

1. Introduction

Diabetes mellitus is a metabolic disorder that is characterized by chronic hyperglycemia (high blood sugar) resulting with carbohydrate, fat and protein metabolism disturbances (Ali *et al.*, 2006). Diabetes mellitus can be classified into (type-1) diabetes also known as insulin-dependent diabetes mellitus (IDDM) and (type-2) diabetes which is known as non-insulin dependent diabetes mellitus (NIDDM) (Oh *et al.*, 2005). In addition, World Health Organization (WHO) also recognizes the third form of diabetes namely gestational diabetes which occurs during pregnancy and has similar signs and symptoms like diabetes (type-2), but with different causes and population distribution (WHO, 1999). Other well characterized forms of diabetes are latent autoimmune diabetes of adults (LADA or type-1.5) (Dineen, 2006).

1.1 History of Diabetes Mellitus

The term diabetes is derived from the Greek word ‘**diabainein**’ which means passing through as extreme urine production is one of the major symptoms of diabetes. In addition, during 1675 Thomas Wills added the name mellitus which means sweet taste in Latin. In 1776, Matthew Dobson proved that the sweet in urine was due to the production of excess kind of sugar in the urine and blood of people with diabetes (Dobson, 1776). Indians in the ancient times also used ants to test for diabetes (which they referred to as sweet urine disease); they used to observe whether ants were attracted to urine or not. Pathogenesis of diabetes was only understood experimentally in 1900. In 1910, Sir Edward Albert Sharpey-Schafer discovered that people who lacked a particular single chemical produced by the pancreas developed diabetes and the single compound was named as insulin (Patlak, 2002).

Insulin is derived from Latin word, ‘insula’ meaning ‘island in reference to insulin-producing islets of Langerhans’ (Patlak, 2002). Sir Frederick Banting, Charles Best and colleagues purified insulin from

bovine at the University of Toronto and this led to the availability of insulin, which was found to be effective treatment for diabetes (Banting, 1922).

1.2 Classification of diabetes mellitus

1.2.1. Type-1 (Insulin dependent diabetes mellitus)

Type-1 diabetes or insulin-dependent diabetes mellitus (IDDM) is an organ specific autoimmune disease which results from damaged β -cells that are situated in pancreas whose primary function is to produce insulin (Jobon *et al.*, 2006). Type-1 is also known as childhood or juvenile diabetes as most people develop it at childhood. The risks associated with this type of diabetes include congenital anomalies such as central nervous system, cardiac and skeletal muscle malformations which usually occur during neonatal stages (Wasserfall and Atkinson, 2006). Type-1 diabetes is the 2nd most common type of diabetes after type-2. It has been shown to be caused as a result of an autoimmune reaction to antigens of β -cells produced by pancreas. Three types of autoimmune exist (Achenbach and Ziegler, 2005; Wasserfall and Atkinson, 2006).

1) Islets cell surface antibodies

These are polyclonal antibodies that react with all the cells of islets i.e. (α , β , and pancreatic polypeptide cells). About 80% of type-1 diabetics have these autoantibodies.

2) Islets cell cytoplasm antibodies

These antibodies are directed against islets cell cytoplasm and about 90% of type-1 diabetics have these antibodies.

3) Specific antigens targets of islets cells

These antibodies are directed to glutamine acid decarboxylase (GAD) and about 80% of type-1 diabetics have these antibodies.

1.2.1.1 Pathogenesis of IDDM (Insulin Dependent Diabetes Mellitus)

Destruction of pancreatic β cells by the autoimmune antibodies leads to a deficiency of insulin secretion. Metabolic disturbances associated with IDDM results from loss of insulin. Type-1 diabetics suffer from excessive production of glucagons, a 29 amino acid polypeptide which is responsible for carbohydrate metabolism. This therefore, results in exaggerated metabolic defects. Patients suffer from ketoacidosis, a formation of ketone bodies. Usually in this case 'somatostatin' is administered to suppress glucagon levels in type-1 diabetics. On the other hand, this may have negative effect in patients since it results in impaired ability of the patient to secrete glucagon when there is a decrease in blood glucose levels (hypoglycaemia) (Achenbach and Ziegler, 2005).

One of the major problems associated with type-1 diabetes is that there is a defect in the in the ability of target tissues to respond to insulin (Bailers, 2002). Uncontrolled lipolysis leads to formation of free fatty acids in the plasma and this is due to insulin deficiency. In peripheral tissues such as skeletal muscles, glucose metabolism is suppressed as a result of the formation of free fatty acids. Action of insulin is impaired leading to a decrease in expression of a number genes necessary for target tissues to respond normally for insulin such as glucokinase in liver and glucose transporter 4 (GLU 4) class of glucose transporters in adipose tissues. IDDM leads to an increase in the in hepatic output causing a further damage to glucose and carbohydrate metabolism. Insulin stimulates the storage of food energy just after the meal which is normally stored as glycogen in liver cell as well as skeletal muscles. Insulin

also stimulates liver cells to produce triglycerides and this process is normally disturbed in type-1 diabetics (Bailers, 2002).

1.2.2 Type-2 or Non-insulin dependent diabetes mellitus

Type-2 diabetes (T2DM) is a complex metabolic disorder that is associated with β -cell dysfunction and has varying degrees of insulin resistance (Oh *et al.*, 2005; Deneen, 2006). Type-2 diabetes is usually the product of two distinct abnormalities: β -cell function and decreased insulin sensitivity (Robertson, 2006). It is mostly a genetic disease which is associated with strong familial association and high concordance rates in identical twins (Nelson *et al.*, 1975). Most type-2 diabetics are obese and these people normally have insulin resistance on liver, muscles and adipose tissues which are the major sites of insulin. However, only minority of obese patients develop diabetes, and 20% of type-2 diabetics are not obese, emphasizing that obesity does not cause diabetes rather it contributes to the phenotypic expression of genes that predispose individuals to type-2 diabetes.

1.2.3 Pathogenesis of NIDDM (Non Insulin Dependent Diabetes Mellitus)

The disease results in combination of several genetic determinants that may affect insulin production or insulin sensitivity (Hoppener and Lips, 2006). Any gene mutation or metabolic disturbance leading to defect in insulin secretion, insulin action, glucose transport, or enzyme associated with glucose metabolism can theoretically result in hyperglycemia or clinical diabetes (Tseng, 2004). An example for genetic subtypes of T2DM involves mutations in glucokinase, which phosphorylates glucose to glucose-6-phosphate, leading to impaired glycolysis (Frajans *et al.*, 2001). The impaired glucose transport into skeletal muscle and adipose tissues can result from a variety of mechanisms involving insulin receptor defects. (Le Roith and Zick, 2001). Over expression of tumor necrosis factor

α (TNF α) in muscle cells has been implicated as an inducer of insulin resistance by increasing the serine phosphorylation of insulin receptor substrates (IRS-1) and (IRS-2), resulting in the reduction in the ability of the IRS molecules to dock with receptor and interact with downstream pathway (Le Roith and Zick, 2001). Interleukin-6 (IL-6) has also been found to play an important role in the induction of insulin resistance in adipocytes (Lagathu *et al.*, 2003; Rotter *et al.*, 2003). Chronic treatment with IL-6 to adipocytes can diminish expression of β -subunit receptor, IRS-1 and GLUT4, resulting in reduced glucose transport (Legathu *et al.*, 2003). Insulin-induced activation of β -subunit of insulin receptor, extracellular signal-regulated kinases (ERK-1) and (ERK-2) are also inhibited by IL-6. Although the expression of p38 mitogen activated protein kinases (MAPK) phosphorylation is increased in skeletal muscle in patients with T2DM, the insulin-stimulated p38 MAPK phosphorylation is only noted in non-diabetic subjects, but not in patients with T2DM (Koistinen *et al.*, 2003). Another mechanism leading to hyperglycemia in patients with type-2 diabetes involves the inability to produce hepatic glucose. Enhanced phosphoenopyruvate carboxykinase (PEPCK), an enzyme catalyzing the rate limiting step in gluconeogenesis activity leading increased hepatic glucose production in patients with T2DM (Consoli *et al.*, 1990). Decreased glycogen synthesis has also been reported in patients suffering from T2DM (Le Roith and Zick, 2001). Pancreatic β cell dysfunction has also been demonstrated in patients with type-2 diabetes (Kahn, 2000). Progressive formation of amyloidosis with loss of β cells is always major pathological factor found in patients with T2DM (Marzaban *et al.*, 2003).

1.3 Complications associated with diabetes mellitus

Studies and clinical trials indicate that hyperglycemia is the main cause of complications associated with diabetes mellitus (Lopez-Candales, 2005); however complications are less common and severe in people who control blood glucose levels. One of the major complications of diabetes is the formation of glycosylated end products (AGE) (Vlassara and Palace, 2002). These end products will react with other

proteins to generate free radicals in diabetic patients hence increasing permeability and thickening of blood vessel walls with loss of elasticity (Vlassara and Palace, 2002; Bonnefont-Rousselot, 2000).

1.3.1 Complications relating to diabetes can be divided into two forms

1.3.1.1 Acute complications

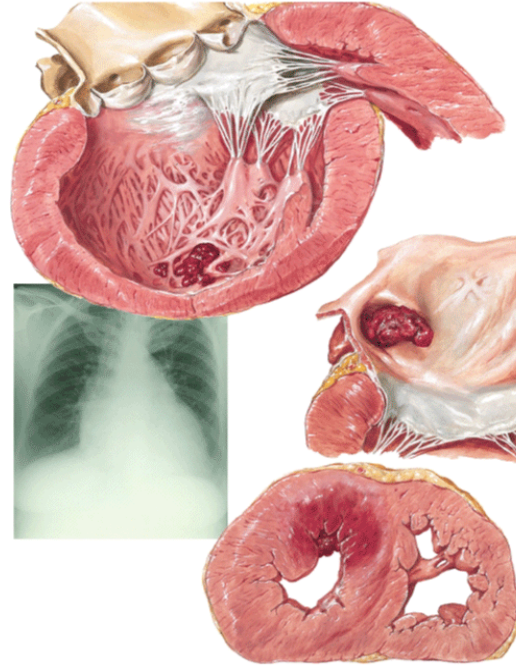
Acute complications include diabetic ketoacidosis, abdominal pain, dehydration, accelerated breath where the patient requires medical emergency. Hypoglycemia or abnormally low sugar that is normally caused by drugs used for diabetes can develop as a result of acute complications. Patients may be agitated, sweaty and may have symptoms of sympathetic activation of Autonomic Nervous System (ANS). Conscious can be lost leading to seizures eventually coma/ brain damage ultimately death may result.

1.3.1.2 Chronic complications

Elevated blood glucose can result in damage of blood vessels. Examples of chronic complications are diabetic retinopathy, coronary artery disease (atherosclerosis, figure 1.1: B), renal failure, limb amputation and eventually premature death (Ortiz-Andrade, 2007). Macrovascular disease leads to cardiovascular complications such as coronary artery disease (angina or myocardial infraction), ischemic stroke and muscle wasting. Diabetic foot (figure 1.1: A) may also result in skin ulcer and infection by gangrene. This is a common cause of adult amputations.



A)



B)

Figure 1.1: Complications that arise as result of diabetic complications, A) diabetic foot that may lead to amputation, B) Atherosclerosis (enlargement of the heart arteries which may lead to cardiac infarction in diabetics)

1.4 Diagnosis of diabetes mellitus

1.4.1 Laboratory diagnosis of Diabetes mellitus

Diagnosis is established exclusively by the demonstration of increased concentration of glucose in the blood. Oral glucose tolerance test has been used for years as a sole diagnostic criterion (Greenberg and Sacks, 2002). There are 3 most recommended criteria of international committee of diabetic experts based on review of epidemiology data, relationship between blood glucose and the impact it has on the microvascular complications. They are:

1. The most evident symptoms of diabetes are thirst, increased urination (polyuria), unexplained loss of weight and increased plasma glucose levels of 200mg/dl (11.1mol/ L).
2. Fasting plasma glucose level of 126mg/dL or (70mmol/L) or higher after overnight (at least 8 hours) fast.
3. Two-hour plasma glucose equals 200mg/dL (11.1mmol/L) or higher during a standard 75g oral glucose tolerance test (Ahmed and Goldstein, 2006). Fasting glucose measurement which is above 126 mg/dL or 7.0mmol/l is considered diagnostic for diabetes mellitus. The diagnostic criteria established by an Expert Committee of the American Diabetes Association (ADA) in 1996 and ratified by WHO is outlined in table 1.1.

Table 1.1: Diagnostic thresholds for diabetes and lesser degrees of impaired glucose regulation (Dineen, 2006)

Category	Fasting plasma glucose	2-hour plasma glucose
Normal	6.1 mmol/litre (110mg/dl)	7.8 mmol/litre (140mg/dl)
IFG	6.1-6.9 mmol/litre (110-125mg /dl)	-
IGT	-	7.8-11.0 mmol/litre (140-199 mg/dl)
Diabetes	≥7.0 mmol/litre (≥ 126mg/dl)	≥11.1 mmol/litre (≥200mg/dl)

IFG: Impaired fasting glucose

IGT: Impaired glucose tolerance

When both tests are performed, IFG or IGT should be diagnosed only when diabetes is not diagnosed by the other tests.

1.5 Epidemiology of Diabetes Mellitus

A global epidemic of diabetes mellitus is predicted for the first quarter of the twenty first century (Greenberg and Sacks, 2002). Diabetes is amongst the five leading causes of deaths these days (WHO, 1994). Table 1.2 illustrates statistics for T2DM.

Table 1.2: Showing non-insulin dependent diabetes statistics worldwide from 1994-2010

	1994		2000	2010
World Region	Population	NIDDM	NIDDM	NIDDM
World	5,638,219	98,868	157,338	215,616
Africa	698,388	4,766	10,881	16,956
Asia	3,879,000	46,864	86,563	126,224
North America	286,041	13,402	15,094	16,787
Latin America	483,862	11,315	14,790	18,179
Europe	510,673	16,044	20,225	24,391
Former USSR	284,654	5,735	8,844	11,946
Oceania	3,346,376	742	941	1,133

***NIDDM (Non-insulin diabetes mellitus)**

1.6 Therapeutic Intervention and limitations associated with α -glucosidase and α -amylase inhibitors

1.6.1 Action of Digestive Glycosidase

In humans digestion of starch occurs in several stages (Breyer and Luo, 1999; Ferey-Roux *et al.*, 1998). Initially, salivary α -amylase breaks down polymeric starch into shorter oligomers. Upon reaching the gut the partially digested starch is then extensively hydrolysed into shorter oligosaccharides by alpha amylase synthesized in the pancreas. This results into formation of mixture of oligosaccharides including maltose, maltotriose and a number of α (1-6) and α (1-4) oligoglucans which then pass through the mucous layer to the brush border membrane (Breyer and Luo, 1999; Ferey-Roux *et al.*, 1998). At this point a number of alpha glucosidases function to further degrade the oligosaccharides to glucose. The glucose is then absorbed and enters the blood stream by means of a specific transport system where it is distributed throughout the body. Glucose then enters into glycolytic pathways where it converted into energy; it can be stored in a form of glycogen for future use (Ryberg, 2000).

1.6.2 Alpha glucosidase and amylase enzymes

One of the therapeutic approaches for reducing postprandial hyperglycemia in patients with diabetes is to prevent absorption of carbohydrates after a meal (Ortiz-Andrade, 2007). Transportation of food into the blood requires smaller molecules (monosaccharides) such as glucose whereas starches, oligosaccharides and disaccharides must be broken down into individual monosaccharides before they can be absorbed in the duodenum and the upper part of the jejunum (Ortiz-Andrade, 2007). This digestion is facilitated by the enteric enzymes such as pancreatic α -amylase (Figure 1.2A) and intestinal α - glucosidase (Figure 1.2B) (Ortiz-Andrade, 2007; deMelo and Gomes, 2006).

Glucosidase and amylase inhibitors have been found to be very effective in reducing postprandial glucose by suppressing the absorption of glucose and these are effective in the treatment and management of diabetes and obesity (deMelo and Gomes, 2006). Current interest in these compounds has been extended to a diverse range of diseases including lysosomal storage disorders and cancer; special attention has been given to those compounds with anti-HIV and diabetic activity (deMelo and Gomes, 2006).

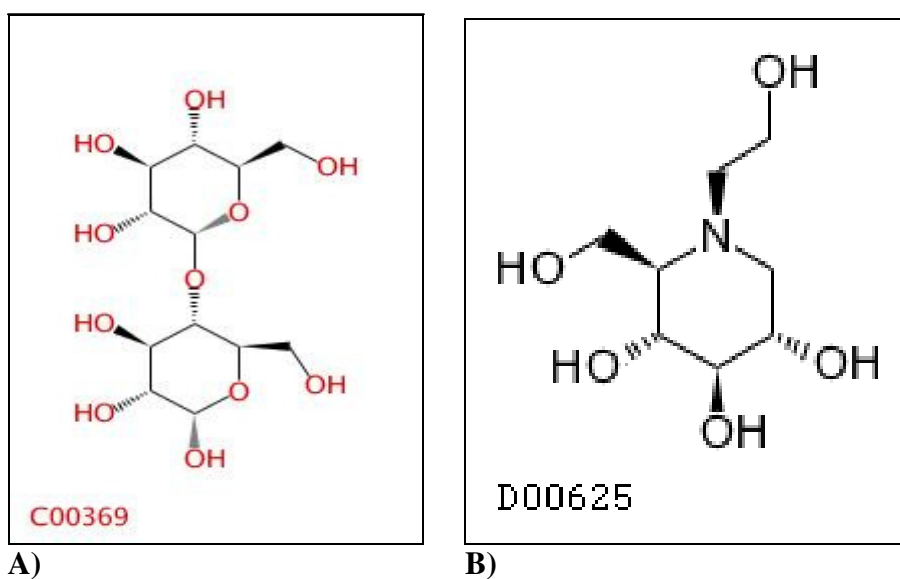


Figure 1.2: Chemical structures of alpha-amylase (A) and alpha glucosidase (B)

1.6.2.1 Alpha glucosidase and alpha amylase inhibitors

There are many pharmaceutical drugs aimed at accomplishing the role of lowering the blood glucose levels hence preventing the onset of complications. A few examples of alpha glucosidase inhibitors are: acarbose (Figure 1.3) and miglitol. These drugs interfere with the action of α -glucosidase that is present in the brush border situated in the small intestine. These drugs function locally in the intestine. They result in abdominal bloating and discomfort, diarrhea and flatulence. Additionally the α -glucosidase

inhibitors result in the reduction in digestion and absorption of glucose into systemic circulation (Skrha, 2007).

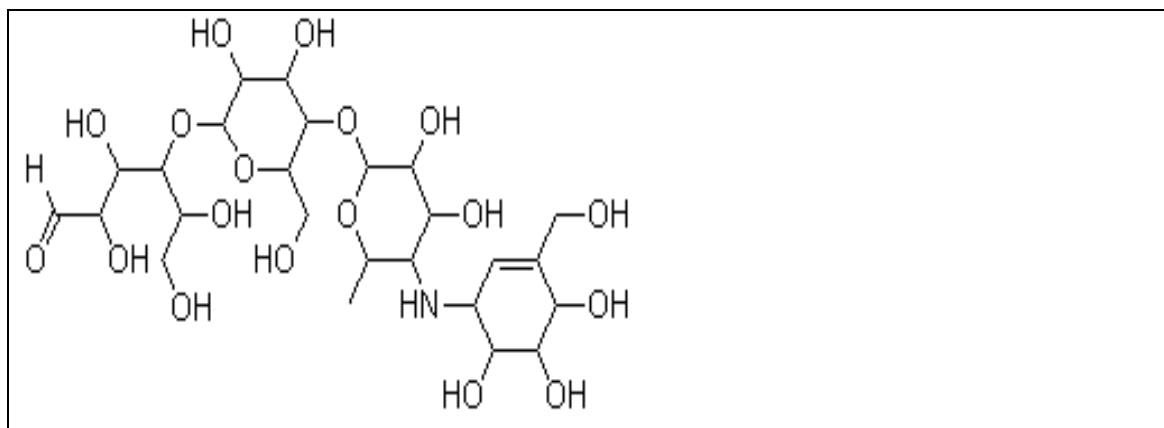


Figure 1.3: Chemical structure of acarbose (Precose)

1.6.3 Sulfonylureas

These drugs are known as endogenous insulin secretagogues since their primary role is to induce the pancreatic release of endogenous insulin and consequently these drugs are effective only when residual pancreatic β cell activity is present (Pleuvry, 2005). These drugs do not have significant effects on circulating triglycerides, cholesterol and lipoproteins that are characteristics in diabetic patients (Skrha, 2007). It is also noted that most sulfonylureas can cross the placenta and cause hypoglycemia in newborns. They can stimulate appetite hence result in weight gain (Pleuvry, 2005).

1.6.4 Biguanides e.g. metformin

In Medieval times, a prescription of a plant known as *Galega officinalis* (goat's rue) was said to relieve symptoms of intense urination that accompanied diabetes mellitus (Witters, 2001). The active constituent that was responsible for lowering of blood glucose levels was discovered to be 'galegine' or 'isoamylene guanidine' (Cusi and DeFronzo, 1998). It was observed that guanidine and certain derivatives were too toxic for the treatment of diabetes mellitus; this resulted in existence of biguanides (two linked guanidine rings) which were useful and three biguanides became available for diabetes therapy in the 1950's.

Phenformin and buformin, with the former becoming quite popular in the 1960's were withdrawn from the pharmacopoeia in the early 1970's due to the appearance of frequent lactic acidosis and increased cardiac mortality (Cusi and Defronzo, 1998). Metformin which is a less lipophilic biguanide became available for the treatment of DM even after 20 years of use in Europe. In 1995, it was approved for use in United States of America (Witters, 2001).

These drugs increase the glucose uptake by the skeletal muscles, thus reducing insulin resistance (Plevry, 2005). This class of drugs functions to lower glucose level by enhancing insulin receptors to increase the absorption of sugars however, these drugs are not ideal to people with liver diseases since the major site the major site of action of metformin is the liver and contraindication in people with liver disease may result (Skrha, 2007).

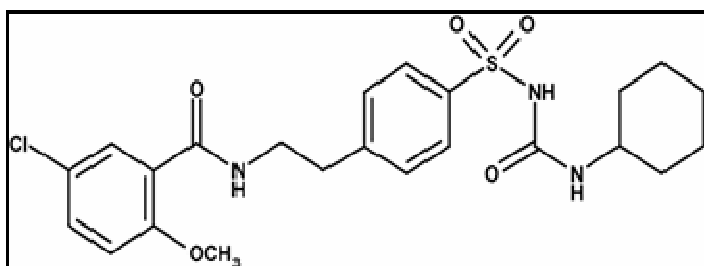


Figure 1.4: Chemical structure of metformin

1.6.5 Meglitinides e.g. prardinic and starlic

Mechanism of action of these drugs involves their binding to a receptor in the pancreatic β -cell that is different from receptors for the sulfonylureas. Additionally the drugs have no effect on circulating levels of plasma lipids (Skrha, 2007). Other drugs that are used for the treatment of diabetes mellitus are illustrated in figure 1.5, table 1.2.

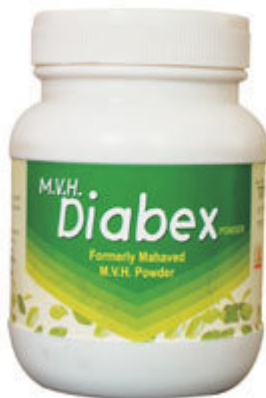


Figure 1.5: Drugs used for the treatment of diabetes mellitus

Table 1.2: Drugs used for the treatment of diabetes mellitus (Li *et al.*, 2004)

Drug group	Example (s)
Insulin	There are many kinds of preparations e.g. Pumps, injections etc
Sulfonyreas (SU)	Tolbutamide (D ₈₆₀ , Orinase), Glibonese (Glyburide, HB419)
Biguanide (BG)	Phenformin (Phenethyldiguanidi Hydrochloridum, Diabenide, DBI), Dimethylbiguanide (FluamineMetformin, Diabex, Mellitin, Metformin
Alpha glucosidase inhibitors (α -GDI)	Glucobay (Acarbose), Voglibose, Miglitol, Emiglitate, Glyset, Precose
Aldose reductase inhibitor (ARI)	Tolrestat, Alredase, Epslstat, Kinedak, Imirestat
Thiazolidinediones (TZD)	Troglitazone, Rosigitazone, Pioglitazone, Englitazone
Carbomoylmethyl benzoic acid (CMBA)	Repaglinide
Insulin-like growth factor (IGF)	IGF-1
Others	Dichloroacetic acid

1.6.6 Insulin

1.6.6.1 Chain of events upon the discovery of insulin

In 1955 ‘Fred Sanger’ was the first to have the amino acids sequence of insulin sequenced (Sanger, 1988), this resulted in him earning Nobel Prize in 1958. It was also the first peptide hormone, circulating

in minute amounts, to be measured using radioimmunoassay (Bersin and Yallow, 1961). In 1967, Don Steiner was the first to determine the pathway behind the biosynthesis of insulin in pancreatic beta cells, specifically as a proinsulin precursor (Steiner and James, 1992). Three-dimensional structure of insulin was discovered by Dorothy Crowfoot Hodgkin and colleagues in 1969 using X-ray crystallographic methods (Adams *et al.*, 1969). In addition, insulin was also the first protein to be synthesized in microorganisms by recombinant DNA technology in the late 1970's. This authenticated the design of insulin in order to optimize the molecule's pharmacodynamic profile for therapeutic purposes.

1.6.6.2 Structure and function

Insulin is the anabolic hormone that is secreted by pancreatic β -cells of the 'Islets of Langerhans' in the pancreas in response to increased circulation levels of glucose and amino acids (Nathanson and Nystrom, 2009). It has two aspects of action, on one aspect it acts as metabolic hormone, while the other aspect is characterized by acting as a growth factor (Mitsuru *et al.*, 2009). The main function of insulin is to maintain normal glucose homeostasis by reducing hepatic glucose production via gluconeogenesis and glycogenolysis. This hormone also aid in promoting glucose uptake primarily by the skeletal muscle and to a lesser extent adipose tissue. This improved glucose uptake involves insulin-stimulated translocation of isoform of the facilitative glucose transporter GLUT-4 to the cell surface, as well as activation of the rate limiting step in glycogen synthesis in muscle cells by insulin (Shulman, 2000).

1.6.6.3 Glucose homeostasis

Homeostasis of glucose is maintained by the highly coordinated interaction of three physiological processes namely, insulin secretion, tissue glucose uptake and hepatic glucose production. The body keeps the supply of glucose to the cells by maintaining a constant concentration of glucose in the blood (Brunner *et al.*, 2009). Normal glucose homeostasis in the body is represented by the balance between

intake (glucose absorption from the gut), tissue utilization (glycolysis, pentose phosphate pathway, tricarboxylic acid cycle, glycogen synthesis) and endogenous production (glycogenolysis and gluconeogenesis) (Meyer *et al.*, 2002). Fatty acids and glucose are considered as the most important fuels. The glucose is mostly used by the brain cells and muscles therefore, to ensure the continuous supply of glucose to the brain and other muscles, metabolic fuels are stored for use in time of need (Brunner *et al.*, 2009). Insulin-an anabolic hormone and some insulin-like growth factors maintain glucose homeostasis (Dunger, 1995). There are several catabolic hormones such as glucagon, catecholamines, cortisol and growth factors that oppose the action of insulin and these hormones are known as anti-insulin or counter-regulatory hormones (Gerich and Campbell, 1988).

1.6.6.4 The role of insulin and glucagon

Insulin is a 51 amino acid protein that is secreted by the β -cells of the pancreatic islets in response to increased blood glucose levels just after a meal. There are several ways by which insulin decreases the plasma glucose levels: 1) the uptake of glucose into tissue, 2) intracellular glucose metabolism, 3) glycogen synthesis (Figure 1.6). The β -cells that are situated in the pancreas's islets of Langerhans secrete insulin (Brunner *et al.*, 2009). There are four main targets of insulin action: the liver, the muscles, adipose tissue and the brain. Upon the binding of insulin to its receptor, a series of reactions which affect glycogen synthesis and glucose transport take place (Kahn, 1988). Glucagon is a small single chain, 29 amino-acid peptide that is secreted by the α -cells of the pancreas. Glucagon mobilizes the fuel reserves for the maintenance of the blood glucose levels after meals by inhibiting glucose-utilization pathways and the storage of metabolic fuels. Its main target is the liver where it stimulates glycogenolysis whilst inhibiting glycogen synthesis, glycolysis and lipogenesis (Brunner *et al.*, 2009). This balance between insulin and glucagon action is a key factor in the control of metabolism. During diabetes mellitus etiology this process is disturbed.

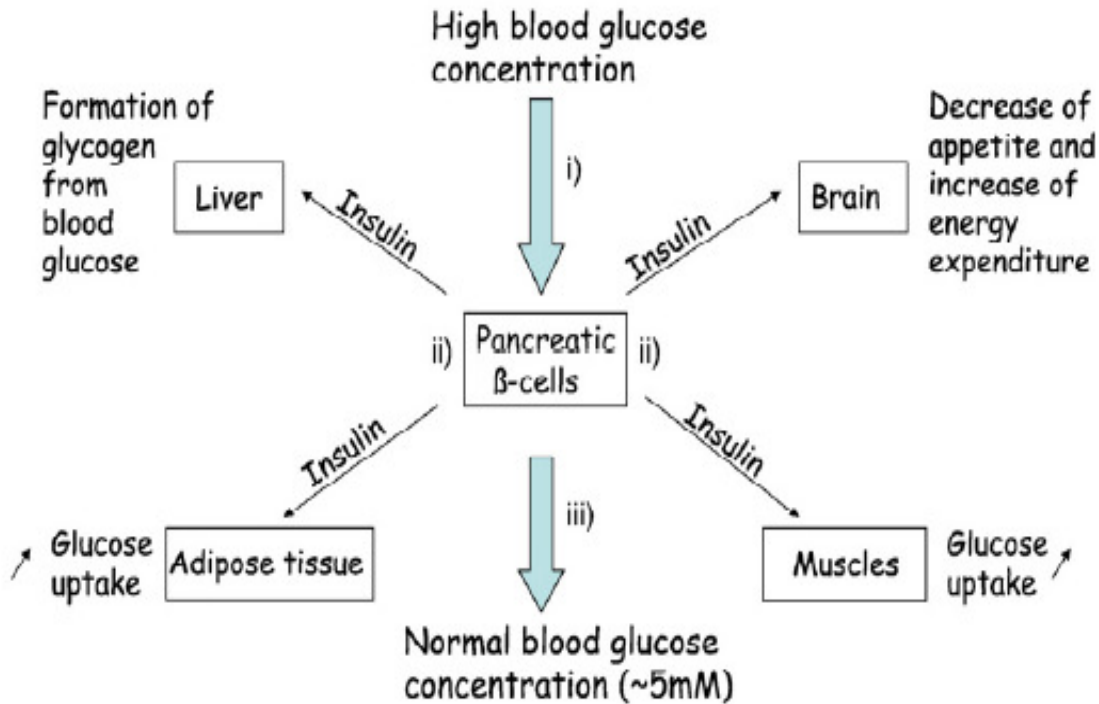


Figure 1.6: Role of insulin for the control of glucose homeostasis. 1) Increase of blood glucose induces the secretion of insulin by the β -cells 11) The action of insulin in the different insulin targeted tissues allows the return to the normal glucose concentration (Brunner *et al.*, 2009).

1.7 Antioxidants and diabetes mellitus

Oxidation is the transfer of electrons from one atom to another and represents an essential part of aerobic life and our metabolism, since oxygen is the ultimate electron acceptor in the electron flow system that produces energy in the form of Adenosine-5'-triphosphate (ATP). However, problems may arise when the electron flow becomes uncoupled (transfer of unpaired single electrons), generating free radicals. Examples of oxygen-centered free radicals, known as reactive oxygen species (ROS), include superoxide (O_2^-), peroxy (ROO^\cdot), alkoxy hydroxyl, and nitric oxide. The hydroxyl (half-life of 10^{-9} s) and the alkoxy (half-life of seconds) free radicals are very reactive and rapidly attack the molecules in nearby cells. The damage caused by them is

necessary and is dealt with by repair processes. On the other hand, the superoxide anion, lipid hydroperoxides, and nitric oxide are less reactive. In addition to these ROS radicals, in living organisms there are other ROS non radicals, such as the singlet oxygen (O_2^-), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl). ROS may be very damaging since they can attack lipids in cell membranes, proteins in tissues or enzymes, carbohydrates, and DNA, to induce oxidations, which cause membrane damage, protein modification (including enzymes), and DNA damage. This oxidative damage is considered to play a causative role in aging and several degenerative diseases associated with it, such as heart disease, cataracts, cognitive dysfunction, diabetes and cancer (Pietta, 2000).

Humans have antioxidants systems to protect themselves against free radicals. These systems include some antioxidants produced in the body (endogenous) and those that are obtained from diets (exogenous). Endogenous antioxidants include enzymatic defense, catalase, superoxide dismutase etc. Exogenous antioxidants on the other hand include vitamins A, E, C., carotenoids, and nitrogen containing compounds. Complications that arise in diabetes are as a result of damage that is caused by ROS. Plants offer a wide range of antioxidants that can be beneficial for the management of diabetes (Pietta, 2000).

2. Use of plants against diabetes mellitus

Plants have always provided mankind with all his needs in terms of shelter, clothing, food, flavours and fragrances as not the least, medicines. Plants have formed the basis of sophisticated traditional medicines systems among which are Ayurvedic (Indian), Unani and Chinese and the plants which were used by these medicinal systems are still in use today. Amongst the lesser known medicinal systems is African

partly because African medicine is verbal; some of the precious information of plants with medicinal value is lost and was not recorded.

The search for new molecules nowadays is changing slightly. Previously plants have been used as crude extracts which consisted of numerous active compounds. Some of these compounds may act synergistically, while at times they can have antagonist effects. The focus is now more on ethnobotany and ethnopharmacognosy in which chemists isolate and purify different sources and classes of compounds (Gurub-Fakim, 2006).

Complications associated with diabetes are the major source of morbidity and mortality in patients (Gurub-Fakim, 2006). People are now turning to alternative medicines for chronic illnesses. Plants have always been used for diabetes mellitus. Drugs that treat diabetes are derived directly or indirectly from plants (WHO, 1999). Some plant/ plant products act by lowering blood glucose levels while others work by inhibiting the absorption of glucose from the gut hence prevent the shoot of glucose in the blood just after the meal (Gurub-Fakim). Ethnobotanical information has reported that approximately 800 plants have some anti-diabetic properties, quite a few number of herbs have shown anti-diabetic results when tested using the available technology (Olarcon-Aguilera, 2000). Amongst the plant-derived actives that have shown some anti-diabetic activity include alkaloids, glycosides, polysaccharide, peptidoglycans, steroids, carbohydrates, amino acids and inorganic ions. It is also indicated that screening alpha glucosidase/ amylase inhibitors from plants is increasing (Gurub-Fakim, 2006), however a large number of these plants have not yet being studied in detail for their pharmacological activities. It can be concluded that plants are potential source of anti-diabetic drugs but this fact has not gained enough momentum in the scientific community.

3. Objectives of the study

- To discover and characterize new α -glucosidase and α -amylase inhibitors for the management of diabetes mellitus from natural sources (plants) using bioassay guided fractionation.
- To determine the chemical structure and activities of possible pure compounds isolated by bio-assay guided fractionation.
- To evaluate the efficacy of isolated compounds from plants for diabetes mellitus.
- To investigate oxidant scavenging activity of plant extracts and pure compounds.

4. Structure of the dissertation

Chapter 1:

This chapter gives detailed information on diabetes mellitus. Classification, complication, diagnosis, epidemiology as well as therapeutic intervention of diabetes is discussed in detail.

Chapter 2:

Selected plants for the treatment of diabetes mellitus have been briefly discussed in this chapter. A closer look at the plants chemistry, medicinal value as well as description is further discussed.

Chapter 3:

Inhibitory effect of plant extracts on α -glucosidase and α -amylase has been argued. The antioxidant and radical scavenging capacity of plants extracts have been discussed. In addition, cytotoxicity of selected plant, *T. sericea* against primary vervet monkey kidney cells (VK) is discussed.

Chapter 4:

Isolation, purification and identification of compounds from *T. sericea* using column chromatography and nuclear magnetic resonance and mass spectra have been discussed in this chapter.

Chapter 5:

This chapter displays the inhibitory activities of purified compounds on α -glucosidase and α -amylase. Furthermore, cytotoxicity and antioxidant properties of purified compounds are discussed.

Chapter 6:

This chapter gives a brief overview of all the findings in the study. Recommendations are also looked at in this chapter.

Chapter 7:

Acknowledgements

Chapter 8:

Appendices

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2. Introduction

Traditional medicine derived mainly from plants play a pivotal role in the management of diabetes mellitus (Ahmed *et al.*, 2004; Karunayake and Tennekoon, 1993). World Health Organization (WHO) has recommended the evaluation of the plants used in the treatment of diabetes, as they are considered to be effective, non-toxic, with lesser side effects and are said to be excellent candidates for oral therapy (Day, 1998).

Metformin, a well known antidiabetic drug has been used for the treatment of diabetes mellitus for centuries. Biguanides are a group of plant-derived compounds that are responsible for antidiabetic property in metformin. It is therefore, proposed that plants may contain similar antidiabetic compounds that can be used to lower blood glucose levels. Plants for the present study have been selected based on phytochemistry and ethnobotanical information. Different plant parts (leaves, root bark, and stem bark) were investigated, however, based on literature review these plant parts have not been validated scientifically as yet. The aim of this study therefore, was to investigate antidiabetic and antioxidant property of selected plants. A brief description, distribution, medicinal use and phytochemistry of each plant is discussed. Further details of isolated compounds of plants are illustrated in the appendix B (from dictionary of natural products).

2.1 Selected plants

2.1.1 *Psidium guajava* L

Family: Myrtaceae

Common name: **guava, guajava, kuawa**

2.1.1.1 Description

A small tree to 33 ft (10inch) with spreading branches, the guava is easy to recognize because of its smooth, copper-coloured bark that flakes off showing the greenish layer beneath; and also

because of the attractive “bony” aspect of its trunk which may in time attain a diameter of 10 inch (25cm). Young twigs are quadrangular and downy. The leaves are aromatic when crushed and are evergreen. The fruit gives off a strong, sweet, musky odor when ripe, may be round, ovoid or pear shaped, 2-4 in (5-10 cm) long. When immature before ripening, the fruit is green, hard, gummy within and very astringent (<http://www.hort.purdue.edu/newcrop/morton/>)

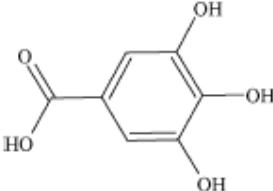
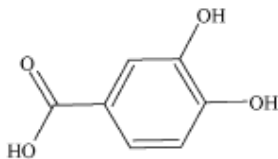
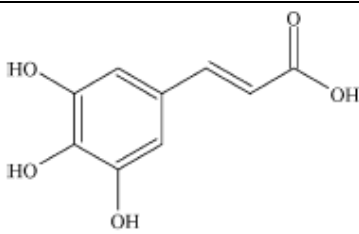
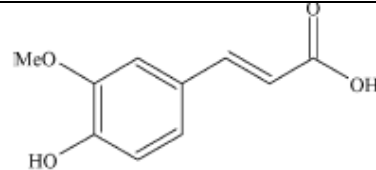
2.1.1.2 Distribution

The guava has been cultivated and distributed by man, birds, animals for so long that its place of origin is uncertain, but it is believed to be an area extending from southern Mexico into or through Central America. It is common throughout all warm areas of tropical America and the West Indies (since 1526), the Bahamas, Bermuda and Southern Florida where it was reportedly introduced in 1847 and was common over more than half the state by 1886. Early Spanish and Portuguese colonizers were quick to carry it from the New World to the East Indies and Guam. It was soon adopted as a crop in Asia and in warm parts of Africa. It occurs throughout the Pacific Islands (<http://www.hort.purdue.edu/newcrop>)

2.1.2.3 Phytochemistry

Guava is rich in tannins, phenols, triterpenes, Flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fiber and fatty acids. The leaves of *P.guajava* are rich in flavonoids, α -pinene, β -pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, β -bisabolene, cineol, caryophyllene oxide, β -copanene, farnesene, humulene, selinene, cardinene and curcumene (Zakaria and Mohd, 1994; Li *et al.*, 1999; Rattanachaikunsopon, and Phumkhachorn, 2007).

2.1.2.3.1 Other compounds isolated from *P. guajava***Table 2.1:** Constituents of *P.guajava* isolated from the leaves, roots and bark and their uses (Perez Guti'erez *et al.*, 2008)

Structure	Use
 <p>Gallic acid</p>	Cardioprotective effects against Ischemia-reperfusion. Antioxidant
 <p>Protocatechiuc acid</p>	Antioxidant
 <p>Caffeic acid</p>	Antibacterial and Antioxidant,
 <p>Ferulic acid</p>	Antioxidant, antimutagenic/anticarcinogenic effect and anti-inflammatory

2.1.1.4 Medicinal uses

External use

A decoction of the bark and leaves/ flowers is used topically for wounds, ulcers and skin sores. The roots, bark, leaves and immature fruits because of the astringency, are commonly employed to halt gastroenteritis, diarrhea and dysentery throughout the tropics.

Internal use

It has been used for sore throat, vomiting, stomach pains and also to regulate menstrual periods (Rattanachaikunsopon, and Phumkhachorn, 2007).



Figure 2.1: *Psidium guajava*

<http://images.search.yahoo.com/search/images?p=PSIDIUM+GUAJAVA&toggle>

2.1.2. Terminalia sericea Burch. Ex DC

Family: Combretaceae

Common names: **silver cluster-leaf** (Engl), **vaalboom** (Tswana), **muxonono** (N.Sotho), **mususu** (Tsonga and Venda), **amangwe** (Zulu)

2.1.2.1 Description

Terminalia is a medium sized deciduous or semi deciduous shrub that can grow about 5-8 meters in height (Eldeen *et al.*, 2006). It has an erect trunk and wide spreading crown with bark being grey to pale brown and coarsely fissured (Palmer and Pitman, 1972). Leaves are silver haired and crowned near the branch tips with cream colored flowers that have unpleasant smell. Fruits are about 30mm long with two wide papery wings surrounding the thickening central part.

2.1.2.2 Distribution

The plant is mostly dispersed in sand savanna areas in further North parts of Southern Africa (Van Wyk, 1995).

2.1.2.3 Phytochemistry

Compounds so far isolated from *Terminalia sericea* include a triterpene sericoside resveratrol-3-*O*- β -D-rutinoside, a hydroxystilbene glycoside (Moshi and Mbwambo, 2004).

2.1.2.4 Medicinal uses

Tswana, a South African tribe use root decoction as a remedy for stomach disorders and diarrhea. Decoction and infusion are used for eye lotion and for the treatment of pneumonia. The bark is used for diabetes mellitus and for wound infections (Van Wyk, 1997).



Figure 2.2 : *Terminalia sericea*

<http://www.nparks.gov.sg/nursery/uploadfiles/terminaliasericea03p408.jpg>

2.1.3 Artemisia afra Jacq. Ex Willd

Family: Asteraceae

Common names: **wild/ African wormwood** (Eng); **wilde als** (Afri); **umhlonyane** (Zulu and Xhosa); **lengana** (Tswana)

2.1.3.1 Description

Artemisia afra grows in thick, bushy and slightly disorderly clumps. The stems are thick, woody at the bases but softer and thin towards the top. *A. afra* can grow up to two meters high. Numerous side branches appear from the main stems. Leaves are thinly separated, with the upper surface being dark green while the undersides and the stems are covered with small white hairs, which gives the shrub

the characteristic overall grey color. Flowers bloom in late summer from March to May. The flowers are yellow and small with diameter between 3-4mm (Van Wyk and Gericke, 2000).

2.1.3.2 Distribution

This plant is distributed in Cedeberg Mountains in the Cape, and can spread as far as tropical east Africa, and north of Ethiopia. It prefers to grow at altitudes between 202-440 m on damp slopes, along stream sides and forest margins (Van Wyk *et al.*, 1997).

2.1.3.3 Phytochemistry

Volatile oil which contains largely 1, 8-cineole, α and β thujone, camphor and borneol are largely distributed in *Artemisia* leaves (Graven *et al.*, 1992). ‘Thujone’, has been associated with toxicity during overdose and long use. Terpenoids of eudesmadien and germacratien types as well coumarins and acetylenes are also present in *Artemisia afra* (Dictionary of natural products, 1996).

2.1.3.4 Medicinal uses

Artemisia afra is used for a wide variety of ailments including cough, fever, cold and loss of appetite, colic, headache, earache and diabetes (Van Wyk *et al.*, 1997). *A. afra* has been used to treat inflammatory diseases such as rheumatism, fever and diabetes (Hallowell and Gutteridge, 1989).



Figure 2.3 : *Artemisia afra*
<http://www.herbalsmokeshoppe.com/dagga/afra1.gif>

2.1.4 Aloe ferox Mill

Family: Aloaceae

Common names: **Bitter/ Red Aloe** (Engl); **Bitteraalwyn** (Afri); **iNhlaba** (Zulu); **ikhala** (Xhosa)

2.1.4.1 Description

Aloe ferox is a tall single stemmed plant that can grow up to 2-3 meters with leaves arranged in a rosette. After the old leaves have dried, they form a ‘petticoat’ on the stem. The leaves are dull green; sometimes they may be slightly blue with spines present in the upper and lower surfaces. The flowers are carried in a large umbrella-like flower head. The colour of the leaves ranges from yellow-orange to bright red. Flowers emerge between May and August but in colder parts of the country this may be delayed until September (Van Wyk *et al.*, 1997).

2.1.4.2 Distribution

Aloes are distributed ranging over 1000km from the southwestern Cape through the southern Kwazulu Natal. They are found in the southern corner of Free State and Lesotho (Van Wyk *et al.*, 1997).

2.1.4.3 Phytochemistry

The Aloes contain anthrone C-glucoside aloin (barbaloin). Glycoproteins present in Aloes are responsible for wound healing. Aloeresin was recently isolated from Cape Aloes (Manito *et al.*, 2003). Aloeresin A have been reported to demonstrated dose-dependent alpha glucosidase inhibitory activity with IC₅₀ values of 11.94 and 2.16mM against intestinal sucrose and maltase respectively (Jong-Anurakkun *et al.*, 2008).

2.1.4.4 Medicinal Uses

Cape aloes have been used for centuries as laxatives. The leaves and roots are boiled in water and are taken for the treatment of arthritis, eczema, conjunctivitis, hypertension, diabetes and stress. The powdered cape aloe when mixed with Vaseline is applied topically to herpes and shingles (Van Wyk *et al.*, 1997).

2.1.5 *Euclea natalensis* A.DC

Family: Ebeaceae

Common names: **Natal guarri** (Engl); **Natalgwari** (Afri); **Umzimane** (Zulu)

2.1.5.1 Description

The plant grows in coastal forests as a shrub or tree and can reach a maximum height of 10m. At a young age, the bark is white bit turns darker and fissured with age. Flowers are small, scented and greenish-white (this is usually noted in May to January) are borne in abundant on branched beads leaves are covered with dense, rust-colored hairs. Attractive fruits to birds are spherical in shape with size between 7-10mm and they usually turn red when ripe (<http://www.up.ac.za/academic/botany/garden/species/1>).

2.1.5.2 Distribution

Euclea natalensis is widely distributed in tropical and subtropical Africa. In Southern Africa it is predominantly found in the East Coast (Port Elizabeth) up to Mozambique extending to Swaziland, Botswana and Zimbabwe.

2.1.5.3 Phytochemistry

Several secondary metabolites have been isolated from *E. natalensis*. Nine of these compounds are naphthoquinones. In addition other compounds that have been isolated from this plant include dihydroxyrunic acids (lactone derivatives), triterpenoids and one tetrahydroxyflavone arabinopyranoside (Lall *et al.*, 2006; Van der Kooy *et al.*, 2006). *Euclea natalensis* is rich in pentacyclic terpenoids and triterpenoids (Hutchings *et al.*, 1996). Some triterpenes have been reported to exert antidiabetic activities or potent α -glucosidase inhibitory activities (Luo *et al.*, 2008).

2.1.5.4 Medicinal uses

Zulu people use the roots for bronchitis, pleurisy, chronic asthma and urinary tract infections. Local inhabitants of South Africa are reported to also use the roots for headaches and toothaches (Lall *et al.*, 2006). Roots of *E. natalensis* have shown some antibacterial activity against *Mycobacterium tuberculosis* and some gram-negative bacteria (Lall *et al.*, 2006; Khan *et al.*, 1978).

2.1.6 Warburgia salutaris (Bertol.f.) Chiov.

Family: Canellaceae

Common names: **pepper-bark tree** (Engl.), **peperbasboom** (Afri), **mulanga**, **manaka** (Venda), **shibaha** (Tsonga), **isibhaha** (Zulu)



Figure 2.4 : *Aloe ferox*
http://perso.orange.fr/h.jung/aloes/Aloe_ferox3.jpg

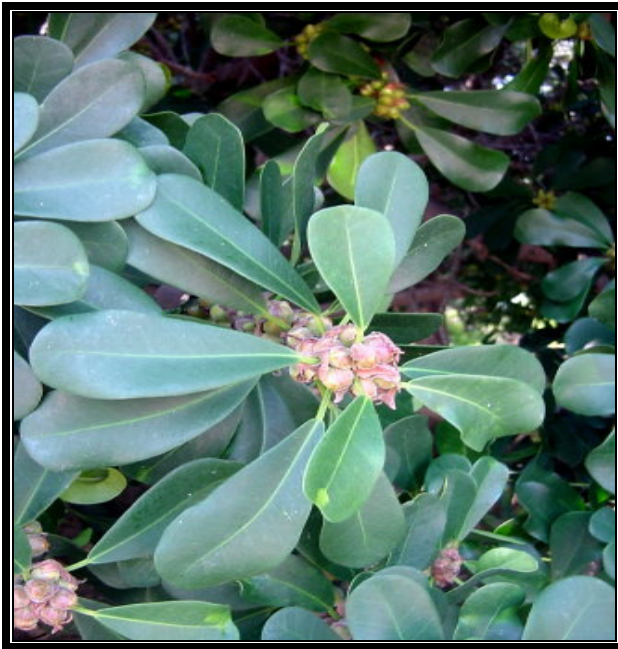


Figure 2.5 : *Euclea natalensis*
http://www.calflora.net/southafrica/images/ficus_natalensis.jpg

2.1.6.1 Description

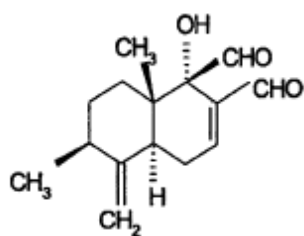
Pepper-bark tree is about 10m in height with rough, molted bark, which is reddish in the inner side. Leaves are rectangle in shape, 60mm long with glossy green on top while they are paler below. The flowers are small, greenish yellow in color with round green fruits (Codd, 1976).

2.1.7.2 Distribution

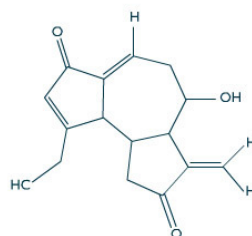
The plant is found in Northeastern parts of South Africa.

2.1.6.3 Phytochemistry

Fractionation of the ethyl acetate extract of the stem bark of *W. salutaris* yielded a known sesquiterpenoid, muzigadial (Rabe and van Staden, 2000). Another sesquiterpene lactone was isolated from *Warbugia salutaris*. The compound demonstrated *in vitro* activity against chloroquine sensitive strain D 10 ($IC_{50} = 0.9\mu\text{g/ml}$) and chloroquine resistant strains (1.2 $\mu\text{g/ml}$) and RSA 11 ($IC_{50} = 0.96\mu\text{g/ml}$) of *Plasmodium falciparum* (Sekhoacha *et al.*, 2007). Other compounds isolated from *W. salutaris* include: warburganal, polygadial, isopolygadial, salutarisolide and mukaadial (Mashimbye *et al.*, 1999).



A)



B)

Figure 2.6: A) Structure of muzigadial; B) Sesquiterpene lactone isolated from *W. salutaris*

2.1.6.4 Medicinal use

Warbugia salutaris has been overexploited by the collectors in the wild for traditional medicinal purposes. The stem bark is most widely sought for the traditional herbal market leading to the species endangered status (Rabe and van Staden, 2000). A bark decoction is taken for colds, influenza, sinus, and other respiratory complaints. Powdered bark on the other hand is applied to sores and used as snuff whilst it is also used for the treatment of malaria (Mander *et al.*, 1995).



Figure 2.7 : *Warbugia salutaris*
<http://www.plantzafrika.com/plantwxyz/warburg.htm>

2.1.7. *Sclerocarya birrea* A.Richi.) Hochst. subsp. caffra

Family: Anacardiaceae

Common names: **marula** (Eng.); **morula** (Northern Sotho); **mufula** (Tshivenda); **ukanyi** (Tsonga)

2.1.7.1. Description

Marula is a medium sized to large deciduous tree with erect trunk and rounded crown. Separate trees bore female and male flowers that produce pollen and fruits for which the tree is famous for. The

fruits are green on the tree but turn yellow after falling-usually from February to June. Compound leaves are mostly crowded at the end of branches (<http://www.plantzafrica.com/plantqrs>).

2.1.7.2. Distribution

The marula is widespread in Africa from Ethiopia in the north to KwaZulu-Natal in the south. In South Africa it is more common in the Baphalaborwa area in Limpopo. It occurs naturally in various types of woodland, on sandy soil or on occasionally sandy loam (<http://www.plantzafrica.com/plants>).

2.1.7.3 Phytochemistry

The bark is known to possess 3.5-20.5% tannin, 10.7% tannin matter and traces of alkaloids (Watt and Breyer-Brandwijk, 1962). The fruit is rich in ascorbic acid and juice extracts yield 33 sesquiterpene hydrocarbons, while kernels yield 54-60% of non-drying oil and 28% protein and iodine (Pretorius *et al.*, 1985; Watt and Breyer-Brandwijk, 1962). The oil-rich seeds contain 64% oleic acid, myristic, stearic and amino acids with prevalence of glutamic acid and arginine. The gum is rich in tannins, whereas the leaves are rich in both tannins and flavonoids (Busson, 1985). A number of investigators have shown that coumarins, flavonoids, terpenoids, and a host of other secondary plant metabolites present in *Sclerocarya birrea*, including arginine and glutamic acid, showed hypoglycemic effects in various experimental animal models (Akah and Okafor, 1992).

2.1.7.4. Medicinal use

Zulu, a South African tribe use bark decoctions as enemas for diarrhea, the bark decoctions are also taken in 300 ml doses for dysentery (Watt and Breyer-Brandwijk, 1962). Bark is also being used for the treatment of proctitis; the Venda people use the bark for treating fevers, stomach ailments and

ulcers (Mabogo, 1990). Roots are used for sore eyes in Zimbabwe whereas in East Africa, roots are ingredient in alcoholic medicine taken to treat internal ailments (Kokwaro, 1976).

2.1.8 *Spirostachys africana* Sond

Family: Euphorbiaceae (spurge family)

Common names: **Tamboti** (Engl.), **tambotie** (Afri.), **umThombothi** (Zulu), **Modiba** (N.Sotho), **Muonze** (Venda)

2.1.9.1 Description

Spirostachys africana is medium sized, semi deciduous tree that can grow up to 18m. The bark is dark brown to black, thick, rough and neatly cracked into rectangular blocks. It consists of alternative leaves that are up to 70 x 35 mm, these leaves are often visible among the older, green leaves in spring. The flowerheads are 15-30mm long bearing mostly male and female flowers. The flowers of females are attached at the base of each spike. The fruit is characterised by the capsule that is three lobed and opens when ripe (<http://www.plantzafrica.com/plantqrs/spirostachafri.htm>).

2.1.8.2 Distribution

This plant is distributed mostly in KwaZulu-Natal in the South up to Tanzania, it can also be found in all southern African countries except Lesotho (<http://www.plantzafrica.com/plant>).

2.1.8.3 Phytochemistry

Two diterpenoids and nonditerpenoids were isolated from the plant. Other active constituent present in *S. africana* is stachenol (Munkombwe *et al.*, 1997).

2.1.8.4 Medicinal Uses

The leaves of *S. Africana* are used for stomach ulcers, acute gastritis, eye washes, headaches, rashes, emetic, renal ailment, purgatives, blood purification, diarrhoea, and dysentery (Elgarashi *et al.*, 2003).



Figure 2.8 : Image of *Sclerocarya birrea*

<http://www.plantzfrica.com/plantqrs/sclerobirr.htm>



Figure 2.9 : *Spirostachys africana*

http://en.wikipedia.org/wiki/Spirostachys_africana

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