BIOACTIVE COMPOUNDS FROM SELECTED MEDICINAL PLANTS USED IN ANTIDIABETIC TREATMENT

by

NASIPI ZAMANALA MNGENI

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Supervisor: Dr F Wewers
Co-supervisor: Dr MG Matsabisa

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DECLARATION

I, **Nasipi Zamanala Mngeni**, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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ABSTRACT

The continued use and popularity of plant-based traditional medicine demands scientific validation of the therapeutic potential of the medicinal plants used in disease management and treatment. These medicinal plants are to be evaluated for phytochemical constituents and pharmacologically screened for their bioactivity and include the isolation and identification of their bioactive compounds. The diabetes tea and its eight individual plants constituents were collected from Sing Fefur Herbs in McGregor, Western Cape. The plant material was ground to a fine powder form using a milling machine. The powdered plant material was sequentially extracted with hexane, 1:1 DCM, DCM:MeOH, MeOH and water.

The antioxidant activity of the tea and its plants was evaluated with comparison to the antioxidant activity of brewed rooibos tea in literature. The concentration of antioxidants in the plants and the tea were found to be significantly high. The ORAC assay results of the water extracts were significantly higher than that of rooibos tea in all plants. *Salvia africana-caerulea* water extract ORAC results were 14147.10±1.02 µmol TE/g and this is 10 times better than the brewed rooibos tea results of 1402±44.1 µmol TE/g. The alpha-amylase enzyme inhibition assay showed no significant results while the alpha-glucosidase enzyme inhibition assays showed significant results in some of the extracts. The highest inhibitory activity towards alpha-glucosidase was found in the *Urtica urens* hexane extract and the *Thymus vulgaris* hexane extract (69.66% and 68.43%, respectively). This observation suggests that alpha-glucosidase enzyme is inhibited mostly by the less polar or medium polarity chemical components of the plant extracts.

The crude plant extracts that showed significant activity in the antidiabetic bioassays were further subjected to cytotoxicity assay to ascertain the safety of extracts. The *T. vulgaris* DCM extract, *Salvia officinalis* DCM extract and *Salvia officinalis* hexane extract showed a cell growth inhibition of 54.91%, 62.14% and 63.87% at 100 µg/ml, respectively. The *Salvia africana-caerulea* DCM extract showed a cell growth inhibition of 59.10% at 50 µg/ml and 62.14% at 100 µg/ml. In the cytotoxicity analysis *Salvia africana-caerulea* DCM extract is the only extract that showed cell viability below 50% for both concentrations. Phytochemical screening of selected methanolic and aqueous extracts of the diabetes tea and the *Salvia africana-caerulea* showed the presence of alkaloids, sugars, flavonoids, glycosides, proteins & amino acids, phenolics & tannins and saponins.

Furthermore isolation, purification and analysis of two *Salvia africana-caerulea* crude extracts (DCM and DCM:MeOH) were done in order to try and obtain pure compounds. The

compound characterization was done through the use of chromatographic techniques. Thin layer chromatography (TLC), flash chromatography and column chromatography resulted in the generation of 29 fractions. Spectroscopic techniques utilized for chemical structural elucidation for compounds of interest included Liquid chromatography mass spectrometry and Nuclear Magnetic Resonance Spectroscopy. Of all the fractions generated, **DM 23** was the purest and its structural elucidation was attempted.

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ABBREVIATIONS

^{1}H NMR	-	Hydrogen Nuclear Magnetic Resonance Spectroscopy
^{13}C NMR	-	Carbon Nuclear Magnetic Resonance Spectroscopy
1D NMR	-	1-Dimensional Nuclear Magnetic Resonance Spectroscopy
2D NMR	-	2-Dimensional Nuclear Magnetic Resonance Spectroscopy
ABTS	-	2, 2'- Azino-bis(3ethylbenothia zoline-6-sulfonic acid)
APTS	-	3-aminopropyltriethoxysilane
CI	-	Colour Interference
$CDCl_3$	-	Diuterated chloroform
$CHCl_3$	-	Chloroform
COSY	-	Correlation Spectroscopy
DCM	-	Dichloromethane
DMSO	-	Dimethyl sulfoxide
DNSA	-	3,5-Dinitrosalicylic acid
EDTA	-	Ethylenediaminetetraacetic acid
EtOAc	-	Ethyl acetate
FRAP	-	Ferric Reducing Atioxidant Power
FTIR	-	Fourie- Transform Infrared Spectroscopy
H_2SO_4	-	Sulphuric acid
Hex	-	Hexane
HRMS	-	High Resolution Mass Spectroscopy
HMBC	-	Heteronuclear Multiple Bond Correlation Spectroscopy
HSQC	-	Heteronuclear Single Quantum Coherence Spectroscopy
HPLC	-	High Performance Liquid Chromatography
LC-MS	-	Liquid Chromatography- Mass Spectroscopy
LRMS	-	Low Resolution Mass Spectroscopy
MeOH	-	Methanol
MS	-	Mass Spectroscopy
m/z	-	Mass to charge ratio
MTT	-	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium Bromide
ND	-	Not Detected
ORAC	-	Oxygen Radical Absorbance Capacity
Rf	-	Retention Factor
TLC	-	Thin Layer Chromatography
UV	-	Ultraviolet
v/v	-	volume to volume

CHAPTER ONE

1.1 Introduction

This research is part of a broader research of the Indigenous Knowledge System Lead Programme, Department of Pharmacology of University of Free State (UFS) and the Cape Peninsula University of Technology (CPUT) for the integration of African Traditional Medicines (ATM) into the Chemistry and Pharmacology research with the aim of finding new drug chemical leads based on medicinal plants used in the traditional management and treatment of diabetes. This part of the research was based on the extraction of eight plants that form a diabetes tea currently being used for the treatment and management of diabetes mellitus in South Africa. The diabetes tea is manufactured by Sing Fefur organic herbs. Their farm is situated in a small town called McGregor in the Western Cape. The diabetes tea is used by the local people in McGregor and is also currently available in some pharmacies and selected green grocer stores in the Western Cape, Eastern Cape and Kwa-Zulu Natal.

The diabetes tea is prepared from a mixture of eight plants and it is taken to treat and/or manage diabetes. The main aim of this study was to determine the antidiabetic active compounds found in the tea. This was done by extracting the tea in totality as well as the individual plants to determine the plant(s) exhibiting the most significant antidiabetic activity. The extracts were tested *in vitro* diabetes assays for anti-diabetic activity. The plant extracts that exhibited the most significant activity were analyzed and the active compounds were identified.

1.2 Background

There is currently no scientific data available on this tea despite it being sold and made freely available to people for the treatment and management of diabetes. There is no scientific basis for the efficacy and safety of the tea including its chemical composition to justify its widely accepted antidiabetic claims. Diabetes is a serious and life-threatening chronic disease condition. This study aims to provide scientific evidence whether that the tea has health benefits and an impact on the treatment and management of diabetes mellitus. It will further identify the most active compounds within the tea and perhaps contribute to the development of new antidiabetic drug discovery.

The World Health Organization (WHO) estimates that 80% of the world population relies on traditional medicine for primary healthcare. Consequently WHO acknowledges the

importance and potential of plant-based medicine and considers the practice as one of the surest means of achieving total health care coverage of the world's populations. There is however a major drawback of traditional medicine which is the lack of authentication or scientific proof of its efficacy and safety. Medicinal plants research therefore can be said to originate from the need to validate and improve traditional medicine as well as to discover new potential drugs and drug leads (Eddouks, 2012).

1.3 Problem statement

There is an increase in the number of people with diabetes in the world due to factors such as lifestyle and other pressures. Most people do not know that they have diabetes until it's too late. Currently there are a few anti-diabetic medications and there is an increase in resistance to the available ones. It has also been found that there is an increase in the number of people who are turning to the use of traditional medicines for the treatment and management of diabetes. According to WHO there is an urgent need to find new alternative cures or treatments that are affordable, safe and easily available for diabetes, and traditional medicine is providing great leads (WHO 2000). This tea is one of the traditional medicines being used to manage and treat diabetes in some parts of South Africa. Therefore there is a need to investigate its efficacy and safety towards the treatment and management of diabetes. This will be conducted through the *in vitro* bioassays and the isolation and identification of chemical compounds present in the tea.

1.4 Research objectives

1.4.1 To extract the tea and the eight constituent plants (*Trigonella foenum-graecum*, *Achillea millefolium*, *Agothosma betulina*, *Salvia officinalis*, *Salvia Africana-caerulea*, *Taraxacum officinale*, *Thymus vulgaris* and *Urtica urens*)with solvents of different polarity and to screen the resultant crude extracts for classes of secondary plant metabolites using phytochemical screening tests.

1.4.2 To investigate the inhibitory activity of the crude extracts against selected diabetes related carbohydrate metabolizing enzymes (alpha amylase, alpha glucosidase and antioxidants) using *in vitro* enzyme inhibition bioassays.

1.4.3 To isolate and qualitatively identify the classes of secondary plant metabolites present in the active extract(s) by means of standard phytochemical screening tests, fractionation and chromatographic techniques.

1.4.4 To isolate and chemically characterize the selected active compound(s) using ` chromatographic and spectroscopic techniques.

1.5.1 Research questions

1.5.1 What classes of secondary plant metabolites are present in the tea?

1.5.2 Does the tea (or its constituent plants) exhibit antioxidant activity?

1.5.3 Which plant extracts possess inhibitory against selected metabolizing enzymes?

1.5.4 Does the tea (or its constituent plants) have cell inhibition activity against the cytotoxicity assay?

1.5.5 Can the active compound(s) in the tea (or its constituent plants) be isolated and chemically characterized?

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Throughout their existence human beings have relied on nature for their basic needs for the production of food-stuff, shelters, clothing, means of transport, fertilizers, flavours and fragrances, and, not the least medicine (Gurib-Fakim, 2006). Plants have been used as medicine for the cure of a wide range of diseases long before recorded history. Medicinal plants are believed to be as old as mankind; and have been used for healing for centuries (Phillipson, 2001; Petrovska, 2012; Al-Snafi, 2016). Plants constitute the basis of sophisticated traditional medicine systems that have been known for thousands of years and continue to provide mankind with new remedies (Gurib-Fakim, 2006). Plants have been an essential resource for the treatment of diseases from minor ailments to the more major and acute diseases like cancer, malaria, diabetes and even HIV/AIDS.

2.2 South African perspective

Studies project that about 80% of the people in Southern Africa use traditional medicine. These traditional medicines are often used in conjunction with conventional medications therefore raising the possibility of synergistic or antagonistic drug interactions. This claim has been further verified by the South African Department of Health which has also estimated that 80% of South Africans consult traditional healers before consulting modern medical practitioners (South Africa, Department of Health, 2007). It is therefore undoubtedly important that the safety, efficacy, and quality of traditional medicines be evaluated. This will assist in new drug development and alternative medicine (Maduna, 2006; Eddouks et al., 2002). Though modern medicine has great advantages, traditional medicine has evolved greatly over the years and its recognition has provided a wider choice of healthcare that is in line with people's needs and also advancement scientifically (Pefile, 2005; Matomela, 2004).

South African traditional health practitioners are highly respected members of their communities and play a vital role in the healthcare of a large percentage of South Africans. Traditional health practitioners are considered to be an important national health resource. They share the same cultural beliefs and values as their patients. As a result traditional remedies have emerged as an integral part of health care in South Africa and many other

African countries. Currently in South Africa, traditional healers have no formal recognition as health care professionals. Unlike other countries such as China and India, South Africa has limited information regarding the scientifically validated safety and efficacy of traditional medicines.

South Africa is believed to be greatly blessed with a vast diversity of medicinal herbs and plants. It has an estimation of over 30 000 species of higher plants. The Cape floral kingdom has the most abundance of these species with nearly 9000 species and it is the most diverse temperate flora on earth, resembling the tropical rainforest in terms of species richness. South Africa has a remarkable biodiversity and cultural diversity and it is not surprising that approximately 3000 species of plants are used as medicines, and of these, some 350 species are the most commonly used and traded medicinal plants (van Wyk et al., 2009). Medicinal plants are an important characteristic of the daily lives of various people and a significant part of South African cultural heritage. This rich heritage of traditional remedies is still in use today. Many people within southern Africa use these remedies daily, for illnesses ranging from fatigue to diabetes and HIV/AIDS. Herbal medicines have been used for thousands of years in many different cultures. The high cost of modern medication has also contributed to an increased use of herbal medicines. This has stimulated research into various uses and applications of herbal medicines (Maduna, 2006).

Plants are used as a primary source of all the medicines in the world and they continue to provide mankind with new remedies. Natural products and their derivatives represent more than 50% of all drugs in clinical use in the world. Higher plants contribute no less than 25% to the total of all drugs in clinical use (Fabricant & Farnsworth, 2001). New anti-cancer drugs such as taxol and vincristine have been developed from the following plant species: *Taxus* and *Catharanthusroseus,* respectively. In South Africa, a large part of the day-to-day medicine is still derived from plants and large volumes of plants or their extracts are sold in the informal and commercial sectors of the economy. The South African medicinal plants have contributed to the world medicine through the following plants: Cape aloes (*Aloe ferox*), buchu (*Agathosmabetulina*) and devil's claw (*Harpagophytumprocumbens*), but local equivalents exist for many of the famously used plants around the world. There is a growing interest in natural and traditional medicines as a source of new commercial products. Medicinal plants are something of the future, not of the past (van Wyk et al., 2009).

The rich cultural diversity of the people of southern Africa is reflected in the formal and informal systems of medicines that are presently practised in different parts of the country. The informal oral medical systems of the Khoi-San people, the Nguni and the Sotho-

speaking people have not been systematised, and are passed on by word of mouth from generation to generation. These medical systems and their herbal, animal and mineral *material medica* have ancient origins which may date back to Palaeolithic time. The formal systems of medicine, which are well documented and systematised, were made known over the last three hundred years by European and other settlers, and are exemplified by today's modern Western medicine, also referred to as Western biomedicine or allopathic medicine (van Wyk et al., 2009).

Each and every system of medicine is based on the art and science of establishing the cause of the disease, treating the disease, and maintaining health in the broadest sense of physical, spiritual, social and psychological well-being. Different cultures have found solutions to the preventative, promotive and curative aspects of health that resonate with the world view of that culture. Western medicines are believed to diagnose a disease in terms of the symptoms without looking at the root cause of the disease and looks at illness as caused by germs. But an African traditional health practitioner will seek understanding why the patient became ill in the first place, and the treatment administered will address the perceived cause; usually in addition to specific therapies the signs and symptoms of the condition (van Wyk et al., 2009).

Little is however known about ancient African traditional medicine systems, in particular, the use of herbal medicine by ancient African people because African tradition systems of medicines are poorly recorded and remain so to date (Okigbo & Mmeka, 2006). African traditional systems of medicine were once thought to be primitive and they were wrongly challenged by foreign religions dating back during the colonial rule in Africa and subsequently by the conventional or orthodox medical practitioners (Okigbo & Mmeka, 2006). Despite suppression by colonial rulers and the negative attitudes of orthodox medical practitioners, traditional healers and herbalists continued to use herbal remedies (Okigbo & Mmeka, 2006). Through consultations with African traditional practitioners, and subsequent scientific testing has led to the establishment that African medicinal plants such as *Acacia senegal* (Gum Arabic), *Agathosma betulina* (buchu), *Aloe ferox* (Cape Aloes), *Aloe vera* (North African Origin), *Artemisia afra* (African wormwood*), Aspalanthus linearis* (Rooibos tea), *Catha edulis* (Khat), *Commiphora myrrha* (Myrrh), *Harpagophytumpro cumbens* (Devils Claw), *Hypoxis hemerocallidea* (African potato) and *Catharanthus roseus* (Rosy Periwinkle) indeed have medicinal properties (van Wyk et al., 1997; Okigbo & Mmeka, 2006).

2.3 Diabetes mellitus

The World Health Organization (2000) defines diabetes mellitus as a metabolic disorder that is characterized by chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. According to Jarald et al. (2008) diabetes can also be defined as a disease where the body either produces little insulin/ceases to produce insulin, or the body becomes progressively resistant to its action.

Diabetes mellitus is a horrible disease that exists in all parts of the world and it is becoming a severe threat to people's wellbeing and among all types of diabetes, type 2 remains the main complication (Jarad et al., 2008; Patel et al., 2012).

2.4 Types of Diabetes Mellitus

2.4.1 Type 1 diabetes mellitus

This type of diabetes is also referred to as the insulin dependent mellitus or juvenile-onset diabetes. It is more prevalent amongst young people and is hereditary (Pietropaolo, 2001; Patel et al., 2012). It results from a complete deficiency of insulin, frequently triggered by chronic autoimmune disease (Kukreja and Maclaren, 1999) that is a consequence of a complex interaction of both genetic environmental factors. It is prevalent in 10% of diabetic patients, islet β-cells destruction usually leads to absolute insulin deficiency. This means the patients becomes entirely reliant on exogenous insulin to prevent ketosis and thereby preserve life (Jarald et al., 2008).

2.4.2 Type 2 diabetes mellitus

This diabetes is universally known as the non-insulin dependent diabetes mellitus. It is likely to occur in adult patients aged 40 years and above. It's a polygenic disorder with obesity related insulin resistance playing a major role in its onset and progression. It is characterized by excessive hepatic glucose production, decreased insulin secretion from pancreatic β-cells, and insulin resistance in peripheral tissue such as muscle adipose and liver. This type of diabetes accounts for more than 85% of cases worldwide. It is a heterogeneous type, ranging from insulin resistance to insulin deficiency. Type 2 diabetes is a multifactorial disease with both a genetic component and an important non-genetic component(s) (Jarald et al., 2008; Afolayan et al., 2010; Hoosseyni et al., 2012).

2.4.3 Gestational diabetes

This type of diabetes is diagnosed in pregnancy, including pre-existing diabetes and diabetes which develops during pregnancy (Jerald et al., 2008).

2.5 Prevalence of diabetes mellitus

The number of people with diabetes is escalating due to population growth, aging, urbanization, and increasing pervasiveness of obesity and physical inactivity. Quantifying the prevalence of diabetes and the number of people affected by diabetes, now and in the future, is therefore important so as to allow sensible planning and allocation of resources.

Many countries face large increases in the number of people suffering from diabetes. The World Health Organization estimated that about 30 million people suffered from diabetes in 1985 and the number increased to more than 171 million in 2000. The 5th edition of the IDF atlas showed that in 2011 there were 366 million people suffering from diabetes worldwide. The conservative South African estimate is that 6.5% of adults aged 20-79 years have diabetes, but age-adjusted prevalence of up to 13% has been described in urban populations as far as 1994. The effects of urbanisation and unhealthy lifestyles are major contributors to the increasing prevalence of diabetes and obesity. The Demographic and Health survey in 2003 demonstrated that 30% of South Africans are obese or overweight (SEMDSA, 2012).

Type 2 diabetes is a major public health problem and it accounts for more than 90% of all diabetes cases. The insidious and initially asymptotic nature of this disease results in patients not seeking early medical care. About 30-85% of type 2 diabetes cases remain undiagnosed and at the time of diagnosis approximately 10% of patients already have complication of the disease. Globally, it is estimated that 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population. This reflects an increase in associated risk factors such as being overweight or obese. Over the past decade, diabetes prevalence has risen faster in low- and middle-income countries than in high-income countries (WHO, 2016).

2.6 Treatment of Diabetes Mellitus

There is no cure for diabetes; therefore one has to maintain good health which needs a lifelong commitment to blood glucose control. The self-care necessities of diabetes or any

chronic disease can be both psychologically and financially hard to adjust to and many patients resist the necessity to follow a regular, day-in and day-out care plan. The consequence of this is that people seek alternative treatments that seem easier or more natural that allow them to avoid dealing with the realities of the chronic disease. Herbal medicines are acquiring great popularity in the treatment of diabetes, with a variety of plant-derived preparations being endorsed as capable of controlling blood sugar levels. Plants contain natural antioxidants (tannins, flavonoids, vitamins C and E, etc.) that can preserve β-cell function and therefore prevent diabetes (Aslan et al., 2010; Nasri et al., 2015; Baharvand-Ahmid et al., 2016).

The current available treatments for diabetes have several adverse effects; therefore there is a need for the development of safe and effective treatment modalities for this disease. Although a vast number of synthetic drugs were developed for the treatment of diabetes mellitus; the safety and efficacy treatment paradigm is yet to be achieved (Patel et al., 2012).

2.7 Current drugs used in the treatment of diabetes mellitus

The pathogenesis of diabetes mellitus and its management by the oral administration of hypoglycemic agents have stimulated great interest in recent years. Control over hyperglycemia can be potentially achieved by different mechanisms: (1) an increase in insulin secretion; (2) a decrease in nutrient ingestion; (3) an increase in peripheral glucose uptake and (4) a decrease in hepatic glucose production. Currently there are various types of oral antidiabetic treatment agents available for diabetes mellitus. These include the sulfonylureas, meglitinides and meglitinide analogues, biguanides, alpha-glucosidase inhibitors, and thiazolidinediones, which may be used individually as monotherapy or in combination to achieve glycemic control. Various insulin preparations also exist and are suitable for patients in whom oral antidiabetic agents do not provide satisfactory glycemic control (Adam et al., 2012; Bedekar et al., 2010).

2.7.1 Sulfonylureas

The sulfonylurea (such as glyburide, glipizide, and glimepiride) are the most frequently prescribed agents in this class. They lower blood glucose levels by stimulating insulin secretion when they bind to receptors on pancreatic β-cells and initiate the closing of adenosine triphosphate sensitive channels (Gerich, 2001, Luna, 2001).

2.7.2 Meglitinides and Meglitinide Analogues

The meglitinides (such as repaglinide, meglitinide analogues, and nateglinide) lower blood glucose levels by stimulating the secretion of insulin from pancreatic beta cells. This is done by the closure of the same potassium channels affected by sulfonylureas. The differences in their binding characteristics compared with the sulfonylureas is that they produce a more rapid onset of action and shorter duration of action (Gerich, 2001; Luna, 2001).

2.7.3 Biguanides

The biguanides, (such as metformin) utilize their antihyperglycemic action primarily by lowering hepatic glucose production. These can also improve the effectiveness of insulin-stimulated glucose uptake by peripheral tissues through a reduction in glucose toxicity (Gerich, 2001; Luna, 2001).

2.7.4 Alpha-Glucosidase Inhibitors

The alpha-glucosidase inhibitors (such as acarbose and miglitol) work by reversibly inhibiting the enzymes that hydrolyse polysaccharides to glucose in the intestinal brush border. The resultant delay in carbohydrate breakdown and glucose absorption reduces postprandial hyperglycemia (Gerich, 2001; Luna, 2001).

2.7.5 Thiazolidinediones

The thiazolidinediones, such as rosiglitazone and pioglitazone, work by increasing insulin sensitivity in hepatic, adipose, and muscle tissue. Thiazolidinediones are peroxisome proliferator-activated receptor agonists that increase transcription of insulin-responsive genes at target tissue sites. The resultant increase in gene products has been associated with an increase in insulin-mediated peripheral glucose uptake, a decrease in endogenous glucose production, and a lowering of plasma free fatty acids (Gerich, 2001; Luna, 2001).

2.7.6 Insulin

The use of insulin is typically reserved for diabetes patients in whom therapies such as dietary modification, exercise, and oral antidiabetic agents have failed to maintain glycemic control (Gerich, 2001).

2.8 Medicinal plants and herbs for diabetes

Although there are many drugs available to control and to treat diabetic patients, total recovery from diabetes has not been reported as yet. Plants provide a potential source of hypoglycemic drugs and are widely used in several traditional systems of medicine to prevent diabetes. Therefore plants are used as an alternative to the synthetic drugs currently used to treat diabetes. A large number of medicinal plants have been studied for their beneficial use in different types of diabetes. The effects of these plants either delay the developments of diabetic complications and/or correct the metabolic abnormalities using a variety of mechanisms. A considerable number of plants were subjected to clinical trials and were found effective. Moreover, during the past few years many phytoconstituents responsible for antidiabetic effects have been isolated from hypoglycemic plants (Jarald et al., 2008; Kavishankar et al., 2011).

Jarald et al. (2008) further states that more than 800 plants are described to have antidiabetic properties. Ethno-pharmacological investigations show that more than 1200 plants are utilized in traditional medicine for their assumed hypoglycemic activity. History has disclosed that the knowledge of the system of diabetes mellitus existed with the Indians since prehistoric age and that the earliest reference (1000 BC in the Ayurvedic literature) is found in mythological form where it is said to have originated by eating Havisha, a special food, which used to be offered at times of yagna organized by Dakshaprajapati. Ayurvedic antidiabetic herbs had been reported to improve digestive power, increase one of the gastric secretions, gets easily digested in the body and decreases the output of overall body fluids such as sweat, urine etc.

Plant-based products have been popular all over the world for centuries. In diabetes, some herbal alternatives are proven to provide symptomic relief and assist in the prevention of secondary complications of the disease while others have been proven to help in the regeneration of β-cells and in overcoming resistance. Some herbs not only maintain normal blood sugar level but also have been reported to possess antioxidant activity and cholesterol-lowering action.

The management of type 2 diabetes mellitus requires a drug that will be able to lower the blood sugar level and restore the liver glycogen level simultaneously. The current modern medicine sphere has no report or record of any drug that possesses such activity or properties. The hypoglycemic effect of certain herbal extracts have however been confirmed in human and animal models of type 2 diabetes to possess the above-mentioned activity.

Conventional drugs have been derived from the active molecules of these medicinal plants. Metformin which is a less toxic biguanides and less potent oral glucose-lowering agent, was developed from *Galega officianalis* and it is currently used for the treatment of diabetes. There are lots of oral medications for diabetes, but only one medication (metformin) is approved for use in children and it has been originated from herbs (Jarald et al., 2008, Patel et al., 2012).

Momordicacharantia and *Gymnemasylvestre* have been extensively tested and have been reported to show effective hypoglycemic activity in both humans and animal studies (Tanira, 1994; Grover et al., 2004, Neeraj & Madhu, 1989).

According to Khan et al. (2003) cinammon has been reported to improve glucose and lipids of people with type 2 diabetes. The following medicinal plants are reported to be most effective and are commonly used in relation to diabetes and its complications: *Gentianaolivieri, Bauhinia forficata, Eugenia jambolana, Lectucaindica, Mucunapruriens, Tinosporacordifolia, Momordicacharantia, Aporosalindleyana, Myrtuscommunis*and *Terminiliapallida*. *Momordica charantia, Pterocarpus marsupium* and *Trigonella foenum graecum.* The plants have been reported to be beneficial in the treatment of type 2 diabetes (Patel et al., 2012; Bnouham et al., 2006; Chang et al., 2013; Eddouks, 2002; Joseph & Jini, 2013).

2.9 Secondary plant metabolites

Plants continuously synthesize natural products that are often called phytochemicals. These phytochemicals can be divided into two, namely the primary and secondary metabolites. These are structurally diverse compounds and are classified into these groups according to the basis of their role in the plant (Gurib-Fakim, 2006). The abundant macromolecules of primary metabolism are present in all plants and are directly involved in plant development and growth. The examples of primary plant metabolites are carbohydrates, amino acids, proteins, nucleotides and lipids (Croteau et al., 2000). The secondary metabolites are said to be responsible for the pharmacological and medicinal activities of medicinal plants and have no direct involvement in the development and growth of the plant but may play a significant role in protecting the plant from disease. The secondary metabolites with medicinal properties are found only in a few species of plants. In previous years secondary metabolites were regarded as by-products of primary metabolism and were considered as non-essential components. Research has shown evidence that some of these secondary metabolites function as defensive compounds against herbivores and pathogens while others serve in

the mechanical support, in attracting pollinators and fruit dispersers, in absorbing harmful ultraviolet radiation, or reducing the growth of nearby competing plants (Prachersky & Gang, 2000; van Wyk & Wink, 2004; Croteau, 2000). There are secondary plant metabolites with reported medicinal properties. These include, but are not limited to polysaccharides, waxes and fatty acids, alkaloids, terpenoids, phenolics (simple phenolics and flavonoids) and glycosides and their derivatives (Marles & Farnsworth, 1995). Some of these secondary metabolites will be briefly discussed in this chapter.

Based on the large chemical and pharmacological research done; numerous bioactive compounds have been found in traditional medicinal plants used for the treatment and management of diabetes mellitus. These bioactive compounds include polysacchsrides, peptides, alkaloids, xanthone, flavonoids, lipids, phenolics, coumarins, iridoids, alkyl disulphides, inorganic ions and guanidines are reported to have antidiabetic activity (Bernhoft, 2008). Some groups of secondary plant metabolites have been used to classify plants in a field known as chemotaxonomy; this is due to their limited occurrence. The interest in secondary plant metabolites most significantly stems from their biological activity on other organisms especially animal cells. These form the major active ingredients of medicinal and poisonous plants. Thus their chemistry, structure, elucidation, isolation, biological activity and synthesis are major focus areas for natural product chemistry and other related fields.

2.10 Chemical constituents with antidiabetic activity

Although there are known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat diabetes. Many traditional plant treatments for diabetes are of major use worldwide. Plant drugs and herbal formulations are frequently considered to be less lethal and have less or are free from side effects than the synthetic drugs which tend to be very toxic. Based on the WHO recommendations, hypoglycemic agents of plant origin used in traditional medicine are important. According to Malviya et al. (2010) the recognised antihyperglycemic effects of most plants are due to their ability to reinstate the function of pancreatic tissues by causing an increase in insulin output or a decrease in the intestinal absorption of glucose. Treatment with herbal drugs has shown an effect on protecting β-cells and smoothing out fluctuation in glucose levels. Largely, very little is known about the biological specific modes of action in the treatment of diabetes, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc. that are frequently associated with having antidiabetic effects. The research for alternate treatments/remedies from the medicinal plants for the treatment of diabetes

mellitus continues throughout the world as this disease poses many challenges (Mukesh & Namita, 2013; Baharvand-Ahmadi et al., 2016).

An extensive and diverse variety of plants have been stated in the literature reviewed to prevent and treat diabetes. Several phytochemicals or bioactive compounds, including alkaloids, flavonoids, glycosides, glycolipid, galactomannan, polysaccharides, peptidoglycan, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids, saponins, dietary fibres and inorganic ions affect various metabolic cascades, which directly or indirectly affect the level of glucose in the human body. These have produced effective hypoglycemic, anti-hyperglycemic and glucose suppressive activities. The effective activities of these bioactive compounds were achieved by either increase in serum insulin level or increase in the production of insulin from pancreatic β-cells, inhibit glucose absorption in the gut, stimulate glycogenesis in liver or increase glucose utilization by the body. These compounds are believed also to possess antioxidant, hypolipidemic, anticataract activities, they also restored enzymatic functions, repair and regenerate the pancreatic islets and alleviation of liver and renal damage (Noor et al., 2013).

2.10.1 Carbohydrates

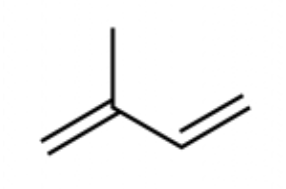
The plant-derived carbohydrates and related compounds with medicinal and therapeutic potential include fibre, cellulose and its derivatives, starch and its derivatives, dextrins, fructans, mucillages (uronic acid containing polymers), pectins (polysaccharide complexes formed from partially methoxylatedpolygalactouronic acid) and gums. Many kinds of polysaccharides have been isolated from traditional Chinese medicines for antidiabetes, most of which performed a good effect. Examples are panaxan, laminaran, coixan, pachymaran, anemarn, moran, lithosperman, trichosan, saciharan, ephedran, abelmosan, atractan (Li et al., 2004). In addition to their use as bulking agents in pharmaceuticals, carbohydrates have been recognized to have useful pharmacological properties (Gurib-Fakim, 2006). Several polysaccharides exhibit immuno-modulatory, anti-tumour, anticoagulant (e.g. heparin), hypoglycaemic or antiviral activities. The various carbohydrate products currently in market include fibre, cellulose and its derivatives, starch (glucose polymers) and its derivatives, dextrins, fructans (fructose polymers; e.g. inulin), algenic acids, agar and gums (Gurib-Fakim, 2006).

2.10.2 Terpenoids

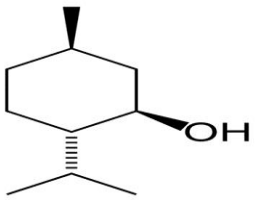
Terpernoids are also referred to as isoprenoids. This is the second class of metabolites and it is the largest and diverse group of secondary metabolites, of which, triterpenoid and saponins are the promising compounds with potential to be developed into new drugs for anti-diabetes. They are structurally characterized by a basic skeleton constructed from repeating 5 carbon units (isoprene) which are usually joined in a head-tail manner. In plants terpenoids are known to be involved in defence, wound scaling and thermo tolerance of plants as well as in the pollination of seed crops. Research has also associated terpenoids with the responsibility for the flavour of fruits, the fragrance of the flowers and the quality of agricultural products. Terpenoids are classified as monoterpenes (C10), sesquiterpenes (C15), diterpene (C20), triterpenes (C30) and tetraterpenes (C40) on the basis of the number of isoprene units (Gurib-Fakim, 2006; Li et al.; 2004). In **Figure 2.1** an example of Isoprene (2 methylbuta -1, 3 diene) is shown.

a) *Monoterpenes* are the most simple of constituents in the terpene series and are C10 compounds. They arise from the head to tail coupling of two isoprene units. They are commonly found in essential oils. Iridoids and pyrethrins are included in this group (Gurib-Fakim, 2006).

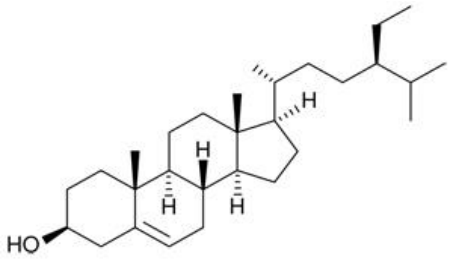
b) *Sesquiterpenes* are also constituents of essential oils of many plants, e.g. bisabolol, humulene and caryophyllene. Sesquiterpene lactones are well known as bitter principles. They occur in families like the Asteraceae. These compounds possess a broad range of activities due to the a-methylene-lactone moiety and epoxides. Their pharmacological activities are anti-bacterial, anti-fungal, anthelmintic, anti-malarial and molluscicidal. Examples are Santonin, which is used as an anthelmintic and as an anti-malarial (Gurib-Fakim, 2006).



Isoprene



Menthol



β-sitosterol

Figure 2.1: Some common terpenoids found in medicinal plants (Guanawardena, n.d; Giancaspro, n.d; Foodscience, 2015; Jacoby, 2005; Quellen, n.d)

c) *Monoterpenes* are the most simple of constituents in the terpene series and are C10 compounds. They arise from the head to tail coupling of two isoprene units. They are commonly found in essential oils. Iridoids and pyrethrins are included in this group (Gurib-Fakim, 2006).

d) *Sesquiterpenes* are also constituents of essential oils of many plants, e.g. bisabolol, humulene and caryophyllene. Sesquiterpene lactones are well known as bitter principles. They occur in families like the Asteraceae. These compounds possess a broad range of activities due to the a-methylene-lactone moiety and epoxides. Their pharmacological activities are anti-bacterial, anti-fungal, anthelmintic, anti-malarial and molluscicidal. Examples are Santonin, which is used as an anthelmintic and as an anti-malarial (Gurib-Fakim, 2006).

e) *Diterpenes* constitute a vast group of C20 compounds arising from the metabolism of 2E-, 6E-, 10E-geranylgeranyl pyrophosphate. They are present in animals and plants. These compounds have some therapeutic applications. For example, Taxol and its derivatives are anti-cancer drugs. Other examples are Forskolin, which has anti-hypertensive

activity. Zoapatanol is an abortifacient while Stevoside is a sweetening agent. Taxol is the most famous diterpene (Gurib-Fakim, 2006).

f) *Triterpenes* are C30 compounds arising from the cyclization of squalene. The basic skeleton arises from the cyclization of 3*S*-2,3-epoxy,2,3-squalene. Oleanane is an example of a pentacyclictriterpenes and testosterone of a steroid. Tetracyclic terpenes and steroids have similar structures but have different biosynthetic pathway. Steroids contain a ring system of three 6-membered and one 5-membered ring because of the profound biological activities encountered, many natural steroids together with a considerable number of synthetic and semi-synthetic steroidal compounds are employed in medicine (e.g. steroidal saponins, cardioactive glycosides, corticosteroid hormones and mammalian sex hormones).The pharmaceutical applications of triterpenes and steroids are considerable. Cardiac glycosides have been used in medicine without replacement by synthetic drugs (Gurib-Fakim, 2006).

Triterpenoids and steroidal glycosides, referred to collectively as saponins, are bioactive compounds that exist naturally in most plants and are reported to possess potent hypoglycaemic activity. An example of this is charantin, a steroidal saponin isolated from Momordicacharantia is known to have insulin-like activity that is responsible for its hypoglycaemic effect. The charantin stimulates the release of insulin and blocks formation of glucose in the blood stream, this may help in the diabetes treatment more especially in insulin dependent diabetes. The following saponins were isolated from antidiabetic plants from India: Lactucain C and furofuran lignin, lactucaside, β-sitosterol, andrographolide, gymnenic acid IV, etc. (Mukherjee et al., 2006).

2.10.3 Phenols

Phenolic compounds are water-soluble pigments and are common in plants. They are widely distributed in plants and are responsible for colour development, pollination and protection against UV radiation and pathogens. The phenolic compounds also contribute to the colour and astringency of some foods. They are characterized by the presence of one or several hydroxyl (OH) groups attached to an aromatic ring. The structures of phenolic compounds are diverse and exist either as a compound with one aromatic ring (simple phenols) or complex compounds (polyphenols) with different functional groups attached to them. Simple phenolic compounds have a monocyclic aromatic ring with an alcoholic, aldehydic or carboxylic group. They may also have a short hydrocarbon chain. Examples of phenols from plants: Capsaicin isolated from *Capsicum sp.*, is a vanillyl amide of isodecenoic acid and is

marketed as an analgesic; Eugenol is widely used in dentistry due to its anti-bacterial and anti-inflammatory and local anaesthetic activities (Gurib-Fakim, 2006). The classification of phenols depends on the number of atoms in the basic skeleton and the major groups are flavonoids, phenolic acids and tannins. **Table 2.1** shows examples of phenolic compounds.

Table 2.1: Examples of different classes of phenolic compounds

No. of Carbons	Basic Carbon Skeleton	Class of Phenols
6	C6	simple phenols & benzoquinones
10	C6-C4	naphthoquinones
14	C6-C2-C6	anthraquinones
15	C6-C3-C6	flavonoids
n>	$(C6-C3-C6)_n$	tannins

Phenolic compounds can be classified according to their structure into two broad classes: the non-flavonoids and the flavonoid phenolic compounds. The subsequent subsection will look at flavonoid phenolic compound.

2.10.4 Flavonoids

This is the largest and most complex group of polyphenolic compounds which is structurally characterized by a C_6-C_3-C_6 carbon skeleton referred to as a the flavan nucleus (containing a three ring structure with two aromatic centers (rings A and B) and a central oxygenated heterocyclic ring (C) as shown in **Figure 2.2** of the basic structure of flavonoids below.

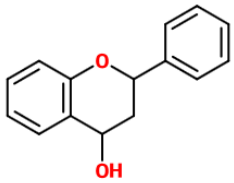


Figure 2.2: Basic structure of flavonoids (Jia et al., 2013)

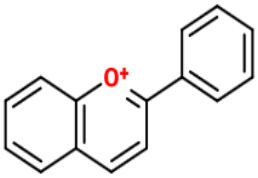
According to van Wyk (2009) the different types of flavonoids are the: flavones, flavanols, flavonones, anthocyanidins, chalcones, etc. These compounds represent another beneficial

group of naturally occurring compounds with hypoglycaemic potentials. **Figure 2.3** shows some known flavonoids that are of pharmacological importance. Some flavonoids have hypoglycemic properties because they improve altered glucose and oxidative metabolism of diabetes states (Mukherjee et al., 2006).

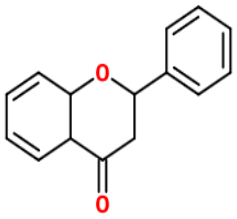
Flavonoids have attracted interest due to the discovery of their pharmacological activities such as anti-inflammatory, analgesic, anti-tumour, anti-HIV, anti-infective (anti-diarrhoeal, anti-fungal), anti-hepatotoxic, anti-lipolytic, anti-oxidant, vasodilator, immunostimulant and anti-ulcerogenic. Biologically active flavonoids comprise of hesperidin and rutin for decreasing capillary fragility and quercetin for its anti-diarrhoeal activity (Dweck, 2009; Gurib-Fakim, 2006; Peterson & Dwyer, 1998; Havsteen, 2002; Mankil et al., 2006).

Some flavonoids have been isolated from traditional Chinese medicines for anti-diabetes. Most flavonoids showed a mechanism to improve the function of β-cells of pancreatic islets. Examples of these isolated flavonoids from Indian and Chinese plants include: kakonein, 7-(6-O-malonyld-glucopyranosyloxy)-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one, flavone C-glycoside, icariin, neomyrtillin, sappanchalcone, caesalpin-*P*, 3-deoxysappanone, protosappanin A, brazilin, swerchirin [from Swertiachirayita (Roxb ex Flem) Karst], hyperin (from Tiliacordata Mill.), Quercetin, naringenin, chrysin, hespesperidin, naringin, genistein, daidzein, proanthocyanidins, epigallocatechingallate (from green tea), etc. (Li et al., 2004).

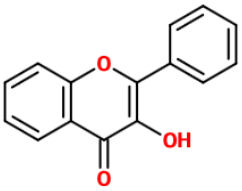
The most common alkaloids are the two caffeines from coca plants and nicotine from the tobacco plant *Nicotina tabacum*. Alkaloids are known to have neuroactive properties and have been used for the treatment of ailment related to the central nervous system, malaria and cancer. Due to their toxic, narcotic and addictive nature, their use is regulated or restricted and most alkaloids are rarely used in their pure form rather semi-synthetic analogues are used.



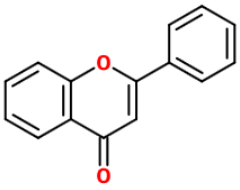
Anthocyanidin



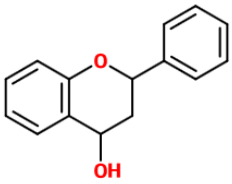
Flavanon



Flavonol



Flavon

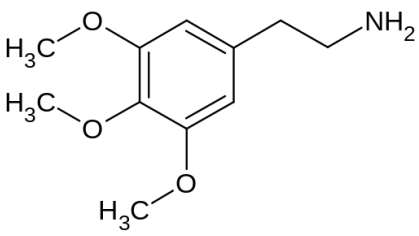


Flavan-4-ol

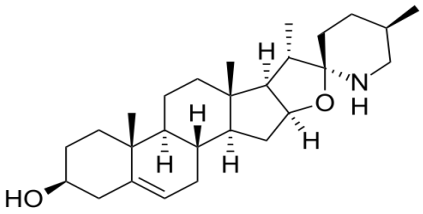
Figure 2.3: Structures of some flavonoids of pharmacological interest (Thovhogi, 2009)

2.10.5 Alkaloids

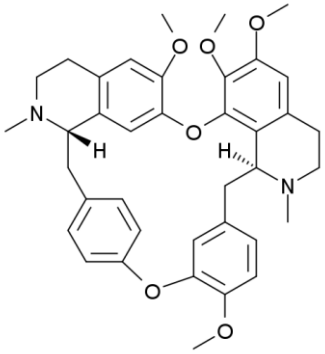
The alkaloids refer to a cyclic organic compound containing nitrogen atoms. Alkaloids are principally found in plants and to a lesser extent in microorganisms and animals. They are distributed in about 20% of all flowering plants (Bandaranayake, 2002). Alkaloids are divided into several subgroups based on their structures: non-heterocyclic alkaloids and heterocyclic alkaloids, which are further divided into 12 major groups according to their basic ring structure. Mescaline is an example of a non-heterocyclic or pseudo-alkaloid, Tetrandrine is another example of a bisbenzylisoquinoline alkaloid while Solasodine is a triterpene alkaloid shown in **Figure 2.4**.



Mescaline



Solasodine



Tetrandrine

Figure 2.4: Example of known alkaloids (Stafford, 1983; Patel et al., 2013; Meng et al., 2004)

Various alkaloids have been isolated from numerous Indian and Chinese medicinal plants, and investigated for possible hypoglycaemic activity. A few compounds were isolated toward diabetes. These are the known antidiabetic alkaloids: berberin, anisodamine, vindoline, vindolinine, leurosine, aconitine, hanfangchin A (from *Stephaniatetradra S. Moore*) and multiflorine (from *L. hirsutus*) (Li et al., 2004; van Wyk et al., 2009; Mukherjee et al., 2006). The structures of some well-known pharmacologically active alkaloids are shown in **Figure 2.5**.

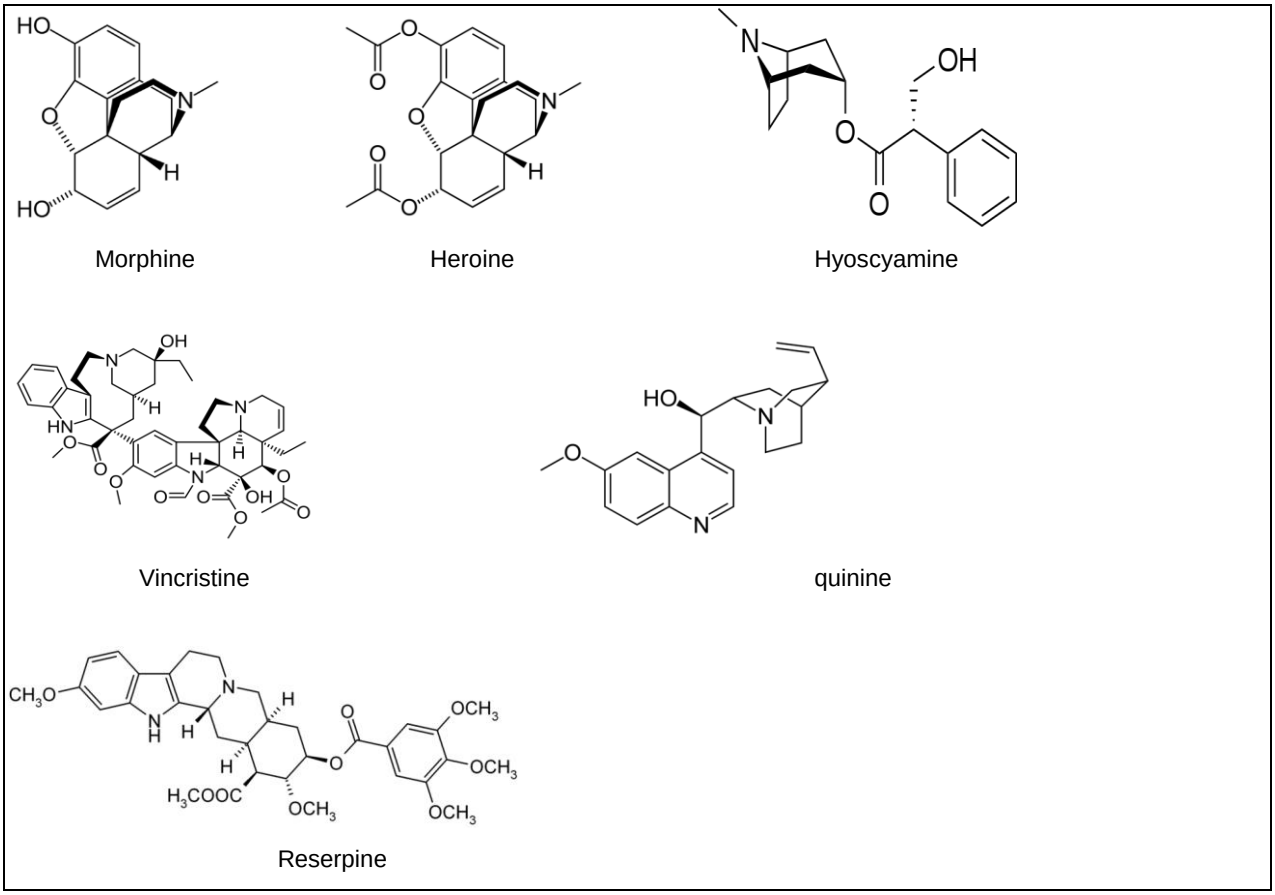


Figure 2.5: Structures of some of the well-known pharmacological active alkaloids (Freemantle, 2002; The role of chemistry in history, 2008; Pubchem, n.d.; Giolitti, 2012; Siravegna, 2011)

2.11 Antioxidants

Antioxidants are molecules that are capable of inhibiting the oxidation of other molecules. They protect cells against injury originated by unstable molecules, such as singlet oxygen molecules, superoxide molecules, hydroxyl radicals and peroxy nitrite radicals. The antioxidant molecules are able to slow down or avoid the oxidation process of other micro molecules. Oxidation is a chemical reaction that transfers an electron or hydrogen from a substance to and oxidizing agent. This reaction produces free radicals and can create toxic

metabolites which damage tissues or cells by a sequence of chain reactions. Antioxidants are compounds that terminate these reactions by eliminating free radical intermediates and impeding other oxidation reactions by being oxidized themselves and thus protecting cells against the destructive effects of the oxygen species (Vijay & Vimukta, 2014; Zatalia & Sanusi, 2013, Farzaneh et al., 2015).

Although oxidation reactions are crucial for life, they can also be damaging hence; plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione lycopene, beta-carotene, carotenoids, selenium, flavanoids and natural vitamins together with vitamin C, vitamin A and vitamin E. Antioxidant enzymes including glutathione S. transferase, superoxide dismutase, catalase, peroxidise, in addition to proteins diminish the accessibility of peroxidise such as iron, copper ions and heme protein. The difference among antioxidant molecules and reactive oxygen species results in endothelial damage or cellular damage. Oxidative stress that originates from reactive oxygen species is emerged as an important element of many human disorders or diseases. Therefore antioxidants are intensely studied in the field of medicine, predominantly as management for inflammation related disorders and its complications like diabetes mellitus, cardiac arrest, cancer and neurodegenerative diseases (Vijay & Vimukta, 2014; Zatalia & Sanusi, 2013; Doupis & Veve, 2007).

2.12 Role of antioxidants in diabetes mellitus

Oxidative stress is known as a state of overload due to imbalanced information and elimination of highly reactive micro molecules including oxygen species (ROS) and nitrogen species (RNS). In diabetes mellitus oxidative stress results from various enzymatic pathways, non-enzymatic pathways and mitochondrial pathways. The most predominant factor behind the development of prooxidant-antioxidant in diabetes mellitus is the auto-oxidation of glucose that leads to the generation of high energy particles. The link between diabetes and oxidative stress can be further explained by the fact that oxidative stress is a result of an imbalance between the formations of oxygen derived free radicals and diabetes mellitus is related with augmented generation of free radicals and diminished antioxidant capacity (Zatalia & Sanusi, 2013; Vijay & Vimukta, 2014).

The induction of oxidative stress is a main process in the commencement of diabetic complications and various antioxidants have been developed recently to manage oxidative stress in diabetes. It is still unknown how oxidative stress hastens the development of diabetic complications. Great amount of work has been done towards the protective effect of

antioxidants in various human ailments, and it has been established that antioxidants might be useful in management of diabetes mellitus and its complications (Vijay & Vimukta, 2014).

2.13 Classification of antioxidants

Antioxidants are classified into two groups' i.e. natural antioxidants and synthetic antioxidants:

2.13.1 Natural antioxidants

They are also known as the chain breaking antioxidants. They act in response with lipid free radicals and change them into highly stable end products. They are mainly the following:

a) Vitamins: these are vital components essential for metabolic activities, for example ascorbic acid, alpha-tocopherol, vitamin B and its subtype.

b) Mineral antioxidants: they act as co-factors of important enzymatic antioxidants and play a dynamic role in metabolism of several macromolecules, for instance carbohydrates, nucleic acid etc. Examples are selenium, copper, iron and manganese.

c) Phytochemicals: these include Flavonoids: phenolic complexes that give colours to the vegetables, fruits, grains, seeds, leaves, flowers and bark. Catechins: major bio-active antioxidants occurring in green tea, sesamol and black tea. Carotenoids: a fat soluble pigment that occurs in carrot and transforms to vitamin A in case of nutritional deficiency of vitamin A. Lycopen: a red pigment is an important phyto-constituent of certain vegetables and fruits including tomatoes and is known for its cancer fighting ability,

2.13.2 Synthetic antioxidants

This is a phenolic group of compounds that performs the important function of arresting free radicals, diminishing oxidative stress and impeding the chain reactions through various biological actions. Examples of these antioxidants are: butylated hydroxyl anisole (BHA); butylated hydroxyl toluene (BHT); tertiary butyl hydroquinone (TBHQ); esters of gallic acid (propyl gallate) and ethylenediaminetetraacetic acid; nordihydroguaretic acid.

2.13.3 Sources of antioxidants

Antioxidants are abundantly present in leafy vegetables, fruits and natural food sources. Beta-carotene, a common biologically active antioxidant, is found in various coloured fruits and green leafy vegetables, such as orange, sweet potatoes, carrots, squash, apricots, pumpkins, collard green spinach, kale and mangoes.

2.14 The diabetes tea composition

Table 2.2 lists the different plants that make up the diabetes tea. The parts used in each plants are listed and their traditional uses according to literature.

2.15 Pharmacological investigations of plant material

Reviews of literature involving research of medicinal plants advise that scientists follow more or less the same general strategy to study plant materials for their pharmacological properties (Kinghorn and Balandrin, 1993; Heinrich et al., 2004).

2.15.1 Selection of plant species

Any plant species and plant parts collected randomly can be studied using available phytochemical methods. Nonetheless, a more guided approach is often preferred than the random selection approach (Kinghorn and Balandrin, 1993; Harborne, 1998; Heinrich et al., 2004). The plant material to be investigated can be selected on the basis of some specific traditional uses (ethno-botanical bio-prospecting approach). Extracts that are prepared from medicinal plants and that are used as traditional remedies to treat certain diseases are more expected to contain biologically active compounds that are of medicinal interest (Heinrich et al., 2004). In other instances, the plant can be selected based on chemo-taxonomical data. In this approach, knowledge that a particular group of plants comprise a certain class of natural products may be used to foretell that taxonomically related plants may contain structurally similar compounds (Heinrich et al., 2004).

Sometimes plant materials can be selected following a combination of the above mentioned approaches. The use of a literature database early in the selection process can provide some preliminary information on the type of natural products already isolated from the plant and the extraction methods employed to isolate them (Heinrich et al., 2004).

Table 2.2: List of the eight plants constituents of the diabetes tea

Name of plant	Parts used	Medicinal Uses	References
Trigonella foenum - graecum	Seeds	Allergies, Appetite loss; Cholesterol; Diabetes Gastric Disorders, Lung Infections, Mucus Excessive, sore throat, Abscesses, Anaemia, Asthma, Boils, Body Odour , Bronchitis, cancer, swollen eyes, Fevers, Gallbladder Problems, Heartburn, Inflammation,	(Jung et al., 2006) (Yadav, et al., n.d) (Moskovitz, 2012) (Vats et al., 2002)
Achillea millefolium	Above ground portion	Anorexia; appetite loss; colds; chickenpox; cramps; fever; eczema; measles; internal bleeding; earache; skin irritation	(Potrich, et al., 2010); (Huo, et al., 2013); (Moskovitz, 2012)
Agothosma betulina	Dried leaves	Antiseptic; aromatic; carminative; diuretic; kidney tonic; urinary antiseptic; uterine stimulant	(van Wyk et al., 2009); (Aromatics); (Moskovitz, 2012) (Street & Prinsloo, 2013)
Salvia officinalis	Leaves, small stem and flowers	antifungal; anxiety; blood clots; colds; diabetes; diarrhea; migraine; memory loss; menopause; worms; fever; flu; indigestion	(Baricevic et al., 2001) (Lima et al., 2005); (Moskovitz, 2012)
Salvia africana-caerulea	Leaf and flowers	Coughs; Colds; Bronchitis; Diarrhoea; Flatulence; Colic; Indigestion; Uterine - normalizes the female reproductive system.	(Moskovitz, 2012)
Taraxacum officinale	All parts	Antifungal; diuretic; detoxifying; anti-inflammatory; anti-cancer; hypoglycemic effect	(Schieber & Schütz, 2006); (Moskovitz, 2012); (Yarnell & Abascal, 2009)
Thymus vulgaris	Flowering aerial part	Acne; arthritis; asthma; bronchitis; cough; tuberculosis; headache; stomach ache; colic; diarrhea	(Shabnum & Wagay, 2011); (Ozcan & Chalcath, 2004); (Özgüven & Tansi, 1998); (Moskovitz, 2012)
Urtica Urens	Whole plant	Skin problem; burns; eczema; inflammation; cystitis	Moskovitz, 2012

There is also another approach that is referred to as the information driven approach; it exploits a combination of ethobotanical, chemotaxonomic and random approaches together with a data base that contains all relevant information concerning a particular plant species (Kinghorn and Balandrin, 1993; Harborne, 1998; Heinrich et al., 2004). The database is used to prioritize which plants should be extracted and screened for biological activity. This approach is favoured by large organizations (particularly pharmacological companies) interested in screening thousands of samples for bioactivity as it may reduce costs by a process known as dereplication – the process of avoiding the repeated discovery of common or known drugs (Heinrich et al., 2004).

The part of the plant to be collected depends on where the metabolites of interest (if they are known) are accumulated. This is why aerial (e.g. leaves stem, flowering tops, fruit, seed, and bark) and underground (e.g. tubers, bulbs, roots) parts can be collected separately. The collection of plant materials can also be influenced by factors such as the age of the plant and environmental conditions (e.g. temperature, rainfall, amount of daylight, soil characteristics and altitude) (Williams et al., 1996; Harborne, 1998). Therefore, it becomes essential to take this into consideration for the re-collection purpose, in order to ensure a reproducible profile (nature and amount) of metabolites (Satyajit et al., 2006). Correct identification of the plant to be collected is equally important. To ensure this a plant taxonomist or a botanist should be involved in the detailed authentication of the plant (i.e. classification into its class, order, family, genus and species) (Satyajit et al., 2006). Any feature related to the collection, such as the name of the plant, the identity of the parts collected, the place and date of collection, should be recorded as part of the voucher (a dried specimen pressed between sheets of paper) deposited in a herbarium for future reference (Harborne, 1998; Satyajit et al., 2006).

2.15.2 Extraction of plant materials

Plant materials are commonly extracted by means of liquid solvents. This type of extraction is known as the “solid-liquid solvent extraction”. The well-known solid-liquid solvent extraction process for plant materials involves drying and grinding of the plant paterial, choosing a suitable extraction solvent and extraction procedure (Starmans and Nijhuis, 1996; Jones and Kinghorn, 2005).

2.15.3 Drying and grinding the plant material

After the plant material has been collected, it needs to be dried as soon as possible. Mostly the sample is left to dry on trays at ambient temperature and in a room with adequate ventilation (Heinrich et al., 2004; Satyajit et al., 2006). The dry conditions are important to prevent microbial fermentation and subsequent degradation of metabolites. Plant materials should be sliced into small pieces and distributed evenly to facilitate homogeneous drying. Protection from direct sunlight is advised to minimize chemical reactions (and formation of artifacts) induced by ultraviolet rays (Satyajit et al., 2006). To facilitate the drying process, the material can be dried in an oven. This can also minimize reactions (e.g. hydrolysis of glycosides) that can occur as long as there is some residual moisture present in the plant material. The dried material should be stored in sealed containers in a dry and cool place. Storage for prolonged periods should be avoided as some constituents may be decomposed (Heinrich et al., 2004; Jones and Kinghorn, 2005). After drying, plant materials are commonly grinded into a fine powder. Grinding of plant materials into smaller particles facilitates subsequent extraction procedures by rendering the sample more homogeneous, increasing the surface area, and facilitating the penetration of solvents into cells (Harborne, 1998; Satyajit et al., 2006). Mechanical grinders (e.g. hammer and cutting mills) are employed to shred the plant material into various particle sizes.

2.15.4 Choice of suitable extraction solvent

Solvent extraction as a method of separation has long been known to chemists but it has only been in recent years that it has been recognized among analysts as a powerful separation technique. The choice of the extraction solvent is dependent mainly on the polarity and consequently the solubility of the bioactive compound(s) of interest. The successful determination of biologically active compounds from plant material is largely depends on the type of solvent used in the extraction procedure. A good solvent in plant extractions is known to have the following properties: low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action and an inability to cause the extract to complex or dissociate.

The quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process and potential health hazard of the extractants are the main factors that affect the solvent choice during the extraction process (Eloff, 1998). The choice of solvent is also further influenced by what is

intended with the extract. It is important that the solvent is non-toxic and should not interfere with the bioassay. The targeted compounds to be extracted in a plant will also play a vital role in the choice of solvent to be used (Ncube et al., 2007; Das et al., 2010). Many traditional procedures use water as a solvent of extraction but organic solvents of varying polarities are often used (either alone or combinations) in modern methods of extraction to exploit the various solubilities of plant constituents. Different solvent systems are available to extract the bioactive compound from natural products. The extraction of hydrophilic compounds uses polar solvents such as methanol, ethanol or ethyl-acetate. For extraction of more lipophilic compounds, dichloromethane or a mixture of dichloromethane/methanol in ratio of 1:1 are used. In some instances, extraction with hexane is used to remove chlorophyll (Cos et al., 2006). The polarity and chemical profiles of most of the common extraction solvents have been determined (Kaul et al., 1985; Ayaffor et al., 1994; Perett et al., 1995; Eloff, 1998; Cowan, 1990) and are summarized in **Table 2.3**.

Thus, if the polarity or the solubility of the compound(s) of interest is known, information in **Table 2.3** can be used to select a suitable extractant solvent or a mixture of two or more solvents of different polarity. If the polarity of the compounds of interest is not known, the powdered plant material can be extracted simultaneously with a mixture of different proportions of two or more solvents of different polarity. Alternatively, the powdered plant material can be extracted sequentially with solvent of different polarity in what is known as a sequential extraction procedure (Bruneton, 1999).

2.15.5 Choice of extraction procedure

Extraction is the fundamental first step in the analysis of medicinal plants, because it is essential to extract the desired chemical components from the plant materials for further separation and characterization. The basic operation include steps such as pre-washing, drying of plant materials or freeze drying, grinding to obtain a homogenous sample and often improving the kinetics of the analytic extraction and also increasing the contact of sample surface with the solvent system. Proper actions must be taken to assure that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples. If the plant was selected on the basis of traditional uses (Fabricant and Farnsworth, 2001), then it is needed to prepare the extract as described by the traditional healer in order to mimic as closely as possible the traditional ‘herbal’ drug.

Table 2.3: Polarity and chemical profiles of the most commonly used extractive solvents

Polarity	Solvent	Extracted phytochemical	Reference
Low	n-Hexane	Fatty acids, waxes and terpenoids	Ayoffor et al., (1994); Cowan, (1990)
	Chloroform	Fatty acids, waxes, flavonoids and terpenoids	Bruneton, (1990); Perett et al. (1995); Cowan, (1990)
Medium	Dichloromethane	Less polar and polar flavonoids, tannins and terpenoids	Bruneton, (1999); Scalbert et al. (2005); Cowan, (1990)
	Ethyl-acetate	Less polar and polar flavonoids, tannins and terpenoids	Bruneton, (1999); Scalbert et al. (2005)
	Acetone	Less polar and polar flavonoids, tannins, terpenoids, glycosides and phenol	Eloff, (1998); Bruneton, (1999); Scalbert, (2005)
High	Ethanol	Polar flavonoids, tannins, glycoside, saponins, polyphenols; polyacetylenes, alkaloids and sterols	Cowan, (1990); Bruneton, (1999)
	Methanol	Anthocyanins; terpenoids, saponins; tannins, xanthoxylines, totarol, quassinoids, lactones, flavones phenones, polyphenols, carbohydrates, lecithin, glycosides, alkaloids and phenylpropanoids	Cowan, (1990); Bruneton, (1999); Scalbert et al., (2005)
	Water	Cabohydrates, lecithin, amino acids, polypeptides, phenolic acid, phenylpropanoids, polar flavonoids, glycosides, alkaloids, anthocyanines, starches, tannins, saponins, terpenoids, polypeptides and lectins	Kaul et al., (199); Jones & Kinghorn (2005); Cowan, (1990)
	Aqueous acid or base	Alkaloids	Bruneton, (1999)

As the target compounds may be non-polar to polar and thermally labile, the suitability of the methods of extraction must be considered. Various methods, such as sonification, heating under reflux, soxhlet extraction and others are commonly used for the extraction of plant samples (United States Pharmacopeia and National Formulary, 2002; Pharmacopoeia of the People's Republic of China, 2000; The Japanese Pharmacopeia, 2001). In addition, plant extracts are also prepared by maceration or percolation of fresh green plants or dried powdered plant material in water and/or organic solvent systems.

The other modern extraction techniques include solid-phase micro-extraction, supercritical-fluid extraction, pressurized-liquid extraction, microwave-assisted extraction, solid-phase extraction, and surfactant-mediated techniques, which possess certain advantages.

These are the reduction in organic solvent consumption and in sample degradation, elimination of additional sample clean-up and concentration steps before chromatographic analysis, improvement in extraction efficiency, selectivity, and kinetics of extraction. The ease of automation for these techniques also favours their usage for the extraction of plant materials (Huie, 2002).

2.16 Screening medicinal plants for biological activity

The medicinal plant (usually selected on the basis of information obtained from traditional healers and herbalist) are collected, dried, powdered and extracted with a suitable solvent (usually either water or alcohol) and screened for hypoglycaemic activity. Screening tests commonly used to assess the antidiabetic/hypoglycaemic activity of medicinal plants can generally be grouped into three main categories: in vivo bioassay (whole animal bioassay); in vitro cell based bioassay (mechanism-based bioassay) and sub-molecular enzyme (inhibition based bioassay). For the purpose of this study, the focus will be on the sub-molecular enzyme bioassay category which are the bioassays used to determine antidiabetic activity of extracts in this study.

2.16.1 Sub-molecular enzyme inhibition-based assays

Some antidiabetic agents are known to exert their blood glucose lowering effects through inhibition of specific carbohydrate metabolizing enzymes. For this reason several researchers (Hara and Honda, 1990; Kim et al., 2005; Ali et al., 2006; Bhandari et al., 2008) have investigated the ability of plant extracts to inhibit the activities of enzymes such as α-amylase, α-glucosidase, hexokinase (glucokinase) and glucose 6-phosphatase by means of

in vitro sub-molecular enzyme inhibition assays. A review of the literature on enzyme inhibition-based assays suggests that a typical *in vitro* enzyme inhibition-based assay involves three distinct steps (**Figure 2.6**).

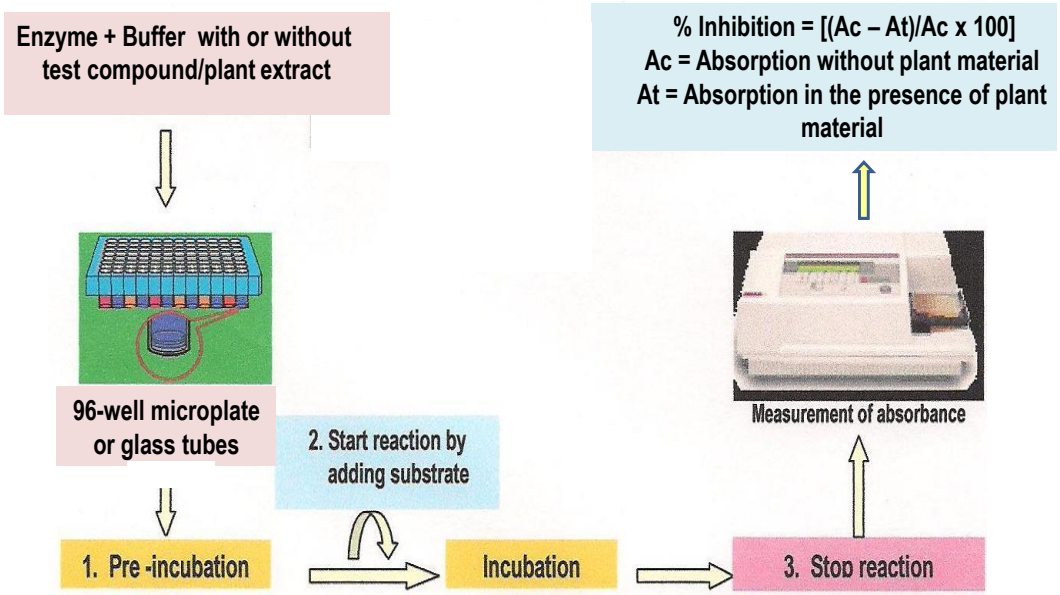


Figure 2.6: Three distinct steps of an in *vitro* inhibition based assay (Thovhogi, 2009)

Initially, the enzyme is pre-incubated in a suitable buffered solution with or without the test compound. In addition to the incubation buffer, the test solution may comprise of numerous other reagents such as sulfhydryl compounds, metals, protein cofactors and stabilizing agents that are needed by the enzyme. This pre-incubation step allows a maximum chance for the enzyme to interact with the test substance before the reaction is initiated. The second stage is the commencement of the reaction. This is most often done by automated or manual addition of the substrate to each tube or well. Finally, the reaction must be terminated if it is a single-time point readout and the amount of the product formed or the loss of the substrate must be determined. Stopping the reaction can be done by a variety of ways depending upon the particular enzyme. One general way is to denature the enzyme by addition of a denaturing agent, for example trichloroacetic acid or a rapid increase in temperature. If a metal ion is required for the activity of the enzyme, the reaction may be stopped by the addition of a chelating agent such as EDTA to sequester the metal ion. Once the reaction is stopped, absorption readings are made against a blank by means of a spectrophotometer and the percentage inhibition calculated.

2.17 Cytotoxicity analysis of medicinal plant extracts

Drug discovery processes entail the test of a potential extract on *vero* cell lines. This determination is called cytotoxicity tests and enables the extract to be developed into a

clinically acceptable drug. This provides a screening system to determine whether the compounds being examined are not more toxic to normal biological processes than the positive effects they demonstrate (Gebhardt, 2000).

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay is widely used for *in vitro* measurements of the metabolic viability of cell cultures subjected to different culture conditions. Traditionally the determination of cell growth is done by counting the viable cells after staining with the yellow MTT dye. The MTT dye is reduced to a water insoluble colourless crystal in the mitochondria of living cells. The crystals are then converted into a purple coloured formazan product upon addition of dimethyl sulfoxide (DMSO). The unviable cells cannot reduce this dye hence there will be no purple stain. The amount of product formed is measured by a spectrophotometer via an absorbance at a certain wavelength (usually between 500 and 600 nm). This reduction is a result of active mitochondrial reductase enzymes, and therefore conversion can be directly associated to the number of viable cells. The assay offers the following advantages: (a) sensitivity and reproducibility, (b) ease with which it can be performed and quantified, (c) rapidity (Ferrari *et al.*, 1990).

2.18 Chemical studies on medicinal plants

The isolation, purification and analysis of the active extracts to get pure compounds are done using various chromatographic techniques such as Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC). Moreover, spectroscopic techniques such as, Liquid Chromatography Mass Spectrometer (LC-MS), Nuclear Magnetic Resonance (NMR), Gas Chromatography Mass Spectrometer (GC-MS), Infra-red (IR), and Ultra Violet/visible Spectroscopy (UV/Vis) are utilized for the chemical analysis of medicinal plants.

2.18.1 Phytochemical Studies

Phytochemistry can be expressed as a link between chemistry and botany. Phytochemistry is concerned with the study of the chemical composition of plants or natural compounds and the explanation of the various plant process in which chemical phenomena are concerned.

2.18.2 Qualitative phytochemical analysis

Phytochemical screening performed on extracts using standard procedures provided by Trease & Evans (1989) and Tiwari (2011) show protocols on how to determine the presence

of secondary plant metabolites such as alkaloids, reducing sugars, anthraquinones, flavonoids, terpenoids, saponins, tannins, glycosides and gums.

2.18.3 Quantitative phytochemical analysis

Evaluation of crude drugs also involves the determination of ash values and extractive values of the drug.

a) *Ash values*: The determination of Ash values is useful for detecting low-grade products, exhausted drugs and excess of sandy or earthy material. Different ash values are calculated for such purposes e.g. total ash, acid-insoluble ash, water-soluble ash and sulphated-ash.

   The total ash value is useful for detection of the crude drug mixed with various minerals e.g. sand, soil, calcium oxalate, chalk powder or other drugs with different inorganic contents to improve their appearance. The maximum temperature used for total ash is not more than 450 °C. It is because of the fact that at higher temperatures, volatile alkali chlorides may be lost (Kumar, 2015).

   Acid-insoluble ash means that the ash is insoluble in dilute hydrochloric acid. Its value is higher than total ash. The majority of crude drugs contain calcium oxalate whose quantity varies frequently. Therefore, total ash of a crude drug vary within wide limits for specimen of genuine drug e.g. for Rhubarb, total ash ranges from 8-40% and in this case, determination of acid-insoluble content is more preferable. The calcium oxide formed due to incinerated oxalate is soluble in hydrochloric acid. The insoluble ash is then weighed. In this way, excessive earthy matter that is usually present in the root, rhizome and leaves can be determined. Water-soluble ash is used to detect the presence of material which is exhausted by water (Kumar, 2015).

   Sulphated-ash is produced after treating the drug with sulphuric acid to get sulphate salts. The percentage ash is calculated with reference to air-dried sample. The temperature used for the procedure is more than 600 °C.

b) *Extractive values*: Water-soluble extracts are used for the determination of crude drugs containing sugars, plant acids, glycosides, tannins and mucilage and alcohol-soluble extracts are used for determination of resins, certain glycosides whilst ether-soluble extracts are used for drugs containg fats and volatile principles.

c) The significance of the extractive value are:

- They are useful for the evaluation of crude drugs especially when the constituents of the drug cannot be readily estimated by any other means.
- They also help to indicate the nature of chemical constituents present in the drug.
- They also assist in the identification of adulterants in the crude drug.

2.19 Chromatographic Techniques

Chromatography is a separation technique in which analytes in a mixture are separated based on their interaction with the two phases, mobile phase and stationary phase (Christian, 2004). The separation is dependent on the differences in the physical properties of analytes like molecular size, charge and solubility. Chromatography is divided into gas and liquid based on the mobile phase utilized. Chromatographic techniques have been influential in the separation of natural products (Henrich, 2004). In this section chromatographic techniques that have been used in the study are discussed.

2.19.1 Open Column chromatography and Flash Chromatography

a) Open column chromatography:

This is a form of a solid-liquid chromatography technique used for the isolation of phytochemicals. This technique is one of the most traditional techniques applicable for the purification of different groups of compounds. The sample mixture is loaded at the top of a glass column that is packed with an adsorbent (stationary phase) and eluted with a solvent (mobile phase). Due to differences in chemistry, analytes separate as they go down the column forming bands which are collected as fractions. These fractions are continuously monitored using TLC and similar fractions are combined and concentrated. Depending on the sample, the column can be eluted in one of two ways. In the isocratic elution a single solvent or solvent mixture is used whereas in gradient elution, a series of solvents with increasing polarity/elution strength are used gradually to elute the column. Advantages of open column chromatography include simplicity and low cost. However, it lacks automation, and is very tedious, labour intensive and time consuming.

b) Flash Chromatography:

Figure 2.7: A flash chromatography instrument (Sigma Aldrich, 2005)

This is a type of preparative purification method used for the rapid isolation of compounds including reaction mixtures, where the target (synthesized) molecule must be separated from excess reagents, by-products, and side-products; natural products-compound of interest have be separated from matrix and impurities.

Sample range varies from several mg to over 150 g with linear flow rates up to 15 cm/min and pressure of 10-100 psi. The Supelco Versa Flash System shown in **Figure 2.7** was used in the experiments. It fits a wide range of cartridge sizes and cartridge change-out is very convenient. Materials in contact with sample are stainless steel, Teflon®, PEEK, and polypropylene, which are inert to most of the samples.

2.19.2 Thin Layer Chromatography

This is a form of chromatographic method in which the stationary phase is a thin layer of a solid absorbent encrusted on a solid support (it uses glass or aluminium plates pre-coated with the sorbent e.g. silica gel or plastic. The different TLC plates have varying thickness depending on the amount to be loaded. The mobile phase is a liquid. The compound mixture is spotted on the plate at around 2 cm from the bottom and developed in a developing tank containing the solvent. As the capillary forces draw the solvent up the plate, the analytes separate at different rates depending on their solubility and retention by the stationary phase. Several reagents are available for visualization of the separated materials. Different spots equivalent to different analytes are characterized by their retention factor (Rf) values. This is

defined as a measure of the distance travelled by the analytes in reference to the distance moved by the solvent.

TLC has the advantage of being a highly cost-effective qualitative technique in as much as a large number of samples can be analyzed or separated simultaneously. The few drawbacks include poor detection and control of elution compared to HPLC (Gurib-Fakim, 2006). Thin-layer chromatography, combined with both biological and chemical detection methods, is an effective and inexpensive technique for the study of plant extracts. It can thus be performed both in sophisticated laboratories and in small laboratories which only have access to limited equipment (Marston, 2011).

2.19.3 High Performance Liquid Chromatography

This method is very popular and widely used for the analysis and isolation of bioactive natural products. The analytical sensitivity is further enhanced depending on the detector that is being used. The detectors can be UV detection such as a photodiode array (PDA), which enables the acquisition of UV spectra of eluting peaks between 190 nm to 800 nm. PDA UV detection has the advantage of detecting compounds with poor UV characteristics and this is particularly useful in the analysis of natural products such as terpenoids or polyketides, which may not necessarily have chromophores that will rise to a characteristic UV signature.
Coupled with electronic library searching of compounds along with the fingerprinting of biologically active extracts, HPLC becomes a very powerful quality control technique of herbal medicines. It has now become a tool of choice for the analysis of a majority of natural products in the pharmaceutical industry. It suffers from one drawback in the sense that it is expensive both from the machine and consumable view-points (Gurib-Fakim, 2006; Sticher, 2007).

2.20 Structure elucidating techniques

Once the biological evaluation has been performed and the separation of the natural product has been achieved, then attempts for the structural elucidation of the compound can be done. Structure elucidation depends on classical spectroscopic techniques such as: Nuclear Magnetic Resonance spectroscopy, Infra-Red spectroscopy, and Liquid Chromatography-Mass Spectrometry (Cordell, 1995; Gurib-Fakim, 2006).

2.20.1 Infra-red Spectroscopy

IR spectroscopy is one of the most common spectroscopic techniques used in chemistry. It is the absorption measurement of different IR frequencies by a sample positioned in the path of an IR beam. The main aim of IR spectroscopic analysis is to determine the chemical functional groups in the sample. It is based on the principle that different functional groups absorb characteristic frequencies of IR radiation. Using various sampling accessories, IR spectrometers can accept a wide range of sample types such as gases, liquids, and solids. Thus, IR spectroscopy is an important and popular tool for structural elucidation and compound identification.

2.20.2 Nuclear Magnetic Resonance Spectroscopy

NMR spectroscopy is the most comprehensive technique in organic chemistry. It gives detailed structural information that is useful for the complete identification of both simple and complex compounds. Under radio frequency, nuclei with non-zero spin values (^{1}H, ^{13}C, ^{31}P, ^{15}N) in a static magnetic field absorb energy with frequencies characteristic of the nuclei. The NMR spectrum is a plot of frequencies of absorbed energy against the peak intensities.

2.20.3 Liquid Chromatography-Mass Spectroscopy

LC-MS is a routine technique with the development of electrospray ionisation (ESI) providing a simple and robust interface. It can be applied to a wide range of biological molecules and the use of MS and stable isotope internal standards allows highly sensitive and accurate assays to be developed although some method optimisation is required to minimise ion suppression effects. Fast scanning speeds allow a high degree of multiplexing and many compounds can be measured in a single analytical run. LC-MS is starting to play a significant role in several areas of clinical biochemistry and compete with conventional liquid chromatography and other techniques such as immunoassay (Pitt, 2009).

CHAPTER THREE

METHODOLOGY

3.1 Introduction

This chapter outlines all the experimental methods, procedures, techniques and materials used for the identification of the active compounds in the selected medicinal plants used for the treatment and management of diabetes mellitus. This chapter has been divided into two sections: the biological studies and chemical studies.

3.2 General experimental

Reagents were bought from Sigma Aldrich and standards were freshly prepared on the day of analysis. All plasma/serum were kept on ice when not handled during the analysis.
Crude extracts for all the assays were treated as follows: 50 mg of the sample in a 50 ml screw-cap tube was weighed. 50 ml water or methanol was added. This was mixed until dissolved and sonicated where necessary. The samples were then centrifuged at 4000 rpm for 5 mins. The supernatant was used directly after a suitable dilution.

3.2.1 Plant Collection

The study was based on the extraction of a tea being used for the treatment and management of diabetes mellitus, and the eight plants making up the tea. The tea is manufactured by Sing Fefur organic herbs, situated in a small town called McGregor in the Western Cape, South Africa. The plant material was collected from their farm. The plants collected were *Trigonella foenum-graecum*, *Achillea millefolium*, *Agothosma betulina*, *Salvia officinalis*, *Salvia africana-caerulea*, *Taraxum offinale*, *Thymus vulgaris* and *Urtica urens*.

3.2.2 Extraction techniques

10 g of the plant material was extracted sequentially with solvents of increasing polarity (i.e. hexane, DCM, 1:1 DCM:MeOH; MeOH; H_2O). The plant material was first extracted with hexane until no colour change is observed on the solvent. The combined hexane extracts were filtered and evaporated by means of a rotary evaporator. The residue obtained from the filtration of the hexane extract was then extracted with DCM and the combined extracts were filtered and evaporated. The residue obtained from filtration of the DCM extract was then extracted with 1:1 DCM:MeOH and the combined extracts filtered and evaporated. The

obtained residue from the filtrate was extracted with MeOH and the combined extracts were filtered and evaporated.

Another 10 g of the plant material was extracted sequentially using distilled water. The combined aqueous extracts were frozen and freeze-dried to obtain the final extracts.

3.3 Biological Studies

The following biological studies were done using a modified method of Marnewick et al (2011) at the Cape Peninsula University of Technology Antioxidant Research Laboratory.

3.3.1 Flavanols plate reader

The analysis made use of 4-dimethylaminocinnamaldehyde which reacts with flavanols to form a characteristic light blue colour that is measured at 640 nm. Since very slight amounts of turbidity interfere with the determination, samples showing visible turbidity were clarified by centrifugation. Alternately, turbid samples were first filtered.

3.3.2 Oxygen Radical Absorbance Capacity Assay (ORAC)

The ORAC method was performed using a fluorescence spectrophotometer until zero fluorescence occurs. The results were reported as the ORAC value, which refers to the net protection area under the quenching curve of β-PE (fluorescein) in the presence of an antioxidant. The ORAC value was calculated by dividing the area under the sample curve by the area under the Trolox curve with both areas being corrected by subtracting the area under the blank curve. One ORAC unit was assigned as being the net protection area provided by 1 μM Trolox in the final concentration. When the area under the curve for the sample is compared to the area under the curve for Trolox, the result is given in Trolox equivalents.

The ORAC method was adapted to be able to analyze the lipid-soluble antioxidant samples by introducing randomly methylated beta-cyclodextrin (RMCD) in a 50% acetone:water mixture. This mixture made lipid-soluble antioxidants soluble in phosphate buffer. The ORAC method is unique in its analysis in that it takes into account the inhibition time and degree of inhibition into a single quantity by measuring the area under the curve. The ORAC method is not affected by dilution.

3.3.3 Phenolic

The analysis made use of the Folin Ciocalteu reagent with gallic acid as the standard to measure total polyphenols in a sample.

3.3.4 ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay)

The radical cation can be monitored by measurement of one of its characteristic absorption maxima at 640, 734 and 820 nm. Antioxidants added to this system can either scavenge the $ABTS^{\bullet+}$ formed or interfere in the radical generating process. Measurement of the absorbance at a specific time after addition enables the calculation of a percentage scavenging.

3.3.5 Flavonols plate reader

The analysis makes use of quercetin as the standard for measuring total phenolics at 360 nm. The extracts are tested for the total phenolics in Flavonol plate reader at a wavelength of 360 nm.

3.3.6 Ferric Reducing Antioxidant Power Assay (FRAP)

The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method, employing an easily reduced oxidant present in stoichiometric excess. At low pH, reduction of a ferric tripyridyltriazine, Fe(III)-TPTZ complex to the ferrous form, which has an intense blue colour, can be monitored by measuring the change in absorption at 593 nm. The reaction is non-specific, in that any half-reaction that has a lower redox potential, under reaction conditions, than that of the ferric/ferrous half-reaction will drive the ferric to ferrous reaction. The change in absorbance, therefore, is directly related to the reducing power of the electron donating antioxidants present in the reaction mixture.

3.4.1 Alpha-glucosidase

The α-glucosidase was dissolved in 100 mM phosphate buffer at pH 6.8 and this was used as the enzyme extract. *P*-Nitrophenyl- α -D-glucopyranoside was used as the substrate. The plant extracts are prepared in concentration ranges (100 µg/ml – 5 µg/ml or lower). The different plant extract concentrations were mixed with 320 µL of 100 mM phosphate buffer at pH 6.8 and incubated at 30 $^{\circ}$C for 5 mins. 3 ml of 50 mM sodium hydroxide was added to the

mixture. The absorbance was read at 410 nm. The control samples were prepared without any plant extracts (Sindhu et al., 2013).

Acarbose was used as reference α-glucosidase inhibitor; Acarbose is obtained from the fermentation process of a microorganism Actinoplanes *utahensis.*

3.5 Alpha-amylase

The alpha-amylase assay was a modification of the methods from Keerthana et al. (2013). 600 µl of different concentration dilutions of plant extracts (100 µg/ml – 5 µg/ml or lower) were added to 1.2 ml of starch in phosphate buffer (pH 6.9). The phosphate buffer had 6.7 mM NaCl added to it. The reaction was initiated by adding 600 µl of Porcine/human pancreatic amylase (from Sigma) and it was then incubate at 37 $^{\circ}$C for 30 min. From the above mixture 600 µL was taken and 300 µL of DNSA (3,5 dinitrosalicylic acid). (DNSA = [1 g of DNS, 30 g of sodium potassium tartrate and 20 ml of 2 N NaOH]) was added to it and the final volume was made up to 100 ml with distilled water. It was kept in a boiling water bath for 15 min. The reaction mixture was diluted with 2.7 ml of water and the absorbance was read at 540 nm.

For each concentration the following were made:

- Blank tubes by replacing the enzyme with 600 µl distilled water.
- Control represents 100% enzyme without extracts.
- Repeat each concentration 3 to 4 times.

3.6 Cytotoxicity

The cells were seeded into a 96-well plate at 6000 cells/well in 200 µl aliquots in RPMI 1640 (Roswell Park Memorial Institute 1640) medium was originally developed to culture human leukemic cells in suspension as a monolayer. The formulation is based on the RPMI 1640 media utilizing a bicarbonate buffering system and alterations in the amounts of amino acids and vitamins. The RPMI 1640 medium is now used for the culture of normal and neoplastic leukocytes (Sigma Aldrich, 2016, and Thermo Fisher Scientific, 2016):10% FBS (Fetal Bovine Serum). The plate was incubated at 37 ^{0}C for 24 hrs to allow cells to attach. The plant extracts were then screened for cytotoxicity using concentrations ranging from 1-250 µg/ml. A positive control was used in concentrations ranging from 10-100 µM. After the 24 hour incubation period the growth medium was removed and 200 µl aliquots of the plant extracts were added to it and the plates were incubated for a further 48 hrs. After the 48 hours

incubation period the medium was replaced with 200 µl MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium Bromide). The MTT was then removed after further 4 hrs of incubation at 37 °C and the product was dissolved in a 100 µl/well DMSO. Plates were agitated for 60 seconds and absorbance was measured at 540 nm (Deutschlander et al., 2009).

3.7 Chemical studies

The isolation, purification and analysis of the active extracts to get pure compounds were done using various chromatographic techniques such as TLC and HPLC. Moreover, spectroscopic techniques such as, LC-MS and NMR spectroscopy were utilized.

3.7.1 Secondary plant metabolites

Phytochemical screening was performed on the extracts using standard procedures provided by Trease & Evans (1989) and Tiwari et al. (2011). These were used to test for secondary plant metabolites such as alkaloids, steroids, reducing sugars, anthraquinones, flavonoids, terpenoids, saponins, tannins, glycosides and gums. The phytochemical screening was done on the methanol and aqueous extracts of each plant by making use of the modifications of the methods by Trease et al., (1989) and Harbone et.al. (1984), as detailed below:

3.7.2 Detection of phytosterols

Liberman-Burchard test

Total steroid constituents were determined by adding 1 ml chloroform, 2 ml of concentrated sulphuric acid and 1 ml acetic anhydride to each extract. The solution was shaken; the presence of a reddish violet colour indicated the presence of steroids (Trease & Evans, 1989).

Salkowiski test

2ml of each extract solution was extracted in 1 ml of chloroform and 1 ml of concentrated sulphuric acid was added and the solution was shaken. The presence of a reddish-blue colour in the chloroform layer and green fluorescence in the acid layer indicated the presence of steroids.

3.7.3 Detection of flavonoids

4 ml of the extract solutions were hydrolyzed with 10 % v/v sulphuric acid and were allowed to cool. The solutions were extracted with diethyl ether and divided into 3 portions. Each of the portions was diluted with 1 ml ammonia, 1 ml sodium bicarbonate and 1 ml sodium bicarbonate and 1 ml of 0.1 N sodium hydroxide. The development of a yellow colour indicates the presence of flavonoids.

3.7.4 Detection of proteins and amino acids

Biret test: 1 ml of 40% NaOH (v/m) was mixed with two drops of 1% copper sulphate and was added to the extract solutions. A violet colour indicates the presence of proteins.

Ninhydrin test: Two drops of a freshly prepared 0.2 % Ninhydrin reagent were added to each extract solution and heated. A blue colour indicates the presence of proteins, amino acids or peptides.

Xanthoprotein test: The extract solutions were treated with 1 ml of concentrated ammonia. An orange colour indicates the presence of aromatic amino acids.

3.7.5 Detection of glycosides

The legal test: Each extract solution was dissolved in pyridine, sodium nitroprusside solutions and made alkaline. A pink-red colour indicates the presence of glycosides.

Baljet test: Sodium picrate solution was added to each extract solution and the development of a yellow to orange colour indicates the presence of glycosides.

Borntrager's test: 2 ml of diluted sulphuric acid were added to the extract solutions. The extract solutions were boiled and the samples were filtered. The filtrates were extracted with chloroform. The organic layers were separated. To the organic layers, a few drops of ammonia were added. The presence of a pink to red colour in the organic layer indicates a positive test for glycosides.

3.7.6 Detection of pentose

2 ml of each extract solutions were dissolved in concentrated hydrochloric acid phloroglicinol (1:1) and heated in a test tube for 5 min. The development of a red colour confirms the presence of pentose sugars.

3.7.7 Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (potassium mercuric iodide). The formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (iodine in potassium iodide). The formation of a brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of potassium bismuth iodide). The formation of a red precipitate indicates the presence of alkaloids.

3.7.8 Detection of phenols

Ferric chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. The formation of a bluish black colour indicates the presence of phenols.

3.7.9 Detection of saponins

Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of a 1 cm layer of foam indicates the presence of saponins.

3.7.10 Detection of tannins

Method 1: 1 ml of the extract was added in 2 ml of water in a test tube. 2 to 3 drops of diluted ferric chloride solution were added and observed for green to blue-green (cathetic tannins) or a blue-black (gallic tannins) colour.

Method 2: 2 ml of the aqueous extract was added to 2 ml of water, 1 to 2 drops of diluted ferric chloride solution were added. A dark green or blue green coloration indicates the presence of tannins.

3.7.11 Detection of reducing sugars

5-8 drops of Fehling's solution were added to 2 ml of plant extracts and then boiled. The formation of a red-brick precipitate indicated the presence of reducing sugars.

3.7.12 Detection of triterpenoids

0.5 ml of the plant extract was mixed with 2 ml of $CHCl_3$ in a test tube. 3 ml of concentrated H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish brown coloration was formed in the presence of triterpenoids, as positive result.

3.7.13 Detection of anthraquinones

2 ml of diluted sulphuric acid was added to the extract solutions. The extract solutions were boiled and the samples were filtered. The filtrates were extracted with chloroform. The organic layers were separated. To the organic layers, a few drops of ammonia were added. The presence of a pink to red colour in the organic layer indicates a positive test for anthraquinones.

3.7.14 Detection of gums

10 ml of extract solution was mixed with 25 ml of alcohol with constant stirring. A white precipitate indicated the presence of gums.

3.8 Quantitative analysis

The ash value of crude medicinal product was determined in three different methods to measure the total ash, acid-insoluble ash and the water-soluble ash.

3.8.1 Total ash

4 g of the crude medicinal product was weighed into a silica crucible that was preheated at 4500 °C and weighed. The crude medicinal product was spread evenly as a fine layer on the

bottom of the crucible. The crucible was incinerated by gradually increasing the temperature to make the crude medicinal product a dull red hot until it was free of carbon. The crucible was cooled and weighed. The percentage total ash was calculated from the air dried crude medicinal product.

3.8.2 Acid-soluble ash

The pre-weighed total ash obtained was boiled with 25 ml of hydrochloric acid for 5 mins. The insoluble ash was collected using an ashless filter paper; the solute was washed with hot water. The insoluble ash was transferred into a silica crucible which was ignited and weighed. The procedure was repeated until constant weight was obtained. The percentage of the insoluble ash based on the weight of the air dried crucible.

3.8.3 Water soluble Ash

The total ash obtained was boiled with 25 ml of water for five minutes. The insoluble ash was collected on an ashless filter paper and washed with hot water. The insoluble ash was transferred into a silica crucible ignited for 15 mins at a temperature exceeding 4500 °C, and weighed. The procedure was repeated until constant weight was obtained. The weight of the insoluble ash was subtracted from the weight of the total ash. The difference in weight was due to the water-soluble ash. The percentage soluble ash was calculated from the total ash.

3.8.4 Sulphated Ash

The silica crucible was heated to redness for 10 mins, cooled in desiccators and weighed. The crude medicinal product was weighed accurately and transferred into a crucible, ignited gently until charred. The residue was cooled and moistened with 1 ml of concentrated sulphuric acid. It was heated gently until there were no white fumes and ignited at 8000 °C ± 250 °C until all black particles had disappeared.

3.8.5 Determination of extracted values

The determination of extracted values is useful as a means of evaluating crude drugs which cannot be readily estimated by other means. Extractive values can be estimated using water and alcohol soluble extractives. Extractive values also determine the nature of the constituents present in a crude drug. These extractive values give approximate measures of their chemical contents.

3.8.6 Alcohol-soluble extractive value

5 g of plant material was weighed and macerated with 100 ml of ethanol in a closed container for 20 hrs. The sample was shaken for 6 hrs and allowed to stand and soaked for 18 hrs. After the sample was filtered, 25 ml of the filtrate was evaporated in a flat bottomed shallow dish dried at 1050 °C and weighed. The percentage alcohol extractive material was calculated with reference to the crude drug.

3.8.7 Water-soluble extracted value

5 g of plant material was weighed and macerated with 50 ml of chloroform water at 800 °C separately for 24 hrs. The sample was shaken for 6 hrs and allowed to stand and soaked for 24 hrs. After the sample was filtered, 25 ml of the filtrate was evaporated in a flat bottomed shallow dish dried at 1050 °C and weighed. The percentage water soluble extractive material was calculated based on the total crude drug extracted.

3.9 Chromatographic techniques

This section focuses on the various chromatographic techniques used in the study. The DCM and 1:1 DCM:MeOH extracts of the *Salvia africana-caerulea* plant showed significant results in the biological assays and phytochemical studies. The extracts were therefore subjected to further purification and identification using chromatographic techniques.

3.9.1 Fractionation of plant extracts

a. Flash chromatography method:

0.3944 g of the DCM: MeOH extract of the *Salvia africana-caerulea* was dissolved in 5 mL of MeOH/EtOAc (50:50) and adsorbed onto 5 g of silica powder. The dried powder was brought onto a 50 g silica column and eluted on the flash chromatography system using a step gradient of (1) hexanes: EtOAc (5:1), (2) hexanes: EtOAc (3:1), (3) hexane: EtOAc (3:1), (4) 100% EtOAc, (5) EtOAc: MeOH (5:1), (6) EtOAc: MeOH (3:1) and (7) 50% EtOAc, 50% MeOH. Large fractions of 400 ml of solvent per gradient step were collected. Flash fractions were dried by rotary evaporation (Eldridge et al., 2002).

The fractions collected were 32 and pooled into 16 major fractions on the basis of their TLC profiles. The fractions were subjected to NMR, and high resolution mass spectroscopic analysis.

b. Open column chromatography

1.0800 g of the *Salvia africana-caerulea* DCM extract was dissolved in 5 mL of MeOH/EtOAc (50:50) and adsorbed onto 5 g of silica powder. The dried powder was brought onto a 50 g silica column and eluted on the open column chromatography system using a step gradient of (1) hexanes: EtOAc (5:1), (2) hexanes: EtOAc (3:1), (3) hexanes: EtOAc (1:1), (4) 100% EtOAc, (5) EtOAc: MeOH (5:1), (6) EtOAc: MeOH (3:1) and (7) EtOAc: MeOH (1:1). Large fractions of 400 ml of solvent per gradient step were collected. The collected fractions were continuously monitored with TLC and similar fractions were combined and dried by rotary evaporation (Eldridge et al., 2002). The fractions collected were 38 and pooled into 17 major fractions on the basis of their TLC profiles. The fractions were subjected to NMR, and high resolution mass spectroscopy analysis.

3.9.2 Thin layer chromatography

The crude extracts of the diabetes tea and the eight plants were subjected to bioassays to determine their antidiabetic activity and cytotoxicity. The plant with extract(s) that showed significant biological activity in all the tests performed was then used for further purification and isolation. All plants showed significant results in all the preliminary tests done but for the purpose of this study *Salvia africana-caerulea* which commonly known as Wild sage was chosen because of the limited research and studies done on the plant. The DCM and 1:1 DCM:MeOH extracts of *Salvia africana-caerulea* were the extracts that were used for further purification and isolation. The extracts were fractionated and the fractions were then subjected to TLC analysis to determine the purity of the fraction.

3.9.3 TLC of crude extracts

The hexane, DCM, 1:1 DCM:MeOH, MeOH and H_2O extracts of the *diabetes tea* and *Salvia africana-caerulea* were subjected to TLC analysis to screen for the presence of different groups of phytochemicals. Hexane is reported to extract fatty acids and waxes; DCM extracts terpenoids and non-polar compounds; MeOH extracts polar compounds; and H_2O extracts very polar compounds and carbohydrates.

3.9.4 Sample preparation for crude extracts

The dry extracts were reconstituted in their different solvents. The extracts were sonicated and slowly stirred using a vortex to ensure homogeneity.

3.9.5 TLC plate development for crude extracts

Preliminary screening tests were performed to identify optimum solvent systems for use as mobile phase. Common solvent systems for a specific group of phytochemicals previously used in the laboratory were tried and modified accordingly to give optimum separation of the extracts. All the crude extracts of the *diabetes tea* (hexane, DCM, DCM:MeOH (1:1), MeOH and H_2O) and *Salvia africana-caerulea* (hexane, DCM, 1:1 DCM:MeOH, MeOH and H_2O) were screened using different ratios (1:1; 1:2 and 1:3) of hexane and ethyl acetate.

Samples were spotted on the 20 x 20 cm aluminum backed TLC plates (Merck Silica 60 F_{254}). The samples were each spotted using a micropipette. Each extract was spotted on separate plates. In each plate the samples were spotted 0.5 cm apart and developed for a distance of 7-8 cm in a closed development tank saturated with mobile phase.

3.9.6 TLC of fractions

The DCM and 1:1 DCM:MeOH extracts of *Salvia africana-caerulea* were used for further purification and isolation.

3.9.7 Sample preparation of fractions

The fractions were prepared using the procedure highlighted in section 3.9.1.

3.9.8 TLC plate development for fractions

Extensive TLC screening tests were performed on the crude extract to identify optimum solvent systems for use as mobile phase. The extracts were then fractionated using column chromatography and flash chromatography as highlighted in section 3.9.1. The fractions were done using ethyl acetate and hexane at different ratios. The step gradient elution used was (1:5) Hex: EtOAc; (1:3) Hex: EtOAc; (1:1) Hex: EtOAc; 100% EtOAc; (1:5). The fractions were then spotted on a 20 x 20 cm aluminium backed TLC plates (Machenery Nagel Silica gel 60 F_{254}). The fractions were each spotted using a micropipette.

In each plate the samples were spotted 0.5-1 cm apart and developed for a distance of 7-8 cm in a closed development tank saturated with mobile phase.

3.10 Nuclear Magnetic Resonance (NMR)

A 600 MHz Bruker Advance spectrometer was used to record the 1H NMR, COSY, HMBC, HMQC (600 MHz) and 13C, APT (150 MHz) experiments in either $CDCl_3$-d1, (δH = 7.24; δC = 77.2), acetone-d6, (δH = 2.04; δC = 29.8) and methanol- d4, (δH = 4.87 and 3.31; δC = 49.2) with tetramethylsilane (TMS) as internal standard. Chemical shifts were expressed as parts per million (ppm) on the delta (δ) scale and coupling constants (j) are accurate to 0.01 Hz.

3.11 Infra-red Spectroscopy

Solid state FT-IR spectra were recorded as neat compounds on a Bruker Tensor 27 spectrometer.

3.12 Liquid Chromatography- Mass Spectroscopy (LC-MS)

High resolution mass spectra data (HRMS) and low resolution mass spectra data (LRMS) were collected using a Waters Micromass LCT Premier TOF-MS spectrometer. All samples were dissolved and diluted to 2 ng/µL and infused without additives. The sample (DM 23) was infused into the MS and data collected in ES^+ modes.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Introduction

Although medicinal plants have long been used as medicines by humankind, the practise still faces a lot of controversy due to efficacy and safety concerns (Nyika, 2009). Due to this WHO highly advocates for strengthened efforts on medicinal plant research with great emphasis on supplying scientific explanations on plant-based medicine and isolation of new bioactive compounds that can be used in new drug development (Gurib-Fakim, 2006). This study focused on a diabetes tea and its eight constituent plants. The tea is widely used in South Africa to manage and treat diabetes mellitus, but little is however known about the chemical composition of the diabetes tea.

This study qualitatively and quantitatively assessed and evaluated the phytochemical profiles of the diabetes tea and its constituent plants. This was coupled with the antioxidant activity, antidiabetic activity, cytotoxicity and isolation of some compounds from selected extracts. This chapter provides a detailed presentation and discussion of the results obtained in the study.

4.2 Extraction yields

Five solvents of increasing polarity (hexane, DCM, DCM:MeOH, MeOH and water) were used to sequentially extract phytochemicals from the eight plants and the diabetes tea. The percentage yields (w/w) were calculated. The percentage yields reflect the quantity of phytochemicals extracted relative to the weight of the dry plant material used. It is a factor of numerous variables including the extraction solvent and extraction method used. Depending on their selectivity and polarity, different solvents extract different amounts of varying groups of phytochemicals.

Table 4.1 and **Table 4.2** present the different percentage yields of the eight plants and the diabetes tea in different solvents. Taking into consideration that secondary metabolites generally account for about 10% of dry plant material, the yields recorded are relatively high. There are plants that show very low extraction yields in certain solvents which may need different extraction methods or extraction solvents from the one used in order to extract a significant percentage of phytochemicals.

Table 4.1: Percentage yields of the hexane, DCM; 1:1 DCM:MeOH and MeOH extracts of plants

Plant	**Mass weighed** g	**HEXANE Extract** %	**DCM Extract** %	**1:1 DCM:MeOH extract** %	**MeOH extract** %
*Trigonella foenum graecum (*fenugreek)	10.1847	0.06	0.01	0.12	0.09
Achillea millefolium (yarrow)	10.1808	1.78	2.28	14.57	6.16
Agothosma Betulina (Buchu)	10.2699	1.50	1.97	70.93	3.11
Salvia officinalis (Garden sage)	10.1617	5.87	3.16	3.27	3.62
Salvia africana-caerulea (wild sage)	10.0238	5.31	1.84	7.45	3.77
Taraxum offinale (dandelion)	10.4564	6.34	0.36	11.68	3.14
Thymus vulgaris (garden thyme)	10.4264	4.10	3.15	11.74	9.59
Urtica Urens (small nettle)	10.1636	3.19	1.92	9.58	4.06
Diabetes tea	10.4495	3.19	1.68	1.69	3.13

The sequential extraction procedure allows for the selective and efficient extraction of different groups of phytochemicals. In all the plant material used water and DCM:MeOH extracted the highest percentage of phytochemicals.

Table 4.2: Percentage yields of the water extracts of the plant material

Plant	Mass weighed g	Mass of water extract g	Water extract %
Trigonella foenum graecum	10.21	0.6716	6.58
Achillea millefolium	10.36	1.6455	15.88
Agothosma Betulina	10.63	1.3895	13.07
Salvia officinalis	10.60	1.3299	12.55
Salvia africana-caerulea	10.37	0.8404	8.10
Taraxum offinale	10.24	1.7064	16.66
Thymus vulgaris	10.95	0.8163	7.45
Urtica Urens	10.25	1.6869	16.46
Diabetes tea	10.44	2.1993	21.07

Trigonella foenum graecum: The extraction method used did not give a satisfactory percentage yield results. The percentage yield from the solvents were very low which shows that the method of extraction needs to be revisited. The results obtained showed that water extracts the highest percentage of phytochemicals with a percentage of 6.58%. The other solvents had very low extraction percentages that were below 1% which can be attributed to the poor choice of the extraction method. A study done by Kumar et al. (2014) showed that when the *Trigonella foenum graecum* seeds were extracted by soaking them in double distilled water at room temperature for hours and later boil with sufficient double distilled water while stirring, the percentage yield was 36% which is much higher than the water extract yield obtained in this study. In another study (Bukhari et al., 2008) the dry seeds were grounded and extracted with different solvents using the soxhelt extraction method. The percentage yield obtained for the different solvents were as follows: hexane (9.68%); DCM (12.96%) and MeOH (25.89%). This method used the same amount of sample used in this study but obtained higher yields due to the different extraction method used.

Achillea millefolium: Results obtained show that for water extracts the highest percentage of phytochemicals was 15.88%. This was followed by DCM:MeOH and MeOH at 14.57% and 6.16%, respectively. Hexane and DCM have relatively low yields compared to the other solvents which show that both solvents extract the least phytochemicals. In a review done by Fiume (2001) *A. millefolium* was subjected to a cold extraction using a mixture of ethyl alcohol and water and about 50-60% of the solid extract was obtained. This study shows that water and methanol extract high amounts of phytochemicals.

Agothosma betulina: The results obtained show that the water extracts the highest percentage of phytochemicals with a percentage of 13.07%, followed by DCM:MeOH at 7.93%. Hexane, MeOH and DCM have relatively low percentage yields compared to the other solvents which show that they both extract the least amount of phytochemicals.

Salvia officinalis: The results obtained show that water extracts the highest percentage of phytochemicals with a percentage of 11.74%, followed by hexane with a percentage of 5.87%. DCM, DCM:MeOH and MeOH have relatively low yields which are slightly above 3% compared to the solvents.

Salvia africana-caerulea: The results obtained show that water extracts the highest percentage of phytochemicals with a percentage of 8.10%, followed by DCM:MeOH at 7.45% and hexane at 5.31%. DCM and MeOH have relatively low percentage yields compared to the other solvents. Fisher (2005) extracted essential oils from the *S. Africana-caerulea* plant using hydrodistillation in a clevenger-type apparatus for 3 hrs. The yield obtained from 335 g of the plant was 0.17%. Hydrodistillation does not use solvents and thererfore results in low yields.

Taraxum offinale: The obtained results show that water extracts the highest percentage of phytochemicals with a high percentage of 16.66%, followed by DCM:MeOH with 11.68% and hexane with 6.34%. DCM and MeOH have relatively low percentage yields compared to the other solvents.

Thymus vulgaris: The results obtained show that the DCM:MeOH solvent extracts the highest percentage of phytochemicals with a high percentage of 11.74%, followed by MeOH and water at 9.59% and 7.45%, respectively. Hexane and DCM have relatively low percentage yields compared to the other solvents. In a study by Porte and Godoy (2008), fresh leaves of thyme were subjected to hydrodistillation for 93 min to extract essential oils. The yield of the essential oil based on the dry mass was obtained as 1.1%.

The essential oils of the *T. vulgaris* plant are known for their medicinal oils and contain a significant number of flavonoids. The highest yields were obtained by the most polar solvents, therefore it can be concluded that T. vulgaris contains polar phytochemical constituents such as sugars, poly-phenols, etc.

Urtica urens: The results show that water extracts the highest percentage of phytochemicals with a high percentage of 16.46%, followed by DCM:MeOH with a percentage of 9.58%. hexane, DCM and MeOH have relatively lower percentage yields compared to the other solvents. In a study by Steenkamp et al., (2004) the percentage yield obtained was very low. The extracts were obtained using 1 g of plant which was added to deionized water and boiled and another 1 g was added to 10 ml of MeOH and macerated for 24 hours. The extraction method and extraction solvents used in this study were favourable as they gave significant amounts of yield. The lowest yield was the DCM solvent yield. The more polar solvents extracted the highest percentage yield so it can be concluded that the plant contains polar phytochemical constituents such as poly-phenols, terpenoids, sugars, tannins, saponins, alkaloids etc.

Tea: The results show that water extracts the highest percentage of phytochemicals with a high percentage of 21.07%. Other solvents show relatively lower percentage yields compared to water.

4.3 Biological Studies

4.3.1 Antioxidant results

The tables below show the results of the antioxidant analysis of the plants and the tea mixture. The results obtained showed that the plants and tea mixture contain antioxidants. While in some plants there were colour interferences (CI) and no detection (ND) the results were favourable and showed that these plants contained a significant amount of antioxidants. The flavonoids showed colour interference in all the extracts and therefore they were not detected.

4.3.2 Antioxidant concentrations of *T. foenum graecum* extract

The antioxidant analysis of *Trigonella foenum graecum* extracts is presented in **Table 4.3**, below. The ABTS assays showed no detection of antioxidants for all extracts. The flavonoids and FRAP assays showed no detection of antioxidants for all other extracts except for the water extract.

The non-detection of the antioxidant compounds in the extract could be attributed to the extraction method used to extract the seeds or impurities in the plant extract. Burkhan et al. (2008) showed that the ethanol and methanol extracts of the seeds showed a significantly high total phenolic content, total flavonoid, ferric reducing antioxidant power (FRAP) and radical scavenging activity (DPPH). Ethanol and methanol are highly polar and contain high yields of phenolic compounds therefore antioxidant activity could be correlated with polyphenols present in the extracts. In this study the DCM and water extracts showed the highest antioxidant concentration in the ORAC assay with the values 1251.30±2.22 µmol TE/g and 1327.01±1.63 µmol TE/g, respectively. In comparison with the ORAC value of brewed rooibos tea (1402±44.1 µmol TE/g), the extracts showed lower concentrations (Marnewick et al., 2011).

Table 4.3: Antioxidant analysis of the *T. foenum-graecum* extracts

Extracts	ORAC µmol TE/g	Phenols mg GAE/g	Flavonols mg QE/g	FRAP µmol AAE/g	ABTS µmol AAE/g
MeOH	20.83 (7.41)*	40.19 (7.47)*	ND	ND	ND
DCM	1251.30 (2.22)*	3.11 (5.53)*	ND	ND	ND
1:1 DCM:MeOH	228.11 (1.81)*	9.32 (3.31)*	ND	ND	ND
Water	1327.01 (1.63)*	5.35 (3.31)*	1.79 (2.71)*	5.01 (1.43)*	ND

ND: Not detected; *RSD; TE: troxol equivalent; GAE: gallic acid equivalent; QE: quercetin equivalent; AAE:ascprbic acid equivalent

4.3.3 Antioxidant concentrations of *A. millefolium* extracts

Table 4.4 below shows the results of the antioxidant analysis of *A. millefolium*. The results give proof that the plant possesses a significant amount of antioxidants. The overall results were favourable and showed that yarrow can contribute significantly to its antioxidant activity and consequently its antidiabetic properties. The water extract showed the most significant antioxidant concentration in the ORAC assay with a value of 5617.01±4.11 µmol TE/g followed by the MeOH extract which had a concentration of 1031.73±1.26 µmol AAE/g in the FRAP assay. The values were compared to those of rooibos tea in a study by Marnewick et al. (2011), the ORAC value of 1402±44.1 µmol TE/g and for the FRAP assay the value was 260.2±24.8 µmol AAE/g. The ORAC value of the water extract was significantly higher than the ORAC value of the rooibos tea while the FRAP assay results of the MeOH were significantly lower than the rooibos FRAP results.

Table 4.4: Antioxidant analysis of the *A. millefolium* extracts

Extracts	ORAC µmol TE/g	Phenols mg GAE/g	Flavonols mg QE/g	FRAP µmol AAE/g	ABTS µmol AAE/g
MeOH	754.77 (4.11)*	65.13 (2.96)*	78.65 (4.64)*	1031.73 (1.26)*	261.8 (7.99)*
DCM	409.85 (7.62)*	229.89 (5.73)*	42.60 (4.97)*	ND	ND
1:1 DCM:MeOH	438.65 (3.08)*	3.08 (5.95)*	79.08 (7.08)*	156.81 (7.96)*	6.09
Water	5617.01 (1.19)*	17.60 (2.84)*	59.93 (6.95)*	194.17 (7.49)*	109.2 (3.17)

ND: Not detected; *RSD; TE: troxol equivalent; GAE: gallic acid equivalent; QE: quercetin equivalent; AAE:ascprbic acid equivalent

4.3.4 Antioxidant concentrations of *A. betulina* extracts

The results in **Table 4.5** give evidence that the plant possesses a significant concentration of antioxidants and therefore has a potential to have antidiabetic activity. The MeOH and 1:1 DCM:MeOH extracts showed no detection of antioxidants in the ABTS assay. Moolla et al. (2007) reported that Agothosma betulina demonstrated antimicrobial activity, antioxidant activity and anti-inflammatory activity. Although the plant showed antioxidant activity, the activity was weak and with extracts having IC_{50} values > 100 µg/ml. The detected antioxidant

activity in the extracts is significant and different from the weak antioxidant activity on the study done by Moolla et al. (2007). The plant extract showed favourable amounts of antioxidants with the DCM and water extracts showing the highest concentrations. The DCM extract showed a concentration of 14333.62±7.37 µmol TE/g and the water extract had a concentration of 7207.29±1.59 µmol TE/g in the ORAC assay. Both extracts show a significantly higher concentration of antioxidants compared to the brewed rooibos tea which showed a concentration of 1402±44.1 µmol TE/g (Marnewick et al., 2011).

Table 4.5: Antioxidant analysis of the *A. belutina* extracts

Extracts	ORAC µmol TE/g	Phenols mg GAE/g	Flavonols mg QE/g	FRAP µmol AAE/g	ABTS µmol AAE/g
MeOH	1228.22 (2.68)*	24.63 (0.77)*	38.77 (1.15)*	169.81 (9.60)*	ND
DCM	14333.62 (7.37)*	49.39 (3.89)*	210.76 (2.12)*	29.87 (7.77)*	36.1 (2.98)*
1:1 DCM:MeOH	974.64 (6.89)*	81.61 (4.88)*	52.96 (3.23)*	173.22 (5.83)*	ND
Water	7207.29 (1.59)*	20.60 (5.63)*	46.33 (2.25)*	242.26 (4.35)*	81.5 (2.85)*

ND: Not detected; *RSD; TE: troxol equivalent; GAE: gallic acid equivalent; QE: quercetin equivalent; AAE:ascprbic acid equivalent

4.3.5 Antioxidant concentrations of the *S. officinalis* plant

Table 4.6 shows the results of the antioxidant analysis of *S. officinalis*. It can be deduced from the results that the plant possesses a significant amount of antioxidants that has the potential to contribute towards the antidiabetic activity of the diabetes tea. In a study by Lima (2006) the antioxidant properties of sage were investigated using in vitro studies. *S. officinalis* alcoholic extracts revealed strong antioxidant activity. The antioxidant activity of *S. officinalis* extracts were established using methods such as: the accelerated antioxidation of Methyl linoleate, the ability to scavenge DPPH and ABTS free radicals as well as by the oxygen radicals' absorbance capacity (ORAC) assay. The DCM and water extracts showed a concentration of 13993.57±3.20 µmol TE/g and 6161.90±0.77 µmol TE/g in the ORAC assay, respectively. The MeOH extract showed significant concentrations in the FRAP, ORAC and ABTS assays with the following values 876.86±1.23 µmol AAE/g, 2484.49±1.71 µmol TE/g and 274.1±4.12 µmol AAE/g, respectively. The 1:1 DCM:MeOH extract showed a

concentration of 300.04±7.69 µmol AAE/g. The concentrations obtained were compared to brewed rooibos concentrations 1402±44.1 µmol TE/g (ORAC), 260.2±24.8 µmol AAE/g (FRAP) and 173.5±12.8 µmol AAE/g (ABTS) (Marnewick et al., 2011). This proves that the results obtained in the current study are significant as they show that the sage plant possesses antioxidant concentration higher than that of the rooibos tea. It has been reported in various studies that the leaves of *S. officinalis* are well known for their phenolic structure-based antioxidant potency.

The main sage phenolic diterpenes show high antioxidant activity in various studies. These are carnosic acid, which is known for its instability and degradation derivatives carsonol, rosmanol; its isomer epirosmanol 7-methyl-epirosmanol as well as rosmanol 9-ethyl ether (Baricevic & Bartol, 2000). Rosmarinic acid is also reported to account for the antioxidant activity in sage plants. A squalene derivatives triterpenoid ursolic acid and its isomer oleanolic acid act as anti-inflammatories and inhibit tumorigenisis in mouse skins. These compounds form up to 4% of sage leaves on dry weight basis (Baricevic & Bartol, 2000).

Table 4.6: Antioxidant analysis of the *S. officinalis* extracts

Extracts	ORAC µmol TE/g	Phenols mg GAE/g	Flavonols mg QE/g	FRAP µmol AAE/g	ABTS µmol AAE/g
MeOH	2484.49 (1.71)*	46.07 (1.99)*	111.94 (5.36)*	876.83 (1.23)*	274.1 (4.12)*
DCM	13993.57 (3.20)*	48.84 (3.27)*	71.44 (5.88)*	19.34 (8.73)*	36.0 (4.69)*
1:1 DCM:MeOH	841.42 (0.88)*	18.09 (2.56)*	59.00 (0.49)*	300.04 (7.69)*	163.3 (4.20)*
Water	6161.90 (0.77)*	29.31 (1.78)*	68.16 (2.50)*	156.03 (3.40)*	78.1 (3.76)*

ND: Not detected; *RSD; TE: troxol equivalent; GAE: gallic acid equivalent; QE: quercetin equivalent; AAE:ascprbic acid equivalent

4.3.6 Antioxidant concentrations of the *S. africana caerulea* plant

The results in **Table 4.7** of the antioxidant analysis of *S. africana caerulea* show that the plant possesses a significant amount of antioxidants. It must be noted that the flavanoids were not detected for all extracts due to colour interferences. In the DCM extract the

Flavonols, FRAP and ABTS assays showed no detection of the antioxidants due to colour interferences. The water extract showed a concentration of 14147.10±1.02 µmol TE/g and the DCM extract has a concentration of 12723.47±2.88 µmol TE/g for the ORAC assays; this is significantly higher than the brewed rooibos tea concentration of 1402±44.1 (Marnewick et al., 2011). The results obtained from the FRAP assay are all significantly higher than the brewed rooibos concentration of 260.2±24.8 µmol AAE/g (Marnewick et al., 2011).

The overall results were significant and showed that *S. africana caerulea* as a as a plant that is found in the tea can contribute significantly to its antioxidant concentration and consequently its antidiabetic properties.

Table 4.7: Antioxidant analysis of the *S. africana caerulea* extracts

Extracts	ORAC µmol TE/g	Phenols mg GAE/g	Flavonols mg QE/g	FRAP µmol AAE/g	ABTS µmol AAE/g
MeOH	1693.05 (1.28)*	101.08 (3.20)*	59.52 (1.05)*	1010.94 (0.16)*	545.2 (0.12)*
DCM	12723.47 (2.88)*	158.59 (4.68)*	ND	ND	ND
1:1 DCM:MeOH	564.46 (0.98)*	29.92 (2.91)*	55.23 (7.71)*	681.94 (3.60)*	337.8 (7.42)*
Water	14147.10 (1.02)*	79.81 (4.10)*	85.46 (2.68)*	495.25 (1.04)*	143.7 (7.72)*

ND: Not detected; *RSD; TE: troxol equivalent; GAE: gallic acid equivalent; QE: quercetin equivalent; AAE:ascprbic acid equivalent

4.3.7 Antioxidant concentrations of the *T. officinale* extract

The antioxidant analysis results of *T. officinale* in **Table 4.8** show that the plant possesses a significant amount of antioxidants. The flavanoids were not detected due to colour interferences. The DCM extract shows no activity on the flavonoids, FRAP and ABTS assays. The overall results were not significant when compared to a study of rooibos tea by Marnewick et al. (2011). The extracts showed significantly lower concentrations of antioxidants in all the assays with the exception of the water extract which had a concentration of 1504.52±2.50 µmol TE/g in the ORAC assay and the MeOH extract with a concentration of 271.93±2.93 µmol AAE/g. The ORAC and FRAP results of the brewed rooibos tea were 1402±44.1 µmol TE/g and 260.2±24.8 µmol AAE/g, respectively. Therefore

the water and MeOH extracts gave better results. In a study by Hu & Kitts (2003), dandelion flower extracts, in particular the EtOAc fraction, scavenged the reactive oxygen species (ROS) and prevented DNA from ROS-induced damage in vitro. The suppression of the oxidative stress was attributed to luteolin and luteolin 7-O-glucosidase which are present in the plant. It was further concluded that a standardized dandelion flower extract suppresses both ROS and RNS in chemical and biological models (Hu & Kitts, 2004). The free-radical-scavenging capacity of the dandelion flower extracts were attributed to the total phenolic content in the extracts. The literature shows that the dandelion plant is a rich source of antioxidants that have potential antidiabetic activity.

Table 4.8: Antioxidant analysis of the *T. officinale* extracts

Extracts	ORAC µmol TE/g	Phenols mg GAE/g	Flavonoids mg QE/g	FRAP µmol AAE/g	ABTS µmol AAE/g
MeOH	1301.20 (2.70)*	99.28 (2.77)*	56.91 (5.44)*	271.93 (2.93)*	142.2 (5.80)*
DCM	363.73 (4.94)*	45.22 (8.11)*	ND	ND	ND
1:1 DCM:MeOH	270.09 (3.34)*	0.69 (6.74)*	4.72 (5.85)*	233.82 (1.87)*	10.9 1.66
Water	1504.52 (2.50)*	19.12 (2.47)*	39.94 (2.77)*	128.42 (5.12)*	59.6 (1.75)*

ND: Not detected; *RSD; TE: troxol equivalent; GAE: gallic acid equivalent; QE: quercetin equivalent; AAE:ascprbic acid equivalent

4.3.8 Antioxidant concentrations of the *T. vulgaris* extracts

Table 4.9 shows the results of the antioxidant analysis of *T. vulgaris*. It is evident from the results obtained that the plant possesses a significant concentration of antioxidants. The overall results were favourable and showed that *T. vulgaris* can contribute significantly to the antioxidant activity and consequently its antidiabetic properties of the diabetes tea. The water extract and the 1:1 DCM:MeOH extract showed the most significant concentrations of 2827.36±1.33 µmol TE/g and 1789.81±0.98 µmol TE/g in the ORAC assay while brewed rooibos tea had a concentration of 1402±44.1 µmol TE/g in a study by Marnewick et al. (2011). The MeOH extract had a concentration of 716.60±2.17 µmol AAE/g and this is significantly higher than the 260.2±24.8 µmol AAE/g of the brewed rooibos tea (Marnewick et al., 2011).

Table 4.9: Antioxidant analysis of *T. vulgaris* extracts

Extracts	ORAC µmol TE/g	Phenols mg GAE/g	Flavonols mg QE/g	FRAP µmol AAE/g	ABTS µmol AAE/g
MeOH	537.84 (1.42)*	210.51 (1.37)*	120.71 (3.25)*	716.60 (2.17)*	493.7 (5.03)*
DCM	1440.41 (1.14)*	29.85 (3.05)*	55.73 (5.88)*	108.42 (4.27)*	ND
1:1 DCM:MeOH	1789.81 (0.98)*	53.80 (2.87)*	26.11 (2.10)*	249.15 (7.80)*	414.3 (3.58)*
Water	2827.36 (1.33)*	21.57 (7.69)*	61.15 (4.06)*	223.71 (1.14)*	59.1 (4.05)*

ND: Not detected; *RSD; TE: troxol equivalent; GAE: gallic acid equivalent; QE: quercetin equivalent; AAE:ascprbic acid equivalent

4.3.9 Antioxidant concentrations of the *U. urens* extracts

The water extracts showed a concentration of 2194.89±2.34 µmol TE/g (**Table 4.10**). This is significantly higher than the 1402±44.1 µmol TE/g of brewed rooibos tea (Marnewick et al., 2011). The overall results of the extracts did not show any significant concentrations.

Table 4.10: Antioxidant analysis of the U. *urens* extracts

Extracts	ORAC µmol TE/g	Phenols mg GAE/g	Flavonols mg QE/g	FRAP µmol AAE/g	ABTS µmol AAE/g
MeOH	241.99 (8.15)*	10.60 (10.78)*	8.73 (0.85)*	39.98 (1.58)*	ND
DCM	909.57 (7.37)*	18.17 (5.69)*	91.29 (1.72)*	ND	ND
1:1 DCM:MeOH	335.44 (5.76)*	22.05 (3.55)*	93.38 (7.68)*	50.20 (7.03)*	ND
Water	2194.89 (2.34)*	6.68 (4.79)*	2.93 (3.01)*	50.44 (3.57)*	ND

ND: Not detected; *RSD; TE: troxol equivalent; GAE: gallic acid equivalent; QE: quercetin equivalent; AAE:ascprbic acid equivalent

4.3.10 Antioxidant concentrations of the *diabetes tea*

Table 4.11 shows the antioxidant results of the various extracts of the diabetes tea. The water extract showed a concentration of 6356.21±5.12 µmol TE/g which was the highest concentration compared to all other extracts. The MeOH, DCM and 1:1 DCM:MeOH extracts showed a concentration of 348.91±1.15 µmol AAE/g, 1032.86±5.48 µmol AAE/g and 300.14±9.16 µmol AAE/g, respectively. The study by Marnewick et al. (2011) of the brewed rooibos tea showed the concentration as 260.2±24.8 µmol AAE/g, therefore the extracts can be said to possess a higher concentration of antioxidant in the FRAP assay compared to brewed rooibos tea. The water extract had a significantly higher concentration of antioxidants in the ORAC assay with a value of 6356.21±5.12 µmol TE/g compared to the 1402±44.1 µmol TE/g of brewed rooibos tea (Marnewick et al., (2011). Overall results were favourable and showed that the tea contained a significant amount of antioxidants.

Table 4.11: Antioxidant analysis of the diabetes tea extracts

Extracts	ORAC µmol TE/g	Phenols mg GAE/g	Flavonols mg QE/g	FRAP µmol AAE/g	ABTS µmol AAE/g
MeOH	809.23 (1.18)*	30.96 (0.84)*	34.17 (4.64)*	348.91 (1.15)*	154.0 (0.09)*
DCM	617.38 (5.09)*	38.27 (4.27)*	191.29 (1.69)*	1032.86 (5.48)*	587.3 (2.84)*
1:1 DCM:MeOH	940.83 (3.91)	39.62 (4.94)*	50.36 (4.51)*	300.14 (9.16)*	143.6 (1.54)*
Water	6356.21 (5.12)*	21.49 (3.00)*	35.37 (2.34)*	131.66 (3.62)*	71.2 (3.23)*

ND: Not detected; *RSD; TE: troxol equivalent; GAE: gallic acid equivalent; QE: quercetin equivalent; AAE:ascprbic acid equivalent

4.3.11 Antioxidant activity of the plant extracts

The antioxidant activity of the tea and its constituent plants was assessed and compared to that of brewed rooibos tea in literature. The concentration of antioxidants in the plants and the tea was found to be significantly high. The ORAC assay results of the water extracts were significantly higher than that of rooibos tea in all plants. Polyphenols in tea contribute significantly to the antioxidant activities of tea extracts.

The potential medicinal uses of this diabetes tea are supported by the presence of the above-mentioned antioxidants and polyphenolic compounds. Hence, the need to exploit the potential of the tea and its plants in pharmaceutical industries arises.

4.4 Alpha- amylase enzyme inhibition

Pancreatic alpha-amylase is a key enzyme of dietary carbohydrate digestion in humans. Inhibitors of these enzymes may be active in retarding carbohydrates digestion and glucose absorption to suppress postprandial hyperglycemia (Jung et al., 2006). The plant extract were tested for the alpha-amylase enzyme inhibition and the results are presented in **Table 4.12** below. Acarbose was used as the control and the percentage inhibition of the plant extracts was compared to that of acarbose.

4.4.1 Alpha-amylase enzyme inhibition by the plant extracts

The different plant extracts (water, 1:1 MeOH, DCM:MeOH, DCM and hexane) investigated in the current study demonstrated very low or no inhibitory activity of the alpha-amylase. The alpha-amylase enzyme inhibition was only done on the water extract for the *T foenum-graecum* due to low amounts of extraction yields from the other solvents. The diabetes tea water extract tested showed no results due to colour interferences. The negative enzyme inhibition shows that there was no inhibition activity by the extracts. The percentage inhibition of the extract was compared to that of a reference drug, acarbose. All alpha-amylase percentage inhibitions lower than the alpha-amylase percentage inhibition of acarbose was considered to be insignificant. This observation suggests that the extracts do not inhibit the alpha-amylase enzyme. It was further observed in the current study that some of the plant extracts under investigation showed no inhibition of the alpha-amylase enzyme.

The reference drug arcabose used was not a potent inhibitor of alpha-amylase under the current study conditions. This observation is consistent with other reports that either described acarbose as a very weak inhibitor of alpha-amylase enzyme activity (IC50 of about 1 mg/ml) or no inhibition of α-amylase at all (Youn, 2004). Only the Salvia officinalis hexane extract showed 43.42% activity at a concentration of 100 µg/ml. This indicated that this extracts may be more beneficial in preventing postprandial hyperglycemia than acarbose (Kim et al., 2005). Furthermore, the mode of inhibition of acarbose towards alpha–amylase has been reported to be mixed non-competitive (Youn, 2004).

Table 4.12: Extracts of the diabetes tea and its constituent plants tested for the inhibition of alpha-amylase inhibition

Plants	*%Inhibition Acorbose (control)*	*% Inhibition hexane extract*		*% Inhibition DCM extract*		*%Inhibition DCM:MeOH extract*		*% Inhibition MeOH extract*		*% Inhibition Water extract*	
	100 µg/ml	*50 µg/ml*	*100 µg/ml*	*50 µg/ml*	*100 µg/ml*	*50 µg/ml*	*100 µg/ml*	*50 µg/ml*	*100 µg/ml*	*50 µg/ml*	*100 µg/ml*
T. foenum-graecum	43.50	-	-	-	-	-	-	-	-	-8.89	-6.28
A. millefolium	42.97	6.41	12.20	4.77	6.84	3.86	3.52	1.21	5.50	4.17	5.09
A. betulina	44.98	-10.81	-4.04	-6.15	-9.10	1.12	-15.38	7.45	-0.84	1.37	-2.83
S. officinalis	40.88	21.79	43.42	13.95	21.52	4.97	15.17	2.60	3.97	9.44	20.68
S. africana-caurelea	43.50	5.26	9.82	6.68	8.50	-4.20	4.14	-7.94	-8.19	-9.67	-4.43
T. officinale	48.74	2.35	8.52	5.10	2.51	2.04	-1.46	-2.33	-2.33	2.73	1.57
U. urens	44.57	0.77	1.48	-4.56	0.65	0.19	0.37	0.46	-12.65	11.76	-13.54
Diabetes tea	49.21	1.67	13.55	4.51	2.65	0.36	1.94	2.58	3.72	-	-

4.5. Alpha-glucosidase enzyme inhibition

The main risk factor in the development of type 2 diabetes is postprandial hyperglycaemia. Alpha-glucosidase inhibitors that reduce postprandial hyperglycaemia have a key role in the treatment of type 2 pre-diabetic states and also have the potential to reduce the progression of diabetes. In the past two decades some synthetic alpha-glucosidase inhibitors, such as acarbose, miglitol and voglibose, have been developed and have received considerable attention. Due to the side effects of these synthetic α-glucosidase, e.g. liver disorders, flatulence and abdominal cramping, some current studies have focused on isolation and identification of natural alpha-glucosidase inhibitors from plant and food products. These natural glucosidase inhibitors could be used to develop physiologically functional foods or lead compounds for antidiabetic treatment (Liu et al., 2011). The possibility of clinical use of such inhibitors for diabetic or obese patients has been attempted by acarbose, which has shown to effectively reduce the intestinal absorption of sugars in humans (Jun et al., 1997). The plant extracts were all tested for the inhibition of the alpha-glucosidase enzyme and results are presented in **Table 4.13**. Acarbose was used as the control.

4.5.1 Alpha-glucosidase enzyme inhibition by the plant extracts

The different plant extracts under investigation inhibited the alpha-glucosidase enzyme variably. The alpha-glucosidase enzyme inhibition was only done on the water extract for the T foenum-graecum due to low amounts of extraction yields from the other solvents. The diabetes tea water extract was tested for the alpha-amylase enzyme inhibition and no results were obtained due to colour interferences. The negative enzyme inhibition shows that there was no inhibition activity by the extracts. The percentage inhibitions of the extracts were compared to the percentage inhibition of the reference drug used in the study which is acarbose. The inhibitions that were significantly lower compared to acarbose were considered to be insignificant. The hexane extracts of most of the plants including the diabetes tea showed the most significant inhibition of the alpha-glucosidase enzyme. The extracts showed inhibition very similar to that of acarbose and in some case the extracts were better inhibitors compared to acarbose. The highest inhibitory activity towards alpha-glucosidase was found in the U. urens hexane extract and the T. vulgaris hexane extract (69.66% and 68.43%, respectively).

Table 4.13: Extracts of the diabetes tea and its constituent plants tested for the inhibition of alpha-glucosidase inhibition

Plants	% Inhibition Acorbose (control)	% Inhibition hexane extract		% Inhibition DCM extract		% Inhibition DCM:MeOH extract		% Inhibition MeOH extract		% Inhibition Water extract	
	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
T. foenum-graecum	50.62	-	-	-	-	-	-	-	-	-3.48	8.08
A. millefolium	39.98	30.49	62.58	30.49	38.30	23.23	34.41	25.07	42.42	26.78	32.65
A. betulina	51.34	28.19	57.14	23.27	50.63	3.83	8.46	16.58	23.33	9.66	18.94
S. officinalis	49.60	50.88	67.99	38.27	46.93	18.92	22.59	19.89	16.61	44.67	45.18
S. africana-caurelea	50.62	16.77	50.52	11.82	57.04	5.99	13.43	-0.22	16.99	8.58	15.40
T. officinale	50.62	8.28	15.76	5.59	8.10	14.51	11.42	15.52	12.58	17.57	13.61
T. vulgaris	39.98	57.44	68.43	28.48	57.75	31.74	35.41	18.80	30.86	18.46	29.44
U. urens	51.34	29.40	69.66	29.40	37.91	-2.98	33.14	7.13	-7.393	-6.48	-1.94
Diabetes tea	49.60	15.40	57.14	42.23	54.84	7.03	10.07	-8.21	-2.93	-	-

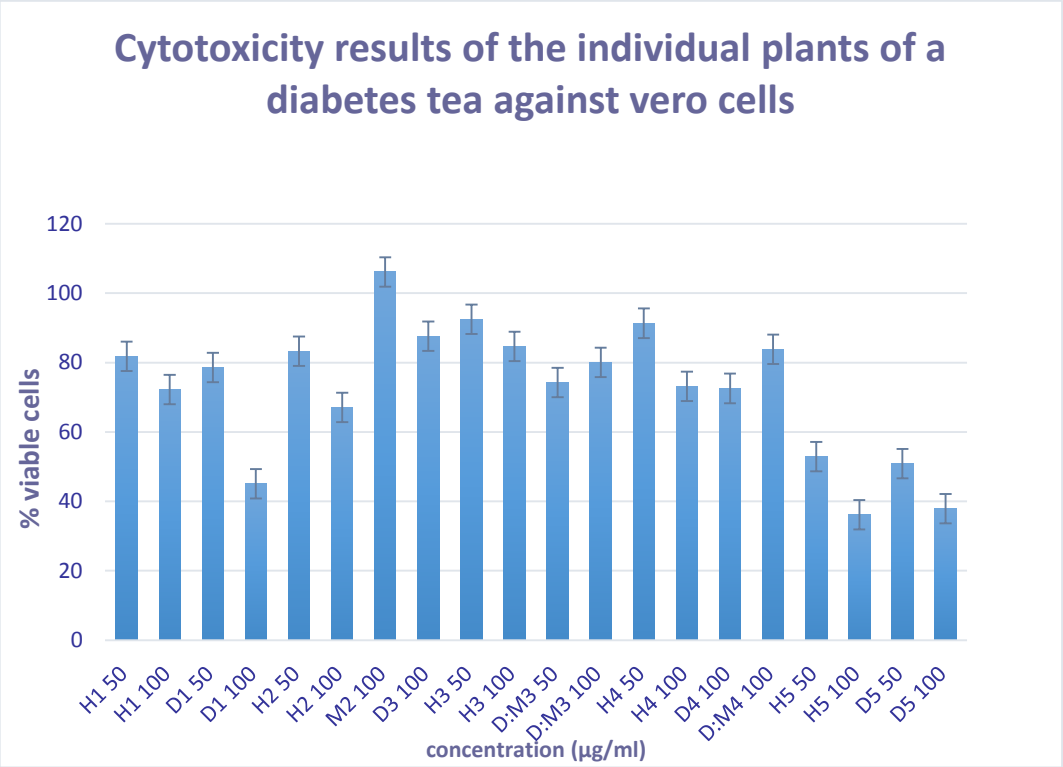
This observation suggests that alpha-glucosidase enzyme is inhibited mostly by the less polar or medium polarity chemical components of the plant extracts. This is further demonstrated by the DCM extracts of the plants which also have significant inhibition of the alpha-glucosidase enzyme. The results of other similar *in vitro* studies have attributed the alpha-glucosidase inhibitory activity of some plant material extracts to the presence of flavonoids, polyphenols as well as their glycoside derivatives (Jung et al., 2006). It is reasonable to suggest that the alpha-glucosidase inhibitory of the plant extracts observed in the current study could also be due to the presence of less polar flavonoids and/or other phenolic compounds.

4.6 Cytotoxicity

The cytotoxicity analysis was only done for extracts that showed significant results for the antioxidant, alpha-amylase and alpha-glucosidase extracts. This means the inhibition of the extracts were higher than that of the control used. **Figures 4.17** and **4.18** show the results obtained in the cytotoxicity analysis of the plants and tea. The plants were numbered from 1-7 as shown in **Figures 4.17** and **4.18**. The nomenclature used in **Figures 4.17** and **4.18** for the extracts is related to the solvents used in the extraction; i.e. D1 will refer to the *T. vulgaris* DCM extract, H2 will refer to the *A. millefolium* hexane extract, D:M3 refers to *A. betulina* 1:1 DCM:MeOH extract, M7 refers to *S. africana-caerulea* methanol extract,etc.

4.6.1 Plant extracts tested for cytotoxicity

Figure 4.1 shows the cytotoxicity of the plants in the diabetes tea mixture. The different extracts were investigated using two concentrations of each extract. These concentrations were 50 µg/ml and 100 µg/ml. The extracts inhibited the *vero* cell growth variably (as observed in the graph in **Figure 4.1**). The D1 (*T. vulgaris* DCM extract), H5 (*S. officinalis* DCM extract) and D5 (*S. officinalis* hexane extract) at 100 µg/ml are the only extracts that showed cell viability that is less than 50%. The D1 (*T. vulgaris* DCM extract), H5 (*S. officinalis* DCM extract) and D5 (*S. officinalis* hexane extract) showed a cell viability of 45.09%, 37.86% and 36.13% at 100 µg/ml concentration, respectively. These plant extracts can be considered to be less toxic compared to the other plant extracts. The D5 (*S. officinalis* hexane extract) and the H5 (*S. officinalis* DCM extract) showed a cell viability of 50.87% and 52.89% at 50 µg/ml concentration, respectively. This shows that the extracts are slightly toxic. Cell viability of 50% and above is considered to be significantly toxic (Ferrari *et al*., 1990).

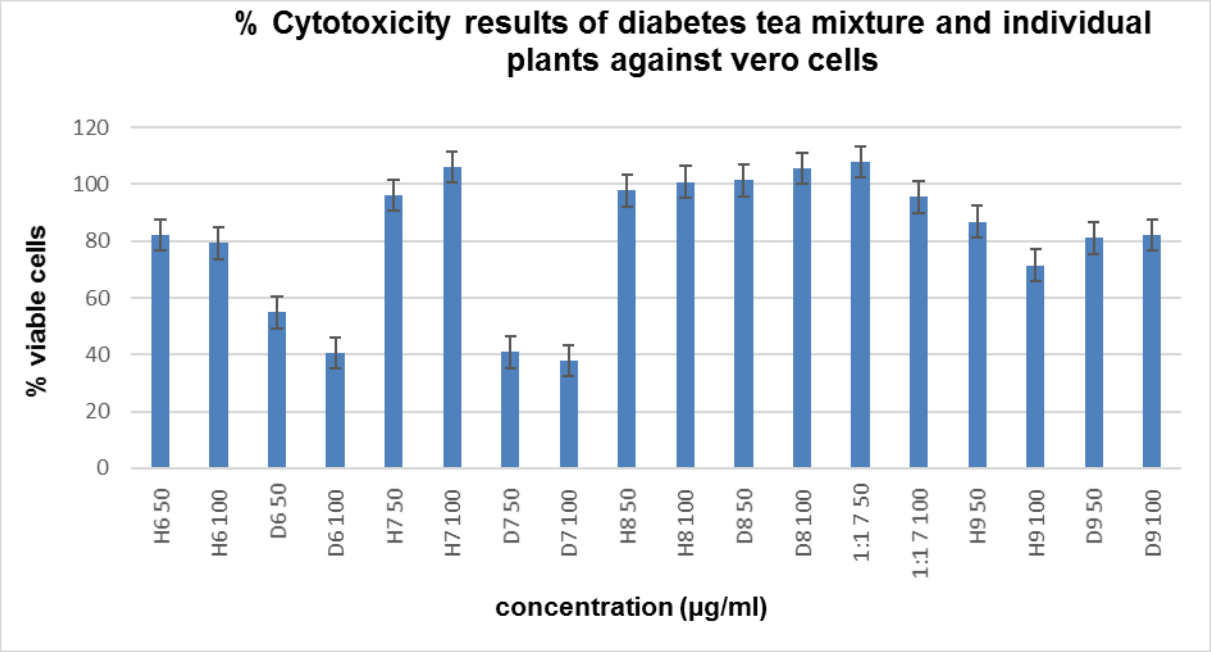


*D-DCM; H-hexane; D:M-1:1 DCM:MeOH; M-MeOH

Figure 4.1: Cytotoxicity of selected extracts of *T. vulgaris (1); A. millefolium (2); U. urens; A. betulina (3); S, officinalis (5)*

4.6.2 Diabetes tea and plant extracts tested for Cytotoxicity

The different extracts were investigated using two concentrations of each extract. These concentrations were 50 µg/ml and 100 µg/ml. The extracts inhibited the *vero* cell growth variably (as observed in the graph in **Figure 4.2**). The D6 (Diabetes tea DCM extract) and D7 (*S. africana-caerulea* DCM extract) at both concentrations showed cell viability that is less than 50% or slightly above 50%. The D6 (Diabetes tea DCM extract) showed cell viability of 54.90% at 50 µg/ml and 40.62% at 100 µg/ml. This shows that the extract is slightly toxic at lower concentrations. The D7 (*S. africana-caerulea* DCM extract) showed cell viability of 40.90% at 50 µg/ml and 37.82% at 100 µg/ml. This shows that the D7 plant extract is not toxic in both concentrations.



*D-DCM; H-hexane; 1:1-1:1 D:M; M-MeOH

Figure 4.2: Cytotoxicity of selected extracts of *diabetes tea (6); S. africana-caerulea (7); T. fornum-graecum (8); T. officinalis (9)*

4.7 Chemical studies

4.7.1 Phytochemical screening results

The methanol and water extracts of the diabetes tea and *S. africana-caerulea* were screened for the presence of phytochemicals. The *S. africana-caerulea* was the plant further studied because the DCM extract was the only extract that showed cell growth inhibition greater than 50% in both concentrations used in the cytotoxicity assay. The preliminary phytochemical tests performed were of qualitative type and from phytochemical investigations it appeared that alkaloids, flavanoids, gums, proteins and amino acids, phenolics, tannins, saponins, reducing sugars, deoxy sugars and glycosides were the phytochemical constituents which were found in both methanol and water extracts of the diabetes tea and *S. africana-caerulea,* as shown in **Table 4.14**. In both the diabetes tea and *S. africana caerulea* it was observed that pentose was only observed in the water extract but not in methanol. Which could be ascribed to its solubility in water. It was also observed that both the methanol and water extract of the wild sage plant did not contain gums.

Furthermore, no anthraquinones, steroids and triterpenoids were observed in both solvent extracts. According to the phytochemical constituents found in this tea, it can be presumed that the tea exhibits anti-bacteria activity, anti-inflammatory activity, anti-viral, anti-allergies, antioxidant, anti-tumor, anti-carcinogen, stimulate immune system and anti-diabetes, because these are biological properties that are observed when these phytochemical constituents are present in plants as confirmed by Savithramma (2011).

Table 4.14: Phytochemical studies of the water and methanol extracts from the diabetes tea and *S. africana caerulea*

Phytochemical Constituents	Diabetes tea		Wild Sage	
	Methanol	Water	Methanol	water
Alkaloids	+	+	+	+
Anthraquinones	-	-	-	-
Deoxy sugars	+	+	+	+
Flavanoids	+	+	+	+
Gums	+	+	-	-
Glycosides	+	+	+	+
Pentose	-	+	-	+
Proteins and amino acids	+	+	+	+
Phenolics and tannins	+	+	+	+
Phytosterols	-	-	-	-
Reducing sugars	+	+	+	+
Saponins	+	+	+	+
Triterpenoids	-	-	-	-

*+: positive test (phytochemicals detected); -: negative test (no detection of the phytochemicals)

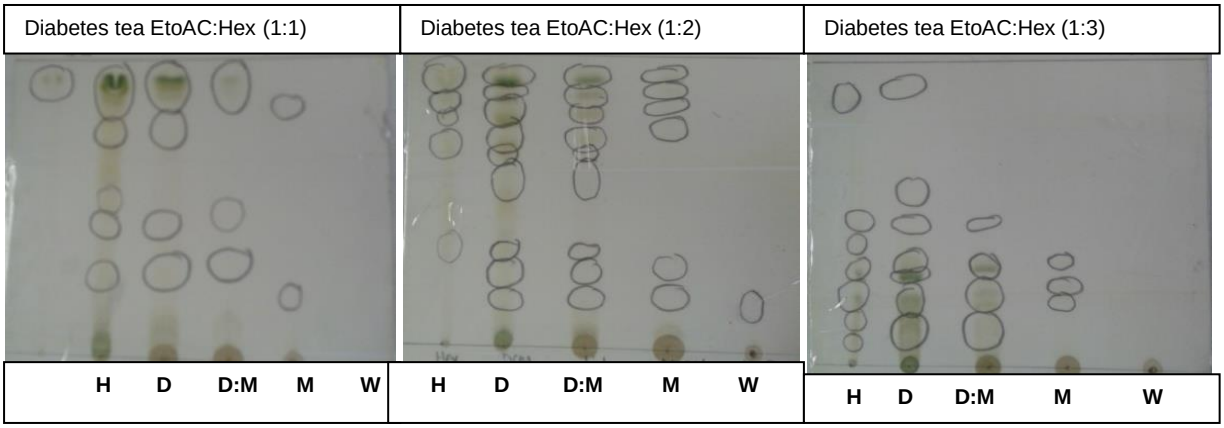
Table 4.15 below shows the total ash of the diabetes tea to be 10.29, 1.64, 9.45 and 11.49% w/w for total ash, water-soluble, acid-soluble and sulphate-soluble ash, respectively.
The diabetes tea alcohol-soluble and water-soluble extractive values were 7.74 and 1.11% w/w, respectively. The table also shows that the total ash of the wild sage plant was found to be 1.89, 2.55, 2.79 and 6.23% w/w for total-ash, water-soluble, alcohol-soluble and sulphate-soluble ash, respectively. The *S. africana-caerulea* alcohol and water-soluble extractive values were 7.04 and 1.62% w/w, respectively. It appears that both powder ashes are more soluble to sulphate compared to the other ashes. According to the extractive values there were more alcohol soluble constituents which were found in these plants compared to the water soluble constituents.

Table 4.15: Ash values from the diabetes tea and *S. africana-caerulea* extracts

Plant	Ash value % w/w				Extractive values % w/w	
	Total ash	Water soluble ash	Acid-soluble ash	Sulphate ash	Alcohol extractive	Water extractive
Diabetes tea	10.29	1.64	9.45	11.49	7.74	1.11
S. africana-caerulea	1.89	2.55	2.79	6.23	7.04	1.62

4.7.2 Thin Layer Chromatography (TLC)

The hexane, DCM, DCM:MeOH (1:1), MeOH and H_2O crude extracts of the *diabetes tea and Salvia africana-caerulea* were subjected to TLC analysis. The diabetes tea crude extracts were spotted on a TLC plate using hexane and ethyl acetate at different ratios. All these ratios showed that the extracts had a rich variety of compounds. The spots obtained were of different colours which showed that they represented different compounds. The Rf values obtained in the different mobile phase ratios showed that there were common compounds that were eluted by all the different mobile phases. The water extract showed poor elution in the selected mobile phase. The recommendation is that further mobile phases should be tried and the sample preparation for the TLC must be investigated further to ensure better elution and therefore better results.



(a) **(b)** **(c)**

Figure 4.3: TLC of the Diabetes tea crude extracts hexane (H), DCM (D), 1:1 DCM:MeOH (D:M), MeOH (M) and Water (W) using ethyl Acetate EtOAc and hexane using ratios (a) 1:1; (b) 1:2 and (c) 1:3

Table 4.16: Rf values of TLC of the diabetes tea

Extract	**Rf values**		
	1:1 (EtOAc: hexane)	**1:2 (EtOAc: hexane)**	**1:3 (EtOAc:hexane)**
Hexane	0.90	0.31; 0.69; 0.78; 0.82; 0.90	0.06; 0.15; 0.25; 0.31; 0.38; 0.46; 0.88
DCM	0.08; 0.18; 0.26; 0.34; 0.42; 0.52; 0.62; 0.74; 0.90	0.10; 0.25; 0.31; 0.43; 0.53; 0.63; 0.69; 0.80; 0.86; 0.91	0.16; 0.21; 0.25; 0.35; 0.45; 0.56; 0.91
DCM:MeOH (1:1)	0.065; 0.13; 0.27; 0.42; 0.65; 0.75; 0.90	0.11; 0.16; 0.25; 0.31; 0.50; 0.55; 0.85; 0.91	0.09; 0.14; 0.19; 0.23; 0.31; 0.45;
MeOH	0.1; 0.29; 0.47; 0.90	0.11; 0.18; 0.28; 0.73; 0.81; 0.89; 0.93	0.20; 0.25; 0.33
H_2O	0.09; 0.83	0.14	

S. africana-caerulea crude extracts TLC: **Table 4.17** below shows the Rf values obtained for the *S. africana-caerulea* crude extracts. The extracts were spotted on a TLC plate and different ratios of hexane and ethyl acetate were used to establish the suitable solvent to use for fractionation. The TLC plates showed a significant number of compound in each extract.

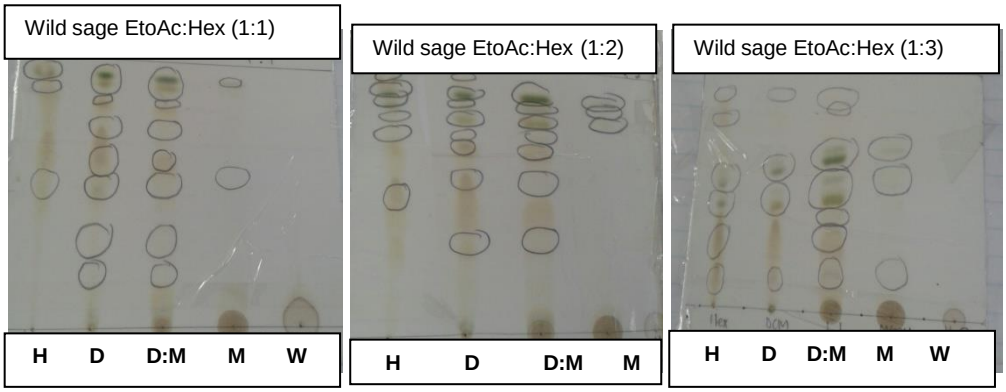


Figure 4.4: TLC of the *S. africana-caerulea* crude extracts (hexane (H), DCM (D), 1:1 DCM:MeOH (D:M), MeOH and Water (W))using Ethyl Acetate (EtOAc) and hexane using ratios 1:1; 1:2 and 1:3

Table 4.17: Rf values of TLC of the *Salvia africana-caerulea*

Extract	Rf values		
	1:1 (EtOAc: hexane)	**1:2 (EtOAc: hexane)**	**1:3 (EtOAc:hexane)**
Hexane	0.50; 0.66; 0.88; 0.94	0.29; 0.39; 0.50; 0.65; 0.71; 0.78; 0.83; 0.88; 0.91	0.13; 0.28; 0.40; 0.51; 0.78; 0.76
DCM	0.19; 0.31; 0.50; 0.60; 0.69; 0.76; 0.91; 0.95	0.30; 0.40; 0.46; 0.55; 0.65; 0.71; 0.78; 0.83; 0.88; 0.94	0.13; 0.25; 0.31; 0.43; 0.48; 0.56; 0.79; 0.90
DCM:MeOH (1:1)	0.16; 0.29; 0.50; 0.59; 0.71; 0.79; 0.89; 0.93	0.33; 0.45; 0.55; 0.66; 0.73; 0.78; 0.84; 0.88	0.16; 0.54; 0.66
MeOH	0.13; 0.54; 0.89; 0.94	0.78; 0.84; 0.88;	0.20; 0.25; 0.33;
H_2O		0.14	

4.7.3 TLC profile of fractions

The DCM and 1:1 DCM:MeOH crude extracts of *S. africana-caerulea* were fractionated using the techniques and protocols highlighted in section 3.9.8.The wild sage DCM extract had 15 fractions and the wild sage 1:1 DCM:MeOH had 14 fractions. The fractions were all subjected to TLC screening to determine the purity of the fractions. The majority of these fractions showed very poor purity and the mass obtained was very low. The fractions were labelled **DM 1** to **DM 29**. According to the TLC screening **DM 23** showed only 1 spot indicating that the fraction could be a pure compound. The Rf value of the spot observed in **DM 23** was 0.54.

Although it can be accepted that the fraction is pure it must be taken into note that the TLC procedure can miss other compounds depending on the different mobile phases used and the different elution times of different compounds.

4.8 Structure elucidation of isolated compounds

Isolation of compounds was attempted from the fractions of both the DCM and 1:1 (DCM:MeOH) since the crude extracts of these showed the best activity in the biological tests done. These fractions were subjected to different spectroscopic techniques (FT-IR, ^{1}H-NMR, ^{13}C-NMR and 2D-NMR experiments (COSY and HSQC)) for structure elucidation.

4.8.1 Nuclear Magnetic resonance (NMR)

NMR is one of the best methods that can be used to obtain structures on non crystalline compounds. All fractions (**DM 1- DM 29**) were subjected to the ^{1}H-NMR and ^{13}C-NMR but because of the poor purity of these fractions no significant structural information could be deduced.

DM 23 which showed potential of being a pure compound was subjected to 2D-NMR experiments (COSY and HSQC). The results obtained showed that the **DM 23** extract was not pure and therefore no structure was obtained from the NMR data. Further purification of the fraction was not done because of the small quantity available. The recommendation is to do extractions on a larger amounts and therefore increase the extraction yield which will probably result in greater amounts of fractions.

4.8.2 Mass spectroscopy (MS)

The **DM 23** fraction was subjected to low resolution mass spectroscopy (LRMS) and high resolution mass spectroscopy (HRMS). This was done to get an indication of the compounds present in the fraction since the NMR could not be interpreted. The LRMS did not give any significant information on the structure of the compound. **Table 4.18** shows the HRMS results that suggested four structural formulas of the potential compound that could be present in the **DM 23** fraction. **DM 23** was isolated as a yellowish to brown sticky substance (gum). The high resolution mass spectroscopy showed a $[M^+ Na]^+$ peak at m/z 369.1670 (calculated for $C_{20}H_{26}0_5Na$ 369.1678) which could suggest the compound with the chemical formula $C_{20}H_{26}0_5Na$.

Table 4.18: HRMS data of the DM 23 compound

Calculated mass	**Formula**
369.1669	$C_2H_{14}N_{22}Na$
369.1678	$C_{20}OH_{26}O_5Na$
369.1683	$C_5H_{22}N_{12}O_6Na$
369.1656	$CH_{18}N_{18}O_4Na$

The information obtained from the HRMS suggests that the isolated compound is a terpenoid. Although there's no specific name for the **DM 23** compound, the literature reviewed has shown that similar compounds to **DM 23** have been isolated from plant material. In a study by Yan et al. (2008) two new diterpenes were isolated from *Isodon nevorsus* and one of these diterpenes was isolated as colourless needles with a molecular formula $C_{20}H_{26}O_5$ HRESI MS m/z 369.1681 $[M^+ Na]^+$ (calculated for $C_{20}H_{26}O_5Na$, 369.1678). The heteroplexisolide (B)4, a yellowish oil with HRESI MS m/z 369.1688 $[M^+ Na]^+$ (calculated for $C_{20}H_{26}O_5Na$, 369.1678); Heteroplexisolide (C)5, yellowish oil with HRESI MS m/z 369.1680 $[M^+ Na]^+$ (calculated for $C_{20}H_{26}O_5Na$, 369.1678); and Heteroplexisolide (D)6, yellowish gum with HRESI MS m/z 369.1687 $[M^+ Na]^+$ (calculated for $C_{20}H_{26}O_5Na$, 369.1678); were isolated from the *Heteroplexis micocephala* (Fan et al., 2009).

CHAPTER FIVE

5.1 Conclusion

The set objectives of the study were explored and from the obtained results the following conclusions could be drawn:

- Substantial amounts of non-polar, medium polar and polar phytochemicals are extractable from the diabetes tea and its eight constituent plants. The highest yields are extractable with water, 1:1 MeOH:DCM and MeOH. The tea shows the highest yields when extracted with water. This shows that the traditional way used by the traditional healers does extract quiet a high amount of phytochemicals. The high yield of water and methanol could suggest that the plants have high levels of polar phytochemicals or it could be attributed to the solvents' broad spectrum of being capable of extracting high molecular weight compounds like tannins and sugars.

- The preliminary phytochemical screening of the extracts revealed the accumulation of non-polar, medium polar and polar phytochemicals. The accumulation of different phytochemical groups shows qualitative and quantitative variations between different plants in the diabetes tea. The chemical profiles of the polar extracts (water and methanol) of the diabetes tea and Wild Sage accumulated the same compounds such as alkaloids, sugars, flavonoids, glycosides, proteins & amino acids, phenolics & tannins and saponins.

- The amount and the composition of ash remaining after combustion of plant material varies considerably according to the type of plant and extraction method. Ash usually represents the organic part of the plant. The ashing destroys the organic material present in the plant. The percentage of total ash content was highest in the diabetes tea mixture compared to the *Salvia africana-carulea.*

- The antioxidant results of the crude extracts of the diabetes tea and its eight constituent plants revealed that there was a variation in antioxidant activity in the different plants. The plant extracts showed a variation in the concentration of the different antioxidants. The presence of the antioxidant activity in the plants suggests that the diabetes tea has potential antidiabetic activity. The crude extracts of the diabetes tea and the eight plants showed poor inhibition activity against the alpha-amylase while some plants showed some significant inhibition of the alpha-glucosidase enzymes.

The hexane extract of *Salvia africana-caerulea officinalis* is the only plant extract that showed significant inhibition at 100 µg/ml.

- In the cytotoxicity assay the extracts inhibited the *vero* cell growth variably. The *Salvia africana-carulea* DCM extract is the only extract that showed cell inhibition above 50% for both the 50 µg/ml and 100 µg/ml concentrations.

- About 29 compounds were obtained from the two extracts of *Salvia africana-carulea*. The compounds could not be fully characterized due to their poor purity. **DM 23** had shown a potential of being pure and therefore was subjected to NMR and LC-MS for structural elucidation. The compound could not be fully characterized which then suggested that it was not in a pure form and therefore required further purification. Although this compound could not be successfully characterized preliminary spectroscopic data suggest the presence of a terpenoid skeleton.

5.2 Recommendations

- Literature reviewed has listed *Trigonella foenum graecum* as one of the plants used worldwide for the treatment of diabetes but in this study the plant showed little to no activity in the bioassays tested. The percentage yields obtained from the plant were very low and therefore a recommendation to review the sample preparation, extraction procedure and extraction solvents in order to optimize the extraction yields of the plant.

- The synergy of the eight plants that make up the diabetes tea needs to be further studied as this could add value to the medicinal properties of the diabetes tea and its plants.

- Further purification of the compounds obtained and their successful characterization needs to be done. The compounds need to be subjected to antidiabetic assays to determine their antidiabetic activity.

REFERENCES

Abo, K., Fred-Jaiyesimi, A. & Jaiyesimi, A. 2008. Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. *Journal of Ethnopharmacology, 115*:67-71.

Adam, Z., Khamis, S., Ismail, A. & Hamid, M. 2012. Ficus deltoidea: Apotential Alternative Medicine for Diabetes Mellitus. *Evidence-Based Complementary and Alternative Medicine, 1*:1-12.

Afolayan, A. & Sunmonu, T. 2010. In vivo studies of antidiabetic plants used in South African Herbal Medicine. *Journal Of Clinical Biochemistry And Nutritio*, 98-106.

Ali, H., Huoghton, P. & Soumayanath, A. 2006. Alpha amylase inhibitory activity of some Malysian plants used to treat diabetes with particular reference to Phyllanthus amarus. *Journal Ethophamacology, 107(3)*:449-455.

Aslan, M., Orhan, D. & Ergun, F. 2010. Hypoglycemic activity and antioxidant potential of some medicinal plants traditionally used in Turkey for diabetes. *Journal of Ethopharmacology, 128*:384-389.

Al-Snafi, A.E. 2016. Chemical constituents and pharmacological effects of *Clerodendum inerme*-A Review. *Sikkim Manipal University Medical Journal, 3:*129-152.

Ayoffor, J., Tehvenden, M. & Nyasse, B. 1994. Novel active diterpenoids from afromomum aulacocapus. *Journal Natural Products, 57*:923-977.

Baharvand-Ahmid, B., Bahman, M., Tajeddini, P., Naghidi, N. and Rafieian-Kapaei. 2016. An ethno-medicinal study of medicinal plants used for the treatment of diabetes. *Journal of Nephropathology, 5(1):*44-50.

Bailey, C. & Day, C. 1989. Traditional plant medicines as treatment for diabetes. *Diabetes Care, 12(8)*:553-564.

Bandaranayake, W. 2002. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wetlands Ecology and Management, 10*:421-452.

Baricevic, D. & Bartol, T. 2000. *The biological properties/pharmacological activity of the Salvia Genus.* Ljubljana, Solvenia: Harwood Academic Publishers.

Baricevic, D., Sosa, S., Della Loggia, R., Tubaro, A., Simonouska, B. & Kransa, A. 2001. Tropical anti-inflammatory activity of *Salvia officinali L.* leaves: the relevance of ursolic acid. *Journal of Ethnopharmacology, 75*:125-132.

Bedekar, A., Shah, K. & Koffas, M. 2010. Natural products for type II diabetes treatment. *Advances in Applied Microbiology, 71*:21-66.

Bernhoft, A. 2008. Bioactive compounds in plants-benefits and risks for man and animals. *The Norwegian Academy for Science and Letters,* 11-17.

Bhandari, M., Jong-Anurakkun, N., Hong, G. & Kwalat, J. 2008. Alpha amylase glucosidase and alpha amylase activities of Nepalese medicinal herb Pkhanbhed (Bergenia ciliate Haw). *Food Chemistry, 106*:247-252.

Bnouham, M., Ziyyat, A., Mekhfi, A. & Tahri, A. 2006. Medicinal plants with potential antidiabetic activity-A review of ten years of herbal medicine research (1990-2000). *Int. J. Diabetes & Metabolism*, 1-25.

Bukhari, S.B., Bhanger, M.I. & Memon, S. 2008. Antioxidant activity of extracts from fenugreek seed (Trigonella foenum graecum). *Pak. J. Anal. Environ. Chem, 9(2):*78-83.

Bruneton, J. 1993. *Pharmacognosie, phytochimie, medicinal plants.* Paris: Tec and Doc-Lavoisier.

Bruneton, J. 1999. *Pharmacognosy, phytochemistry and medicinal plants.* England, UK: Intercept ltd.

Chan, E.W. 2014. Antioxidant and Antibacterial Properties of Green, Black, and Herbal Teas of Camellia Sinensis. *Pharmacognosy Research, 3(4*):266-272.

Chang, C., Lin, Y., Bartolome, A., Chen, Y., Chiu, S. & Yang, W. 2013. Herbal Therapies for type 2 diabetes mellitus: chemistry, biology and potential application of selected plants and compounds. *Evidence-Based Complementary and Alternative Medicine,* 1-33.

Christian, G.D. 2004. Analytical Chemistry. *John Wiley Inc*: USA.
Cordell, A. 1995. Changing strategies in natural product chemistry. *Phytochemistry, 40(6)*:1585-1612.

Cos, P., Vlietinck, A.J., Berghe, D.V. & Maes, L. 2006. Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept. *Journal of Ethnopharmacology, 106:*290-302.

Cowan, M. 1990. Plant products as antimicrobial agents. *Clinical microbiology review, 12*:564-582.

Croteau, R., Kutchan, T. & Lewis, N. 2000. Natural Products (Secondary Metabolites). *American Society of Plant Physiologist*, 1250-1318.

Das, K., Tiwar, R.K.S. & Shrivastava, D.K. 2010. Techniques for evaluation of medicinal plant products as antimicrobial properties agent: current and future trends. *Journal of Medicinal Plant Research*, 4(2):104-111.

Department of Health (South Africa). 2007. The status of traditional medicine in Africa. [Online] http://www.doh.gov.za/calmh3/docs/thestatusoftraditionalmedicineinafrica.pdf. [02 April 2016].

Deutschlander, M., van de Venter, M., Roux, S., Louw, J. & Lall, N. 2009. Hypoglyceamic activity of four plant extracts traditionally used in South Africa for diabetes. *Journal Ethopharmacology,*1-6.

Doupis, J. & Veve, A. 2007. Antioxidants, Diabetes, and Endothelial Dysfunction. *US endocrine disease*, 61-65.

Dweck, A. 2009. The internal and external use of medicinal plants. *Clinics in Dermatology, 27*:148-158.

Eddouks, M. 2012. Management of Diabetes in Africa: The Role of Traditional Medicines. *Pharmaceutical Regulatory Affairs:Open Access,1:*3.

Eddouks, M., Maghrani, M., Lemhadri, A., Ouahidi, M. & Jouad, H. 2002. Ethopharmacological survey of medicinal plants used for the treatment of diabetes mellitus,hypertension and cardiac dideases in the siouth-east region of Morocco (Tafilalet). *Journal of Ethnopharmacology, 82*:97-103.

Eldridge, G.R., Vervoot, H.C., Lee, C.M., Cremin, P.A., Williams, C.T., Hart, S.M., Goering, M.G., O'Neil-Johson, M. & Zeng, L. High-throughput method for the production and analysis of large natural product libraries for drug discovery*. Anal. Chem.*, 74:3963-3971.

Eloff, J. 1998. Wahich extractant should be used for screening and isolation of antimicrobial components from plants. *Journal Ethnopharmacology, 60*:1-8.

Fabricant, D. & Farnsworth, N. 2001. The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives, 109(1)*:69-75.

Fan, X., Zi, J., Zhu, C., Xu, W., Cheng, W.,Yang, S., Guo, Y. & Shi, J. 2009. Chemical constituents of *Heteroplexis micocephala*. *J. Nat. Prod., 72*:1184-1190.

Farzaneh, V. and Carvallo, I.S. 2015. A review of the health benefit potentials of herbal infusions and their mechanism of action. *Industrial Crops and Products Journal, 65:*247-258

Ferrari, M., Fornasiero, M.C. & Issetta, A.M. 1990. MTT colorimetric assay for testing macrophage cytotoxic activity in vitro. *J. Immunol. Methods, 131(2):*72-165.

Fiume, M.Z. 2001. Final report on the safety assessment of yarrow (*Achillea Millefolium*) extracts. *International Journal of Toxicology, 20:*79-84.

Fisher, V.F. 2005. Indigenous Salvia species- an investigation of the antimicrobial activity, antioxidant activity and chemical composition. Master of Science in Medicine, University of Witwatersrand, Johannesburg.

Foodscience. 2015. Phytosterols in foods. [Online] https://www.foodscience-avenue.com/2015/05/phytosterols-in-foods.html. [11 June 2016].

Freemantle, M. 2002. Morphine. Chemical & Engineering News. [Online] https://www.pubsacs.org/cen/coverstory/83/8325/8325morphine.html. [11 June 2016].

Gebhardt, R. 2000. In vitro screening of plant extracts and phytopharmaceuticals: Novel approach for the elucidation of active compounds and their mechanism. *Planta Medica, 66(2):*99-105.

Gerich, J. 2001. Matching treatment to physiology in type 2 diabetes . *Clinical Therapeutics, 23(5):7-28* .

Giancaspro, I.G. (n.d). Menthol. (USP 28-NF28 Page 1207) https://www.newdruginfo.com/pharmacopeia/usp28/v28/v28230nf2350-m4853.html. [11 June 2016].

Giolitti, A. 2012. Reserpine: The treatment of Hypertention as a cause of Depression. https://www.flipper.diff.org/app/items/399 . [11June 2016].

Grover, J., Vats, V., Yadav, S. & Biswas, N. 2004. Anti-cataract activity of Ptserocarpus marsupium bark ang Trigonella Foenum-Graecum seed extracts in olloxan diabetic rats. *Journal of Ethnopharmacology, 93*:289-294.

Grover, J., Yadav, S. & Vats, V. 2006. Medicinal plants of India with anti-diabetic potential. *Journal of Ethnopharmacology*, 81-100.

Gunawardena, G. (n.d). Isoprene rule. Ochempal. https://ochempal.org/indexphp/alphabetical/i-j/isoprene-rule/ . [11 June 2016].

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

Hara, Y. & Honda, M. 1990. The inhibition of alpha amylase by tea polyphenols. *Agricultural and Biological Chemistry*,1939-1945.

Harborne, J. 1998. Phytochemical Methods: a guide to mordern techniques.2ND edi. Imprint. London.

Harborne, J. 1984. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. *Chapman and Hall Ltd. London*, 149-188.

Havsteen, B. 2002. The biochemistry and medicinal significance of flavonoids. *Pharmacology & Therapeutics, 96*:67-202.

Heinrich, M., Barnes, J., Gibbons, S. & Williamson, E. 2004. *Fundamentals of pharmacognosy and phytotherapy.* Churchill Livingstone: Elseriever Science.

Hosseyni, E., Kashaani, H. & Asadi, M. 2012. Mode of action of medicinal plants on diabetic disorders. *Life Science Journal, 9(4)*:2776-2783.

Hu, C. & Kitts, D.D. 2003. Antioxidant, prooxidant and cytotoxicity activities of solvent-fractionated dandelion (Traxacum officinalis) flower extracts in vitro. *Journal of Agricultural and Food Chemistry,* 51:301-310.

Hu, C & Kitts, D.D. 2004. Lteolin and luteolin 7-O-glucosidase from dandelion flower suppress NOS and COX-2ln Raw 2647 cells. *Molecular & Cellular Biochemistry*, 265:107-173.

Huie, W.C. 2002. A review of modern sample preparation techniques for the extraction and analysis of medicinal plants. *Anal. Bioanal. Chem., 372(1)*:23-30.

Huo, C., Li, Y., Shang, Q., Wang, Y., Shang, Q. & Qin, F. 2013. Cytotoxic flavonoids from the flower of Achillea millefolium. *Chemistry natural compouds*, 958-962.

Jacoby, M. 2005. Taxol. *Chemical & Engineering News*. [Online] https://pubs.acs.org/cen/coverstory/83/8325/8325/taxol.html. [11 June 2016].

Jarald, E., Joshi, S. & Jain, D. 2008. Diabetes and Herbal Medicines. *Iranian Journal Of Pharmacology and Therapeutics, 7(1)*:97-106.

Jia, L. & Liu, F.T. 2013. Why bortezomib cannot go with green. *Cancer Biology & Medicine*, *10*:206-213.

Jin-Ming, K., Ngoh-Khang, G., Lian-Sai, C. & Tet-Fatt, C. 2003. Recent advances in traditional plant drugs and orchids. *Acta Pharmacologica Sinica, 24(1)*:7-21.

Jones, W. & Kinghorn, A. 2005. Natural product isolation: extraction of plant secondary metabolites. *Methods in Biotechnology, 20*:323-351.

Joseph, B. & Jini, D. 2013. Antidiabetic effects of Momordica charantia (bitter lemon) and its medicinal potency. *Asian Pac. J. Trop. Dis., 3(2)*:93-13.

Jun, W., Jun, K. Hideyuki, K. & Ryoya, N. 1997. Isolation & Identification of α-Glucosidase Inhinitors from Tochu-cha (*Eucommia ulmoides*). *Biosci. Biotech. Biochem., 61(1)*:177-178.

Jung, M., Park, M., Lee, H., Kang, Y., Kang, E. & Kim, S. 2006. Antidiabetic agents from medicinal plants. *Current Medicinal Chemistry, 13*:1203-1218.

Kaul, T., Middletown, J. & Ogra, P. 1985. Antiviral effects of flavonoids in human viruses. *Journal of Medical Virology, 15*:71-75.

Kavishankar, G., Lakshmidevi, N., Mahadeva, M., Prakash, H. & Niranjana, S. 2011. Diabetes and medicinal plants-A review. *Int. J. Pharm. Biomed. Sci., 2(3)*:65-80.

Kazeem, M.K., Akanji, M.A., Hafiur, R.M. & Choudhary, M.I. 2012. Antiglycation, antioxidant and toxicological potential of polyphenol extract of alligator pepper, ginger and nutmeg from Nigeria. *Asian Pacific Journal of Tropical Biomedicine*, 2(9):727-732.

Keerthana, G., Kalaivan, M. & Sumathy, A. 2013. In vitro alpha amylase inhibitory and antioxidant activities of ethanolic leaf extracts of Croton Bonplandianum. *Asian Journal of Pharmaceutical and Clinical Research, 8(4)*:36-62.

Khan, A., Safdar, M., Khan, M., Khattak, K. & Anderson, R. 2003. Cinnamon improves glucose and lipids of people with type 2 diabetes. *Diabetes Care, 26(12)*:3215-3218.

Kim, Y., Jeong, Y., Wang, M., Lee, W. & Rhee, H. 2005. Inhibitory effects of pine bark extracts on alpha glucosidase activity and posprandial hyperglycemia. *Nutrition, 21*:756-761.

Kinghorn, A. & Balandrin, M. 1993. Human medical agents from plants. Journal of *American Chemistry Society, 534:*48-55*.*

Kukreja, A. & Maclaren, N. 1999. Autoimmunity and diabetes. *J. Clin. Endocrinal Metab., 54*:4377-4378.

Kumar, D., Singhal, S., Bansal, S. & Gupta, S.K. 2014. Extraction, isolation and evaluation of Trigonella foenum-graecum as a mucoadhesive agent for nasal gel drug delivery. *Journal Network Professional Association, 27(1*):40-45.

Li, W., Zheng, H., Bukuru, J. & De Kimpeb, N. 2004. Natural medicine used in the traditional Chinese medical system for therapy of diabetes mellitus. *Journal of Ethnopharmacology, 92*:1-21.

Lima, C., Andrade, P., Seabra, R., Fernandes-Ferreira, M. & Pereira-Wilsona, C. 2005. The drinking of Salvia officinalis improves liver antooxidant status in mice and rats. *Journal of Ethnopharmacology, 97*:383-389.

Lima, C.F.M. 2006. Effects of Salvia officinalis in the liver: Relevance of glutathione levels. PhD Degree. Escola de Ciencias, Universidade do Minho.

Liu, L., Deseo, M.A., Morris, C., Winter, K.M. & Leach, D.N. 2011. Investigation of α-glucosidase inhibitory activity of wheat bran and germ. *Food Chemistry*, *126*:553-561.

Luna, B. 2001. Oral agents in the management of type 2 diabetes mellitus. *American Family Physician, 63(9)*:1747-1756.

Maduna, P. 2006. The Role Of Traditional Medicine In The Treatment Of Diabetes Mellitus. *Continuing Medical Education, 24(10)*:574-577.

Malviya, N., Jain, S. & Malviya, S. 2010. Antidiabetic Potential of medicinal plants. *Acta Poloniae Pharmaceutical-Drug Research, 67(2)*:113-118.

Marles, R. & Farnsworth, N. 1995. Antidiabetic plants and their active constituents . *Phytomedicine, 2(2)*:137-189.

Marnewick, J.L., Rautenbach, F., Venter, I., Neethling, H., Blackhurst, D.M., Wolmarans, P. and Macharia, M. 2011. Effects of rooibos (Aspalathus linearis) on oxidative stress and biochemical parameters in adults at risk for cardiovascular disease. *Journal of Ethnopharmacology, 133:*46-52.

Marston, A. 2011. Thin Layer Chromatography with biological detection in phytochemistry. *Journal Chromatography A, 1218*:2676-2683.

Matomela, N. 2004. Recognition for traditional medicine. [Online] Available: http://www.buanews.gov.za. [02 April 2016].

Meng, L., Zhang, H., Haywara, L., Takemura, H., Shao, R.G. & Pommier, Y. (2004). Tetrandrine Induces Early G, arrest in Human colon Cacinoma Cells by Down Regulating the Activity and Inducing the Degradation of G-S-Specific Cyclin-Dependent Kinases and by Inducing p53 and p21. *American Association Cancer Research*, *64*:9086-9092.

Moolla, A., van Vuuren, S.F., van Zyl, R.L. & Vljoen, A.M. 2007. Biological activity and toxicity profile of 17 Agothosma (Rutaceae) species. *South African Journal of Botany*, *73(4):*597.

Mukesh, R. & Namita, P. 2013. Medicinal plants with antidiabetic potential-A review. *American-Eurasian J. Agric. & Environ. Sci, 13(1)*:81-94.

Mukherjee, P., Maiti, K., Mukherjee, K. & Houghton, P. 2006. Leads from Indian medicinal plants with hypoglycemic potentials. *Journal of Ethnopharmacology 106*:1-28.

Nasri, H., Shizard, H., Baradaran, A. and Rafieian-Kopaei, M. 2015. Antioxidant plants and diabetes mellitus. *J. Res. Med. Sci., 20:*491-502.

Ncube, N.S., Afoayan, A.J. & Okoh, A.I. 2008. Assessment techniques of antimicrobialproperties of natural compounds of plant origin: Current methods and future trends. *African Journal of Biotechnology*, 7(12):1797-1806.

Neeraj, T. & Madhu, S. 1989. Indigenous plant drugs for diabetes mellitus. *Indian Council Research*,1-32.

Noor, A., Bansal, V. & Vijayalakshmi, M. 2013. Current update on antidiabetic biomolecules from key tarditiona lndian medicinal plants. *Current Science, 104(6)*:721-727.

Nyika, A. 2009. The ethics of improving African traditional medical practice: Scientific or African traditional method. *Acta Tropica, 112*:32-36.

Okigbo, R. & Mmeka, E. 2006. An appraisal of phytomedicine in Africa. *King Mongkut's Institute of Technology Ladkrabang, 2(6)*:83-94.

Oscan, M. & J,C. 2004. Aroma profile of Thymus vulgaris L. growing in wild Turkey. *Bulgarian Journal of Plant Physiology, 3:*68-73.

Osguven, M. & Tansil, T. 1998. Drug yield and essential oils of Thymus vulgaris L. as influenced by ecological and ontogenetical variations. *Tr. J. of Agriculture and Forestry, 22*:537-542.

Patel, D., Kumar, R., Laloo, D. & Hemalatha, S. 2012. Natural medicines from plant source used for therapy of diabetes mellitus: and overview of its pharmacological aspects. *Asian Pacific Journal of Tropical Disease*, 239-250.

Patel, D., Prasad, S. & Kumar, R. 2012. An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pacific Journal of Tropical Biomedicine,* 320-330.

Patel, K.; Singh, R.B. & Patel, D.K. 2013. Medicinal significance, pharmacological activities, and analytic aspects of solasodine: A concise report of current scientific literature. *Journal of Acute Disease*, 92-98.

Pefile, S. 2005. South African legislation on traditional medicine. [Online] Available: http://www.scidev.net/en/content/overview. [02 April 2016].

Perret, S., Whitfield, P., Sanderson, L. & Bartlet, A. 1995. The plant Millenttia thoningii (leguminoceae) as a topic antihistosomal agent. *Journal of Ethnopharmacology, 47*:49-57.

Peterson, J. & Dwyer, J. 1998. Flavonoids: Dietary occurrence and biochemical activity. *Nutrition Research, 18(12):*1995-2018.

Petrovska, B.B. 2012. Historical review of medicinal plants' usage. *Pharmacognosy Review, 6(11)*:1-5.

Pietropaolo, M. 2001. Pathogenesis of diabetes: our current understanding. *Clinical cornerstone diabetes, 4(2)*:1-21.

Phillipson, J. 2001. Phytochemistry and Medicinal plants. *Phytochemistry, 56*:237-243.

Pitt, J. 2009. Applicatios of Liquid Chromatography Mass Spectroscopy in Clinical Biochemistry. *Clin. Biochem, 30*:19-31.

Pitt, J. 2009. Principles of Liquid Chromatography-Mass Spectroscopy in clinical biochemistry. *Clinical Biochemistry Rev*,19-34.

Potrich, F., Allemand, A., Mota da Silva, L., Cristina dos Santosa, A., Baggioa, C. & Freitas, C. 2010. Antiulcerogenic activity of hydroalcoholic extract of Achillea millefolium L: involvement of the antioxidant system. *Journal of Ethnopharmacology, 130*:85-92.

Porte, A. & Godoy, R.L.O. 2008. Chemical composition of Thymus vulgaris L. (thyme) essential oil from Rio de Janeiro State (Brazil). *Journal of the Serbian Chemical Society, 73(3)*:307-310.

Prachersky, E. & Gang, D. 2000. Genetic & Biochemistry of Secondary Metabolites in Plants: An Evolutionary Perspective. *Trends in Plant Science*, *5(10)*:439-445.

Pubchem. (n.d). Hyoscyamine. Open chemistry database. [Online] https://pubchem.ncbi.nlm.nib.gov/compound/hyoscyamine. [11 June 2016].

Quellen, S. (n.d). Beta-carotene. Ingredients. [Online] https://www.scitoys.com/ingredients/beta-carotene.html. [11 June 2016].

Romero, M.A., Ovejero, G., Rodriguez, A., Gomez, M. & Agueda, I. 2004. Omethylation of phenol in liquid phase over basic zeolites. *Ind. Eng. Chem*. Res., *43*:8194-8199.

Satyajit, D., Sarker, S., Latigf, A., & Gray, I. (2006). *Natural product isolation: second edition.* ISBN 1-59259-9: Human press.

Savithramma, N., Ling, R. & Suhrulatha, D. 2011. Screening of Medicinal Plants for Secondary Metabolites. *Middle-East J. Sci. Res., 8(3)*:579-584.

Scalbert, A., Johnson, I. & Saltmarsh, M. 2005. Polyphenolic antioxidants and beyond. *Am. J. Clin. Nutri., 81*:2155-2175.

Scheiber, A., Schuts, K. & Carle, R. 2006. Taraxacum-A review on its phytochemical profile. *Journal of Ethonopharmacology, 107*:313-323.

Shabnum, S. & Wagay, M. 2011. Essential oils of Thymus vulgaris L. and their uses. *Journal of Research and Development, 11*:83-94.

Sigma Aldrich. 2005. Versaflash-high throughput flash purification. Sepelco Sigma Aldrich. [Online].https://www.sigmaaldrich.com/content/dam/sigmaaldrich/docs/sigma/general_information/versaflash_brochure.pdf.[11 June 2016].

Sindhu, S., Varbhavi, K. & Anshi, M. 2013. In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts . *European Journal of Eperimental Biology, 3 (1)*:128-132.

Siravegna, G. 2011. Vinca Alkaloids. *Chemotherapy.* [Online]
. http://www.flipper.diff.org/app/items/info/3506 [11 June 2016].

Stafford, P. 2014. Synthetic compounds similar to mescaline. Herbal museum: Psychedelic encyclopedia, *1983*:131-133.

Starmans, D.A.J. & Nijhuis, H.H., 1996. Extraction of secondary metabolites from plant material: a review. *Trends Food Sci. Technol., 7*:191–197.

Steenkamp, V.; Mathivha, E.; Gauws, M.C. & van Rensburg, C.E.J. 2004. Studies on antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Africa. *Journal of Ethnopharmacology*, *95*:353-357.

Stitcher, O. 2007. Natural product isolation. *Natural Product Reports*: 517-554.

Street, R. & Prinsloo, G. 2013. Commercially important medicinal plants of South Africa: A Review. *Journal Chemistry*, 1-16.

Tadera, K., Minami, Y., Takamatsu, K. & Matsuoka, T. 2006. Inhibition of α-glucosidase and α-amylase by Flavonoids. J. Nutri. Vitaminol, *52*:149-153.

Tanira, M. 1994. Antidiabetic medicinal plants: a review of the present and future direction. *Int. J. Diabetes, 2(1)*:15-22.

The Japanese pharmacopoeia. 2001. [Online] http://jpdb.nihs.go.jp. [23 December 2016].

The People's Republic of China Pharmacopoeia. 2000. Chemical Press Industry: State Pharmacopoeia Commission.

The Role of Chemistry in History. 2008. Historical significance of heroin.
[Online] https://www.itech.dickinson.edulchemistry/cat=79. [11 June 2016].

The Role of Chemistry in History. 2008. Chemistry of quinine.
[Online] https://www.itech.dickinson.edulchemistry/cat=102 [11 June 2016].

Thovhogi, T. 2009. Effects of the stem bark extracts of scelerocarga birreea on the activities of selected diabetic related carbohydrate metabolizing enzymes. Master of Science Degree, University of Limpopo, Limpopo.
Tiwari, P., Kumar, B., Kaur, M., Kaur, M. & Kaur, H. 2011. Phytochemical screening and extraction: A review. *International Pharmaceutica Sciencia*, *1(1)*:100-106

Trease, G. & Evans, W. 1989. Trease and Evans: Pharmacognosy. *Bailliere Tindale London,* 13.

United States Pharmacopoeia and National Fotmulary (USP 26 NF20). 2002. Rockville, Md: United States Pharmacopoeia Convention.

Uprety, Y., Asselin, H., Dhakal, A. & Julien, N. 2012. Tradional use of mediciinal plant in Boreal forest of Canada: A review and perspectives. *Journal of Ethnomedicine, 8(7)*:1-14.

van Wyk, B. & Wink, M. 2004. *Medicinal Plants of the world 1st edition.* Pretoria: Brisa Publications.

van Wyk, B., van Oudstshoorn, B. & Gerickle, N. 1997. *Medicinal plants of South Africa. Brisa publications*: Pretoria.

van Wyk, B., van Oudtshoorn, B. & Gericke, N. 2009. *Medicinal Plants of South Africa. Bria publiations*: Pretoria, Gauteng, South Africa.

Vats, V., Grover, J. & Rathi, S. 2002. Evaluation of antihyperglycemic and hypoglycemic effect of Trigonella foenum-graecum Linn, Ocium sanctum Linn and Pterocarpus marsupium Linn in normal and alloxanised diabetic rats. *Journal of Ethnopharmacology, 79*:95-100.

Vijay, P. & Vimukta, S. 2014. The role of natural antioxidants in oxidative stress induced diabetes mellitus. *Research Journal Pharmaceutical Science, 3(4)*:1-6.

Wild, S., Roglic, G., Green, A., Sicree, R. & King, H. 2004. Global Prevalence of Diabetes . *Diabetes Care, 27(5)*:1047-1053.

Williams, E., Okpato, D. & Green, A. 2004. Selection, preparation and pharmacological evaluation of plant material*. Wiley and son*.

World Health Organisation. 2000. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. *World Health Organisation Geneva*, 1-80.

World Health Organisation. 2016. Global Report on Diabetes. *World Health Organisation Geneva*, 1-86.

World Health Organisation. 2001. Legal Status of Traditional Medicine and Complementary/Alternative Medicine: A world Review. *World Health Organisation*.

Yadav, R., Kaushik, R. & Gupta, D. (n.d.). The health benefits of *Trigonella Foenum-Graecum*: A review. *International Journal of Engineering Research An Application, 1*:032-035.

Yan, F.L., Guo, L.Q. & Bai, S.P. 2008. Two new diterpenoids and other constituents from *Isodon nervosus*. *Journal of the Chinese Chemical Society, 55*:933-936.

Yarnell, E. & Abascal, K. 2009. Dandelion [*Taraxacum officinale* and *T. Mongolian*]. *Intergrative Medicine, 8(2)*:35-38.

Youn, S.H. & Robyt, J.F. 2004. Study of the inhibition of four alpha-amylase by acarbose and its 4IV-alpha-maltododeaosyl analogues, 3389(19):196-1980.

Zatalia, R. & Sanusi, H. 2013. The role of antioxidants in the pathophysiology, complications, and management of diabetes mellitus. *The Indonesian Journal of Internal Medicine, 45(2)*.