a constant weight was reached.

5.2.3. Alpha-glucosidase inhibitory activity

Alpha-glucosidase inhibitory activity was determined as previously described by **TAO *et al*. (2013)** with slight modifications using 96-well microtiter plates. Briefly, yeast α-glucosidase (0.1 Unit/ml) was dissolved in 0.1 M potassium buffer (pH 6.8), to serve as the enzyme solution. The substrate, 0.375 mM of *p*-nitrophenyl-*α*-D-gluco-pyranoside (pNPG) was prepared in the same buffer (pH 6.8). The sample extracts were dissolved in dimethylsulfoxide (10 mg/ml) (DMSO) to give final assay concentrations of 0.16, 31, 63 and 125 µg/ml, respectively. Each extract (20 µl) and enzyme solution (20 µl) was mixed in the microtiter plate. The reaction was initiated by adding 40 µl substrate. The reaction mixture was incubated at 37 ºC for 40 min.

After incubation, 80 µl (0.2) of sodium carbonate in 0.1 M potassium phosphate buffer (pH 6.8) was added to each well to quench/stop the reaction. The amount of *p*-nitrophenol (pNP) released was quantified using an Opsys MR 96-well microplate reader at 405 nm. The control experiment contained the same reaction mixture, but the sample was replaced with the same volume of phosphate buffer. Acarbose dissolved in DMSO was used as a positive control. The determinations were carried out in triplicate. The percentage inhibition (%) was calculated by using the following equation.

$$\% \text{ Inhibition} = (A_{control}-A_{extract})/A_{control} \times 100$$

Where $A_{control}$ and $A_{extract}$ are the absorbance values of the control and extract respectively at 405 nm. The IC_{50}, which is the extract concentration required for enzyme inhibitory activity was determined for each extract.

5.2.4. Data analysis

Regression analysis for calculating the IC_{50} was done using GraphPad Prism software (version 4.03). A significant difference between means was determined by least significant difference (LSD) test ($p < 0.05$) using Statistica (Southern African Analytic, RSA).

5.3. Results and discussion

Effective control of postprandial hyperglycemia is important in the early intervention and prevention of Type 2 DM **(RATNER, 2001)**. There is pressure to develop new drugs for Type 2 DM and this has been stimulated by the increase in the incidence of the disease world-wide **(NATHAN, 2007)**. Alpha-glucosidase inhibitors play a major role in the management of hyperglycemia by delaying the postprandial increase of the blood glucose after a mixed carbohydrate diet **(PULS *et al.,* 1997)**. The inhibitory effects of *S. frutescens* at different pruning and fertilizer levels against *α*-glucosidase was evaluated to determine the antidiabetic effects of the plants. The results of *α*-glucosidase inhibitory activity can be seen in **Table 5.1**. All treatments showed stronger activity against *α*-glucosidase than the positive control acarbose, with IC_{50}

values ranging from 22.39 µg/ml to 63.31 µg/ml. The lower the value, the higher the quality of enzymatic inhibition. Treatment interaction P2F2 (heading back and 100 kg/ha NPK interaction) was the most potent at 125 µg/ml concentration with inhibitory activity of 72.75% and IC_{50} of 42.4 µg/ml compared to other treatments **(Table 5.2)**. The highest *α*-glucosidase inhibitory activity was obtained from plants that were not pruned (P0), with an IC_{50} value of 22.39 µg/ml, whereas tip-pruning (P1) exhibited the lowest inhibitory activity.

Table 5.1: Alpha-glucosidase inhibitory activity IC_{50} (µg/ml) of *Sutherlandia frutescens* leaf extracts in response to different pruning and fertilizer treatments.

Treatments	IC_{50} (µg/ml)
P0F0	45.12 ± 1.60^{de}
P0F1	22.39 ± 0.80^{f}
P0F2	39.91 ± 2.43^{e}
P1F0	63.31 ± 1.67^{ab}
P1F1	49.31 ± 1.46^{cde}
P1F2	53.11 ± 3.69^{bcde}
P2F0	56.16 ± 7.03^{bcd}
P2F1	59.4 ± 5.36^{bc}
P2F2	42.4 ± 5.24^{d}
Acarbose	103.4 ± 8.90^{a}

Inhibitory concentrations are expressed as mean ± SEM (n=3). Mean values followed by different letters are significantly different (P<0.05). Treatments: P0=No pruning, P1= Tip-pruning, P2= Heading back, F0= No fertilizer, F1=200 kg/ha NPK and F2=100 kg/ha NPK.

Fertilizer treatment ($P < 0.05$) significantly affected the *α*-glucosidase inhibitory activity of the leaves of *S. frutescens* **(Table 5.1)**. Results showed that the application of fertilizers significantly increased *α*-glucosidase inhibitory activity as compared to the zero fertilizer application (0 kg/ha NPK). It shows that *S. frutescens* inhibits the activity of *α*-glucosidase better than acarbose in a smaller concentration. The observed increased ability of the leaves of *S. frutescens* to exhibit strong inhibitory activity against *α*-glucosidase in response to fertilizer treatment might be related to the enhanced synthesis and accumulation of phytochemicals (alkaloids,

phenols, saponins, steroids, tannins and other plant chemical substances) by this plant as a result of fertilizer treatment. The enhanced production of phytochemicals, vitamins and other plant chemical substances by plants as a result of fertilizer treatment has been reported by **DAS *et al*. (2006)** and **KOLOZIEJ (2007)**.

Screening of *α*-glucosidase inhibitors from plants and synthetic sources are increasing **(GHOLAHOSEINAN *et al.,* 2008)**. Previous studies on *α*-glucosidase inhibitors isolated from other medicinal plants suggest that several potential inhibitors belong to the flavonoid class which has features of *α*-glucosidase activities **(KWON *et al.,* 2007b)**. Flavonoids also have beneficial health effects for human beings and several studies have shown that intake of flavonoids improves health and fights off chronic diseases such as cancer, Type 2 diabetes and heart disease **(PAN *et al*., 2010)**. Inhibition of *α*-glucosidase is a key factor in carbohydrate digestion. It hydrolyzes glycosidic bond in polysaccharide chains to monosaccharides such as glucose. Therefore, acarbose as *α*-glucosidase inhibitor can be used in diabetic therapy **(HAKAMATA *et al*., 2009)**.

Table 5.2: Percentage inhibition of α-glucosidase of *Sutherlandia frutescens* leaf extracts in response to different pruning and fertilizer treatments (mean ± SEM, n = 3)

Treatments	Concentration (µg/ml)	Inhibition (%)
P0F0	0.15.62	35.5±1.50[a]
	31.25	47.03±0.03[b]
	62.5	51.78±0.42[c]
	125	64.6±0.03[d]
P0F1	0.16	49.28±0.64[a]
	31.	50.42±0.21[a]
	63	55.67±0.67[b]
	125	63.89±1.67[c]
P0F2	0.16	35.5±2.50[a]
	31	51.35±0.57[b]
	63	53.53±0.32[b]
	125	64.28±0.85[c]
P1F0	0.16	30±2[a]
	31	49.78±0.71[b]
	63	50.96±0.89[bc]
	125	54.78±0.82[c]
P1F1	0.16	19±1[a]
	31	50.64±0.21[b]
	63	56.39±0.53[c]
	125	63.78±1.78[d]
P1F2	0.16	38.5±1.50[a]
	31	46.07±0[b]
	63	50.82±0.10[c]
	125	58±1.07[d]
P2F0	0.16	32.5±0.5[a]
	31	50.35±0[b]
	63	52.07±1.71[bc]
	125	55.82±2.03[c]
P2F1	0.16	30±2a
	31	50.35±0[b]
	63	53.10±0.89[b]
	125	53.82±1.17[b]
P2F2	0.16	15.5±1.5[a]
	31	54.67±1.75[b]
	63	58.71±6.07[b]
	125	72.75±1.60[c]

Treatments: P0=No pruning, P1= Tip-pruning, P2= Heading back, F0= No fertilizer, F1=200 kg/ha NPK and F2=100 kg/ha NPK.

Polyphenolic compounds in plants have long been recognized to inhibit the activities of digestive enzymes because of their abilities to bind proteins **(GRIFFITHS and MOSELEY, 1980)**. Reports by **KWON *et al*. (2007a)** had shown natural *α*-glucosidase inhibitors from plants to be effective and therefore can potentially be used as an effective therapy for postprandial hyperglycemia with minimal side effects. The present study indicated that *S. frutescens* has potential in management of postprandial hyperglycemia.

5.4. Conclusions

The experiment showed that pruning at different levels has little effect on the activity of cancer bush. This was indicated by the higher *α*-glucosidase inhibitory activity in unpruned plants. In the present study, it was observed that the use of fertilizer at 200 kg/ha NPK resulted in higher *α*-glucosidase inhibitory activity as compared to the use of fertilizer at 100 kg/ha NPK. It was also noted that higher rates of fertilizer enhanced the *α*-glucosidase inhibitory activity. The expected bioactive component could be pinitol as literature indicates a clear link between the compound and the antidiabetic activity of *S. frutescens.* This study also suggests that one of the mechanisms by which this plant displayed the antidiabetic potential is by the inhibition of *α*-glucosidase.

6.1. Introduction

Secondary metabolites are plant products with no known nutritional and photosynthetic functions **(HARTMANN, 1991)**. They can be classified based on their chemical structure, composition, solubility in various solutes and or pathways **(KENNEDY and WIGHTMAN, 2011)**.

Plant secondary metabolites are mainly involved as ultra violet ray protectants, defence against herbivores and pathogens, as well as regulators of seed germination **(MAKKAR *et al*., 2007)**. They serve as attractants for pollinators and seed-dispersing animals. They also function as agents of plant-plant and plant-microbe symbiosis. Secondary metabolites are reported to play a major role in the adaptation of plants to the environment and additionally represent a paramount source of pharmaceuticals **(KENNEDY and WIGHTMAN, 2011)**. Due to their diverse biological activities, secondary metabolites have been utilized as medicinal components for centuries. Most pharmaceuticals and cosmetics are based on secondary metabolites **(BOURGAUD *et al*., 2001)**. Phytochemical compounds are divided into three major groups depending on their structures namely: phenolic compounds, terpenoids and alkaloids **(KENNEDY and WIGHTMAN, 2011)**. Secondary metabolites are viewed as potential sources of new natural drugs, antibiotics, insecticides and herbicides **(CROZIER *et al*., 2006)**.

6.1.1. Phenolic compounds

Phenolic compounds are secondary plant metabolites, which are important determinants in the sensory and nutritional quality of fruits, vegetables and other plants **(TOMAS-BARBERAN *et al*., 2001; LAPORNIK *et al*., 2005)**. Simple phenolics have at least one hydroxyl group attached to an aromatic ring while polyphenolics have two or more hydroxyl groups attached to a matrix of aromatic rings. They possess a wide range of biochemical activities such as antioxidant, antimutagenic and antimicrobial activity **(MARINOVA *et al*., 2005)**. Phenolic

compounds are reported to contribute to quality in food production in terms of modifying colour, taste, aroma and flavour, besides health effects **(NDHLALA *et al*., 2007)**.

Flavonoids are the largest group of phenolic compounds. They can be divided into sub-groups namely: flavones, flavonols, flavanones, isoflavones, flavan-3-ols, and anthocyanins **(KENNEDY and WIGHTMAN, 2011)**. The flavones and flavonols are the most widely distributed of all the flavonoids. Flavonoids occur in different plant parts both in a free state and as glycosides. Studies have reported flavonoids to have many biological activities including antimicrobial, mitochondrial adhesion inhibition, antiulcer and antiarthritic **(SULAIMAN and BALACHANDRAN, 2012)**. Flavonoids are categorically beneficial, acting as antioxidants and providing protection against cardiovascular diseases, certain forms of cancer and age cognate degeneration of cell components **(JOHN *et al*., 2014)**.

Due to the increase in importance of medicinal plants, there are several factors that can be investigated to increase the levels of active ingredients. There is no data documented on the effect of pruning and fertilizer on the chemical composition of *S. frutescens*. This study was aimed to investigate the effect of different pruning and fertilizer levels on total phenolic and total flavonoid contents in *S. frutescens* cultivated at the Agricultural Research Council-VOP, Roodeplaat, Pretoria.

6.2. Materials and methods

6.2.1. Plant collection

The leaves of *Sutherlandia frutescens* were collected from all nine treatments as described in **Chapter 5, Section 5.2.1**.

6.2.2. Chemical analysis

6.2.2.1. Preparation of plant extracts

Phenolic compounds were extracted from plant materials as described by **MAKKAR (1999)** with modifications. Dried plant samples (2 g) were extracted with 10 ml of 50% (v/v) aqueous methanol by sonication in a cold water bath for 20 min. The extracts were filtered through Whatman No.1 filter paper. They were transferred into pill vials and used in the determination of total phenolic and flavonoids.

6.2.2.2. Determination of total phenolics

The total phenolic contents were determined using the Folin-Ciocalteu assay as described by **MAKKAR (1999)** with slight modifications. A standard solution of gallic acid (Sigma-Aldrich, USA) was prepared at 0.1 mg/ml. In triplicate, 50 µl of methanolic plant extract were transferred into test tubes and 950 µl of distilled water added, to make a total of 1 ml, followed by 500 µl of 1 N Folin-Ciocalteu phenol reagent and 2.5 ml of 2% (w/v) sodium carbonate. A blank that contained 50% (v/v) aqueous methanol instead of plant extracts was also prepared. The test mixtures were incubated for 40 min at room temperature and after incubation the absorbance was determined at 725 nm using a UV-Visible spectrophotometer (Varian Cary 50, Australia). Total phenolic content was expressed as mg gallic acid equivalents (GAE).

6.2.2.3. Determination of flavonoids

Total flavonoids were analysed as described by **HAGERMAN (2002)** with modifications. In triplicate, 50 µl of each plant extract was made up to 1 ml with methanol in test tubes before adding methanolic-HCl (9:5 v/v) and 2.5 ml 1% (w/v) vanillin reagent. A blank that contained 50% (v/v) aqueous methanol instead of plant extracts were made. After incubation for 20 min at 30 ^{o}C, absorbance at 500 nm was read using a UV-Visible spectrophotometer. A catechin standard curve was prepared from freshly made 1 mg/ml catechin (Sigma-Aldrich-USA) stock solution in methanol.

The amount of flavonoids in the plant extracts was expressed as catechin equivalents (CTE), derived from the standard curve.

6.2.3. Statistical analysis

The data were analyzed using analysis of variance (ANOVA) and a significant difference between means was determined by a least significant difference (LSD) test ($p < 0.05$) using Statistica (Southern African Analytic, RSA).

6.3. Results and discussion

Phenolic compounds are important bioactive components of medicinal plant extracts that exhibit various pharmacological properties **(VUNDAC *et al*., 2007)**. Their role is to provide mechanical strength, response to stress, and defense against pathogens **(GORSHKOVA *et al*., 2000)**. Due to these pharmacological properties, plant phenolic compounds have gained increasing attention in both modern and traditional medicine as therapeutic compounds. The content of phenolic compounds and other phytochemicals present in medicinal plants is mostly influenced by genetic factors, cultivation, environmental conditions, as well as the degree of maturation and the variety of the plant **(KOLEVA *et al*., 2002)**.

The concentration of total phenolic and flavonoid compounds in each of the evaluated plant treatments are presented in **Table 6.1**. There was variation in the levels of total phenolics between different pruning and fertilizer levels. Total phenolics were significantly higher in heading back pruning and 200 kg/ha NPK (P2F1) interactions, with 9.43 mgGAE/g, while the lowest was observed in control plants (P0F0) with 5.10 mgGAE/g. There was a noticeable difference in the total phenolic content from pruned and control plants (P0F0). It was noted that recovery of total phenolics was significantly higher with heading back pruning (P2) conditions. It is assumed that pruning imparted shock to the plants and therefore increased the production of total phenolics. The results of this study confirmed that the content of chemical defense secondary metabolites may increase as a response to pruning and mimicked the effect of herbivory. The increase in the content of secondary metabolites and their distribution in the leaves after pruning may, therefore, be a

defense strategy. The influences of repeated pruning on increasing the content of secondary metabolites may be part of the plant's defense strategy (**GUTTERMAN and CHAUSER-VOLFSON, 2000a)**. The lower stress levels in unpruned plants might have minimized the production of secondary metabolites as, low levels of total phenolics were recorded in this study. The highest total phenolic content was obtained using 200 kg/ha NPK (F1) which probably was due to the improved physical properties of the soil **(BLAISE, 2006)**.

Table 6.1: Total phenolics and flavonoid concentrations of *Sutherlandia frutescens* leaf extracts in response to different pruning and fertilizer treatments.

Treatments	Total Phenolics	Flavonoids
	mgGAE/g	µgCTE/g
P0F0	5.10 ± 1.02^{b}	1.61 ± 0.02^{e}
P0F1	6.15 ± 0.24^{ab}	1.63 ± 0.01^{d}
P0F2	6.46 ± 0.92^{ab}	1.69 ± 0.02^{bcd}
P1F0	8.51 ± 1.26^{ab}	1.69 ± 0.01^{bcd}
P1F1	9.29 ± 1.46^{a}	1.69 ± 0.02^{bcd}
P1F2	6.99 ± 0.34^{ab}	1.74 ± 0.01^{abc}
P2F0	8.84 ± 1.43^{a}	1.75 ± 0.01^{ab}
P2F1	9.43 ± 1.54^{a}	1.76 ± 0.00^{a}
P2F2	7.25 ± 0.66^{ab}	1.68 ± 0.03^{cd}

Treatments: P0=No pruning P1= Tip removal, P2= Heading back pruning, F0= No fertilizer, F1=200 kg/ha NPK and F2=100 kg/ha NPK; GAE = gallic acid equivalents; CTE = catechin equivalent

A study by **SHAIK *et al*. (2010)** revealed the phenolic content was higher in the leaf extracts (*in vitro* and the leaves) as compared to the seed extract of *S. frutescens*. Shoots and leaves were also reported to have higher phenolic content in comparison to other plant parts **(BERNARDI *et al.*, 2008)**. Reasons for the variations in phenolic quantity in *in vitro* and field-grown leaves are said to be the result of endogenous degradation of some of the phenolic compounds, occurring after air exposure, as well as to be the result of an increase in temperature or light exposure during field sampling **(SANTOS-GOMES *et al.*, 2002)**.

Phytochemical compounds at lower concentrations have significant beneficial effects such as antimicrobial, antioxidant, anti-inflammatory, antiviral, antimutagenic and chemopreventive effects. Higher concentrations of phytochemical compounds have been reported to have negative physiological effects such as neurological dysfunction, gastrointestinal toxicity, and reproductive failure **(POLYA, 2003; MAKKAR *et al*., 2007)**.

The concentrations of flavonoids in the evaluated plant extracts, measured as catechin equivalents, are presented in **Table 6.1**. The highest levels of flavonoids (1.76 µgCTE/g) were found in heading back pruning treatments and 200 kg NPK/ha fertilizer interaction (P2F1). The flavonoid content was lower in control plants (P0F0) (1.61 µgCTE/g). Heading back pruning (P2) may have exerted stress on plants which resulted in the synthesis of more flavonoids. Pruning may have triggered the response mechanism in terms of increased flavonoid production **(HERNANDEZ *et al.*, 2009)**. The amount of active components in plants can be influenced by cultivation practices such as fertilizer levels. The use of fertilizers improve soil fertility and it is a logical step towards increasing production of medicinal plants. Flavonoids significantly increased when using 200 kg/ha NPK (F1) fertilizer application, which implies that physical properties of the soil or soil fertility was improved. The synthesis and accumulation of flavonoids can be influenced by other factors such as genotype (species and variety) and ecological conditions such as locality and harvesting period **(JIANG *et al.*, 2007)**. Previous studies by **HERNANDEZ *et al*. (2009)** and **SHAIK *et al*. (2010)** reported flavonoids in the in *vitro* leaf extract to be significantly higher than in the field leaf extract of *S. frutescens*, which may be attributed to differences in light exposure and to the warm temperatures characteristic of growth chamber conditions of *in vitro* plant cultivation. Flavonoids may be responsible for the protective effect against many disease processes, such as cancer **(WANG and MAZZA, 2002)**, cardiovascular and circulatory diseases **(STOCLET *et al*., 2004)** and diabetes **(ISHIGE *et al*., 2001; ABDILLE *et al*., 2005)**.

In this study *S. frutescens* plant extracts exhibited good *α*-glucosidase inhibitory activities but with low amounts of total phenolics and flavonoids. It could be possible that the antidiabetic activities observed could be from other secondary metabolites apart from tested compounds.

6.4. Conclusions

Plant secondary metabolites act as bulwark mechanisms against herbivores and pathogenic attack. The effects of pruning and the applications of fertilizers at different levels on secondary metabolites were shown to vary greatly. This designates the type of bioactive compounds that could be responsible for the antidiabetic activities. Total phenolics and flavonoid levels were higher in heading back pruning and lowest in unpruned plants. High phenolic concentration in pruned plants could suggest that pruning enhances the plant to produce more secondary metabolites. Total phenolics and flavonoids significantly increased when using 200 kg/ha NPK (F1) fertilizer application. *S. frutescens* showed good antidiabetic activities with low amounts of total phenolics and flavonoids. In view of the antidiabetic activity observed, it is required that there should be an assessment of other groups of phytochemical compounds and evaluating those *in vitro* and in *vivo* models. The results indicate the importance of the continuity of phytochemical and cultivation present, especially in the evaluation of biological activities.

CHAPTER 7: General conclusions

Sutherlandia frutescens has a long history of medicinal use in southern Africa among different cultural groups. It has been widely used for treatment of various ailments including diabetes, stress, fever, internal cancers and more recently HIV/AIDS symptoms. Reports have indicated that its medicinal use originates from the Khoi-San and Cape Dutch who used it as a tonic against stomach ailments, internal cancers, as well as treating wounds. It is reported that *S. frutescens* leaves contain primary and secondary metabolites, amino acids (GABA), a non-protein amino acid (L-canavanine), a cyclitol (pinitol) and several flavonols. The plant has a variety of pharmacological activities due to the presence of these compounds, providing a concrete support for its wide range of medicinal uses.

Pruning and fertilizer levels are known to improve productivity of crops over time and in addition, they have also been found to influence the quality of plants. An experiment was conducted to determine the effect of pruning and fertilizer on growth of *S. frutescens*. Results showed no significant interaction effect amongst all parameters measured. However, treatment interaction P2F2 (heading back and 100 kg/ha NPK interaction) and P0F2 (no pruning and 100 kg/ha interaction) significantly improved the leaf area index at week one and week two, respectively. It can be concluded that more effort should be employed by researchers in determining the optimal quantity of fertilizers and pruning techniques for growing *S. frutescens*.

Investigation for nematodes in *S. frutescens* roots identified five genera of plant parasitic nematodes. A wilted plant had large numbers of these nematodes. The study revealed the presence of root-knot nematodes (*M. javanica*) in large numbers by means of morphological characteristics. Juveniles, eggs and females were isolated and observed. Symptoms usually associated with root-knot nematodes were detected on the roots of the wilted plant and in the soils where the wilted plant was growing. This is the first report of *M. javanica* and *S. brachyurus* being associated with *S. frutescens*. In conclusion, plants were infested by nematode communities already present in the soil of the field. These findings will be helpful to researchers developing control methods and priorities for future studies.

The results presented in this study are the first information on pruning and fertilizer on *α*-glucosidase inhibitory activities of *S. frutescens.* Tested extracts of *S. frutescens* exhibited significant *α*-glucosidase inhibitory activity than when compared with acarbose that was used as a positive control. The plant may essentially contain bioactive compounds inhibiting enzyme activity. The study showed pruning to have little or no effect on the *α*-glucosidase inhibitory activity of *S. frutescens*. There was a significant positive effect of fertilizer on *α*-glucosidase inhibitory activity at the application rate of 200 kg/ha NPK. The investigation into the activity of leaf extracts showed it was affected by fertilizer level but not by pruning. *S. frutescens* has potential for application in diabetes mellitus management. However, further research on the effects of cultivation practices on the bioactivity of *S. frutescens* are required.

Another study was conducted to determine the effects of pruning and organic fertilizer on the chemical content of *S. frutescens*. The results showed heading back pruning (P2) encouraged plants to produce more secondary metabolites, while secondary metabolites remained low in unpruned plants. The fertilizer rate at 200 kg/ha NPK significantly increased secondary metabolites, compared to control plants. This was confirmed by high levels of total phenolics and flavonoids recorded with this treatment. The results showed a range of phytochemical levels among different pruning and fertilizer treatments but continuity on the phytochemical and cultivation studies, especially in the evaluation of biological activities is essential.

More information is needed to decide if pruning and fertilizer affect the growth, medicinal content and quality of the plant. The current findings on growth parameters emphasized the need for an appropriate choice of fertilizer level and an adequate pruning method. At this stage, no recommendations can be made in terms of fertilizer applications and/or the best pruning treatment for growing *S. frutescens,* since the treatments did not result in any statistically significant difference for almost all growth parameters measured. For identification of plant parasitic nematodes, above-ground symptoms (stunted growth, wilting and chlorosis) and below-ground symptoms (galls, root rotting, necrosis and small black lesions) were very useful. This study will be helpful to research scholars and nematologists, and will aid in the investigation for an effective biocontrol agent.

In view of bioactivity, an evaluation of other groups of phytochemical compounds is required as there was no direct relationship between the level of phytochemicals and the antidiabetic activity recorded. Low amounts of phenolic and flavonoid content resulted in higher *α*-glucosidase inhibitory activity. Fertilizer alone seemed to increase the *α*-glucosidase inhibitory activity. The results of this study should therefore stimulate further studies of *S. frutescens* using other forms of cultivation in an attempt to increase plant growth and chemical composition, ensuring the effectiveness and successful commercialization of the species.

ABDILLE, M.H., SINGH, R.P., JAYAPRAKASHA, G.K. and JENA, B.S. 2005. Antioxidant activity of the extracts from *Dillenia indica* fruits. *Food Chemistry* 90: 891-896.

ABDOU, A.M., HIGASHIGUCHI, S., HORIE, K., KIM, M., HATTA, H. and YOKOGOSHI, H. 2006. Relaxation and immunity enhancement effects of γ-aminobutyric acid (GABA) administration in humans. *BioFactors* 26: 201-208.

AFRICA, D.L. and SMITH, C. 2015. *Sutherlandia frutescens* may exacerbate HIV-associated neuroinflammation. *Journal of Negative Results in BioMedicine* 14: 14.

AHMED, I. 2006. Diabetes mellitus, Vol. 4, Division of Endocrinology, Diabetes and Metabolic Disease, Department of Medicine, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107, USA: 237-246.

ALABOUVETTE, C. 1993. Naturally occurring disease-suppressive soils. Pest Management: Biologically Based Technologies. In: Lumsden R.D., Vaughn J.L. (Eds), Conference Proceedings Series, ACS Books, Washington: 204-210.

ALBRECHT, C.F., STANDER, M.A., GROBBELAAR, M.C., COLLING, J., KOSSMANN, J., HILLS, P.N. and MAKUNGA, N.P. 2012. LC-MS-based metabolomics assists with high quality assessment and traceability of wild and cultivated plants of *Sutherlandia frutescens* (Fabaceae). *South African Journal of Botany* 82: 33-45.

AMERICAN DIABETES ASSOCIATION. 1997. Report of the expert committee on the diagnosis and classification of diabetes Mellitus. *Diabetes Care* 20: 1183-1197.

AMIR, H. and ALABOUVETTE, C. 1993. Involvement of soil abiotic factors in the mechanisms of soil suppressiveness to fusarium wilts. *Soil Biology and Biochemistry* 25: 157-164.

ANFOSSI, G., MASSUCCO, L.M., CAVALOT, P.P., GIORI, A., TASSONE, F. and TRAVATI, M. 1999. Modulation of human platelet function by L-canavanine: different effects of low and high concentrations. *General Pharmacology* 32: 321-328.

ASREY, A., PATEL, V.B., BARMAN, K. and PAL, R.K. 2013. Pruning affects fruit yield and postharvest quality in mango (*Mangifera indica* L.) cv. Amrapali. *Fruits* 68: 367-380.

BAILEY, C.J. and DAY, C. 1989. Traditional plant medicine as treatments for diabetes. *Diabetes Care* 12: 553-564.

BALANDIER, P., LACOINTE, A., LE ROUX, X., SINOQUET, H., CRUIZIAT, P. and LE DIZE`S, S. 2000. ‘‘SIMWAL’’: a structure-function model simulating single walnut tree growth according to climate and pruning. *Annals of Forest Science* 57: 571-585.

BALLIU, A. and IBRO, V. 2002. Influence of different levels of potassium fertiliser on growth, yield and ascorbic acid content of tomato fruit grown in non-heated greenhouse. *Acta Horticulturae* 579: 385-388.

BATES, S.H., JONES, R.B. and BAILEY, C.J. 2000. Insulin-like effect of pinitol. *British Journal of Pharmacology* 130: 1944-1948.

BARKER, A.V. and BRYSON, G.M. 2007. Nitrogen. In*:* Barker, A.V., Pilbeam, D.J. (Eds), Handbook of Plant Nutrition. CRC Press, Boca Raton: 21-50.

BENTON-JONES, J. 2005. Hydroponics: A Practical Guide for the Soilless Grower (2nd Ed.). CRC Press, Boca Raton, Florida.

BERNARDI, A.P.M., DE MATOS NUNES, J., MARCHIORO, M.K., ROSA, L.M.G., VON POSER, G.L. and RECH, S.B. 2008. Phenolic compounds profiles during *ex vitro* acclimatization of micro-propagated *Hypericum polyanthemum*. *Plant Physiology and Biochemistry* 46: 694-700.

BESSONG, P.O., ROJAS, L.B., OBI, L.C., TSHISIKHAWE, P.M. and IGUNBOR, E.O. 2006. Further screening of Venda medicinal plants for activity against HIV type 1 reverse transcriptase and integrase. *African Journal of Biotechnology* 5: 526-528.

BLAISE, D. 2006. Yield boll distribution and fibre quality of hybrid cotton *Gossypium hirsutum* (L.) as influenced by organic and modern methods of cultivation. *Journal of Agronomy and Crop Science* 192: 248-256.

BOLARÍN, M.C., SANTA-CRUZ, A., CAYUELA, E. and PÉREZ-ALFOCEA, F. 1995. Short-term solute changes in leaves and roots of cultivated and wild tomato seedlings under salinity. *Journal of Plant Physiology* 147: 463-46.

BOURGAUD, F., GRAVOT, A., MILESI, S. and GONTIER, E. 2001. Production of plant secondary metabolites: a historical perspective. *Plant Sciences* 161: 839-851.

BREDA, N.J.J. 2003. Ground-based measurements of leaf area index: a review of methods, instruments and current controversies. *Journal of Experimental Botany* 54: 2403-2417.

BROWN, L., HEYNEKE, O., BROWN, D., VAN WYK, J.P. and HAMMAN, J.H. 2008. Impact of traditional medicinal plant extracts on antiretroviral drug absorption. *Journal of Ethnopharmacology* 119: 588-592.

BROWN, R.H. 1984. Growth of Green Plants. In: Tesar, M.B. (Ed), Physiological Basis of Crop Growth and Development. ASA. Madison, W.I.: 153-173.

BRÜMMERHOFF, S.W.D. 1969. Some constituents of *Sutherlandia microphylla.* [Sommige inhoudstowwe van *Sutherlandia microphylla*]. DSc Thesis. University of the Free State, Bloemfontein. Cambridge: Cambridge University Press.

CALATAYUD, A., ROCA, D., GORBE, E. and MARTINEZ, P.F. 2008. Physiological effects of pruning in rose plants cv. Grand Gala. *Scientia Horticulturae* 116: 73-79.

CHADWICK, W.A., ROUX, S., VAN DE VENTER, M., LOUW, J. and OELOFSEN, W. 2007. Anti-diabetic effects of *Sutherlandia frutescens* in Wistar rats fed a diabetogenic diet. *Journal of Ethnopharmacology* 109: 121-127.

CHAFFY, N. and STOKES, T. 2002. Aids herbal therapy. *Trends in Plant Science* 7: 57-59.

CHINKWO, K.A. 2005. *Sutherlandia frutescens* extracts can induce apoptosis in cultured carcinoma cells. *Journal of Ethnopharmacology* 98: 63-170.

COCKS, M.L., DOLD, A.P. and GRUNDY, I.M. 2004. The trade in medicinal plants from forests in the Eastern Cape Province. In: Lawes, M.J. (Ed), Indigenous Forests and Woodlands in South Africa: Policy, People and Practice. University of KwaZulu-Natal Press: 473-492.

COOKE, D.W. and PLOTNICK, L. 2008. Type diabetes mellitus in pediatrics *Pediatrics Review* 29: 374-384.

CORTAZAR, B., KOYDEMIR, H.C., TSENG, D., FENG, S. and OZCAN, A. 2015. Quantification of plant chlorophyll content using google glass. *Lab On a Chip*, 15: 1708.

COYNE, D.L., NICOL, J.M. and CLAUDIUS-COLE, B. 2014. Practical Plant Nematology: A Field and Laboratory Guide (2nd Ed.). SP-IPM Secretariat, International Institute of Tropical Agriculture (IITA), Cotonou, Benin.

COYNE, D.L. and ROSS, J.L. 2014. Protocol for Nematode Resistance Screening: Root-Knot Nematodes, *Meloidogyne* spp. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria: 27.

CROOKS, P.A. and ROSENTHAL, G.A. 1994. Use of L-canavanine as a chemotherapeutic agent for the treatment of pancreatic cancer. *United States Patent* 5: 552-440.

CROZIER, A., CLIFFORD, M.N. and ASHIHARA, H. 2006. Plant secondary metabolites: occurrence, structure and role in the human diet. Blackwell Publishing, Tokyo.

CUBERA, E. and MORENO, G. 2007. Effect of single *Quercus ilex* trees upon spatial and seasonal changes in soil water content in dehesas of Central Western Spain. *Annals of Forest Science* 64: 355-364.

CUC, N.T.T. and PROT, J.C. 1992. Effect of changing the agricultural environment on ufra occurrence in the Mekong delta. *International Rice Research Newsletter* 17, 25.

CUNNINGHAM, A.B. 1991. The herbal medicine trade: resource depletion and environmental management for a hidden economy. In: Preston-Whyte, E., Rogerson, C. (Eds), South Africa's informal economy. Oxford University Press, pp. 196-206.

DAGIT, R. and DOWNER, J. 2002. To Prune or Not to Prune: Responses of Coast Live Oaks (*Quercus agrifolia*) to Canopy Retention during Transplanting. USDA Forest Service. http://www.californiaoaks.org/ExtAssets/RosiDagitTransplantingOaks.pdf. Accessed 18 November 2016.

DALVI, S. 2003. *Sutherlandia* for immune support. *Positive Health Magazine* 85: 23-25.

DAS, K., DANGA, R. and SHIVANANDA, T.N. 2006. Effect of major nutrients on biomass production and stevioside content of *Stevia rebaundiana* on pot culture study. *Biomedical* 1: 22-25.

DAVIDSON, T.E. 2006. The effect of *Sutherlandia frutescens* 200CH on CD4 and Symptomatology in persons with the human immunodeficiency virus syndrome. Magister Technologiae Thesis (Homeopathy), University of Johannesburg.

DE FRONZO, R.A. 2004. Pathogenesis of Type 2 diabetes mellitus. *Medical Clinics of North America* 88: 787-835.

DOLD, A.P. and COCKS, M.L. 2002. The trade in medicinal plants in the Eastern Cape, South Africa. *South African Journal of Science* 98: 589-597.

EBADI, M. 2007. Pharmacodynamic basis of herbal medicine. Second edition. Taylor & Francis. London.

EVANS, E. 2003. Pruning Shrubs. Consumer Horticulture NC State University.

FARNSWORTH, N.R. and SOEJARTO, D.D. 1991. Global importance of medicinal plants. In: Akerele, O., Heywood, V., Synge, H. (Eds). *The Conservation of Medicinal Plants*. Cambridge University Press, Cambridge: 25-52.

FERNANDES, A.C., CROMARTY, A.D., ALBRECHT, C. and JANSEN VAN RENSBURG, C.E. 2004. The antioxidant potential of *Sutherlandia frutescens*. *Journal of Ethnopharmacology* 95: 1-5.

FREDEEN, A.L., RAO, I.M. and TERRY, N. 1989. Influence of phosphorus nutrition of growth and carbon partitioning in *Glycine max* (L.). *Plant Physiology* 89: 225-230.

FU, X., LI, X.C., SMILLIE, T.J., CARVALHO, P., MABUSELA, W., SYCE, J., JOHNSON, Q., FOLK, W., AVERY, M.A. and KHAN, I.A. 2008. Cycloartane Glycosides from *Sutherlandia frutescens*. *Journal of Natural Products* 71: 1749-1753.

GABRIELSE, V.S. 1996. Pharmacological studies on rooperol, rooperol derivatives and *Sutherlandia* extracts. MSc Thesis. University of Stellenbosch.

GASTAL, F. and LEMAIRE, G. 2002. N uptake and distribution in crops: an agronomical and ecophysiological perspective. J*ournal of Experimental Botany* 53: 789-799.

GHOLAMHOSEINAN, A., FALLAH, H., SHARIFI-FAR, F. and MIRTAJADDINI, M. 2008. The inhibitory effect of some Iranian plants on the alphaglucosidase. *Iranian Journal of Basic and Medical Sciences* 11: 1-19.

GOLDBLATT, P. and MANNING, J.C. 2000. Cape plants: a conspectus of the Cape Flora of South Africa. *Strelitzia* 9. National Botanical Institute. Missouri: Pretoria & Missouri Botanical Garden Press.

GORSHKOVA, T.A., SALNIKOV, V.V., POGODINA, N.M., CHEMIKOSOVA, S.B., YABLOKOVA, E.V., ULANOV, A.V., AGEEVA, M.V., VAN DAM, J.E.G. and LOZOVAYA, V.V. 2000. Composition and distribution of 121 cell wall phenolic compounds in flax (*Linum usitatissimum* L.) stem issues. *Annals of Botany* 85: 477-486.

GREEN, M.H. 1988. Method of treating viral infections with amino acid analogue. *United States Patent* 5: 110-600.

GRIFFITHS, D.W. and MOSELEY, G. 1980. The effect of diets containing field beans of high or low polyphenolic content on the activity of digestive enzymes in the intestines of rats. *Journal of the Science of Food and Agriculture* 31: 255-259.

GROVER, J.K., YADAV, S. and VATS, V. 2002. Medicinal plants of India with antidiabetic potential. *Journal of Ethnopharmacology* 81: 81-10.

GUPTA, R., GABRIELSEN, B. and FERGUSON, F.M. 2005. Nature's medicines: traditional knowledge and intellectual property management. Case studies from the national institutes of health (NIH). *USA Current Drug Discovery Technologies* 2*:* 203-219.

GURIB-FAKIM, A. 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine* 27: 1-93.

GUTTERMAN, Y. and CHAUSER-VOLFSON, E. 2000. The distribution of the phenolic metabolites *barbaloin*, *aloeresin* and *aloenin*, as a peripheral defence strategy in the succulent leaf parts of *Aloe arborescens*. *Biochemical Systematics and Ecology* 28: 825-838.

GYENGE, J.E., FERNA´NDEZ, M.E. and SCHLICHTER, T.M. 2010. Effect of stand density and pruning on growth of ponderosa pines is in NW Patagonia, Argentina. *Agroforestry Systems* 78: 233-241.

HAGERMAN, A.E. 2002. Tannin Chemistry, Miami University, Miami.

HAMAKATA, W., KURIHARA, M., OKUDA, H., NISHIO, T. and OKU, T. 2009. Design and screening strategies for alpha-glucosidase inhibitors based on enzymological information. *Current Topics in Medicinal Chemistry* 9: 3-12.

HAMDEN, M.I.K. and FADNI, O.E. 2010. Effect of different types of organic fertilizers on growth, quality and yield of tomatoes in sandy soil. *Agriculture Research Corporation*. Second RUFORUM Biennial Meeting 20-24 September, Entebbe, Uganda.

HAQ, I. 2004. Safety of medicinal plants. *Pakistan Journal Medicinal Research* 43: 203-210.

HARI, G.S., RAO, P.V., REDDY, Y.N. and REDDY, M.S. 2006. Effect of organic manures in combination with nitrogenous fertilizer on yield and nutrient uptake in paprika (*Capsium annum* L.) under irrigated conditions of northern Telangana zone of Andhra Pradesh. *Crop Research* 31: 230-233.

http://www.africanbotanicals.com. Accessed September 2014.

http://www.medicoherbs.co.za. Accessed February 2014.

http://www.apsnet.org/edcenter/intropp/lessons/Nematodes/Article%20Images/BurrowingNematodeDiscycle.jpg.

http://www.organicafricanredtea.com/sutherlandia.html. Accessed February 2014.

http://www.phytonova.co.za/data_sutherlandia.html. Accessed February 2014.

http://redlist.sanbi.org/species.php?species=269-79. Accessed September 2014.

https://smartsite.ucdavis.edu/access/content/user/00002950/courses/figures/ppn.GIF

HARPER, F. 1983. Principles of Arable Crop Production. Granada Publishing Limited. pp. 336.

HARTMANN, T. 1991. Alkaloids. In: Herbivores; their interaction with secondary plant metabolites, Vol. 1, The chemical participants (2nd Ed.), Rosenthal, G.A., Berenbaum, M.R. (Eds), Academic Press, San Diego, pp. 33-85.

HARTNETT, S.M., OOSTHUIZEN, V. and VAN DE VENTER, M. 2005. Anti-HIV activities of organic and aqueous extracts of *Sutherlandia frutescens* and *Lobostemon trigonus*. *Journal of Ethnopharmacology* 96: 113-119.

HEINRICH, M., BARNES, J., GIBBONS, S. and WILLIAMSON, E.M. 2004. Fundamentals of pharmacognosy and phytotherapy. Churchill Livingstone, Elsevier Science Ltd., United Kingdom.

HERNÁNDEZ, I., ALEGRE, L., VAN BREUSEGEM, F. and MUNNÉ-BOSCH, S. 2009. How relevant are flavonoids as antioxidants in plants? *Trends in Plant Science* 14: 125-132.

HUBER, S.C. 1985. Role of potassium in photosynthesis and respiration. In: Munson, R.D. (Ed), *Potassium in Agriculture*. ASA, CSSA and SSA, Madison, W.I.: 369-369.

HUSSEY, R.S. and BARKER, K.R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporte*r 57: 1025-1028.

ISHIGE, K., SCHUBERT, D. and SAGARA, Y. 2001. Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. *Free Radical Biology and Medicine* 30: 433-446.

JACKSON, W.P.U. 1990. Origins and meanings of names of South African plant genera. Cape Town: University of Cape Town. Printing Department.

JACKSON, N.A., WALLACE, J.S. and ONG, C.K. 2000. Tree pruning as a means of controlling water use in an agroforestry system in Kenya. *Forest Ecology Management* 126: 133-148.

JÄGER, A.K. and VAN STADEN, J. 2000. The need for the cultivation of medicinal plants in Southern Africa. *Outlook on Agriculture* 29: 283-284.

JIANG, P., BURCZYNSKI, F., CAMPBELL, C., PIERCE, G., AUSTRIA, J.A. and BRIGGS, C.J. 2007. Rutin and flavonoid contents in three buckwheat species *Fagopyrum esculentum, F. tataricum*, and *F. homotropicum* and their protective effects against lipid peroxidation. *Food Research International* 40: 356-364.

JOFFE, B.L. and SEFTEL, H.C. 1994. Diabetes mellitus in the Black communities of Southern Africa. *Journal of Internal Medicine* 235: 137-142.

JOHNSON, Q., SYCE, J., NELL, H., RUDEEN, K. and FOLK, W.R. 2007. A randomized, double-blind, placebo-controlled trial of *Lessertia frutescens* in healthy adults. *PLoS Clinical Trials* 2: 16.

JOHN, B., SULAIMAN, C.T., GEORGE, S. and REDDY V.R.K. 2014. Total phenolics and flavonoids in selected medicinal plants from Kerala. *International Journal of Pharmacy and Pharmaceutical Sciences* 6: 406-408.

JULSING, M.K., WIM, J.Q. and KAYSER, O. 2007. The engineering of medicinal plants: Prospects and limitations of medicinal plant biotechnology. In: Kayser, O., Quax, W.J. (Eds), *Medicinal Plant Biotechnology*: From basic research to industrial applications Volume 2. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, pp 3-8.

KATERERE, D.R. and ELOFF, J.N. 2005a. Antibacterial and antioxidant activity of *Sutherlandia frutescens* (Fabaceae), a reputed anti-HIV/AIDS. *Phytomedicine. Phytotherapy Research* 9: 779-781.

KATERERE, D.R. and ELOFF, J.N. 2005b. Management of diabetes in African traditional medicine. In: Soumyanath, A. (Ed), Traditional Medicines for Modern Times-Antidiabetic Plants. CRC Press, pp. 203-218.

KEE, N.L.A., MNONOPI, N., DAVIDS, H., NAUDÉ, R.J. and FROST, C.L. 2008. Antithrombotic/anticoagulant and anticancer activities of selected medicinal plants from South Africa. *African Journal of Biotechnology* 7: 217-223.

KELLY, J.M. and ERICSSON, T. 2003. Assessing the nutrition of juvenile hybrid poplar using a steady state technique and a mechanistic model. *Forest Ecology and Management* 180: 249-260.

KESARI, A.N., GUPTA, R.K., SINGH, S.K., DIWAKAR, S. and WATAL, G. 2006. Hypoglycemic and antihyperglycemic activity of *Aegle marmelos* seed extract in normal and diabetic rats. *Journal of Ethnopharmacology* 107: 374-379.

KENNEDY, D.O. and WIGHTMAN, E.L. 2011. Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. *Advances in Nutrition* 2: 32-50.

KIKUCHI, T., AKIHISA, T., TOKUDA, H., UKIYA, M., WATANABE, K. and NISHINO, H. 2007. Cancer chemopreventive effects of cycloartane-type and related triperpenoids in *in Vitro* and *in Vivo* Models. *Journal of Natural Products* 70: 918-922.

KLEYNHANS, K.P.N., VAN DEN BERG, E., SWART, A., MARAIS, M. and BUCKLEY, N.H. 1996. Plant nematodes in South Africa. Plant Protection Research Institute Handbook No. 8. Pretoria: Business Print SA.

KLEYNHANS, K.P.N. 1997. Collecting and preserving nematodes. Manual for a SAFRINET course in practical Nematology, SAFRINET, the Southern African (SADC) LOOP of Bio NET-INTERNATIONAL, Pretoria.

KOLEVA, I.I., VAN BEEK, T.A., LINSSEN, J.P.H., DE GROOT, A. and EVSTATIEVA, L.N. 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochemical Analysis* 13: 8-17.

KOLOZIEJ, B. 2007. The effect of NPK fertilization on golden rod (*Solidago virgaurea*, L. subsp *Virgauera*) yield and quality parameters. *Herba Polonica* 53: 129-134.

KUMAR, V., COTRAN, R.S. and ROBBINS, J. 1992. Basic pathology, fifth edition, a division of Harcourt Brace and company, Philadelphia, London: 570-573.

KUKREJA, A. and MACLAREN, N.K. 1999. Autoimmunity and diabetes. *Journal of Clinical Endocrinology and Metabolism* 84: 4371-4378.

KWON, O., ECK, P. and CHEN S. 2007a. Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. *FASEB Journal* 21: 366-377.

KWON, Y-I., APOSTOLIDIS, E. and SHETTY, K. 2007b. Evaluation of pepper (*Capsicum annuum*) for management of diabetes and hypertension. *Journal of Food Biochemistry* 31: 370-385.

LAMBERT, K. and BEKAL, S. 2002. Introduction to plant-parasitic nematodes. *The Plant Health Instructor* 1218-01.

LAPORNIK, B., PROŠEK, M. and WONDRA, A.G. 2005. Comparison of extracts prepared from plant by-products using different solvents and extraction time. *Journal of Food Engineering* 71: 214-222.

LARNER, J. 2002. D-chiro-inositol - its functional role in insulin action and its deficit in insulin resistance. *International Journal of Experimental Diabetes Research* 3: 47-60.

LEISTNER, O.A. 2000. Seed plants of southern Africa: families and genera, *Strelitzia* 10. National Botanical Institute, Pretoria.

LEVITT, N. 1996. Diabetes Mellitus in Black South Africans, *International Journal of Diabetes in Developing Countries* 16: 41-42.

LI, X., ATKINSON, R.N. and KING, S.B. 2001. Preparation and evaluation of new L-canavanine derivatives as nitric oxide synthase inhibitors. *Tetrahedron* 57: 6557-6565.

MAI, W.F., MULLIN, P.G., LYON, H.H. and LOEFFLER, K. 1996. Plant-parasitic nematodes: A pictorial key to genera. Ithaca New York: Cornell University Press.

MAKKAR, H.P.S. 1999. Quantification of tannins in tree foliage: A laboratory manual for the FAO/IAEA Co-ordinated Research Project on 'Use of nuclear and related techniques to develop simple tannin assay for predicting and improving the safety and efficiency of feeding ruminants on the tanniniferous tree foliage. Joint FAO/IAEA *Division of Nuclear Techniques in Food and Agriculture*, Vienna.

MAKKAR, H.P.S., SIDHURAJU, P. and BECKER, K. 2007. Plant secondary metabolites. Humana Press Inc., New Jersey.

MANDER, M. 1998. Marketing of indigenous medicinal plants in South Africa. A case study in KwaZulu-Natal. Rome: *Food and Agricultural Organization of the United Nation.*

MANDER, M. and LE BRETON, G. 2006. Overview of the medicinal plants industry in Southern Africa. In: Diederichs, N., (Eds), Commercialising medicinal plants: A southern African guide. Sun Press. Stellenbosch.

MANDER, M., NTULI, L., DIEDERICHS, N. and MAVUNDLA, K. 2008. Economics of the traditional medicine trade in South Africa. *National Health Review.*

MARAIS, M. 2006. South African Plant-Parasitic Nematode Survey (SAPPNS). *Plant Protection News* 67, 6.

MARINOVA, D., RIBAROVA, F. and ATANASSOVA, M. 2005. Total phenolics and total flavonoids in Bulgarian fruits and vegetables, *Journal of the University of Chemical Technology and Metallurgy* 40: 255-260.

MARLES, R.J. and FARNSWORTH, N.R. 1995. Antidiabetic plants and their active constituents. *Phytomedicine* 2: 137-189.

MATHOWA, T., CHINACHIT, W., YANGYUEN, P. and ISARANGKOOL, N.A., AYUTTHAYA, S. 2012. Changes in turf-grass leaf chlorophyll content and some soil characteristics as influenced by irrigation treatments. *International Journal of Environmental and Rural Development* 3: 181-187.

MAYER, R.R., CHERRY, J.H. and RHODES, D. 1990. Effects of heat shock on amino acid metabolism of cowpea cells. *Plant Physiology* 94: 796-810.

MAYFIELD, J. 1998. Diagnosis and classification of diabetes mellitus; New criteria, Bowen Research Center, Indian University Indianapolis, Indiana.

MEDHI, G.K., BARUAH, A. and MAHANTA, J. 2006. Growth and nutritional status of school age children (6-14 years) of tea garden worker of Assam. *Journal of Human Ecology* 19: 83-85.

MENGEL, K. 2007. Potassium. In*:* Barker, A.V., Pilbeam, D.J. (Eds), Handbook of Plant Nutrition. CRC Press, Boca Raton. pp 91-120.

MILLS, E., COOPER, C., SEELY, D. and KANFER, I. 2005. African herbal medicines in the treatment of HIV: *Hypoxis* and *Sutherlandia*. An overview of evidence and pharmacology. *Nutrition Journal* 4: 19.

MOGALE, M.A., LEBELO, S.L., THOVHOGI, N., DE FREITAS, A.N. and SHAI, L.J. 2011. α-Amylase and α-glucosidase inhibitory effects of *Sclerocarya birrea* [(A. Rich.) Hochst.] subspecies *caffra* (Sond) Kokwaro (Anacardiaceae) stem-bark extracts. *African Journal of Biotechnology* 10: 15033-15039.

MOSHE, D. 1998. A biosystematic study of the genus *Sutherlandia* R.Br (Fabaceae, Galgeae). MSc Thesis (Botany), University of Johannesburg.

MOTEETEE, A. and VAN WYK, B-E. 2007. The concept of '*Musa-pelo* and the medicinal use of shrubby legumes (Fabaceae) in Lesotho. *Bothalia* 37: 75-77.

MUCHERU, M., MUGENDI, D., PYPERS, P., MUGWE, J., KUNG'U, J., VANLAHUWE, B. and MERCKX, R. 2014. Enhancing maize productivity and profitability using organic inputs and mineral fertilizer in central Kenya small-hold farms. *Experimental agriculture* 50: 250-269.

NA, H-K., MOSSANDA, K.S., LEE, J-Y. and SURH, Y-J. 2004. Inhibition of phorbol ester-induced COX-2 expression by some edible African plants. *BioFactors* 2: 149-153.

NAFIU, M.O., AKANJI, M.A. and YAKUBU, M.T. 2011. Phytochemical and mineral constituents of *Cochlospermum planchonii* (Hook. Ef. x Planch) *Root. Bioresearch Bulletin* 5: 342-347.

NARAYANAN, C.R., JOSHI, D.D., MUDJUMIDAR, A.M. and DHEKNE, V.V. 1987. Pinitol, a new anti-diabetic compound from the leaves of *Bougainvillea spectabilis*. *Current Science* 56: 139-141.

NATHAN, D.M. 2007. Finding new treatment for diabetes-how many, how fast...how good. *New England Journal of Medicine* 356: 437-440.

NDHLALA, A.R., KASIYAMHURU, A., MUPURE, C., CHITINDINGU, K., BENHURA, M.A. and MUCHUWETI, M. 2007. Phenolic composition of *Flacourtia indica*, *Opuntia megacantha* and *Sclerocarya birrea*. *Food Chemistry* 103: 82-87.

NDHLALA, A.R., STAFFORD, G.I., FINNIE, J.F. and VAN STADEN, J. 2011. Commercial herbal preparations in KwaZulu-Natal South Africa: the urban face of traditional medicine. *South African Journal of Botany* 77: 830-843.

NI, X., QIUNSBERRY, S.S., MARKWELL, J., HENG-MOS, T., HIGLEY, L., BAXENDALE, F., SARATH, G. and KLUCAS, R. 2001. *In vitro* enzymatic chlorophyll catabolism in wheat elicited by cereal aphid feeding. *Entomologia Experimentalis et Applicata* 101: 159-166.

NICOL, J.M. 2002. Important nematode pests of cereals. In: Curtis, B.C. (Eds), Bread Wheat Improvement and Production, (FAO Plant Production and Protection Series) Rome, 345-366.

OFORI, D.A., DARKO, B.O., GYIMAH, A., ADAM, K.A., JIMOH, S.O. and JAMNADASS, R. 2012. Ethnobotany, propagation and conservation of medicinal plants in Ghana. *Ghana Journal of Forestry* 28: 29-38.

OJEWOLE, J.A.O. 2004. Analgesic, anti-inflammatory and hypoglycaemic effects of *Sutherlandia frutescens* R. Br. (variety Incana E. MEY.) [Fabaceae] shoot aqueous extract. *Methods & Findings in Experimental Clinical Pharmacology* 26: 409-416.

OJEWOLE, J.A.O. 2007. Anticonvulsant property of *Sutherlandia frutescens* R. Br (variety Incana E. MEY.) [Fabaceae] shoot aqueous extract. *Brain Research Bulletin* 75: 126-132.

OKONWU, K. and MENSAH, S.I. 2012. Effects of NPK (15:15:15) Fertilizer on some growth indices of pumpkin. *Asian Journal of Agricultural Research* 6: 137-143.

ORTEGA, A. 2003. A new role for GABA: inhibition of tumor cell migration. *Trends in Pharmacological Science* 24: 151-154.

OSTLUND, R.E. and SHERMAN, W.R. 1996. Pinitol and derivatives thereof for the treatment of metabolic disorders. United States Patent no. 5: 8827-8896.

OWUOR, P.O., KAMAU, D.M. and JONDIKO, E.O. 2009. Responses of clonal tea to location of production and plucking intervals. *Food Chemistry* 115: 290-296.

PAN, M-I., LAI, C-S. and HO, C-T. 2010. Anti-inflammatory activity of natural dietary flavonoids. *Food and Function* 1: 15-31.

PANSE, V.G. and SUKHATME, P.V. 1978. Statistical Methods for Agricultural Workers. I.C.A.R., New Delhi, India.

PAPADOPOULOS, A.P. 1998. Seasonal fertigation schedules for greenhouse tomatoes - concepts and delivery systems. *Acta Horticulturae* 458: 123-124.

PARK, S.D., KIM, J.C. and KHAN, Z. 2004. Host status of medicinal plants for *Meloidogyne hapla. Nematropica* 34: 39-43.

PETTY, F., KRAMER, G.L., FULTON, M., MOELLER, F.G. and RUSH, A.J. 1993. Low plasma GABA is a trait-like marker for bipolar illness. *Neuropsycho Pharmacology* 9: 125-132.

PHILLIPS, E.P. and DYER, R.A. 1934. The genus *Sutherlandia* R. Br. *Revista Sudamericana de Botanica* 1: 69-90.

PIETROPOLO, M. 2001. Pathogenesis of Diabetes: Our current understanding. *Clinical Cornerstone Diabetes* 4: 1-21.

POLYA, G.M. 2003. Biochemical targets of plant bioactive compounds. a pharmacological reference guide to sites of action and biological effects. CRC Press, Florida.

PULS, W., KEUP, U., KRAUSE, H., THOMAS, P.H. and HOLFFMEISTER, F. 1997. Glucosidase inhibition: A new approach to the treatment of diabetes obesity and hyperlipoproteinemia. *Naturwissenschafein* 64: 536-537.

RATNER, R.E. 2001. Controlling postprandial hyperglycemia. *American Journal of Cardiology* 88: 26-31.

RENGASAMY, K.R.R., ADEROGBA, M.A., AMOO, S.O., STIRK, W.A. and VAN STADEN, J. 2013. Potential antiradical and alpha glucosidase inhibitors from *Ecklonia maxima* (Osbeck) Papenfuss. *Food Chemistry* 141: 1412-1415.

REID, K.A., MAES, J., VAN STADEN, J., DE KIMPE, N., MULHOLLAND, D.A. and VERSCHAEVE, L. 2006. Evaluation of the mutagenic and anti-mutagenic effects of South African plants. *Journal of Ethnopharmacology* 106: 44-50.

RHEEDER, P. 2006. Type 2 diabetes: the emerging epidemic. *South African Family Practice* 48: 20.

ROBERTS, M. 1990. Indigenous healing plants. Southern Book Publishers, Halfway House, Gauteng, South Africa.

RIOS, J.L. and RECIO, M.C. 2005. Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology* 100: 80-84.

SANCHEZ, C.A. 2007. Phosphorus. In*:* Barker, A.V., Pilbeam, D.J. (Eds), Handbook of Plant Nutrition. CRC Press, Boca Raton. pp 51-90.

SANTOS-GOMES, P.C., SEABRA, R.M., ANDRADE, P.B. and FERNANDES-FERREIRA, M. 2002. Phenolic antioxidant compounds produced by *in vitro* shoots of sage (*Salvia officinalis* L.). *Plant Science* 162: 981-987.

SARKKA, L. and ERIKSON, C. 2003. Effects of bending and harvesting height combinations on cut rose yield in a dense plantation with high intensity lighting. *Scientia Horticulturae* 98: 433-447.

SCHUMANN, G.L. and D 'ARCY, C.J. 2012. Stories of plant diseases. *The American Phytopathological Society*, St Paul, MN, USA, APS, pp 304.

SHAIK, S., DEWIR, Y.H., SINGH, N. and NICHOLAS, A. 2010. Micropropagation and bioreactor studies of the medicinally important plant *Lessertia (Sutherlandia) frutescens* L. *South African Journal of Botany* 76: 180-186.

SHAW, J.E., SICREE, R.A. and ZIMMET, P.Z. 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice* 87: 4-14.

SIA, C. 2004. Spotlight on Ethnomedicine: Usability of *Sutherlandia frutescens* in the treatment of diabetes. *The Review of Diabetic Studies* 1: 145-149.

SEIER, J.V., MDHLULI, M., DHANSAY, M.A., LOZA, J. and LAUBSCHER, R. 2002. A toxicity study of *Sutherlandia* leaf powder (*Sutherlandia microphylla*) consumption. Medical Research Council of South Africa and National Research Foundation.

SIDDIQUI, T., FARRAKH-NAWAZ, M. and AHMED, I. 2010. Effect of different pruning intensities on the growth of *Acacia nilotica* (Kikar). *Agrociencia* 44: 93-97.

SIMON, J.E. 1986. Developing herbs as cash crops in the United States. In: Simon, J.E., Grant, L. (Eds), Proceeding of the first national herb growing and marketing conference. Purdue University, West Lafayette, Indiana, U.S.A. pp 13-33.

SIMPSON, B.B. and OGORZALY, M.C. 2001. Economic botany, plants in our world. McGraw-Hill, Boston, United States of America. pp 529.

SIPES, B.S., CASWELL, E.P., CHEN-SARAH, J. and APT, W.J. 2005. Nematode parasites of pineapple. In: Luc, M., R.A. Sikora and J. Bridge (Eds.). Plant parasitic nematodes in subtropical and tropical agriculture, 2nd Edition. CAB International, Wallingford, United Kingdom, pp. 709-731.

SOLUBO, R. 1972. Studies on white yam (*Discorea rotundata*) II. Changes in nutrient content with age. *Experimental Agriculture* 8: 107-115.

SPARG, S.G. 2003. Medicinal properties and growth of *Merwilla natalensis*. PhD Thesis, University of Natal, Pietermaritzburg, South Africa.

STANDER, B.A., MARAIS, S., STEYNBERG, T.J., THERON, D., JOUBERT, F., ALBRECHT, C. and JOUBERT, A.M. 2007. Influence of *Sutherlandia frutescens* extracts on cell numbers, morphology and gene expression in MCF-7 cells. *Journal of Ethnopharmacology* 112: 312-318.

STIRLING, G.R. 1991. Biological control of plant parasitic nematodes. CAB International, Wallingford.

STOCLET, J.C., CHATAIGNEAU, T., NDIAYE, M., OAK, M.H., EL BEDOUI, J., CHATAIGNEAU, M. and SCHINI-KERTH, V.B. 2004. Vascular protection by dietary polyphenols. *European Journal of Pharmacology* 500: 299-313.

STREET, R.A. and PRINSLOO, G. 2012. Commercially important medicinal plants of South Africa: A review. *Journal of Chemistry*. Volume 2013, Article ID 205048.

SUD, R.G. and BARU, A. 2000. Seasonal variation in the flavins, thearubigins, total colour and brightness of Kangra orthodox tea (*Camellia sinensis* (L) in Himichal Pradesh. *Journal of the Science of Food and Agriculture* 80: 1291-1299.

SULAIMAN, C.T. and BALACHANDRAN, I. 2012. Total phenolics and total flavonoids in selected Indian medicinal plants. *Indian Journal of Pharmaceutical Sciences* 74: 258-260.

SWAFFAR, D.S. 1995. Combination therapy with 5-fluorouracil and L-canavanine: *in vitro* and *in vivo* studies. *Anticancer Drugs* 6: 586-593.

TAI, J., CHEUNG, S., CHAN, E. and HASMAN, D. 2004. *In vitro* culture studies of *Sutherlandia frutescens* on human tumor cell lines. *Journal of Ethnopharmacology* 93: 9-19.

TAO, T., ZHANG, Y., CHENG, Y. and WANG, Y. 2013. Rapid screening and identification of *α*-glucosidase inhibitors from mulberry leaves using enzyme-immobilized magnetic beads coupled with HPLC/MS and NMR. *Biomedical Chromatography* 27: 148-155.

TOBWALA, S., FAN, W., HINES, C.J., FOLKAND, W.R. and ERCAL, N. 2014. Antioxidant potential of *Sutherlandia frutescens* and its protective effects against oxidative stress in various cell cultures. *Complementary and Alternative Medicine* 14: 271.

TOMAS-BARBERAN, F.A., MARTOS, I., FERRERES, F., RADOVIC, B.S. and ANKLAM, E. 2001. HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *Journal of the Science of Food and Agriculture* 81: 485-496.

TAYLOR, G., MACDONALD, A.J.S., STADERNBERG, I. and FREE-SMITH, P.H. 1993. Nitrate supply and the biophysics of leaf growth in *Salix viminalis. Journal of Experimental Botany* 44: 155-164.

UPADHYAY, R.S., OOSTENBRINK, M. and KHAN, A.M. 1972. The effect of different soil type on the density of nematode populations. *Indian Journal of Nematology* 2: 42-53.

VALADABADI, S.A. and HOSSEIN, A.F. 2010. Effects of planting density and pattern on physiological growth indices in maize (*Zea mays* L.) under nitrogenous fertilizer application. *Journal of Agricultural Extension and Rural Development* 2: 40-47.

VAN DER WAL, A.F. 1999. Vegetables and their nematodes. Integrated nematode management and damage Control. University of Natal, Pietermaritzburg, 250-284.

VAN OOSTEN, C. 2006. Hybrid poplar crop manual for the prairie provinces. Sask. For. Cent. Technical sheet series #2006/10. (Available online with updates at http://www.saskforestcentre.ca/uploaded/HybridPoplarManual.pdf). Accessed 8th January 2016.

VAN WYK, B-E. 2008. Learning and an African lifeworld in (higher) education. *Indilinga: African Journal of Indigenous Knowledge Systems* 7: 171-182.

VAN WYK, B-E. and GERICKE, N. 2000. People's plants. A guide to useful plants of southern Africa. Briza Publications, Pretoria.

VAN WYK, B-E. and ALBRECHT, C. 2008. A review of the taxonomy ethnobotany, chemistry and pharmacology of *Sutherlandia frutescens* (Fabaceae). *Journal of Ethnopharmacology* 119: 620-629.

VAN WYK, B-E. and WINK, M. 2004. Medicinal plants of the world. Briza Publications, Pretoria.

VAN WYK, B-E., VAN OUTSHOORN, B. and GERICKE, N. 1997. Medicinal plants of South Africa. Briza Publications, Pretoria.

VALADABADI, A. and ALIABADI FARAHANI, H. 2010. Effects of planting density and pattern on physiological growth indices in maize (*Zea mays* L.) under nitrogenous fertilizer application. *Journal of Agricultural Extension and Rural Development* 2: 040-047.

VILJOEN, P.T. 1969. The oxidation of pinitol and partial identification of a triterpene glycoside for *Sutherlandia microphylla*. MSc Thesis. University of the Free State, Bloemfontein.

VUNDAC, V.B., BRANTNER, A.H. and PLAZIBAT, M. 2007. Content of polyphenolic constituent and antioxidant activity of some stachys taxa. *Food Chemistry* 104: 1277-1281.

WADE, G.L. and WESTERFIELD, R.R. 1999. Basic principles of pruning woody plants. The University of Georgia, Cooperative Extension. Learning for Life. Bulletin: 949.

WANG, J. and MAZZA, G. 2002. Effects of anthocyanins and other phenolic compounds on the production of tumor necrosis factor alpha in LPS/IFN-gamma-activated RAW 264.7 macrophages. *Journal of Agricultural and Food Chemistry* 50: 4183-4189.

WANG, D.Y-C., KUMAR, S. and HEDGES, B.S. 1999. Divergence time estimates for the early history of animal phyla and the origin of plants, animals and fungi. Proceedings of the Royal Society of London B 266: 163-171.

WATSON, D.J.1947. Comparative physiological studies in the growth of field crops, variation in net assimilation rate and leaf area between species and varieties, and within and between years. *Annals of Botany* 11: 41-76.

WELLS, B.G., DIPIRO, J.T., SCHWINGHAMMER, T.L. and DIPIRO, C.V. 2009. Pharmacotherapy handbook. 7th (Ed), McGraw-Hill. New York.

WILD, S., ROGLIC, G. and GREEN, A. 2004. Global prevalence of diabetes, Estimate for the year 2000 and projections for 2030, *Diabetes Care* 27: 1047-1053.

WORLD HEALTH ORGANISATION. 1999. Definition, diagnosis and classification of diabetes Mellitus and its complication, department of non-communicable diseases sarveillane Geneve, Part 1 Diagnosis and Classification of Diabetes Mellitus.

WORLD HEALTH ORGANISATION. 2003. WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants.

WORLD HEALTH ORGANISATION. 2002. Traditional medicine strategy. 2002-2005. World Health Organisation, Geneva.

WORLD HEALTH ORGANISATION. 2004. Launch of “Diabetes Action Now”. WHO publications.

XABA, P. 2007. The cancer bush, a timeless remedy. *Journal of the Botanical Society of South Africa* 93: 234-236.

XABA, P.M. and NOTTEN, A. 2003. *Sutherlandia frutescens* (L.) R. Br. South African National Biodiversity Institute.

YANG, B., WANG, Q., HU, K., YU, S., YIN, Y., FENG, R. and WU, Q. 1991. A survey of root-knot nematodes (*Meloidogyne* spp.) in China. Afro Asian Journal of Nematology 11: 91-93.

YU, Y., LIU, H., ZHU, A., ZHANG, G., ZENG, L. and XUE, S. 2012. A review of root lesion nematode: identification and plant resistance. *Advances in Microbiology* 2: 411-416.

ZHANG, H., YAO, H.Y. and CHEN, F. 2006. Accumulation of gamma-aminobutyric acid in Rice germ using Protease. *Journal of Bioscience, Biotechnology and Biochemistry* 70: 1160-1165.

ZIESLIN, N. and MOR, Y. 1981. Plant management of greenhouse roses. The pruning. *Scientia Horticulturae* 14: 285-293.

ZHILIN, L., SARKER, R.S, NAYAK, S.K., RAVI, I. and LI, J.L. 1997. Physiological effect of nitrogen application on aromatic rice. *Journal of South China Agricultural University* 18: 13-17.