EFFECT OF THE EXTRACTS OF STEM BARK OF *Detarium microcarpum* AND LEAVES OF *Vitex doniana* ON ALPHA GLUCOSIDASE AND ALPHA AMYLASE ACTIVITY

BY

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DECLARATION

I hereby declare that the work in this thesis "**Effect of the Extracts of Stem Bark of *Detarium microcarpum* and Leaves of *Vitex doniana* on Alpha Glucosidase and Alpha Amylase Activity**" was performed by me in the Department of Biochemistry, under the supervision of Prof. D.A. Ameh and Mr. G.C. Njoku. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

Yakubu, Rahinat Nimma _____ _____

M.Sc/SCIE/985/2011-2012 Signature Date

CERTIFICATION

This project titled **"Effect of the Extracts of Stem Bark of *Detarium microcarpum* and Leaves of *Vitex doniana* on Alpha Glucosidase and Alpha Amylase Activity**" meets the regulation governing the award of the degree of Masters in Biochemistry of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research work is dedicated to Almighty Allah and to my most loving and supporting Parents and Siblings; your love knows no bounds.

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ABSTRACT

The *in vitro* and *in vivo* inhibitory effect of the hexane, methanol and aqueous extracts of *Detarium microcarpum* stem bark and *Vitex doniana* leaves on alpha amylase and alpha glucosidase was investigated using standard methods. The qualitative and quantitative phytochemical analysis of the extracts of *Detarium microcarpum* stem bark and *Vitex doniana* leaves showed that the methanolic and aqueous extracts of both plants contained more phytochemicals than the hexane extract of both plants. The aqueous extract of *Vitex doniana* showed a significantly ($P<0.05$) higher flavonoid content (0.87 ± 0.03mg quercertin /g dry sample) than all the other extracts while the methanolic extract of *Detarium microcarpum* showed a significantly ($P<0.05$) higher polyphenol content than the other extracts (0.62±0.13 mg gallic acid/g dry sample). The inhibition studies of the extracts revealed that the aqueous and methanolic extract of *Detarium microcarpum* stem bark were the most potent inhibitors of alpha glucosidase and alpha amylase when compared to the other extracts as they had the lowest IC_{50} for both enzymes. Upon the fractionation of the methanolic extract of *Detarium microcarpum* stem bark with ethyl acetate and n-butanol and testing of the ethyl acetate, butanol and aqueous fractions of the extract, it was revealed that the butanol and aqueous fractions contained similar phytochemicals as the crude extract such as phenols, flavonoids, tannins, saponins and alkaloids but the ethyl acetate fraction didn't contain saponin and triterpenes unlike the crude extract, the butanol and aqueous fractions. In addition, the butanol fraction contained significantly ($P<0.05$) higher flavoniod and polyphenols (0.63 ± 0.12 mg quercertin/g dry sample and 0.87 ± 0.02 mg gallic acid/g dry sample) than the crude extract and the ethyl acetate and aqueous fraction. The fractions showed very strong inhibitory activity against alpha glucosidase as was seen from the IC_{90} of the three fractions (12.19 ± 3.62µg/ml, 7.79 ± 1.36µg/ml, 16.39 ± 2.22µg/ml). The fractions were even more potent than acarbose against alpha glucosidase. In addition, the fractions inhibited alpha amylase in a dose dependent manner with the butanol fraction being the most potent with an IC_{50} of 26.33 ± 0.19µg/ml. From the lineweaver

burk plot of alpha glucosidase in the presence of the butanol fraction, a noncompetitive inhibition was seen for the fraction but a competitive inhibition was observed against alpha amylase in the presence of the butanol fraction. The oral starch and sucrose tolerance test carried out on normal rats in the presence of different doses of the methanolic extract of *Detarium microcarpum* stem bark revealed that a dose of 1000mg/kg body weight extract significantly ($P<0.05$) reduced the blood glucose of the rats in the oral starch tolerance test and a dose of 250mg/kg body weight significantly ($P<0.05$) reduced the blood glucose in the oral sucrose tolerance test. This study revealed that the extracts (hexane methanol and aqueous) of *Vitex doniana* and *Detarium microcarpum* are potent inhibitors of alpha amylase and alpha glucosidase and may prove useful in the management of postprandial hyperglycemia and consequently in the management of type 2 diabetes.

TABLE OF CONTENTS

CONTENT PAGE

LIST OF TABLES

TABLES PAGE

LIST OF FIGURES

FIGURES PAGE

LIST OF APPENDICES

ABBREVIATIONS

ADA; American Diabetes Association

ANOVA; Analysis of Variance

DMSO; Dimethyl sulphoxide

DPP-4; Dipepetidyl Peptidase-4 Inhibitors

EASD; European Association for the Study of Diabetes

GAE; Gallic Acid Equivalent

MGAM; Maltase-glucoamylase

Na_2CO_3; Sodium trioxocarbonate(iv)

pNPG; para-Nitrophenol Alpha-D-GlucoPyranoside

QE; Quecertin Equivalent

TLC; Thin Layer Chromatography

TZD; Thiazolidinedione

UKPDS; United Kingdom Prospective Diabetes Studies

WHO; World Health organization

CHAPTER ONE

INTRODUCTION

Diabetes mellitus consists of a group of metabolic diseases characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action or both (American Diabetes Association, 2008). There are two main types of diabetes mellitus based on their requirement for insulin namely; Type 1 or insulin dependent diabetes mellitus (IDDM) and Type 2 or non-insulin dependent diabetes mellitus (NIDDM) (Lee *et al.,* 2006).

The most prevalent form of diabetes is non-insulin dependent diabetes mellitus (NIDDM/type II) caused by impaired secretion of insulin and /or insulin action resulting in high postprandial glucose levels (hyperglycemia). Hyperglycemia is associated with the incidence and progression of microvascular (diabetic retinopathy, loss of vision and nephropathy) and macrovascular diseases (cardiovascular diseases) that are difficult to manage (Meenakshi *et al*., 2011; Prinya *et al*., 2012).

Hydrolysis of starch by pancreatic α-amylase and hydrolysis of dextrins by intestinal α-glucosidase causes sudden rise in blood glucose levels or hyperglycemia in type 2 diabetes patients. Alpha-amylase (EC 3.2.1.1) hydrolyses alpha bonds of large, alpha-linked polysaccharides, such as starch and glycogen, yielding glucose and maltose (Pugh, 2000). Although found in many tissues, amylase is most prominent in pancreatic juice and saliva, each of which has its own isoform of human α-amylase. Amylase is found in saliva and breaks starch into maltose and dextrin.

Alpha-glucosidase (EC3.2.1.20) which is located in the brush-border surface membrane of intestinal cells, catalyzes the hydrolysis of the alpha glycosidic bond of

oligosaccharides to liberate the monosaccharide units from dietary sources (Gao *et al*., 2008), and this leads to hyperglycemia in type 2 diabetics. Therefore, one of the important therapeutic approaches to decrease postprandial hyperglycemia is to retard absorption of glucose through inhibition of carbohydrate hydrolyzing enzymes such as α- amylase and α-glucosidase. As such inhibition of alpha glucosidase and alpha amylase will help regulate the level of postprandial blood glucose. Inhibition of these enzymes is an effective strategy for the management of type2 diabetes (Kwon *et al*, 2007).

Currently a variety of chemotherapeutic agents are available for management of type 2 diabetes. These agents include hypoglycaemic agents such as acarbose, miglitol and voglibose that competitively and reversibly inhibit α-glucosidase and α-amylase enzyme from intestine as well as pancreas. However, these drugs are associated with gastrointestinal side effects such as abdominal pain, flatulence and diarrhoea in the patients, which might be caused by excessive inhibition of pancreatic α- amylase resulting in fermentation of undigested carbohydrates in the colon by colonic flora (Suzuki *et al*., 2009; Meenakshi *et al*., 2011).

From ancient times, man has been relying heavily on plant based medicine for treatment of varying diseases. Nearly half of medications that are used today have their composition origin from plant. Indeed, plants play an important role in human disease treatment for populations in developing countries, particularly in the areas where it is difficult for most to access health facility because of their remoteness from cities or their low purchasing power. The World Health Organization estimated that about 80% of Africa population use traditional medicine for their primary health care (Shrabana *et al*., 2005).

Detarium microcarpum is an African tree belonging to the Fabaceae family (legumes) (Abdalbasit *et al.,* 2009) and is widely distributed in dry savannah areas of Africa. In African ethnomedicine, different parts of the plant have been reported to possess medicinal activities (Keay *et al*., 1989; Burkell 1995; Abreu *et al*., 1998). Decoction of the stem bark is reported to possess diuretic, anti-inflammatory and anti-parasitic properties whereas its fruits and leaves are used in the treatment of dysentery and syphilis (Iwu, 1993). Water extract of the root is used for leprosy (Collier and Chapman 2001). The anti-viral property of *D. microcarpum* was reported by Olugbuyiro et al. (2009), while cytotoxicity, antibacterial properties, and hypoglycaemic activity were reported by Abreu et al., (1998), Ebi and Afieroho, (2011) and Manosroi et al. (2011) respectively. The seeds and leaves are eaten as condiment and vegetable (Njoku *et. al*., 1999).

Vitex doniana is a plant that belongs to the dicotyledonous family of Verbenaceae and is widely used in Nigerian traditional medicine. In ethnomedicine, *Vitex doniana* is employed in the treatment of a variety of diseases. Hot aqueous extracts of the leaves are used in the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhoea and dysentery (Irvine, 1961). The root and leaves are used for treating nausea, colic and epilepsy (Iwu, 1993). The antihypertensive effect of the extract of the stem bark of *V. Doniana* has been reported (Olusola *et. al.,* 1997). Extracts of the stem bark of *V doniana* have also demonstrated some level of *in vitro* trypanocidal activity against *Trypanosoma brucei brucei* (Atawodi, 2005). The potency of the aqueous extract of *V. doniana* against hyperglycemia was reported to be greater than that of the standard anti-diabetic drug, glibenclamide, at the dose of 0.3 mg/kg body weight (Ezekwesili *et. al.,* 2013). In different parts of Nigeria, the young leaves are used for vegetable sauces and porridge for meals.

1.1 Statement of Research

The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. According to the International Diabetes Foundation (IDF), there are about 387 million people living with diabetes worldwide and this number is expected to rise by 55% in 2035 (IDF, 2013). Also, 80% of diabetes deaths occur in low- and middle-income countries (Mathers and Loncar, 2011). WHO projects that diabetes will be the 7th leading cause of death in 2030 (WHO, 2011). In 2014, an estimated 4.9 million people died from consequences of diabetes (IDF, 2014).

The African continent accounts for approximately 21.5 million people with diabetes (IDF, 2014). Estimates for Africa for 2035 are likely to more than double and reach 41.5 million (IDF, 2014). In Africa, Nigeria has the highest number of people with diabetes with approximately 3.7 million people affected (IDF, 2014).

Diabetes imposes a large economic burden on the individual, national healthcare system and economy. Healthcare expenditures on diabetes accounted for 11.1% of the total healthcare expenditure in the world in 2014 (IDF, 2014). Estimated global healthcare expenditure to treat and prevent diabetes and its complication reached a total of 612 billion US dollars in 2014 (IDF, 2014) and by 2035 this number is projected to exceed 627 billion US dollars (IDF, 2013). Diabetes also imposes economic burdens in the form of loss of productivity and forgone economic growth.

1.3 Justification

Oral chemotherapeutic agents may be effective for glycemic control, but they come with their attendant side effects such as liver disorders, flatulence, abdominal pain, renal

tumours, hepatic injury, acute hepatitis, abdominal fullness and diarrhea (Meenakshi *et al*., 2011; Suzuki *et al*., 2009). Therefore, there is an increasing need for the development of a natural and safe product with minimal side effects since some of these plants are already consumed as food by most people.

Traditional medicinal plants with their various biological constituents have been used effectively by the communities for a long time to treat diseases.

Furthermore, there are various plants across the world, particularly in Nigeria that have been shown to have anti-diabetic potentials, but the mechanism through which these plants act are not known. It is therefore necessary to investigate how these plants exert their anti-diabetic actions in order to be able to explore the full potentials of these plants.

1.4 Aim and Objectives

The aim of this study is to investigate the inhibitory potential of the stem bark of *Detarium microcarpum* and leaves of *Vitex doniana* extracts on α- glucosidase and α-amylase activity.

1.4.1. Specific objectives

- To carry out phytochemical screening on the hexane, methanol and aqueous extracts of the stem bark of *Detarium microcarpum* and the leave of *Vitex doniana*.
- To evaluate the effect of the stem bark of *Detarium microcarpum* and the leaves of *Vitex doniana* extracts on alpha glucosidase.
- To evaluate the effect of the stem bark of *Detarium microcarpum* and the leaves of *Vitex doniana* extracts on alpha amylase.

- To evaluate the effect of the most potent extract on postprandial hyperglycemic wistar albino rats.

1.5 Null Hypothesis

The extracts of the stem bark of *Detarium microcarpum* and the leaves of *Vitex doniana* do not have any effect on the activity of alpha glucosidase and alpha amylase.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitus is a group of metabolic diseases characterised by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The abnormalities in carbohydrate, fat, and protein metabolism that are found in diabetes are due to deficient action of insulin on target tissues, (Craig *et. al.,* 2009). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels.

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action.

Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia.

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome. Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral

neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction.

Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral, arterial and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes.

2.1.1. Classification of diabetes mellitus

Assigning a type of diabetes to an individual often depends on the circumstances present at the time of diagnosis, and many diabetic individuals do not easily fit into a single class (American Diabetes Association, 2012). However there are different types of diabetes mellitus and they include;

- *Type 1 diabetes mellitus* **:** This form of diabetes, which accounts for only 5–10% of those with diabetes, (American Diabetes Association, 2012), previously encompassed by the terms insulin dependent diabetes, type 1 diabetes, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas. Markers of the immune destruction of the β-cell include islet cell auto-antibodies, auto-antibodies to insulin, auto-antibodies to Glutamic Acid Decarboxylase (GAD) (GAD65), and auto-antibodies to the tyrosine phosphatases IA-2 and IA-2b. One and usually more of these auto-antibodies are present in 85–90% of individuals when fasting hyperglycemia is initially detected.

  In this form of diabetes, the rate of β-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults). Some patients, particularly children and adolescents, may present with ketoacidosis as the first

manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual β-cell function sufficient to prevent ketoacidosis for many years; such individuals eventually become dependent on insulin for survival and are at risk for ketoacidosis. At this latter stage of the disease, there is little or no insulin secretion, as manifested by low or undetectable levels of plasma C-peptide. Immune-mediated diabetes commonly occurs in childhood and adolescence, but it can occur at any age, even in the 8th and 9th decades of life.

Autoimmune destruction of β-cells has multiple genetic predispositions and is also related to environmental factors that are still poorly defined. Although patients are rarely obese when they present with this type of diabetes, the presence of obesity is not incompatible with the diagnosis. These patients are also prone to other autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis, and pernicious anemia.

Some forms of type 1 diabetes have no known etiologies. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. Although only a minority of patients with type 1 diabetes fall into this category, of those who do, most are of African or Asian ancestry. Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. An absolute requirement for insulin replacement therapy in affected patients may come and go.

- *Type 2 diabetes mellitus*: This form of diabetes, which accounts for 90–95% of those with diabetes (American Diabetes Association, 2012), previously referred to as non–insulin-dependent diabetes, type 2 diabetes, or adult onset diabetes, encompasses

individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency. At least initially, and often throughout their life time, these individuals do not need insulin treatment to survive. There are probably many different causes of this form of diabetes. Although the specific etiologies are not known, autoimmune destruction of β-cells does not occur. Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. Ketoacidosis seldom occurs spontaneously in this type of diabetes; when seen, it usually arises in association with the stress of another illness such as infection. This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes. Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications. Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the higher blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their β-cell function been normal. Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance. Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal. The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity. It occurs more frequently in women with prior gestational diabetes mellitus and in individuals with hypertension or dyslipidemia, and its frequency varies in different racial/ethnic subgroups. It is often associated with a strong genetic predisposition, more so than is the autoimmune form of

type1 diabetes. However, the genetics of this form of diabetes are complex and not clearly defined.

- *Gestational Diabetes Mellitus (GDM):* For many years, GDM has been defined as any degree of glucose intolerance with onset or first recognition during pregnancy. Although most cases resolve with delivery, the definition applied whether or not the condition persisted after pregnancy and did not exclude the possibility that unrecognized glucose intolerance may have antedated or begun concomitantly with the pregnancy. This definition facilitated a uniform strategy for detection and classification of GDM, but its limitations were recognized for many years. As the ongoing epidemic of obesity and diabetes has led to more type2 diabetes in women of child bearing age, the number of pregnant women with undiagnosed type2 diabetes has increased. After deliberations in 2008–2009, the International Association of Diabetes and Pregnancy Study Groups (IADPSG), an international consensus group with representatives from multiple obstetrical and diabetes organizations, including the American Diabetes Association (ADA), recommended that high-risk women found to have diabetes at their initial prenatal visit, using standard criteria, receive a diagnosis of overt, not gestational, diabetes. Approximately 7% of all pregnancies (ranging from 1 to 14%, depending on the population studied and the diagnostic tests employed) are complicated by GDM, resulting in more than 200,000 cases annually. (American Diabetes Association, 2012).
- *Other specific types of diabetes*:
- *Genetic defects of the β-cell*. Several forms of diabetes are associated with monogenetic defects in β-cell function. These forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before age 25 years). They are referred to as maturity on-set diabetes of the young (MODY) and are characterized by impaired insulin secretion with minimal or no defects in insulin action. They are inherited in an

autosomal dominant pattern. Abnormalities at six genetic loci on different chromosomes have been identified to date. The most common form is associated with mutations on chromosome 12 in a hepatic transcription factor referred to as hepatocyte nuclear factor (HNF)-1α. A second form is associated with mutations in the glucokinase gene on chromosome 7p and results in a defective glucokinase molecule. Glucokinase converts glucose to glucose-6-phosphate, the metabolism of which, in turn, stimulates insulin secretion by the β-cell. Thus, glucokinase serves as the "glucose sensor" for the β-cell. Because of defects in the glucokinase gene, increased plasma levels of glucose are necessary to elicit normal levels of insulin secretion. The less common forms result from mutations in other transcription factors, including HNF-4α, HNF-1β, insulin promoter factor (IPF)1, and NeuroD1.

- *Genetic defects in insulin action*: There are unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to severe diabetes. Some individuals with these mutations may have acanthosis nigricans. Women may be virilized and have enlarged, cystic ovaries. In the past, this syndrome was termed type A insulin resistance. Leprechaunism and the Rabson Mendenhall syndrome are two pediatric syndromes that have mutations in the insulin receptor gene with subsequent alterations in insulin receptor function and extreme insulin resistance. The former has characteristic facial features and is usually fatal in infancy, while the latter is associated with abnormalities of teeth and nails and pineal gland hyperplasia. Alterations in the structure and function of the insulin receptor cannot be demonstrated in patients with insulin resistant lipoatrophic diabetes. Therefore, it is assumed that the lesion(s) must reside in the postreceptor signal transduction pathways.

- *Diseases of the exocrine pancreas:* Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma. With the exception of that caused by cancer, damage to the pancreas must be extensive for diabetes to occur; adrenocarcinomas that involve only a small portion of the pancreas have been associated with diabetes. This implies a mechanism other than simple reduction in β-cell mass. If extensive enough, cysticfibrosis and hemochromatosis will also damage β-cells and impair insulin secretion. Fibrocalculous pancreatopathy may be accompanied by abdominal pain radiating to the back and pancreatic calcifications identified on X-ray examination. Pancreatic fibrosis and calcium stones in the exocrine ducts have been found at autopsy.
- *Endocrinopathies:* Several hormones (e.g., growth hormone, cortisol, glucagon, epinephrine) antagonize insulin action. Excess amounts of these hormones (e.g., acromegaly, Cushing'ssyndrome, glucagonoma, pheochromocytoma, respectively) can cause diabetes. This generally occurs in individuals with pre-existing defects in insulin secretion, and hyperglycemia typically resolves when the hormone excess is resolved. Somatostatinoma- and aldosteronoma-induced hypokalemia can cause diabetes, at least in part, by inhibiting insulin secretion. Hyperglycemia generally resolves after successful removal of the tumor.
- *Drug- or chemical-induced diabetes:* Many drugs can impair insulin secretion. These drugs may not cause diabetes by themselves, but they may precipitate in diabetes in individuals with insulin resistance. In such cases, the classification is unclear because the sequence or relative importance of β-cell dysfunction and insulin resistance is unknown. Certain toxins such as Vacor (a rat poison) and intravenous pentamidine can permanently destroy pancreatic β-cells. Such drug reactions fortunately are rare. There are also many drugs and hormones that can impair insulin action. Examples include

nicotinic acid and glucocorticoids. Patients receiving α-interferon have been reported to develop diabetes associated with islet cell antibodies and, in certain instances, severe insulin deficiency, (Cozzolongo, *et al*., 2006).

- *Infections*: Certain viruses have been associated with β-cell destruction. Diabetes occurs in patients with congenital rubella, although most of these patients have HLA and immune markers characteristic of type 1 diabetes. In addition, coxsackievirus B, cytomegalovirus, adenovirus, and mumps have been implicated in inducing certain cases of the disease.
- *Uncommon forms of immune-mediated diabetes:* In this category, there are two known conditions, and others are likely to occur. The stiff-man syndrome is an autoimmune disorder of the central nervous system characterized by stiffness of the axial muscles with painful spasms. Patients usually have high titers of the GAD autoantibodies, and approximately one-third will develop diabetes. Anti-insulin receptor antibodies can cause diabetes by binding to the insulin receptor, thereby blocking the binding of insulin to its receptor in target tissues. However, in some cases, these antibodies can act as an insulin agonist after binding to the receptor and can thereby cause hypoglycemia. Anti-insulin receptor antibodies are occasionally found in patients with systemic lupus erythematosus and other autoimmune diseases. As in other states of extreme insulin resistance, patients with anti-insulin receptor antibodies often have acanthosis nigricans. In the past, this syndrome was termed type B insulin resistance.
- *Other genetic syndromes sometimes associated with diabetes:* Many genetic syndromes are accompanied by an increased incidence of diabetes. These include the chromosomal abnormalities of Down syndrome, Klinefelter syndrome, and Turner syndrome. Wolfram's syndrome is an autosomal recessive disorder characterized by insulin-

deficient diabetes and the absence of β-cells at autopsy. Additional manifestations include diabetes insipidus, hypogonadism, optic atrophy, and neural deafness.

2.1.2. Categories of individuals with increased risk of diabetes

In1997 and 2003, The Expert Committee on Diagnosis and Classification of Diabetes Mellitus (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1997, Genuth *et. al.*, 2003) recognized an intermediate group of individuals whose glucose levels do not meet criteria for diabetes, yet are higher than those considered normal. These people were defined as having impaired fasting glucose (IFG) (fasting Blood glucose (FBG) levels 100 mg/dl (5.6 mmol/l) to 125mg/dl (6.9mmol/l)), or impaired glucose tolerance (IGT) (2-h values in the oral glucose tolerance test (OGTT) of 140 mg/dl (7.8 mmol/l) to 199 mg/dl (11.0 mmol/l)).

Individuals with IFG and/or IGT have been referred to as having pre-diabetes, indicating the relatively high risk for the future development of diabetes. IFG and IGT should not be viewed as clinical entities in their own right but rather risk factors for diabetes as well as cardiovascular disease. IFG and IGT are associated with obesity (especially abdominal or visceral obesity), dyslipidemia with high triglycerides and/or low HDL cholesterol, and hypertension. Structured lifestyle intervention, aimed at increasing physical activity and producing 5–10% loss of body weight, and certain pharmacological agents have been demonstrated to prevent or delay the development of diabetes in people with IGT; the potential impact of such interventions to reduce mortality or the incidence of cardiovascular disease has not been demonstrated to date.

As A1C (glycated haemoglobin) is used more commonly to diagnose diabetes in individuals with risk factors, it will also identify those at higher risk for developing

diabetes in the future. When recommending the use of the A1C to diagnose diabetes in its 2009 report, the International Expert Committee (International Expert Committee, 2009) stressed the continuum of risk for diabetes with all glycemic measures and did not formally identify an equivalent intermediate category for A1C. The group did note that those with A1C levels above the laboratory "normal" range but below the diagnostic cut point for diabetes (6.0 to 6.5%) are at very high risk of developing diabetes. Indeed, incidence of diabetes in people with A1C levels in this range is more than 10 times that of people with lower levels (Edelman, *et. al.*, 2004; Pradhan *et. al.*, 2007; Shimazaki, *et. al.*,2007; Sato *et. al.*, 2009). However, the 6.0 to 6.5% range fails to identify a substantial number of patients who have IFG and/or IGT. Prospective studies indicate that people within the A1C range of 5.5–6.0% have a 5-year cumulative incidence of diabetes that ranges from 12 to 25% (Edelman, *et. al.*, 2004; Pradhan *et. al.*, 2007; Shimazaki, *et. al.*,2007; Sato *et. al.*, 2009), which is appreciably (three to eight fold) higher than incidence in the U.S. population as a whole (Geiss *et. al.*, 2006). Analyses of nationally representative data from the National Health and Nutrition Examination Survey (NHANES) indicate that the A1C value that most accurately identifies people with IFG or IGT falls between 5.5 and 6.0%. In addition, linear regression analyses of these data indicate that among the non-diabetic adult population, an FBG of 110 mg/dl (6.1 mmol/l) corresponds to an A1C of 5.6%, while an FPG of 100 mg/dl (5.6 mmol/l) corresponds to an A1C of 5.4%

Evidence from the Diabetes Prevention Program (DPP), wherein the mean A1C was 5.9% (SD 0.5%), indicates that preventive interventions are effective in groups of people with A1C levels both below and above 5.9% (Knowler *et. al.*, 2002). For these reasons, the most appropriate A1C level above which to initiate preventive interventions is likely to be somewhere in the range of 5.5–6%. To maximize equity and efficiency of

preventive interventions, such an A1C cut point should balance the costs of "false negatives" (failing to identify those who are going to develop diabetes) against the costs of "false positives" (falsely identifying and then spending intervention resources on those who were not going to develop diabetes anyway). Compared to the fasting glucose cut-point of 100 mg/dl (5.6 mmol/l), an A1C cut-point of 5.7% is less sensitive but more specific and has higher positive predictive value to identify people at risk for later development of diabetes. A large prospective study found that a 5.7% cut-point has a sensitivity of 66% and specificity of 88% for the identification of subsequent 6-year diabetes incidence (Droumaguet *et. al.*, 2006). Receiver operating curve analyses of nationally representative U.S. data (NHANES 1999-2006) indicate that an A1C value of 5.7% has modest sensitivity (39-45%) but high specificity (81-91%) to identify cases of IFP (FPG.100 mg/dl) (5.6 mmol/l) or IGT (2-h glucose. 140 mg/dl). Other analysis suggests that an A1C of 5.7% is associated with diabetes risk similar to the high-risk participants in the DPP. Hence, it is reasonable to consider an A1C range of 5.7 to 6.4% as identifying individuals with high risk for future diabetes and to whom the term pre-diabetes may be applied if desired. Individuals with an A1C of 5.7–6.4% should be informed of their increased risk for diabetes as well as cardiovascular disease and counselled about effective strategies, such as weight loss and physical activity, to lower their risks. As with glucose measurements, the continuum of risk is curvilinear, so that as A1C rises, the risk of diabetes rises disproportionately.

2.1.3. Prevalence of diabetes

The International Diabetes Federation (IDF) estimated in 2014 that 387 million adults, aged 20–79 years, of the world's seven billion population have diabetes (IDF, 2014). This gives a comparative prevalence of 8.3%. Since more than 90% of the global cases

of diabetes are type 2, it is evident that the epidemic is mainly due to the escalation of the causes of type 2 diabetes (WHO, 1997; Motala 2002 ; WHO, 2003). Also, up to 50% of cases of gestational diabetes may end up as type 2 diabetes (American Diabetes Association, 2004). Diabetes is complicated by recurrent infections, varied eye problems, erectile dysfunction, poor obstetric outcome, skin ulcers and gangrene, renal disease, and acute hyperglycaemic emergencies. All the above complications ensure a high mortality and morbidity. The prevention and the delay in the time of onset of type 2 diabetes, will curtail the current global diabetic epidemic.

All nations, rich and poor, are suffering the impact of the diabetes epidemic. The impact is worse in those countries that are socially and economically disadvantaged. Diabetes threatens the achievement of the Millenium Development Goals (MDGs), increases the risk of developing tuberculosis, and is closely linked with other infections (IDF, 2011).

The diabetes epidemic is centred on type 2 diabetes. Only half a million people have type 1 diabetes globally, and only 78 000 children develop the disease every year (IDF, 2011). Type 2 diabetes is associated with many preventable risk and causative factors such as obesity, hypertension, dyslipidaemia, poor diet, physical inactivity, and lack of regular exercise. Other risk factors such as increasing age, family history of diabetes, history of impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), history of gestational diabetes (GDM), and large babies and ethnicity could be anticipated and taken care of by regular screening, national guide lines, the expansion of health insurance, and regular national surveys.

In Africa, the estimated prevalence of diabetes is 1% in rural areas, up to 7% in urban sub-Sahara Africa, and between 8-13% in more developed areas such as South Africa and in population of Indian origin, (Motala, 2002).

The number of diabetes sufferers in Africa remains uncertain, although and IDF estimate from 2000 put the figure at 7.5 million diabetic adults between 20 and 79 years of age. It is thought that this figure is now much larger. The WHO (World Health Organisation) and the IDF (International Diabetes Federation) estimate that the diabetes population will double over the next twenty five years in Africa. This raises enormous healthcare questions, as all African countries are already struggling to cope with the diabetes burden. Awareness is regarded as being poor, and the concentrations of the disease vary considerably between different ethnic groups.

The prevalence in Nigeria varies from 0.65% in rural Mangu (North) to 11% in urban Lagos (South) (Akinkingbe, 1997) and data from the World Health Organization suggests that Nigeria has the greatest number of people living with diabetes in Africa (Wild, *et al.* 2004).

2.2. Postprandial Hyperglycemia

Diabetes is characterized by a high incidence of cardiovascular disease (CVD) (Kannel and McGee, 1979), and poor control of hyperglycemia appears to play a significant role in the development of CVD in diabetes (Laakso, 1999). Recently, there has been increasing evidence that the postprandial state is an important contributing factor to the development of atherosclerosis (Bonora and Muggeo, 2001). In diabetes, the postprandial phase is characterized by a rapid and large increase in blood glucose levels, and the possibility that these postprandial "hyperglycemic spikes" may be relevant to the pathophysiology of late diabetes complications is recently receiving much attention

A large number of epidemiologic studies have documented the strong link between chronic hyperglycemia, typically reflected by glycosylated hemoglobin (A1C), and long term morbidity and mortality in patients with diabetes. Results from a cohort of 879

individuals with type 1 diabetes who were followed for 20 years indicated that A1C was significantly associated with all cause and cardiovascular mortality. Subjects were divided into quartiles based on A1C. The risk of death for subjects in the highest quartile (average A1C 11.4%) was 2.42 times that for those in the lowest quartile (average A1C 8.7%). A similar pattern was seen in the risk of cardiovascular mortality for subjects in the highest quartile, which was 3.28 times that for subjects in the lowest A1C quartile.

Epidemiologic studies also have demonstrated that hyperglycemia increases the risk of microvascular complications. Investigators for the Pittsburgh Epidemiology of Diabetes Complications Study calculated hyperglycemia exposure in A1 months (A1C units above normal × months) in 353 patients with insulin dependent diabetes mellitus. They found that the risks of developing proliferative retinopathy, microalbuminuria, overt nephropathy, and distal symmetrical polyneuropathy all rose with increasing A1 months. Subjects with 1,000 A1 months and above appeared to be at increased risk for developing most microvascular complications; however, the majority of complications arose in individuals with less exposure (Orchard *et al*., 1997). Results from the ARIC study showed that the risk of chronic kidney disease increases progressively with A1C. In this study, A1C concentrations of 6% to 7%, 7% to 8%, and >8% were associated with hazard ratios for chronic kidney disease of 1.4 ×, 2.5×, and 3.7×, respectively, versus an A1C < 6% (Bash *et al.*, 2008).

Several studies have demonstrated the effectiveness of targeting postprandial glucose to decrease the risk of diabetes complications. The Campanian Postprandial Hyperglycemia Study compared the effects of repaglinide and glyburide on PPG, carotid intimamedia thickness, and markers of systemic vascular inflammation in 175

patients with type 2 diabetes. After 12 months, peak PPG was 148 mg/dL in the repaglinide group versus 180 mg/dL in the glyburide group. Regression of carotid intimamedia thickness (a decrease greater than 0.020 mm) was observed in 52% of patients in the repaglinide group versus 18% of those in the glyburide group. Reductions in C-reactive protein and IL6 were significantly greater with repaglinide than with glyburide. These results show that targeting postprandial glucose can promote atheroma regression in patients with type 2 diabetes (Esposito *et al*., 2004).

2.3. Alpha Amylase and Alpha Glucosidase

The digestion of carbohydrates begins in the mouth by the action of salivary α-amylase, which hydrolyzes the α-1,4 bonds in starch, the products of this process are maltose, maltriose, and small dextrins (Robyt, 2008). The starch digestion process continues in the small intestine by the action of pancreatic α-amylase. The digestion process is completed by enzymes in the brush border of the small intestine (maltase, sucrase and lactase, also known as diasaccharidases or alpha glucosidase) which yields the absorbable monosaccharides glucose, fructose and galactose. A small proportion of monosaccharides can be absorbed passively; however, a carrier protein is required to absorb the amount ingested in a normal diet (Grabitske and Slavin, 2009).

Alpha glucosidase (3.2.1.20) (alpha-d-glucoside glucohydrolase) is an exo-type carbohydrase distributed widely in microorganisms, plants, and animal tissues, (Kimura *et. al*. 2004) which catalyzes the liberation of α-glucose from the non reducing end of the substrate. Inhibiting this enzyme slows the elevation of blood sugar following a carbohydrate meal (Lebovitz, 1997). It is a membrane bound enzyme present in the epithelium of the small intestine, which works to facilitate the absorption of glucose by the small intestine by catalyzing the hydrolytic cleavage of oligosaccharides into

absorbable monosaccharides (Irfan, 2002). By the inhibition of α-glucosidase in the intestine, the rate of hydrolytic cleavage of oligosaccharides decreases and the process of carbohydrate digestion spreads to the lower part of small intestine. This spreading of digestion process delays the overall absorption rate of glucose into the blood. This has proved to be one of the best strategies to decrease the postprandial rise in blood glucose and in turn help avoid the onset of late diabetic complications (Irfan, 2002).

To generate dietary glucose from starchy foods, salivary and pancreatic α-amylase and four small intestine mucosal α-glucosidase subunits are employed in the human body. Alpha amylase hydrolyzes starch by an endo mechanism at inner α-1,4 glucosidic linkages and produces linear and branched maltooligosaccharides (Hizukuri *et. al.,* 1962). The mucosal α-glucosidases are two membrane bounded protein complexes, maltase-glucoamylase (MGAM) and sucrase-isomaltase (SI). All four subunits, N and C-terminal subunits of MGAM and SI complexes, hydrolyze α-1,4 glucosidic linkages from the non-reducing end of α-amylase degraded starch molecules and produce free glucose (Dahlqvist and Telenius, 1969; Dahlqvist, 1962; Van Beers *et. al*., 1995) . In addition to its well-known maltase activity (Dahlqvist, 1962), Ct (C-terminal subunit) MGAM was termed glucoamylase due to its activity on longer chain oligomers (Heymann and Gunther, 1994), and Nt (N-terminal subunit) SI was named as isomaltase because of its debranching activity (Dahlqvist *et al*., 1963). The conventional view of starch digestion has suggested that α-amylase is the limiting digestive enzyme that determines digestion rate. The role of the mucosal α-glucosidases has been thought to simply and passively convert the post-α-amylase dextrins to glucose. However, it is reported that recombinant Nt-MGAM α-glucosidase can digest even granular starch and the four individual α-glucosidases digest gelatinized starch molecules to glucose without the aid of α-amylase, albeit at a slow rate for granular starch (Ao *et al*., 2007;

Lin *et al.*, 2012[a]; Lin *et al.*, 2012[b]). One subunit, Ct-MGAM, digested starch molecules in vitro to nearly 80% (Lin *et al.*, 2012[b]). This supports the hypothesis that human mucosal α-glucosidases may act together with α-amylase to digest starch, or at the very least provide an alternative pathway for starch digestion when luminal salivary and pancreatic α-amylase activity is inhibited or reduced because of immaturity and malnutrition (Auricchio *et al*., 1972; Nichols *et al*., 1998). It is proposed that human α-amylase is not required for granular starch digestion, but amplifies its digestion by providing favored substrates for mucosal α-glucosidases (Ao *et al*., 2007)

2.4 Treatment and Management of Diabetes Mellitus.

Different classes of oral diabetes drugs act at different parts of the glucose–insulin pathway. They include agents that increase the amount of insulin secreted by the pancreas, increase the sensitivity of target organs to insulin, and decrease the rate at which glucose is absorbed in the gastrointestinal tract. The American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) have both stated that treatment for type 2 diabetes requires a progressive pharmacological approach to cover all of these aspects.

Most existing antidiabetic drugs were developed without a prior definition of molecular targets. The advances being made in understanding the pathogenesis of type 2 diabetes and the molecules involved provide the opportunity to develop new treatment interventions, the diabetic drugs that are available and are in use right now are listed below;

2.4.1 Insulin secretagogues

- *Sulfonylureas*

Sulfonylureas were the first widely used oral antidiabetic treatments, having been available in the US since 1954. They trigger insulin release by acting directly on the KATP channel of the pancreatic β cells. As insulin secretion is relatively deficient in type 2 diabetes, use of insulin secretagogues is logical for patients in whom the β-cell defect is not too advanced. Treatment with sulfonylureas has been shown to reduce glyclated hemoglobin (HbA1c) by approximately 1–2%. Furthermore, in the UK Prospective Diabetes Study (UKPDS) it was also associated with a significant reduction in microvascular complications, with a trend toward reduction in myocardial infarction but no significant effect on diabetes related or all-cause mortality (UK Prospective Diabetes Study, 1998[a]) Sulfonylureas have the advantage of being available in multiple formulations at low cost with minimal side effects, and with demonstrated efficacy in controlling hyperglycemia. Sulfonylureas are approved for use as monotherapy and in combination with insulin and all other oral agent classes except the rapid-acting secretagogues.

The downsides of sulfonylureas are well-known, and include weight increase and hypoglycemia. Therefore, sulfonylureas are widely regarded as less attractive for first-line therapy in obese patients. Second generation sulfonylureas, including glyburide, glipizide, and glimepiride, are more potent than the first-generation drugs (chlorpropamide, tolbutamide, acetohexamide, and tolazamide) and generally have fewer side effects and are of comparable efficacy, but cause weight gain (Cohen and Harris, 1987). Most sulfonylureas are metabolized hepatically and cleared renally, and are therefore not recommended in patients with advanced liver or kidney disease. Given

the epidemiological association between hyperinsulinemia and cardiovascular disease, there have been concerns that sulfonylureas might increase cardiovascular morbidity (Henry, 1998). Cardiac tissues contain KATP channels similar to those in β cells. However, there was no increase in mortality seen in the UKPDS trial (UK Prospective Diabetes Study, 1998[c]). In addition, the newest member of the sulfonylureas class, glimepiride, binds less strongly in the myocardium and may therefore actually reduce ischemic pre-conditioning (Green and Feinglos, 2006).

- *Rapid-acting Secretagogues*

Rapid- or short-acting secretagogues, also known as meglitinides, have a mode of action that is similar to that of the sulfonylureas. By closing the potassium channels of the pancreatic β cells, they open the calcium channels and enhance insulin secretion. They were developed to have a rapid onset and short metabolic half-life, resulting in preferential targeting of postprandial hyperglycemia and decreased risk for hypoglycemia later on (Nattrass and Lauritzen, 2000). This class consists of repaglinide and, more recently, nateglinide, which is a D-phenylalanine derivative and developed to be even shorter-acting. Meglitinides reduce HbA1c to a similar extent as sulfonylureas (about 1–2%), but require multiple daily doses. In a study involving 576 patients with type 2 diabetes, pharmacotherapy-naïve patients exhibited less weight gain with repaglinide than with sulfonylurea glyburide (2.5 versus 3.6kg, respectively), although treatment-experienced patients did not exhibit this trend (Marbury, *et al*., 1999). However, meglitinides have not been assessed for their long-term effectiveness in decreasing microvascular or macrovascular risk.

2.4.2 Insulin sensitizers

- *Biguanides*

Biguanides reduce hepatic glucose output and increase uptake of glucose by the peripheral tissues, including skeletal muscle. Metformin, the only widely available biguanide, acts primarily by reducing glucose production and thus fasting hyperglycemia in the presence of a sufficient amount of insulin. Metformin only became available in the US in 1995, although it had been marketed in Europe for nearly 20 years (Bailey and Natrass, 1995). Metformin's mechanism of action is not completely understood, but it is typically classified as an insulin sensitizer (DeFronzo, *et al.*, 1991). As with the sulfonylureas, biguanides reduce HbA1c by approximately 1–2% (Inzucchi, 2002). In contrast to sulfonylurea therapy, metformin monotherapy is associated with weight loss (or little to no weight gain) and a lower incidence of hypoglycemia. In the UKPDS, obese patients randomized to metformin gained only 1–2kg compared with gains of 5–7kg in patients receiving sulfonylurea or insulin treatment (UK Prospective Diabetes Study, 1998[a]). The drug also has non-glycemic benefits, including reducing low-density lipoprotein cholesterol and triglycerides and reducing the antifibrinolytic factor plasminogen activator inhibitor. In addition, its lack of β-cell stimulation may have consequent positive effects on other cardiovascular risk factors. The most serious complication of biguanide use is lactic acidosis, which can be fatal. Two of the three drugs in this class (phenformin and buformin) were withdrawn in the 1980s owing to this side effect. Fortunately, the incidence of lactic acidosis with metformin use is low (one case per 33,000 patient-years). Nevertheless, this concern does restrict metformin use to patients with sufficient renal function to avoid drug accumulation. It is contraindicated in patients with cardiac or respiratory insufficiency

or other conditions associated with hypoxia or reduced perfusion, hepatic dysfunction, alcoholism, or a history of metabolic acidosis.

2.4.3 Thiazolidinediones

Introduced in 1997, thiazolidinediones (TZDs, also known as glitazones) bind to peroxisome proliferator-activated receptor gamma (PPARγ), a type of nuclear regulatory protein involved in the transcription of genes that regulate glucose and fat metabolism. This class consists of rosiglitazone, pioglitazone, and troglitazone, although the latter was withdrawn in 2000 owing to a risk for hepatitis and liver damage. As with biguanides, the mechanism of action of the thiazolidinediones is not fully understood, but the two classes of drug are known to work independently of each other. Neither class stimulates pancreatic islet cells to secrete more insulin. The most prominent effect of TZDs is to increase insulin-stimulated glucose uptake by skeletal muscle cells. This results in a reduction in insulin concentrations, often to an even greater extent than with metformin (Hoffmann and Spengler, 1997). Preliminary data also suggest that this drug class may actually prolong β-cell survival (Buchanan *et. al.*, 2000). Unlike other antidiabetic agents, TZDs have a very slow onset of action. Although effects begin to manifest within two weeks of commencing treatment, the maximal benefit is not seen for around three months. Fluid retention is a concern with this class of drugs. A small proportion of patients develop leg oedema, and in vulnerable patients there is an increased risk for heart failure, particularly for those who are also taking insulin. Weight gain is generally similar to that seen with sulfonylurea therapy (i.e. about 1–4kg, with stabilization over six to 12 months), although this may also be caused by fluid retention. In terms of cardiovascular disease in high-risk patients with type 2 diabetes, the Prospective Pioglitazone Clinical Trial in Macrovascular

Events (PROactive) study found a statistically significant association between pioglitazone and a 16% reduction in the secondary end-point of all-cause mortality, non-fatal myocardial infarction, or stroke. However, there is also an increased frequency of heart failure and oedema without heart failure compared with placebo, which detracts from the otherwise positive vascular effects.

In Europe, TZDs are contraindicated in patients with a history of heart failure or who show current evidence of heart failure, particularly those also taking insulin. Recent US guidelines also urge a cautious approach to TZD use in patients with evidence of heart failure. They are also contraindicated in patients with active liver disease, although their effects on the liver are still under investigation; reduced levels of hepatic transaminases have been reported in several studies. Recent meta-analyses indicate concern about the safety of rosiglitazone. Among patients with impaired glucose tolerance or type 2 diabetes, rosiglitazone use for at least 12 months is associated with a significantly increased risk for myocardial infarction and heart failure, without a significantly increased risk for cardiovascular mortality (Singh, *et al*., 2007)

2.4.4 Incretin-based therapies

Incretin-based therapies are the newest additions to the diabetes armamentarium. Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are rapidly degraded by the proteolytic enzyme dipeptidyl peptidase-4 (DPP-4), and thus are only bioavailable for a very short time. Patients with type 2 diabetes usually lack the insulinotropic response to GIP, but while GLP-1 levels may also be reduced in this patient population the response is usually preserved. It should also be noted that not only is insulin secretion glucose dependent, but so too are the glucagonostatic effects of GLP-1 (Dunning *et al.*, 2005). Thus, GLP-1 results in a much

more physiological regulation of α- and β-cell function, which minimizes the risk for hypoglycemia.

- *Incretin Mimetic*

Exenatide, the only currently available incretin mimetic, is the synthetic form of exendin-4, found in the saliva of the Gila monster. It has more than 50% homology with native human GLP-1 and exhibits many of the same biological effects, except that it has a much longer half-life (10 hours versus just a few minutes). Unlike native GLP-1, exenatide is resistant to degradation by DPP-4. Exenatide was approved by the US Food and Drug Administration (FDA) in April 2005 as an injection for use in combination with metformin and/or sulfonylureas.

The pharmacokinetic profile of exenatide reveals that peak plasma concentrations are reached after 2.1 hours through subcutaneous administration in the abdomen, thigh, or upper arm. Its half-life is 2.4 hours, and it is detectable in plasma for up to 10 hours postadministration (Kolterman, *et al*., 2005). It is eliminated primarily by glomerular filtration, followed by proteolytic degradation. In patients with mild or moderate renal impairment, exenatide clearance is also impaired. In patients with severe renal impairment (creatinine clearance less than 30ml/minute), exenatide clearance impairment is more than 10-fold, and use of the drug is not recommended (Linnebjerg, *et al*., 2005; Jason, *et al*., 2006). Furthermore, as exenatide affects the rate of gastric emptying, the timing of other orally available medications will need to be carefully considered. The efficacy and safety of 5 and 10μg exenatide administered twice a day were evaluated in three multicenter, randomized, triple-blind, placebo-controlled phase III trials—the AC2993: Diabetes Management for Improving Glucose Outcomes (AMIGO) studies. All studies were 30 weeks long, and enrolled more than 1,400

patients with type 2 diabetes. Study participants had inadequate glycemic control with metformin (DeFronzo *et al*., 2005), sulfonylurea (Buse *et al.*, 2004) or a combination of both (at maximally effective doses) (Kendall *et al*., 2005). The main efficacy end-point was the change in HbA1c from baseline. Secondary measures included changes in fasting plasma glucose and bodyweight and the percentage of subjects who achieved HbA1c of 7% or less by the end of the trial. Nausea was the most common adverse event in the AMIGO studies, with most episodes being mild to moderate; progressive dose escalation lessened its incidence. Nausea was the reason for a drop-out rate of up to 4% in the 10µg group. Patients also taking sulfonylureas experienced a higher incidence of hypoglycemia than those taking either metformin or placebo. Exenatide has been shown to be non-inferior to insulin as part and glargnine in terms of HbA1c reduction and has helped reduce bodyweight, while insulin therapy was associated with significant weight gain (Heine, *et al.*, 2005; Nauck, *et al*., 2007).Thirty post-marketing reports of acute pancreatitis and six cases of hemorrhagic or necrotizing pancreatitis have been reported in patients taking exenatide. No definitive causal relationship between exenatide and pancreatitis has been established; however, the FDA felt it prudent to issue a safety warning relating to these rare adverse events.There is some evidence that exenatide may exhibit a disease-modifying effect.

Recent preliminary studies by Faradji, et al., (2006) in patients who have undergone pancreatic islet transplants indicated that exenatide enhanced glycemic response and HbA1c levels. It has also been shown to restore the insulin secretion patterns, similar to those observed in subjects with no diabetes (Fehse, *et al.*, 2005). Liraglutide, a human GLP-1 analog, is currently in late-stage clinical development. Preliminary data from clinical trials suggest that liraglutide monotherapy was more effective than glimepiride monotherapy in terms of reducing HbA1c, and was also associated with significant

weight reduction in previously treated patients with type 2 diabetes (Garber, *et al*., 2008).

Additionally, liraglutide plus metformin was as effective as glimepiride plus metformin in patients with type 2 diabetes previously treated with oral antidiabetic monotherapy (Nauck, *et al*., 2008)

2.4.5 Dipeptidyl peptidase-4 inhibitors (DPP-4)

DPP-4 inhibitors are orally available agents that prevent the degradation of GLP-1 and GIP, thus increasing endogenous incretin levels. DPP-4 is rapidly and sustainably inhibited with these agents: it is seen within 30 minutes of administration and lasts for 24 hours. Sitagliptin was the first oral DPP-4 inhibitor to be approved in the US (in October 2006) for use either as monotherapy or in combination with metformin or TZDs; in Europe it is currently indicated for use only in combination with metformin or TZDs. In a trial of 552 patients, sitagliptin monotherapy was shown to reduce HbA1c over a 12-week period by 0.4% (at 25mg once daily [QD]) and 0.6% (100mg QD) from a baseline of 7.7% (Hanefeld, *et al*., 2005). Similar effects were reported in a study involving 743 patients over 12 weeks (Scott, *et al*., 2005). Sitagliptin does not appear to have an effect on bodyweight and it has been shown to be generally well tolerated, with no increased risk for hypoglycemia compared with placebo. Adverse events reported in at least 5% of patients more frequently in the study group include upper respiratory tract infections and nasopharyngitis. Vildagliptin over 12 weeks at 25mg BID was shown to reduce both fasting and prandial glucose, while HbA1c was reduced by 0.6% (Pratley, *et al*., 2006). In a 24-week parallel-group study in drug-naïve type 2 diabetes patients, vildagliptin (50mg or 100mg QD or 50mg BID) significantly reduced HbA1c compared with placebo (Pi-Sunyer *et al*., 2007).

Compared with other medications, DPP-4 inhibitors generally show non-inferiority, although there as have yet been no comparisons with insulin. In a 24-week study on vildagliptin as monotherapy (50mg BID) in 459 patients, DDP-4 inhibition showed a reduction in HbA1c by 1.1% from a baseline of 8.7% and was non-inferior compared with rosiglitazone. Patients on vildagliptin did not gain weight, while those on rosiglitazone did (Rosenstock, *et al*., 2007). A 52-week study using vildagliptin as monotherapy at 50mg BID in 526 subjects with type 2 diabetes showed a reduction in HbA1c of 1% from a baseline of 8.7%, although non-inferiority to metformin was not reached. Overall, vildagliptin is generally well tolerated, with no increased risk for hypoglycemia compared with placebo. Adverse events reported in at least 5% of vildagliptin patients include upper respiratory tract infections, nasopharyngitis, dizziness, influenza, and headache. A number of DPP-4 inhibitors are currently in late-stage clinical development, including alogliptin and saxagliptin. The efficacy and safety of both agents have been evaluated as monotherapy and as combination therapy. Early data suggest that both alogliptin and saxagliptin also effectively lower glucose and HbA1c as monotherapy in treatment-naïve patients (DeFronzo, *et al*., 2008; Rosenstock, *et al*., 2008). These agents also appear to be effective as combination therapy in type 2 diabetes patients inadequately controlled on monotherapy with currently available antidiabetic agents (Nauck *et al*., 2008; Pratley, *et al*., 2008; Allen, *et al*., 2008). In addition, currently available data suggest that they have a similar safety profile to sitagliptin and vildagliptin.

To date, the clinical outcomes of incretin-based therapies have not been directly compared in a type 2 diabetes patient population, and can be attempted only indirectly. However, comparisons based exclusively on the HbA1c effect are of little relevance for the purpose of estimating practical clinical benefit, as populations and designs differ

among studies. Study participants in the exenatide studies typically had longer diabetes duration (4.9–9.9 years) compared with the DPP-4 inhibitor study patients (1.9–6.5 years). One may conclude that DPP-4 inhibitors are a good treatment option for patients with shorter diabetes duration as they do not cause weight gain and have low hypoglycemic risk—important advantages over sulfonylureas or thiazolidinediones. Nevertheless, the effect of exenatide in terms of glycemic control has been demonstrated in insulin non-inferiority studies. Exenatide is the only antidiabetic with an additional weight-reduction effect—a desired goal of treatment in any stage of type 2 diabetes, especially in obese patients (Gallwitz and Bachmann, 2007).

2.4.6 Alpha glucosidase inhibitors

Alpha glucosidase inhibitors such as acarbose acts by competitively inhibiting alpha glucosidase thus the absorbtion of glucose is delayed. Acarbose is especially useful in decreasing postprandial glucose levels. It can be combined with sulphonylureas and biguanides but its hypoglycaemic potency is much less in comparison with these compounds. The starting dose is 25-50mg once daily, which is increased to 50mg two to three times daily. It must be ingested with the first bite of food, as the drug must be present in the small bowel with the food for proper effect. Hypoglycaemia rarely occurs if used as monotherapy. Its side effects include gastrointestinal side effects such as bloating, abdominal discomfort, diarrhoea, flatulence and more serious liver function impairment.

2.4.7 Insulin therapy

Insulin is usually added to an oral agent when glycemic control is suboptimal at maximal doses of oral medications. Some diabetologists prefer to initiate insulin therapy in patients with newly diagnosed type 2 diabetes (DeFronzo, 1999). Weight

gain and hypoglycaemia are common side effects of insulin therapy (Sinha *et. al.*, 1996; UKPD, 1998[a]; UKPD, 1998[b]; UKPD, 1998[c]) Vigorous insulin treatment may also carry an increased risk of atherogenesis (UKPD, 1998[b])

2.5 Alternative Treatment in the Management of Diabetes Mellitus

The earliest recorded treatments for diabetes mellitus involved the use of plants. The Papyrus Ebers of 1550 BC recommended a high-fiber diet of wheat grains and ochre (The Papyrus Ebers, 1937). A multitude of herbs, spices, and other plant materials have been described for the treatment of diabetes throughout the world (Ajgaonkar, 1979; British Herbal Medicine Association, 1979; Day and Bailey, 1988[a]; Day and Bailey, 1988[b]). Since the availability of insulin, folklore medicines for diabetes have almost disappeared in occidental societies, although they continue to be the cornerstone of therapy in underdeveloped regions. Renewed attention to alternative medicines and natural therapies has stimulated a new wave of research interest in traditional practices, and the World Health Organization expert committee on diabetes has listed as one of its recommendations that traditional methods of treatment for diabetes should be further investigated (WHO, 1980; Watt and Woods, 1988).

Traditional antidiabetic plants might provide a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities, or as simple dietary adjuncts to existing therapies. Sulfonylureas and metformin are valuable treatments for hyperglycemia in non-insulin-dependent diabetes mellitus (NIDDM), but they are often unable to lower glucose concentrations to within the normal range, or to reinstate a normal pattern of glucose homeostasis (Bailey, 1988). Use of these therapies is restricted by their pharmacokinetic properties, secondary failure rates, and accompanying side effects (Bailey, 1988).Whereas their modes of action partially

compensate for the metabolic disturbances in diabetic states, they do not necessarily correct the fundamental biochemical lesions (Taylor and Agius, 1988). Even insulin therapy does not reinstate a normal pattern of glucose homeostasis in most NIDDM patients, and over-vigorous insulin treatment may carry an increased risk of atherogenesis and hypoglycaemia (Ginsberg and Rayfield, 1981; Peacock and Tattersall, 1984). Although, an orally active botanical substitute for insulin seems unlikely, new molecules to stimulate endogenous insulin biosynthesis and secretion (and to promote insulin action) are realistic possibilities.

More than 400 different plants and plant extracts have been described as reputedly beneficial for the diabetic patient. Most of these plants have been claimed to possess hypoglycemic properties but most claims are anecdotal and few have received adequate medical or scientific evaluation. Those that have been evaluated may be grouped into three categories: plants from which a reputedly hypoglycemic compound or partially characterized hypoglycemic fraction has been prepared; plants reported to exert a hypoglycemic effect, but the nature of the active principle is unestablished and plants that reputedly exert a hypoglycemic effect, but the scientific evidence is equivocal. These categories exclude the numerous traditional plants for which an independent scientific or medical evaluation has not been published.

2.6 *Vitex doniana* Sweet.

2.6.1 Classification of *Vitex doniana*

Kingdom: Plantae

Phylum: Angiosperms

Sub-phylum: Eudicots

Class: Core eudicots

Sub-class: Rosids

Sub order: Eurosids

Order: Lamiales

Family: Verbenaceae

Genus: *Vitex*

Species: *doniana*

Name: *Vitex doniana* Sweet

Other names: Dinya (Hausa), Ori-nla (Yoruba), Ucha-koro (Igbo)

2.6.2 Description, distribution and habitat of *Vitex doniana*

Vitex doniana is a deciduous small to medium-sized tree that grows up to 25m tall with a heavy rounded crown. The bark is rough, pale brown or light grey with numerous vertical fissures. Branchlets are not hairy.

Leaves are opposite, glabrous, 14-34 cm long, usually with 5-leaflets on stalks 6-14cm long. Leaflets distinctly stalked ovate, obovate to elliptical, 8-22 cm long, 2-9 cm wide. Leaf tips are rounded or emarginated, leaf bases cuneate, dark green above, pale greyish- green below, thickly leathery, with a few scattered stellates hairs on the upper surface, otherwise without hairs.

Flower petals white except on large lobe, which is purple, in dense opposite and axillary cymes. Flowers small, blue or violet, 3-12 cm in diameter, only a few being open at a time. Fruits are obovoid to oblong-ellipsoid drupe 2-3 cm long, green when young, turning purplish-black on ripening and with starchy black pulp. Each fruit contains 1 hard, conical seed that is 1.5-2 cm long and 1-1.2 cm wide (Burkill, 2000). The black plum tree can actually be found all over Africa's tropical regions. Okafor (1980) cites it as being one of Nigeria's nutritionally important savannah trees.

Figure 1.1: *V.doniana* plant (obtained from:

http://www.zelenykurier.sk/image_upd/products/Moxy294nbkv.png)

2.6.3 General uses of *Vitex doniana*

Young leaves of *Vitex doniana* are cooked and eaten as vegetable or in sauces. The blackish pulp of the fruits is edible and sweet, and eaten raw (taste like prunes). It is nutritious, as it has high lipid content and the pulp contains vitamin C, vitamin A and protein are also present in the fruits (Iwu, 1986). Beverage can be made from the fruit juice, and boiled fruits are the basis for alcoholic liquor and wine. It has been reported that syrup similar to honey was produced from the fruit and that physicochemical and sensory results showed that it can be substitute for other syrup as a nutritive sweetener (Egbekun *et. al*., 1996).

Vitex doniana has numerous applications in traditional medicine. The leaf sap is used as an eye drop to treat conjunctivitis and other eye complaints. Leaf decoction is applied externally as a galactogogue and against headache, stiffness, measles, rash, fever, chickenpox and hemiplagia, and internally as a tonic, anodyne and febrifuge, and to treat respiratory diseases. Pastes of pounded leaves and bark are applied to wounds and burns. Leaf infusions are added to alcoholic drinks to make them stronger. A root decoction is administered orally to treat ankylostomasis, rachitis, gastro-intestinal disorder and jaundice. The powdered bark added to water is taken to treat colic, and the bark extract are used to treat stomach complaints and kidney troubles (Adejumo *et al*., 2013).

Dried and fresh fruits are eaten against diarrhoea and as remedy against lack of vitamin A and B. The twigs are used as chewing sticks for teeth cleaning. The stem bark is given to cattle to treat diarrhoea, dysentery and liver problems. Report has showed the use of the stem bark of *Vitex doniana* to control postpartum bleeding after child birth (Ladeji *et al*., 2005) due to its high potassium and phosphorus content. Other reported

uses of the tree include its stem bark extract for the control of hypertension and its anti-hepatotoxic effect and treatment of stomach ache, pains, disorders and indigestion (Ladeji and Okoye, 1996; Ladeji *et al*., 1996).

The anti- hypertensive (Olusola *et al*., 1997) and antidiabetic (Owolabi *et al*., 2011) effects of the stem bark have also been reported. Extract of stem bark of *Vitex doniana* have also demonstrated some level of *in vitro* trypanocidal activity against *Trypanosome brucei brucei* (Atawodi, 2005).

2.6.4 Phytochemical profile of *Vitex doniana*

The medicinal value of a plant depends on its bioactive phytochemical constituents that produce definite physiological action in the body. Some of the most important bioactive phytochemical constituents include alkaloids, flavonoids, phenolics, essential oils, tannins and saponins (Krishnaiah *et al.,* 2009). Several authors have evaluated the phytochemical composition of *V. doniana*. Egharevba et al, (2010) showed the presence of flavonoids, tannins, saponins, anthraquinones, balsam, carbohydrates and resin in methanol, ethylacetate and hexane stem bark and leaf extract of *V. doniana*.

Report on the mineral content of *V. doniana* shows that iron, magnesium, manganese, molybdenum, phosphate, zinc, calcium and sodium are present with the most abundant mineral being calcium (Robert *et al*., 1997).

Many dietary factors have been reported to contribute to the ability of herbs to improve dyslipidemia (Nimenibo-Uadia, 2003). Saponins, among other secondary metabolites were reported to be present in the leaves of *V. doniana* (Egharevba *et. al*., 2010), and this may be responsible for the lipid-lowering effect of the plant juice on blood lipid. Saponins may lower cholesterol by binding with cholesterol in the intestinal lumen, and

carbohydrates resulting in reduction in digestibility of these macromolecules and thus inhibition of microbial growth (Nwogu *et. al*., 2008; Bulter, 1989). The presence of tannins in *V. doniana* has been reported by Suleiman and Yusuf, (2008).

Natural products are important sources for biologically active drugs and wild herbs have been investigated for their antioxidant properties (Gazzaneo *et. al*., 2005). Medicinal plants containing active chemical constituents with high antioxidant property play an important role in the prevention of various degenerative diseases and have potential benefit to the society (Lukmanul *et. al*., 2008). Phenolics are found in *V. doniana* and have been reported to have multiple biological effects, including antioxidant activity (Francois *et. al*., 2011).

Alkaloids are reported to have analgesic, anti-inflammatory and adaptogenic activities which help to alleviate pains, develop resistance against diseases and endurance against stress (Gupta, 1994).

2.6.5 Pharmacological properties of *Vitex doniana*

The value for the proximate composition of *Vitex doniana* leaves were: moisture content 77.03%, ash content 1.65%, fat 2.9%, fiber 2.75%, protein 8.10% and carbohydrate 7.57% (Adejumo *et al*., 2013). *Vitex doniana* contains vitamins, macro and micro nutrients in different proportion. The anti nutritive factors found in the young leaves of *Vitex doniana* include tannins, saponins, alkaloids and trace of cardiac glycosides (Adejumo *et al*., 2013) which can be controlled by boiling. It can therefore be concluded that the young leaf is highly rich in nutrients and contains the nutrients level that fall within the level reported for other popular edible vegetables.

Mustapha et al. (2012) evaluated the effect of ethanolic extract of *Vitex doniana* stem bark on peripheral and central nervous system of laboratory animals. The result showed that the extract has significant ($P<0.01$) local anesthetic effect when compared to xylocaine. Antinociceptive activity of the ethanolic extract was evaluated using acetic acid induced pain and heat. The extract demonstrated significant antinociceptive activities dose dependently when compared to control, the extract also increased the sleeping time together with the pentobarbitone from 72.3±3.07 at a doses (100mg/kg of extract and 35 mg/kg of the pentobarbitone) to 181±0.35 at a dose of 400mg/kg and 35 mg/kg) respectively. Thus this plant could be a good source of psychotherapic agent.

Kadejo et al. (2013) evaluated the *in vivo* antioxidant effect of aqueous root bark, stem bark and leaves of *Vitex doniana* in carbon tetrachloride induced liver damage and non induced liver damaged albino rats. The study showed that there was a significant ($P>0.05$) increase in Thio Barbituric Acid Reactive Substances (TBARS) and a significant ($P>0.05$) decrease in the SOD and CAT of the liver of the CCl_4 induced not treated groups. However, there was no significant ($P>0.05$) difference between the TBARS, SOD, and CAT in the liver of the induced treated groups and the normal control. In the kidney, the study showed that there was no significant ($P>0.05$) difference in TBARS level between the normal and the induced groups. They therefore concluded that application of *Vitex doniana* plant would play an important role in increasing the antioxidant effect and reducing the oxidative damage in that formed both in the liver and kidney.

Ezekwesili et al.,(2012) evaluated the antidiabetic effect of the aqeous extract of *Vitex doniana* leaves and reported that the extract showed significant anti diabetic activity in alloxan- induced diabetic rats. Also, Okpe et al., (2012) reported that the oral

administration of leaf extracts of *V. doniana* at 100mg and 200mg/kg body weight significantly decreased the fasting blood glucose levels in streptozotocin-induced diabetic albino rats, as well as improved hyperlipidemia associated with diabetes.

2.7 *Detarium microcarpum*, Guill and perr.

2.7.1 Classification of *Detarium microcarpum*

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: *Detarium*

Species: *microcarpum*.

Name: *Detarium microcarpum*.

Other names: Taura (Hausa), Ofo (Igbo), Ogbobgo (Yoruba).

2.7.2 Description, distribution and habitat of *Detarium microcarpum*

Detarium microcarpum is an African tree belonging to the Fabaceae family (legumes). Typically, it is found in high rainfall savannah areas, dry forests and fallow lands on sandy or iron rich hard soils as well as scattered trees on farms. It also occurs in dry savannah as a more stunted tree with smaller fruits (Vautier *et al*., 2007) reaching 10 m high and with a dense rounded crown; in wet areas it can grow up to 25 m tall.

The leaves alternate with 3–4pairs of leaflets, short hairy when young, leaflets alternate to subopposite, ovate, oblong to elliptical, 7–11 cm × 3.5–5 cm, base rounded, apex usually emarginate, thickly leathery. Flowers are bisexual, regular, sessile, fragrant;

sepals are elliptical, white or cream, densely pubescent outside; petals are absent; stamens (8–10), free; ovary superior, sessile, single-celled, style slender, stigma terminal, head-shaped. Fruit an ovoid or rounded, indehiscent drupe-like pod, 2.5–4.5 cm in diameter, more or less flattened, glabrous, yellowish when ripe, with greenish mealy pulp, fibrous and sweet, single-seeded. Seed orbicular, 15–20 mm × 6.5–8.5 mm. Seedling with epigeal germination. The root system is horizontal; bole usually straight, cylindrical, 30cm in diameter; bark scaling on older branches, grey, brown or reddish; crown irregular (Kouyate and Van Damme, 2006).

D. microcarpum is classified as a major African medicinal plant. It is commonly known in English as sweet dattock, trees. *D. microcarpum* is known locally in Ghana as *Takyikyiriwa, Twutwiriwa*; in Senegal as *Kpagra, Kpayhga*; in Nigeria *Taura*, (Hausa): *Ofo*, (Igbo): *Ogbogbo*, (Yoruba): Gungorochi, (Nupe): Ejiji (Igala) *Konkehi*, (Fulfulde): *Galapo*, (Kanuri): *Agashidam*, Tiv (Irvine, 1961; Keay *et al* 1964; Dalziel 1955). *D. microcarpum* is found in semi-arid sub-Saharan Africa from Senegal to Cameroon, extending east to the Sudan. It has an irregular distribution, but locally, it is very common.

Detarium microcarpum is naturally distributed in the drier regions of West and Central Africa (Benin, Cameroon, Central African Republic, Chad, Gambia, Ghana, Guinea, Guinea Bissau, Côte d'Ivoire, Mali, Niger, Nigeria, Senegal, Sudan and Togo) (Contu, 2012). Unlike the other species of its family, *D. microcarpum* grows in dry savannah, in humid forest (Kouyaté and Van Damme 2006). It is most common in wooded savannahs or savannahs, semi-cleared dry forest areas and fallows, growing in sandy or hard soils with high iron content (Kouyaté and Lamien 2011).

Figure 2.2**:** *D. microcarpum* (obtained from;

www.westafricanplants.senckenberg.de/images/pictures/detarium_microcarpum_ms_2151_552_ff9925.jpg)

2.7.3 General uses of *Detarium microcarpum*

Fruits, plant bark and leaves are used not only for texture and flavour, but also for their chemical and nutritional properties (Abulude *et. al.,* 2004). The seeds are used as frankincense and to make necklaces for women. The seeds and leaves are eaten as a condiment and vegetable (Kouyate and van Damme 2006). The seed oil was reported to have low biogenic and oxidative rancidity; a desired property in oils meant for consumption, industrial purposes and pharmaceutical applications (Okorie *et. al*., 2010). The kernel of the seed is deep purple brown, and is more or less oily and edible. Nutritionally, the seed which is used as a traditional soup thickener contain lipids, carbohydrates, proteins, crude fibre and the essential elements: Na, K, Mg, Ca, S, P and Fe (Abreu and Relva, 2002; Abreu *et al*., 1998). Saponins, phytates and cyanides are reportedly present as anti-nutrients (Anhwange *et al*., 2004).

The defatted seed yield gum, which have been utilized as a bio-adhesive agent in the formulation of muco-adhesives and sustained release tablets (Okorie, 2004). The gum content of the seeds was reported to be high; Linoleic acid was the predominant fatty acid (Njoku *et al,* 1999). *D. microcarpum* produces timber which can serve as a mahogany substitute. Its hard dark brown wood provides very good quality timber, which is used in carpentry and construction (Vautier *et al.*, 2007). It is also used for good quality charcoal and fuel wood delivering 19 684 kJ kg-1 of calorific power (Kaboré, 2005). It is the most important commercial fuel wood species and is harvested preferentially from the state forests in Burkina Faso (Kaboré, 2005; Savadogo *et al.,* 2007). Its foliage are avoided by animals and the roots used in perfume (Vautier *et al.,* 2007).

Among the Ibos in the south eastern Nigeria, *Detarium microcarpum* is a revered plant mythically believed to be chip of the primal trees that germinate and grow in God's own garden. They are the main object in traditional worship, symbolizing truth, honesty and integrity (Ejizu, 1986).

Throughout western Africa the genus *Detarium* is believed to possess medico-magical powers. In African ethnomedicine, the bark, leaves and roots of *Detarium microcarpum* are widely used throughout its distribution area because of their diuretic and astringent properties. They are prepared as infusions or decoctions to treat rheumatism, venereal diseases, urogenital infections, haemorrhoids, caries, biliousness, stomach-ache, intestinal worms and diarrhoea including dysentery. They are also used against malaria, leprosy and impotence. A decoction of the powdered bark is widely taken to alleviate pain, e.g. headache, sore throat, back pain and painful menstruation. The fresh bark or leaves are applied to wounds, to prevent and cure infections (Kouyate and van Damme, 2006).

The leaves, stems, roots, barks, as well as the fruits have found tremendous usage in treatment of various ailments e.g. tuberculosis, meningitis, itching and diarrhea (Obun *et al.*, 2010). In Burkina Faso, the fruit pulp of *D. microcarpum* is used to treat skin infection. In Mali the bark is used to treat measles, itching, hypertension, nocturia and tiredness, while the decoction of the leaves or roots is used for paralysis, meningitis, tiredness, cramps and difficult delivery (Kouyate, 2005).

In Niger and Togo, the fruit preparation is used for dizziness, while in Benin a decoction of the leaves is used in treating convulsions and fainting. Apart from medicinal uses, the fruit of *D. microcarpum* is sweet and commonly eaten fresh, while

the pulp is used in making cakes, as well as a substitute for sugar. The seeds are used as frankincense to ward off evil spirits (Akah *et al*., 2012).

In West Africa the roots are part of a medico-magical treatment for mental conditions, and for protection against bad spirits. In veterinary medicine the leaves and roots are used to treat diarrhoea in cattle in southern Mali, and in Benin to treat constipation. In Niger cattle are made to inhale the smoke of the leaves to treat fever (Kouyate and van Damme, 2006).

2.7.5 Pharmacological properties of *Detarium microcarpum*

Methanol extract of *D. microcarpum* roots and its fraction significantly reduced blood sugar level in alloxan-diabetic rats without producing hypoglycemia, an effect attributed to the flavonoids abundantly present in the extract, (Okolo *et al.,* 2012).

The methanol crude alkaloids extracted from the bark of *D. microcarpum* plant was found to be very active against *E. coli*, *P.aeruginosa*, *Streptococcus aureus*, *Staphylococcus aureus* and these showed that alkaloids from this plant could be used as broad spectrum antibiotic against diseases caused by the test microbes (Garba *et. al*., 2013). Similarly, ethanol extract of the bark of *D. microcarpum* was shown to exhibit antimicrobial action against some pathogenic organisms including *Pseudomonas aeruginosa, Klebsiella pneumonia, Citrobacter freunditis Staphylococcus. aureus, Streptococcus pyrogenes and Listeria monocytogenens* (Abreu *et. al*.,1998). Kouyate and van Damme (2006) reported some antibacterial action of *Detarium microcarpum* bark extract against some bacteria including *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Ebi and Afieroho (2011) reported that using the aqueous methanol extract of the seeds of *D. microcarpum* a broad spectrum antimicrobial activity was observed against clinical isolates of *Staphylococcus, aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella paratyphi and Candida albicans*. This activity was attributed to the presence of steroidal saponins and flavonoids. Inhibition of the growth of the plant pathogenic fungus-*Cledosporium eucumerinum* by the pulp extract of *D. microcanpum* was reported by Cavin et al. (2006). All these studies point to the potential antimicrobial usefulness of *Detarium microcarpum.*

The flavones present in the methanol extract of *D. microcarpum* was shown by Mahmood et al. (1993) to strongly inhibit HIV-1 or HIV-2 virus. Olugbuyiro et al. (2009) reported the antiviral activity of the fractions of the methanol stem bark extract of *D. microcarpum* using-7 Replicon assay. They demonstrated that the active fraction MTH-1700 selectively inhibited Hepatitis C-Virus (HCV). The extract was also shown to exhibit moderate antitumor activity against breast cancer (Abreu *et. al.,* 1998). Also the bark extract of *D. microcarpum* showed significant molluscicidal activity against *Lymnaea natalensis* (Mahmood *et al.,* 1993).

The clerodane diterpenes isolated from the fruits of *D. microcarpum* (Cavin *et al.,* 2006) was showed to inhibit the growth of the plant pathogenic *Cladosporium cucumerinum* and of the enzyme acetylcholinesterase (AChE). One of the compounds-5α, 8α (2-oxokolavenic acid) was ten times as potent as galanthamine, a clinically useful drug for Alzheimer's disease. Inhibition of AChE is currently the most efficient approach in managing the symptoms of Alzheimer's disease.

The leaves of *D. microcarpum* are commonly used in the northern part of Nigeria to treat snake bite. Studies by Iful (2008) reported that the leaf extract of *D. microcarpum* potently reduced mortality in *Echis carinatus* (carpet viper) venom treated animals. The study also revealed that the extract relaxed the rabbit isolated jejunum and contracted the rat phrenic nerve-diaphragm muscle.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

Quercetin, aluminium chloride, dimethyl sulphoxide (DMSO), hexane, methanol, ethyl acetate, n-butanol, sodium carbonate, starch, maltose, sucrose, dinitro salicyclic acid (DNS), alpha glucosidase from *Saccharomyces cerevisiae* and porcine pancreatic alpha amylase were purchased from Sigma Chemical Company St Louis U.S.A and all other chemicals were of analytical grade.

3.1.2 Plant materials

The stem bark of *Detarium microcarpum* and the leaves of *Vitex doniana* were collected Tudun-Biye in Giwa LGA of Kaduna state and authenticated at the Heberium unit of the Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria where voucher numbers V/N3105 and V/N1162 respectively were obtained.

3.1.3 Preparation of plant extract

The collected plant samples were rinsed in clean water and air dried at room temperature and all foreign matter was removed. The dried samples were pulverised into powder using laboratory mill, the powder obtained were used to prepare the extracts. Five hundred gram of each plant was weighed and successive extraction was carried out on them using maceration technique. The solvents that were used for the extraction are hexane, methanol and water respectively. The powdered plant was soaked in hexane for 48 hours at room temperature, after which it was filtered using muslin

cloth and Whatmann filter paper to obtain the hexane extract, then the residue was then dried and soaked in methanol for 48 hours at room temperature, after which it was filtered using muslin cloth and Whatmann filter paper to obtain the methanol extract. The residue from the methanol extraction was then dried and soaked in distilled water for 24 hours after which it was filtered using muslin cloth and Whatmann filter paper to obtain the aqueous extract. The hexane and methanolic extracts were concentrated using rotary evaporator while the aqueous extract was concentrated to dryness at 40ºC using water bath.

3.1.4 Animals

A total of 55 apparently healthy male wistar rats weighing between 150-200g were obtained and kept in well aerated laboratory cages in the animal house, Department of Pharmacology, Faculty of Pharmacy, Ahmadu Bello University, Zaria. They were allowed to adjust to laboratory environment for a period of two weeks before the commencement of experiment. The animals were fed with Growers' mash from the Vital Feeds Company and water was provided *ad libitum* during the stabilization period. The animals were then divided into extract treatment groups and control groups.

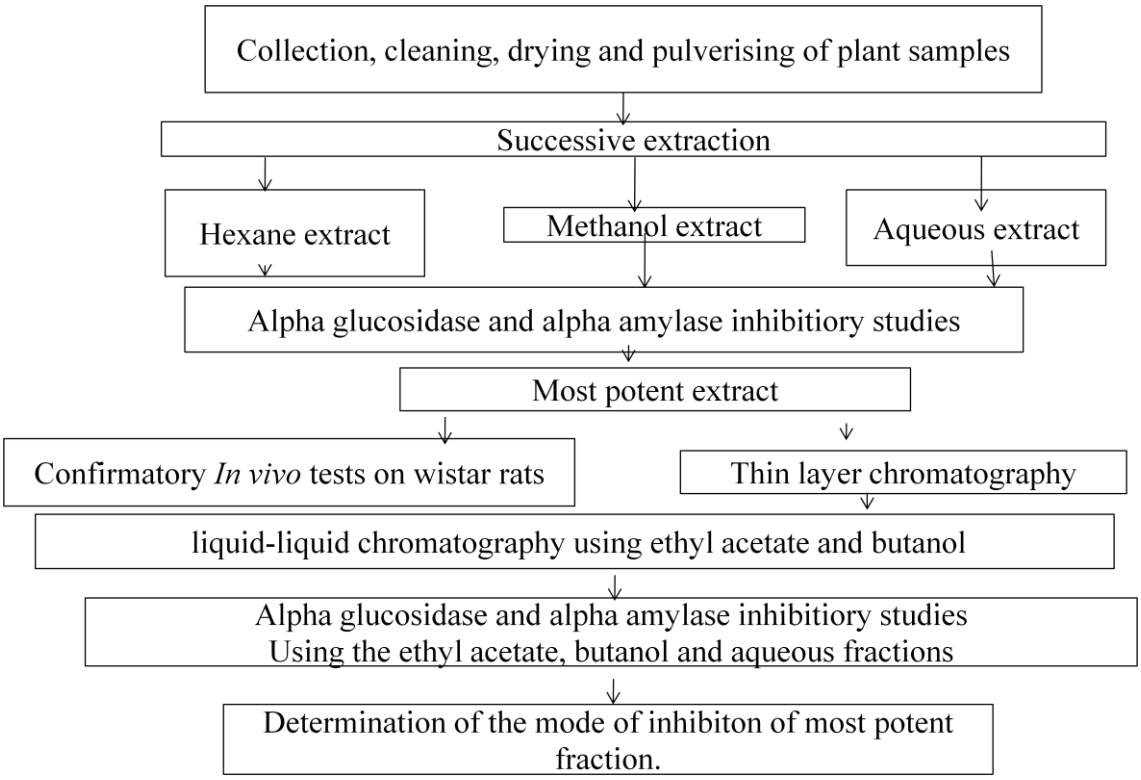


Figure 3.1: Experimental design

3.2 Methodology

3.2.1 Phytochemical screening

Phytochemical screening for the presence of alkaloids, anthraquinones, cardiac glycosides, coumarins, flavonoids, saponins, phlobatannins, tannins and terpenoids were carried out.

3.2.1.1 Test for alkaloids

Forty milligram of the extract was stirred with 8 ml of 1% HCl and the mixture was warmed and filtered (Harborne, 1973). Two milliliter of filtrate was treated separately; (a) with three drops of potassium mercuric iodide (Mayer's reagent) and (b) potassium bismuth (Dragendroff's reagent). Turbidity or precipitation with either of these reagents was taken as evidence for existence of alkaloids.

3.2.1.2 Test for saponins

The ability of saponins to produce emulsion with oil was used for the screening test (Harborne, 1973). Twenty milligram of the extract was boiled in 20 ml of distilled water in a water bath for five minutes and filtered. Ten milliliter of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for froth formation. Three drops of olive oil was mixed with froth, shaken vigorously and observed for emulsion development.

3.2.1.3 Test for triterpenes

Presence of triterpenoids in the extract was carried out by taking 5 ml (1 mg/ml) of the extract and mixing it with 2 ml of chloroform, followed by 3 ml of concentrated H_2SO_4. A reddish brown colouration of the interface confirmed the presence of triterpenoids (Harborne, 1973).

3.2.1.4 Test for cardiac glycosides

Five millilitre (10 mg/ml in methanol) of the extract was mixed with 2 ml of glacial acetic acid having one drop of $FeCl_3$ solution. To the mixture obtained 1 ml of concentrated H_2SO_4 was added to form a layer. The presence of brown ring of the interface indicated deoxy sugar characteristic of cardiac glycosides (Trease and Evans, 1996).

3.2.1.5 Test for flavonoids

Fifty milligram of the extract was suspended in 100 ml of distilled water to get the filtrate. Five millilitre of dilute ammonia solution was added to 10 ml of filtrate followed by few drops of concentrated H_2SO_4. Presence of flavonoids was confirmed by yellow colouration (Sofowara, 1993).

3.2.1.6 Test for tannins

Fifty milligram of the extract was boiled in 20 ml of distilled water and filtered. Three drops of 0.1% $FeCl_3$ was added in filtrate and observed for colour change; brownish green or a blue-black colouration was taken as evidence for the presence of tannins (Sofowara, 1993).

3.2.1.7 Test for reducing sugar

One gram of the extract was placed in a test tube and 10 ml distilled water was added then the mixture was boiled for 5 min. The mixture was then filtered while hot and the cooled filtrate made alkaline to litmus paper with 20 % sodium hydroxide solution. The resulting solution was boiled with an equal volume of Benedict qualitative solution on a

water bath. The formation of a brick red precipitate indicated the presence of reducing compound. (Trease and Evans, 1996)

3.2.1.8 Test for carbohydrates

Three drops of Molisch reagent was added to a small portion of the extract in a test tube and concentrated sulphuric acid was added down the side of the test tube to form a lower layer, a reddish coloured ring at the interphase indicated the presence of carbohydrates (Trease and Evans, 1996).

3.2.1.9 Test for steroids

Three drops of concentrated sulphuric acid was added to the side of a test tube containing a small portion of the extract. A red cheery colour change at the interphase of the extract and the extract indicated the presence of steroids (Trease and Evans, 1996).

3.2.1.10 Test for phenols

Five drops of ferric chloride solution was added to a portion of the extract, a green precipitate indicated the presence of phenolic nucleus (Trease and Evans, 1996).

3.2.1.11 Test for deoxy sugars

A portion of the extract was dissolved in glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube to form a lower layer at the bottom. A purple-brown ring at the interphase indicated the presence of deoxy sugars. (Trease and Evans, 1996)

3.2.2 Quantitative estimation of flavonoid and polyphenols

3.2.2.1 Total flavonoid assay

Method: Flavonoids was determined using the aluminum chloride colorimetric method of Olajire and Azeez, (2011)

Principle: Determination of the total flavonoids using aluminum chloride method is based upon the formation of stable complex between aluminium chloride and keto and hydroxyl groups of flavones and flavonoids.

Procedure: One millilitre each of the plant extracts and standard solution of Quercetin (2-10mg/ml) was added to a 10ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3ml of 5% sodium nitrite ($NaNO_2$) was added. After 5 min, 0.3ml of 10% aluminium chloride ($AlCl_3$) was added. After 6 min, 2 ml of 1M sodium hydroxide (NaOH) was added and the total volume made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured at 510 nm against a blank (1ml of sample was replaced by 1ml of distilled water). Using a quecertin standard curve, the total flavonoids content of the fractions was expressed as mg of Quercetin equivalent (QE) per gram of extract. All samples were analysed in triplicates.

3.2.2.2 Total polyphenol assay

Method: The total phenolic content was determined by the Folin-Ciocalteu spectrophotometric method described by Chun *et al*. (2003)

Principle: The estimation of the total polyphenols was based on a spectroscopic method using the Folin-Ciocalteau reagent. The reduction of phosphomolybdic-phosphotungstic

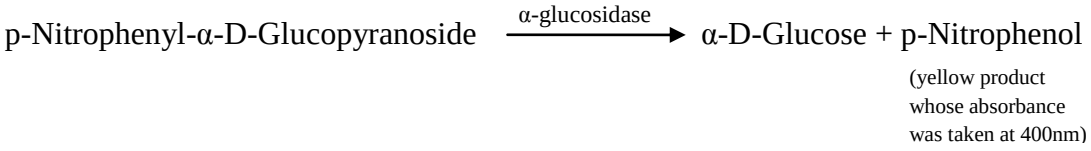
acid Folin reagent to a blue-colored complex in an alkaline solution occurs in the presence of phenolic compounds.

Procedure: One millilitre of extract or standard solution of gallic acid (2-10mg/l) was added into 25 ml volumetric flask containing 9 ml of distilled water. One millilitre of Folin Ciocalteau reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% sodium carbonate (Na_2CO_3) solution was added and the solution was diluted to volume with distilled water and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank (distilled water) was measured at wave length of 750 nm. Using a Gallic acid standard curve, total polyphenol contents were reported as mg Gallic Acid Equivalent (GAE) per gram of extract. All samples were analysed in triplicates.

3.2.3 Determination of alpha glucosidase inhibition

Method: The effect of the plant extracts on α-glucosidase activity was determined according to the method described by Kim *et al*. (2005).

Principle



Procedure: Alpha-glucosidase from *Saccharomyces cerevisiae* (g0660) was used for the assay.

The substrate solution para-nitropheynyl-α-D- glucopyranoside (pNPG) was prepared in 100 mM phosphate buffer, pH 6.9. The substrate (10ml) was prepared by weighing

60.25mg of pNPG into a 10ml volumetric flask and making up the volume to the 10ml mark with phosphate buffer pH 6.9.

The solution of the extracts was prepared by using equal volume of distilled water and dimethyl sulphoxide (DMSO). Exactly 10mg of the extract was weighed into a 10ml volumetric flask and 5ml of DMSO was added to dissolve the extract, after which the volume was made up to the 10ml mark with distilled water to obtain extract stock solution of 1mg/ml. This stock solution was then diluted to obtain the different concentrations used for the assay.

Then 50 µL of α-glucosidase (1U/ml) was added to five tubes containing 20 µL of different concentrations of the extract. After which 30 µL of 5.0 mM (pNPG) was added to start the reaction. The reaction mixture was incubated at 37ºC for 1 hour and stopped by adding 1 ml of 0.1 M Na_2CO_3. The α-glucosidase activity was determined by measuring the yellow colored para-nitrophenol released from pNPG at 400 nm.

The blank solution was prepared by adding the Na_2CO_3 to the reaction mixture before adding the enzyme.

The results were expressed as percentage of the negative control in which the extract was replaced with DMSO and distilled water mixture in equal volume. Acarbose was used as the positive control. All the reactions were conducted in triplicates.

Percentage inhibition calculated as

% Inhibition = [(Abs control-Abs extract)/Abs control] x 100

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically using the concentration/inhibition plots.

3.2.4 Determination of alpha amylase inhibition

Method: This assay was carried out using the procedure of McCue and Shetty (2004).

Principle: Starch + H_2O $\xrightarrow{\alpha\text{-Amylase}}$ Reducing Groups (Maltose)

Reducing groups + dinitrosalicyclic acid $\longrightarrow$ 3-amino-5-nitrosalicyclic acid

(Absorbs different amount of light at 540nm)

Procedure: Porcine pancreatic α-amylase (a6255) was used for this assay. A solution of the enzyme (1mg/ml) was prepared using 0.02M sodium phosphate buffer (pH 6.9).

Starch solution (1%) was also prepared using 0.02M sodium phosphate buffer (pH 6.9) and this was used as substrate.

The solution of the extracts was prepared by using equal volume of distilled water and dimethyl sulphoxide (DMSO). Exactly 10mg of the extract was weighed into a 10ml volumetric flask and 5ml of DMSO was added to dissolve the extract, after which the volume was made up to the 10ml mark with distilled water to obtain extract stock solution of 1mg/ml. This stock solution was then diluted with distilled water to obtain the different concentrations used for the assay.

A total of 50 µL of extract was added to 50 µL of the alpha amylase solution, after which 50 µL of 1% starch solution was added and then incubated at 25 °C for 30 mins. The reaction was terminated after incubation by adding 100 µL of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 mins and cooled to room temperature. The reaction mixture was diluted with 1ml distilled water and the absorbance was measured at 540 nm using spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water. The α-amylase

inhibitory activity was then calculated as percentage inhibition. Acarbose was used as positive control. All the reactions were conducted in triplicates.

% Inhibition = [(Abs control-Abs extracts)/Abs control] x 100.

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

3.2.5 Chromatography

The extract that showed the lowest inhibitory concentration (Methanolic extract of *Detarium microcarpum* stem bark) was fractionated using a suitable solvent system (ethyl acetate and n-butanol). The solvent system to be used was developed from Thin Layer Chromatography studies. This was done with the use of varied ratios of different solvents such as hexane, methanol, ethylacetate, butanol and chloroform.

3.2.5.1 Thin Layer Chromatography (TLC)

TLC was carried out to determine the best solvent system for the liquid-liquid chromatography. A thin layer chromatographic plate pre-coated with silica gel was used. The crude extract was dissolved and applied to the plate. The plates were placed in chromatographic tanks with a mixture of different solvents system. The different solvent systems used include butanol, acetic acid and water in the ratios 8:1:1, 6:2:2, 5:4:1:; hexane and ethyl acetate in the ratio 9:1 and 5:5 and ethyl acetate, methanol and water in the ratio 7:2:1 and 6:2:2 Thereafter the plates were removed, air dried and sprayed.

3.2.5.2 Successive liquid-liquid chromatography

The crude methanolic extract of *D.microcarpum* was subjected to liquid-liquid chromatography to partition the extract into different fractions. Reconstituted extract (250ml) was placed in a separating funnel and 250 ml of absolute ethylacetate and n-butanol were added sequentially at a 1:1 (v/v) solution and rocked vigorously (Abbot and Andrews, 1970). The sample was left standing for 30 min for each solvent on the separator funnel until a fine separation line appeared clearly indicating the supernatant from the sediment before it was collected sequentially. The process was repeated thrice in order to get adequate quantity for each fraction. The ethylacetate, n-butanol as well as the aqueous fractions were evaporated to dryness in a water bath at 40ºC to obtain three fractions respectively. The various fractions obtained were kept in sealed container at 4□C in a refrigerator until used. The fractions were then used to treat alpha amylase and alpha glucosidase in order to ascertain their inhibitory potentials on the enzymes by obtaining their respective minimum inhibitrory concentrations (IC_{50}).

3.2.6 Determination of the mode of α-glucosidase inhibition

The mode of inhibition of the butanol fraction of the methanol extract of *Detarium microcarpum* stem bark on alpha glucosidase was determined using the method described by Ali *et al*. (2006) with slight modifications. Four sets of test tubes were set up for this assay, in the first three sets of test tubes; three different concentrations of the fraction were used. These concentrations were determined from the IC_{50} of the fraction using the percentage inhibition/concentration graph. The concentrations used were half of the IC_{50}, the IC_{50} and twice the IC_{50}. Briefly, 20 µL (the three different concentrations respectively) of the fraction were pre-incubated with 50 µL of α-glucosidase solution for 10 mins at 25 ºC in three sets of tubes. In another set of tubes α-

glucosidase were pre-incubated with 20 µL of phosphate buffer (pH 6.9). Then 30 µL of pNPG at increasing concentrations (0.625mM, 1.25mM, 2.5mM and 5mM) was added to all sets of reaction mixtures to start the reaction. The mixture was then incubated for 60 mins at 37 °C, and 1000 µL of Na_2CO_3 was added to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically using a paranitrophenol standard curve. A double reciprocal plot (1/V versus 1/[S]) where V is reaction velocity and [S] is substrate concentration was plotted. The type (mode) of inhibition of the fraction on α-glucosidase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics.

3.2.7 Determination of mode of α-amylase inhibition

The mode of inhibition of the butanol fraction of the methanolic extract of *Detarium microcarpum* stem bark on alpha amylase was conducted according to the modified method described by Ali *et al*. (2006). Four sets of test tubes were set up for this assay, in the first three sets of test tubes; three different concentrations of the fraction were used. This concentration was determined from the IC_{50} of the fraction using the percentage inhibition/concentration graph. The concentrations used were half of the IC_{50}, the IC_{50} and twice the IC_{50}. Briefly, 50 µL of the fraction (the three different concentrations) was pre-incubated with 50 µL of α-amylase solution for 10 minutes at 25 ºC in three sets of tubes. In another set of tubes α-amylase was pre-incubated with 50 µL of phosphate buffer (pH 6.9). 50 µL of starch solution at increasing concentrations (0.125%, 0.25%, 0.5% and 1%) was added to both sets of reaction mixtures to start the reaction. The mixture was incubated for 30 mins at 25 °C, and then boiled for 5 mins after addition of 100 µL of dinitrosalicylic acid to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically using a maltose

standard curve. A double reciprocal plot (1/V versus 1/[S]) where V is reaction velocity and *[S]* is substrate concentration was plotted. The type (mode) of inhibition of the fraction on α-amylase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics.

3.2.8 Confirmatory *in vivo* tests

3.2.8.1 Starch tolerance test

The animals used for this experiment were grouped into six (6) groups of five (5) animals each as shown below;

- Group 1 : rats + distilled water (1ml)
- Group 2 : rats + acarbose (10mg/kg bw) + starch (3g/kg bw)
- Group 3: rats + methanolic extract of *D. microcarpum* (250mg/kg bw) +starch (3g/kg bw)
- Group 4: rats +methanolic extract of *D. microcarpum* (500mg/kg bw) +starch (3g/kg bw)
- Group 5: rats + methanolic extract of *D. microcarpum* 1000mg/kg bw) +starch (3g/kg bw)
- Group 6: rats + distilled water (1ml) +starch (3g/kg bw)

In this test, overnight-fasted rats were treated with different concentrations of the methanolic extract of *Detarium microcarpum* (250, 500 1000mg/kg body weight), acarbose and distilled water orally. Ten minutes after, the rats were administered starch (3 g/kg body weight) orally and blood was collected via tail puncture for blood glucose

estimation immediately after the administration of the starch solution and at 30, 60 and 120 minutes after starch treatment (Subramanian and Asmawi, 2006). The recorded blood glucose concentrations, peak blood glucose (PBG) and area under curve (AUC) were determined. Whereas the maximum blood glucose concentration for each group was taken as PBG for the group,

$$\text{AUC (mmol/L.h)} = \frac{BG0+BG30\ X\ 0.5}{2} + \frac{BG30+BG60X0.5}{2} + \frac{BG60+BG120X1}{2}$$

Where BG represents the blood glucose concentration measured at time intervals 0, 30, 60 and 120 minutes.

3.2.8.2 Sucrose tolerance test

The animals used for this experiment were grouped into six (6) groups of five (5) animals each as shown below;

- Group 1 : rats + distilled water (1ml)
- Group 2: rats + acarbose (10mg/kg bw) + sucrose (4g/kg bw)
- Group 3: rats + methanolic extract of *D. microcarpum* (250mg/kg bw) +sucrose (4g/kg bw)
- Group 4: rats +methanolic extract of *D. microcarpum* (500mg/kg bw) +sucrose (4g/kg bw)
- Group 5: rats + methanolic extract of *D. microcarpum* 1000mg/kg bw) +sucrose (4g/kg bw)
- Group 6: rats + distilled water (1ml) + sucrose (4g/kg bw)

In this test, overnight fasted rats were treated with different concentrations (250, 500 1000mg/kg body weight) of the methanolic extract of *Detarium microcarpu* stem bark, acarbose and distilled water. Ten minutes after, the rats were administered sucrose (4 g/kg body weight) orally and blood was collected via tail puncture for blood glucose estimation immediately after the administration of the sucrose solution and at 30, 60 and 120 minutes after sucrose treatment (Subramanian and Asmawi, 2006). The recorded blood glucose concentrations, peak blood glucose (PBG) and area under curve (AUC) was determined. Whereas the maximum blood glucose concentration for each group was taken as PBG for the group,

- AUC (mmol/L.h) = $\frac{BG0+BG30\ X\ 0.5}{2} + \frac{BG30+BG60X0.5}{2} + \frac{BG60+BG120X1}{2}$
- Where BG represents the blood glucose concentration measured at time intervals 0, 30, 60 and 120 minutes.

3.3 Statistical Analysis

The results are presented as mean ± standard deviation (SD). Comparisons between the groups were performed by the analysis of variance (ANOVA) (using SPSS 20.0 for Windows Computer Software Package). Significant differences were compared by Duncan's Multiple Range test; a probability level of less than 5% ($P< 0.05$) was considered significant (Duncan, 1955).

CHAPTER FOUR

RESULTS

4.1 Phytochemical Analysis of the Hexane, Methanol and Aqueous Extracts of *Vitex doniana* Leaves and *Detarium microcarpum* Stem Bark

4.1.1 Qualitative phytochemical screening the hexane, methanol and aqueous extracts of *Vitex doniana* leaves and *Detarium microcarpum* stem bark

Table 4.1, shows the results for the qualitative phytochemical screening of the hexane, methanol and aqueous extracts of the leaves of *Vitex doniana* and the stem bark of *Detarium microcarpum*. From the results in Table 4.1, flavonoids, alkaloids and deoxy sugars were detected in the extracts, while phenols were not detected in the hexane extracts of the leaves of *Vitex doniana* and the stem bark of *Detarium microcarpum*. Tannins were also not detected in all the extracts except for the hexane and methanolic extracts of *Detarium microcarpum*. In addition all the extracts contained saponins and triterpenes except for the hexane extracts of the two plants. Carbohydrates and reducing sugars were present in all the extracts except for the hexane extract of *Vitex doniana*. Furthermore steroids were not detected in all the extract except for the hexane extract of the two plants. Finally, cardiac glycosides were also not detected in the extracts except for the methanol and aqueous extracts of the two plants.

Table 4.1. Qualitative phytochemical analysis of the extracts of the stem bark of *Detarium microcarpum* and the leaves of *Vitex doniana*.

	Extracts					
Pytochemicals	**Vd1**	**Vd2**	**Vd3**	**Dm1**	**Dm2**	**Dm3**
Flavonoids	+	+	+	+	+	+
Phenols	-	+	+	-	+	+
Alkaloids	+	+	+	+	+	+
Tannins	-	-	-	+	+	-
Saponins	-	+	+	-	+	+
Triterpenes	-	+	+	-	+	+
Steroids	+	-	-	+	-	-
carbohydrates	-	+	+	+	+	+
Reducing sugars	-	+	+	+	+	+
Cardiac glycosides	-	-	-	-	+	+
Deoxy sugars	-	+	+	+	+	+

Key;
+ indicates phytochemical detected
- Indicates phytochemical not detected

Vd1=hexane extract of *Vitex doniana*
Vd2=methanol extract of *Vitex doniana* leaves
Vd3=aqueous extract of *Vitex doniana* leaves
Dm1=hexane extract of *Detarium microcarpum* stem bark
Dm2=methanol extract of *Detarium microcarpum* stem bark
Dm1=aqueous extract of *Detarium microcarpum* stem bark

4.1.2 Total polyphenol and flavonoid content of the hexane, methanol and aqueous extracts of the leaves of *Vitex doniana* and the stem bark of *Detarium microcarpum*

Table 4.2 shows the results for the total phenols and flavonoid content of the plant extracts. It is seen from the table that the hexane extracts of the leaves of *Vitex donina* and the stem bark of *Detarium microcarpum* do not contain polyphenols, but the total polyphenol concentration in the methanolic extract of the stem bark of *Detarium microcarpum* (0.62±0.13 mg GAE/g of sample) was significantly higher ($P<0.05$) than in the other extracts. The total polyphenols in the aqueous and methanolic extracts of the stem bark of *Detarium microcarpum* (0.55±0.01 and 0.62±0.13 mg GAE/g of sample) were significantly higher ($P<0.05$) than that in the aqueous and methanolic extracts of *Vitex doniana* (0.39±0.02 and 0.48±0.02 mg GAE/g of sample) respectively. As for the concentration of the flavonoids in the extracts, the concentration of flavonoids in the aqueous extract of *Vitex doniana* (0.87 ± 0.03 mg QE/g of sample) was significantly higher ($P<0.05$) than in the other extracts. The hexane extract of *Detarium microcarpum* had the lowest concentration (0.15 ± 0.01 mg GAE/g of sample) of flavonoids when compared to all the other extracts.

Table 4.2, Quantitative estimation of total phenols and flavonoids in the extracts of the stem bark of *Detarium microcarpum* and the leaves of *Vitex doniana.*

Extracts	**TPC (mg GAE/g of sample)**	**TFC (mg QE/g of sample)**
Vd1	0.00 ± 0.00^{a}	0.20 ± 0.02^{b}
Vd2	0.48 ± 0.02^{c}	0.60 ± 0.01^{d}
Vd3	0.39 ± 0.02^{b}	0.87 ± 0.03^{e}
Dm1	0.00 ± 0.00^{a}	0.15 ± 0.01^{a}
Dm2	0.62 ± 0.13^{e}	0.62 ± 0.07^{d}
Dm3	0.55 ± 0.01^{d}	0.32 ± 0.02^{c}

Results are presented as mean ± SD. Values with different superscripts down the column are significantly different from each other at $P<0.05$.

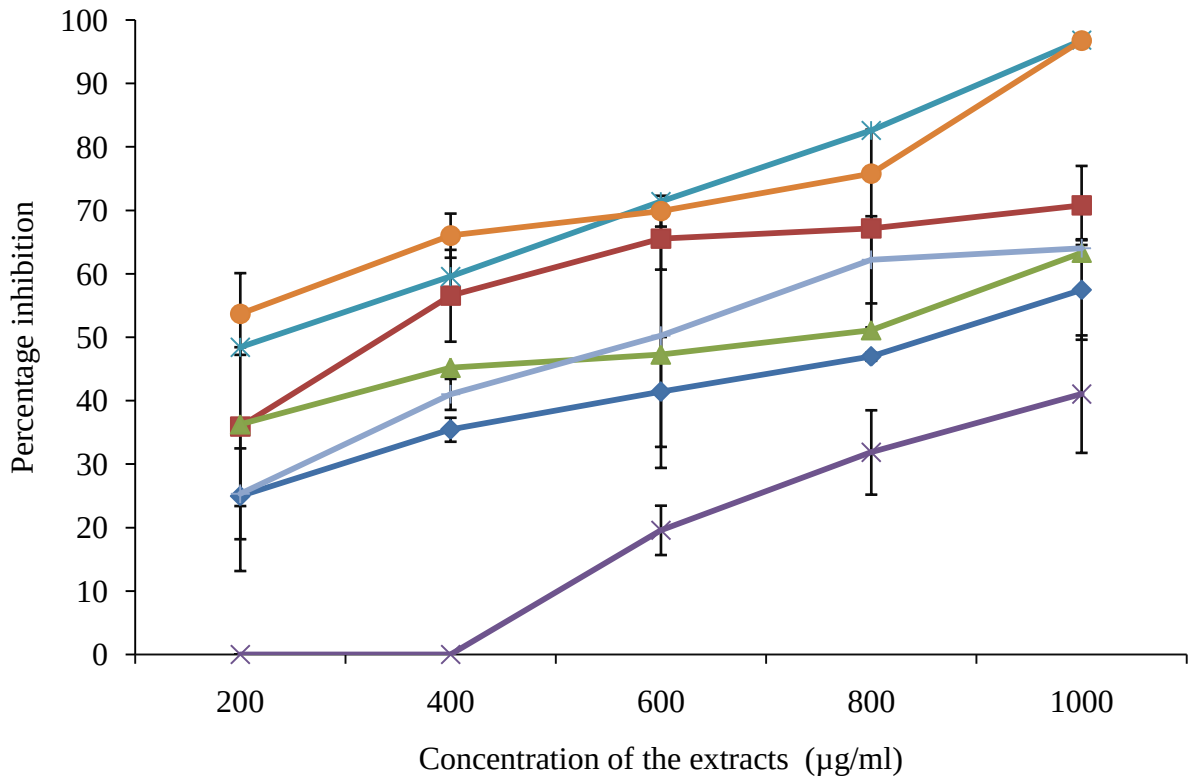
Key;
TPC = total polyphenol content
TFC = total flavonoid content
GAE =gallic acid equivalent
QE = Quercertin equivalent

Vd1=hexane extract of *Vitex doniana*
Vd2=methanol extract of *Vitex doniana* leaves
Vd3=aqueous extract of *Vitex doniana* leaves
Dm1=hexane extract of *Detarium microcarpum* stem bark
Dm2=methanol extract of *Detarium microcarpum* stem bark
Dm1=aqueous extract of *Detarium microcarpum* stem bark

4.2 The *In Vitro* Inhibition of Alpha Glucosidase and Alpha Amylase by the Hexane, Methanol and Aqueous Extracts of *Vitex doniana* Leaves and *Detarium microcarpum* Stem Bark.

4.2.1 Percentage inhibition of alpha glucosidase by the hexane, methanol and aqueous extracts of the leaves of *Vitex doniana* and the stem bark of *Detarium microcarpum*

Figure 4.1 shows the variation of the percentage inhibition of alpha glucosidase as the concentration of the extracts increases. It is seen from the figure that all the extracts had a dose dependent inhibitory effect on the enzyme activity with the aqueous extract of the stem bark of *Detarium microcarpum* showing the highest inhibitory effect against alpha glucosidase when compared to the other extracts and even the standard drug.



Key;

- Hexane extract of *V. doniana* leaves
- Methanol extract of *V. doniana* leaves
- Aqueous extract of *V.doniana* leaves
- Hexane extract of *D. microcarpum* stem bark
- Methanol extract of *D. microcarpum* stem bark
- aqueous extract of *D. Microcarpum* stem bark
- acarbose

Figure 4.1; Variations in percentage inhibition of α glucosidase activity against concentrations of hexane, methanol and aqueous extract of *Detarium microcarpum* stem bark and *Vitex doniana* Leaves.

4.2.2 Percentage inhibition of alpha amylase by the hexane, methanol and aqueous extracts of the leaves of *Vitex doniana* and the stem bark of *Detarium microcarpum*

Figure 4.2 shows the variation of the percentage inhibition of alpha amylase with increasing concentration of the extracts. It is seen from the figure that all the extracts inhibited the enzyme in a dose dependent manner.

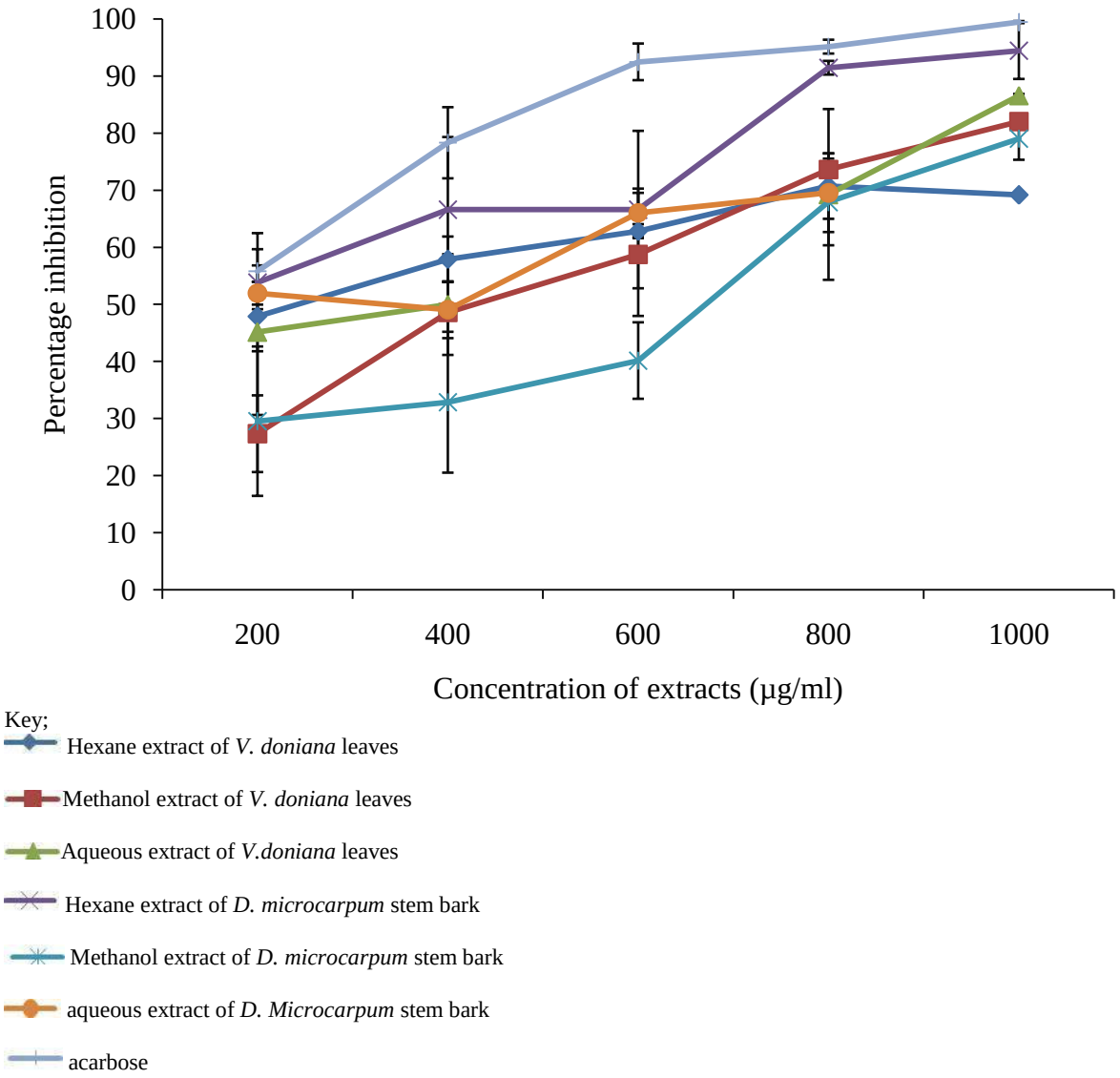


Figure 4.2; Variations in percentage inhibition of α amylase activity against concentrations of hexane, methanol and aqueous extract of *Detarium microcarpum* stem bark and *Vitex doniana* Leaves.

4.2.3 The minimum inhibitory concentration (IC_{50}) of the hexane, methanol aqueous extracts of *Vitex Doniana* leaves and *Detarium microcarpum* stem bark on alpha glucosidase and alpha amylase

Table 4.3 shows the result for the effect of the extracts on alpha glucosidase and alpha amylase. The potency of the extracts was evaluated by determining the minimum inhibitory concentrations (IC_{50}) of all the extracts on both enzymes. From the table, all the extracts had inhibitory activity against both enzymes but the methanolic and the aqueous extract of the stem bark of *Detarium microcarpum* were most potent against both enzymes as they had the lowest IC_{50} for both enzymes (5.88 ± 0.47, 4.63 ± 1.64, 73.98 ± 19.59, 228.16 ± 119.53), these extracts were however more potent against alpha glucosidase, it was observed that there was no significant difference between the IC_{50} of the methanolic and aqueous extracts of the stem bark of *Detarium microcarpum* and the IC_{50} of acarbose (the standard drug) on alpha amylase (228.16 ± 119.53, 51.68 ± 0.00). On the other hand the methanolic and aqueous extracts of the stem bark of *Detarium microcarpum* were even more potent than acarbose against alpha glucosidse. Since the methanolic extract of the stem bark of *Detarium microcarpum* had the least IC_{50} against both enzymes, it was picked for the *in vivo* confirmatory tests and liquid-liquid fractionation and the effect of the resulting fractions were further tested on the enzymes.

Table 4.3; Inhibition of alpha glucosidase and alpha amylase activity by the extracts of the leaves of *V.doniana* and stem bark of *D.microcarpum*

Extracts	**IC_{50} of extract on alpha glucosidase (µg/ml)**	**IC_{50} of extract on alpha amylase (µg/ml)**
Vd1	823.34 ± 95.87^{c}	283.89 ± 28.87^{bcd}
Vd2	224.80 ± 77.41^{b}	501.70 ± 99.35^{d}
Vd3	665.05 ± 148.93^{c}	310.09 ± 209.06^{cd}
Dm1	1175.49 ± 165.36^{d}	$286.56 \pm 154.86^{\mathbf{bcd}}$
Dm2	5.88 ± 0.47^{a}	73.98 ± 19.59^{ab}
Dm3	4.63 ± 1.64^{a}	228.16 ± 119.53^{abc}
Acabose (standard drug)	631.35 ± 99.52^{c}	51.68 ± 0.00^{a}

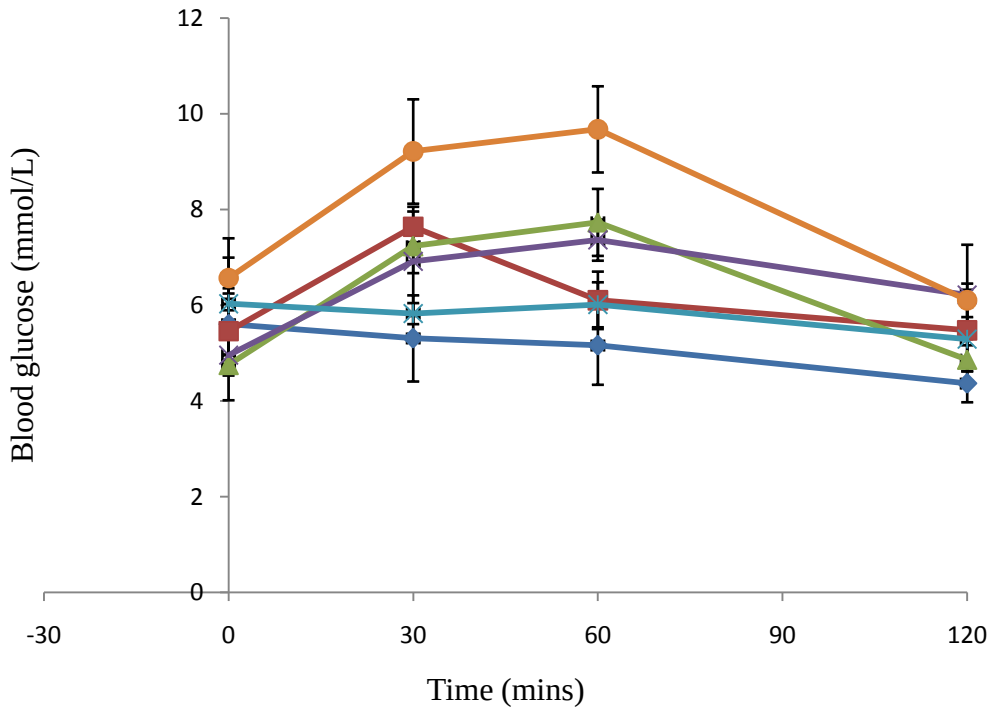
Results are presented as mean ± SD. Values with different superscripts down the column are significantly different from each other at $P<0.05$.

Key;
Vd1; hexane extract of *V. doniana* leaves
Vd2; methanol extract of *V. doniana* leaves
Vd3; aqueous extract of *V.doniana* leaves
Dm1; hexane extract of *D. microcarpum* stem bark
Dm2; methanol extract of *D. microcarpum* stem bark
Dm3; aqueous extract of *D. Microcarpum* stem bark

4.3 The *In Vivo* Inhibition of Alpha Glucosidase and Alpha Amylase by the Methanolic Extract of *Detarium microcarpum* Stem Bark.

4.3.1 Changes in the blood glucose levels of rats following starch tolerance test in the presence of the methanolic extract of *Detarium microcarpum* stem bark

Figure 4.3 shows the changes in the blood glucose level of the rats in mmol/L of the rats that were used for the starch tolerance test in the presence of the methanolic extract of *Detarium microcarpum* stem bark over a period of 120 minutes. From the figure it is seen that the extract reduced the blood glucose of the rats in a dose dependent manner with the group treated with the highest dose of the extract having the most significant ($P<0.05$) reduction. In addition, the extract reduced the blood glucose level even more than the standard drug acarbose.



Key;

group 1= rats + distilled water (1ml)

group 2= rats + acarbose (10mg/kg bw) + starch (3g/kg bw)

group 3 = rats + methanolic extract of D. microcarpum (250mg/kg bw) +starch (3g/kg bw)

group 4 = rats +methanolic extract of D. microcarpum (500mg/kg bw) +starch (3g/kg bw)

group 5 = rats + methanolic extract of D. microcarpum 1000mg/kg bw) +starch (3g/kg bw)

group 6 = rats + distilled water (1ml) +starch (3g/kg bw)

Figure 4.3; Blood glucose response during oral starch tolerance test in normal rats treated with different concentrations of *Detarium microcarpum* stem bark extract and Acarbose.

4.3.2 Area under the curve of blood glucose and peak blood glucose of rats subjected to starch tolerance test

Table 4.4 shows the results for the effect of the methanolic extract of *Detarium microcarpum* on peak blood glucose of rats that were subjected to starch tolerance test. This test was to see the effect of the extract *in vivo* on alpha amylase. The table shows that all the extract treated groups showed significantly lower ($P<0.05$) Peak Blood Glucose (PBG) as compared to the group treated distilled water and starch only. It was also observed that the group treated with 1000mg/kg body weight extract had lower peak blood glucose (6.03 ± 1.37) than the group treated with acarbose (7.64 ± 0.32), this group reduced the peak blood glucose by 37.77% as compared to acarbose that reduced the peak blood glucose by 21.16%. In addition all the extract treated groups also significantly ($P<0.05$) reduced the area under the curve as compared to the group treated with distilled water and starch only. In addition the group treated with 1000mg/kg body weight extract reduced the area under the curve more than the group treated with acarbose though not significantly ($p>0.05$).

Table 4.4 Peak blood glucose and area under the curve for the rats treated with different doses of methanol extract of *Detarium microcarpum* for starch tolerance test.

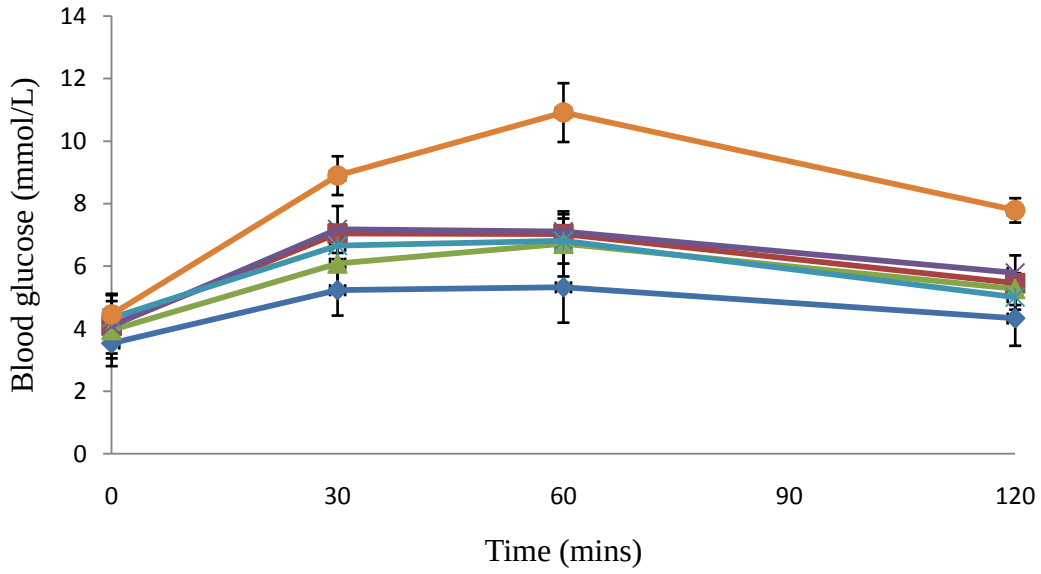
Groups	**Peak Blood Glucose (PBG) (mmol/L)**	**Percentage decrease (%)**	**Area Under the Curve (AUC)(mmol/L.h)**	**Percentage decrease (%)**
1	5.61 ± 0.75^{a}		10.12 ± 1.43^{a}	
2	7.64 ± 0.32^{b}	21.16	12.51 ± 1.09^{b}	24.37
3	7.73 ± 0.70^{b}	20.23	13.04 ± 0.94^{b}	21.16
4	7.36 ± 0.43^{b}	24.05	13.33 ± 1.34^{b}	19.41
5	6.03 ± 1.37^{a}	37.77	11.81 ± 0.35^{b}	28.60
6	9.69 ± 0.31^{c}		16.54 ± 0.49^{c}	

Results are presented as mean ± SD. Values with different superscripts down the column are significantly different from each other at $P<0.05$

Key:
Group 1; rats + distilled water (1ml)
Group2; rats + acabose (10mg/kg bw) + starch (3g/kg bw)
Group 3; rats + methanolic extract of D. microcarpum (250mg/kg bw) +starch (3g/kg bw)
Group 4; rats +methanolic extract of D. microcarpum (500mg/kg bw) +starch (3g/kg bw)
Group 5; rats + methanolic extract of D. microcarpum 1000mg/kg bw) +starch (3g/kg bw)
Group 6; rats + distilled water (1ml) +starch (3g/kg bw)

4.3.3 Changes in the blood glucose levels of rats following sucrose tolerance test in the presence of the methanolic extract of *Detarium microcarpum* stem bark

Figure 4.4 shows the changes in the blood glucose level of the rats in mmol/L of the rats that were used for the sucrose tolerance test in the presence of the methanolic extract of *Detarium microcarpum* stem bark over a period of 120 minutes. All the extract treated groups showed reduced blood glucose levels but not in a dose dependent manner as the group treated with the least concentration of the extract (250mg/kg body weight) showed the most reduction in blood glucose level.



Key;

Group 1= rats + distilled water

Group 2= rats + acabose (10mg/kg bw) + starch (3g/kg bw)

Group 3 = rats + methanolic extract of D. microcarpum (250mg/kg bw) +starch (3g/kg bw)

Group 4 = rats +methanolic extract of D. microcarpum (500mg/kg bw) +starch (3g/kg bw)

Group 5 = rats + methanolic extract of D. microcarpum 1000mg/kg bw) +starch (3g/kg bw)

Group 6 = rats + distilled water +starch (3g/kg bw)

Figure 4.4; Blood glucose response during oral sucrose tolerance test in normal rats treated with different concentrations of *Detarium microcarpum* stem bark extract and acarbose.

4.3.4 Area under the curve of blood glucose and peak blood glucose of rats subjected to sucrose tolerance test

Table 4.5 shows the result for the effect of the extract on the Peak Blood Glucose (PBG) and Area Under the Curve (AUC) for rats that were subjected to sucrose tolerance test. From the table it is seen that all the extract treated groups had significantly ($P<0.05$) lower Peak Blood Glucose than the group treated with distilled water and starch only, it is noteworthy to point out that the group treated with 1000mg/kg body weight had a lower peak blood glucose than the group treated with acarbose. In addition, all the extract treated groups had significantly lower area under the curve than the group treated with starch and water only. Also, the group treated with 1000mg/kg body weight extract had lower area under the curve than the group treated with acarbose, but the difference was not significant ($p>0.05$).

Table 4.5; Peak blood glucose and area under the curve for the rats treated with different doses of methanolic extract of *detarium microcarpum* for sucrose tolerance test.

Groups	**Peak Blood Glucose (PBG) (mmol/L)**	**Percentage decrease (%)**	**Area Under the Curve (AUC) (mmol/L.h)**	**Percentage decrease (%)**
1	5.32 ± 1.13^{a}		10.01 ± 0.79^{a}	
2	7.12 ± 0.17^{b}	34.80	12.67 ± 1.56^{b}	24.81
3	6.72 ± 1.04^{b}	38.46	12.15 ± 1.58^{b}	27.89
4	7.18 ± 0.75^{b}	34.25	12.84 ± 0.50^{b}	23.8
5	6.81 ± 0.72^{b}	37.64	12.03 ± 0.92^{b}	28.61
6	10.92 ± 0.06^{c}		16.85 ± 0.27^{c}	

Results are presented as mean ± SD. Values with different superscripts down the column are significantly different from each other at $P<0.05$

Group 1; rats + distilled water (1ml)
Group2; rats + acabose (10mg/kg bw) + sucrose (4g/kg bw)
Group 3; rats + methanolic extract of D. microcarpum (250mg/kg bw) +sucrose (4g/kg bw)
Group 4; rats +methanolic extract of D. microcarpum (500mg/kg bw) +sucrose (4g/kg bw)
Group 5; rats + methanolic extract of D. microcarpum 1000mg/kg bw) +sucrose (4g/kg bw)
Group 6; rats + distilled water (1ml) +sucrose (4g/kg bw)

4.4 Phytochemical Analysis of the Ethyl Acetate, N-Butanol and Aqueous Fractions of the Methanolic Extract of *Detarium Microcarpum* Stem Bark

4.4.1 Qualitative phytochemical screening of the ethyl acetate, butanol and aqueous fractions of the methanolic extract of *Detarium microcarpum* stem bark.

Table 4.6 shows the result for the qualitative phytochemical screening of the fractions of the methanolic extract of the stem bark of *Detarium microcarpum*. The table shows that all the fractions contain the same phytochemical as the methanolic extract of *Detarium microcarpum* except for the ehtylacetate fraction which did not contain saponins and triterpenes as compared to the methanolic extract of *Detarium microcarpum.*

Table 4.6; Qualitative phytochemical analysis of fractions of methanolic extract of *Detarium microcarpum* stem bark

	Fractions		
Pytochemicals	**EAf**	**n-Bf**	**Af**
Flavonoids	+	+	+
Phenols	+	+	+
Alkaloids	+	+	+
Tannins	+	+	+
Saponins	-	+	+
Triterpenes	-	+	+
Steroids	-	-	-
Carbohydrates	+	+	+
Reducing sugars	+	+	+
Cardiac glycosides	+	+	+
Deoxy sugars	+	+	+

+ indicates presence of phytochemical
- indicates phytochemical not detected

Key;
EAf = Ethyl acetate fraction
n-Bf = n-Butanol fraction
Af = Aqueous fraction

4.4.2 Total polyphenol and flavonoid content of the ethyl acetate, butanol and aqueous fractions of the methanol fraction of *Detarium microcarpum* stem bark

Table 4.7 shows the polyphenol and flavonoid content in the fractions of the methanolic extract of the stem bark of *Detarium microcarpum*. From the table it is seen that the butanol fraction had a significantly higher polyphenol and flavonoid content (0.87 ± 0.02 mg gallic acid/g dry sample and 0.63 ± 0.12 mg quercertin/g dry sample) when compared to the other fractions while the aqueous fraction had a significantly lower polyphenol and flavonoid content 0.15 ± 0.04mg gallic acid/g dry sample and 0.28 ± 0.01mg quecertin/g dry sample respectively) when compared to the other fractions.

Table 4.7; The total polyphenols and flavonoid content in the ethyl acetate, butanol and aqueous fractions of the methanolic extract of *Detarium microcarpum*

Fractions	**TPC (mg GAE/g of sample)**	**TFC (mg QE/g of sample)**
EAf	0.56 ± 0.07^{b}	0.52 ± 0.03^{b}
n-Bf	0.87 ± 0.02^{c}	0.63 ± 0.12^{b}
Af	0.15 ± 0.04^{a}	0.28 ± 0.01^{a}

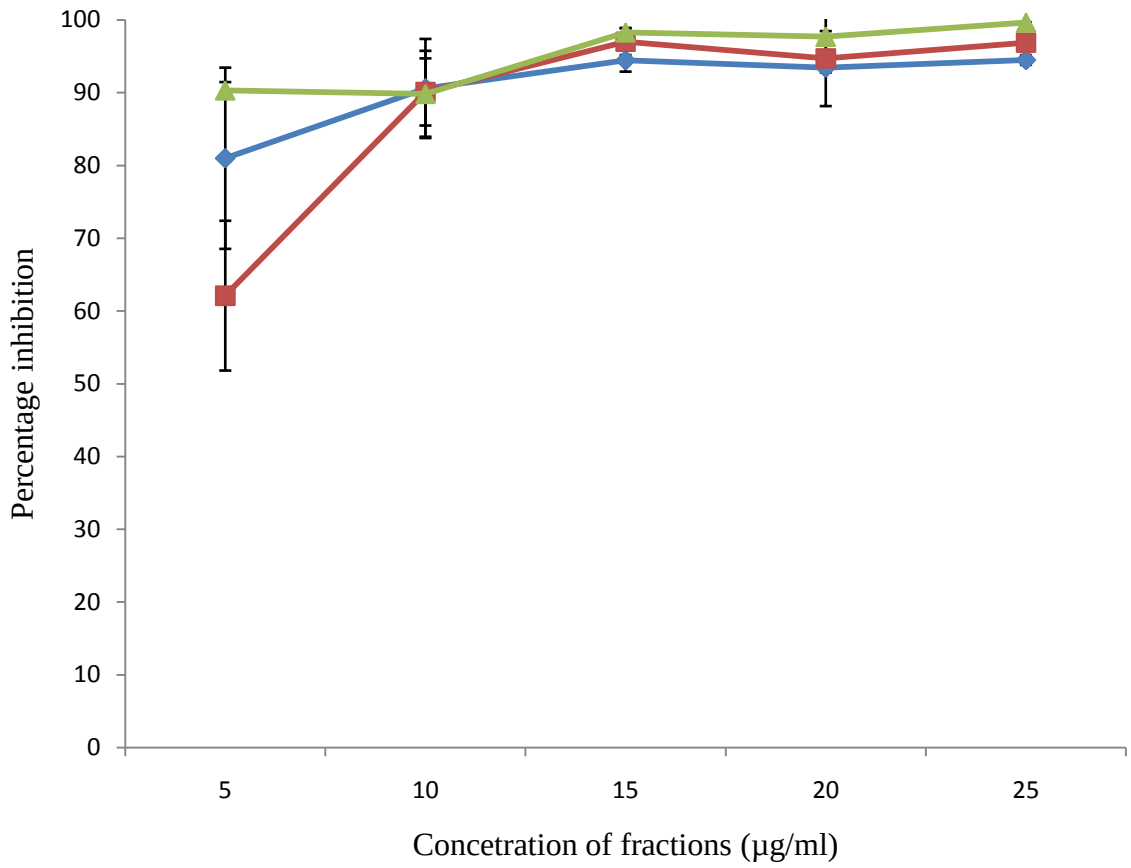
Results are presented as mean ± SD. Values with different superscripts down the column are significantly different from each other at $P<0.05$

Key;
EAf = Ethyl acetate fraction
n-Bf = n-Butanol fraction
Af = Aqueous fraction
TPC =Total Polyphenol Content
TFC = Total Flavonoid Content
GAE = Gallic acid equivalent
QE = Quercertin equivalent

4.5 The *In Vitro* Inhibition of Alpha Glucosidase and Alpha Amylase by the Ethyl Acetate, N-Butanol and Aqueous Fractions of the Methanolic Extract of *Detarium microcarpum* Stem Bark.

4.5.1 The percentage inhibition of the ethyl acetate, butanol and aqueous fraction of the methanolic extract of *Detarium microcarpum* stem bark on alpha glucosidase

Figure 4.5 shows the changes in the percentage inhibition of alpha glucosidase by increasing concentrations of the ethyl acetate, butanol and aqueous fractions of the methanolic extract of *Detarium microcarpum* stem bark. From the figure, it is seen that a concentrations of as low as 5µg/ml of the three fractions inhibited the enzyme by as much as 60% and above.



Key;

Ethyl acetate fraction

Aqueous fraction

N-butanol fraction

Figure 4.5; Variation in percentage inhibition of α glucosidase against increasing concentrations of ethyl acetate, butanol and aqueous fractions of the methanolic extract of *Detarium microcarpum* stem bark.

4.5.2 The percentage inhibition of the ethyl acetate, butanol and aqueous fraction of the methanolic extract of *Detarium microcarpum* stem bark on alpha amylase

Figure 4.6 shows the change in the percentage inhibition of alpha amylase as the concentration of the ethyl acetate, butanol and aqueous fractions of the methanol extract of the *Detarium microcarpum* stem bark increases. From the figure it is seen that the n-butanol fraction of the extract showed the highest inhibition against the enzyme.

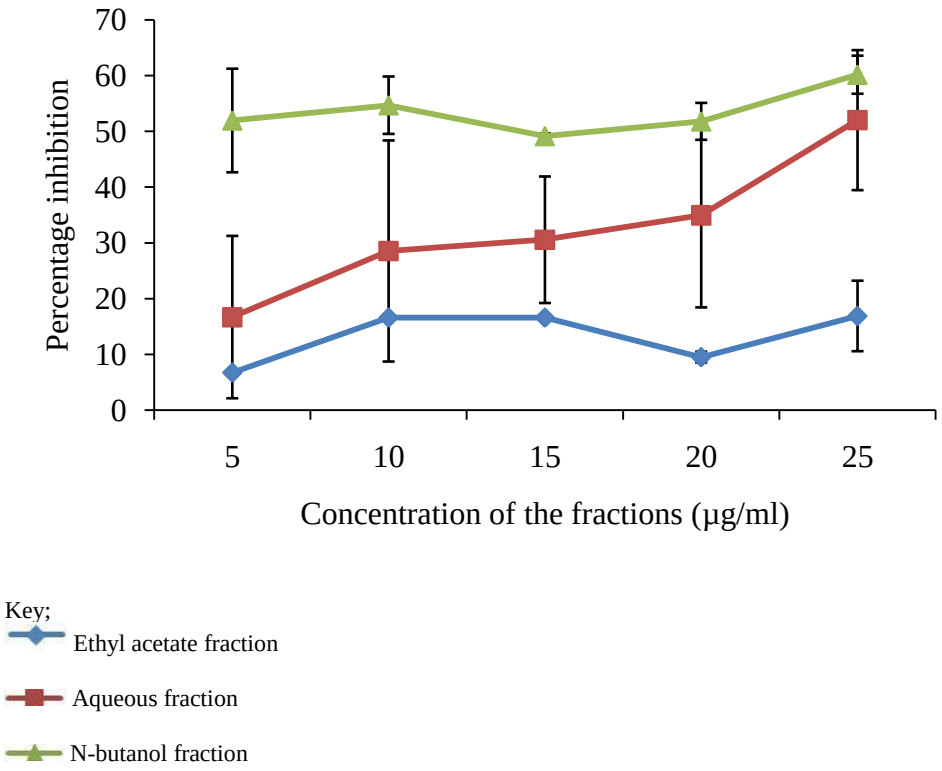


Figure 4.6; Variation in percentage inhibition of α amylase against increasing concentrations of ethyl acetate, butanol and aqueous fractions of the methanolic extract of *Detarium microcarpum* stem bark.

4.5.3 The minimum inhibitory concentration of the ethyl acetate, butanol and aqueous fractions of the methanol extract of *Detarium microcarpum* stem bark on alpha glucosidase and alpha amylase

Table 4.8; shows the result for the IC_{90} of the fractions on alpha glucosidase and the IC_{50} on alpha amylase. The IC_{90} of the fractions on alpha glucosidase was determined instead of the IC_{50} because the fractions inhibited the enzyme by more than fifty percent (50%) at a concentration of as low as 5µg/ml. From the table it is seen that the fractions had lower IC_{90} on alpha glucosidase than the extract although the difference between the extract and the fractions was not significant ($p>0.05$). As for alpha amylase, the IC_{50} of the fractions were determined and the results show that the aqueous and butanolic fractions of the extract had lower IC_{50} than the extract but there was no significant difference ($p>0.05$). The ethyl acetate fraction of the extract on the other hand had a significantly higher IC_{50} than all the other fractions, extract and the standard drug acarbose on alpha amylase. Since the butanol fraction was the most potent aginst both enzymes, its mode of inhibition on the enzymes was therefore studied.

Table 4.8; The effect of the ethyl acetate, butanol and aqueous fractions of methanolic extract of the stem bark of *Detarium microcarpum* on alpha glucosidase and alpha amylase.

Extracts/ Fractions	**IC_{90} of extract/fraction on alpha glucosidase (µg/ml)**	**IC_{50} of extract/fraction on alpha amylase (µg/ml)**
Methanolic extract of *D. microcarpum*	22.82 ± 2.02^{a}	73.98 ± 19.59^{a}
Ethyl Acetate fraction	12.19 ± 3.62^{a}	119.91 ± 37.24^{b}
Butanolic fraction	7.79 ± 1.36^{a}	26.33 ± 0.19^{a}
Aqueous fraction	16.39 ± 2.22^{a}	29.12 ± 4.35^{a}
Acabose (standard drug)	1429.34 ± 95.30^{b}	51.68 ± 0.00^{a}

Results are presented as mean ± SD. Values with different superscripts down the column are significantly different from each other at $P<0.05$

4.6 Mode of Inhibition of Alpha Glucosidase and Alpha Amylase by the Butanol Fraction of the Methanol Extract of *Detarium microcarpum* Stem Bark

4.6.1 Mode of inhibition of alpha glucosidase by the butanol fraction of the methanolic extract of *Detarium microcarpum* stem bark

Figure 4.7 shows that the butanol fraction of the methanolic extract of the stem bark of *Detarium microcarpum* is a non competitive inhibitor since the presence of the inhibitor does not affect the K_m of the enzyme but the V_{max} is reduced from 7.69µM/min in the control reaction to 0.74µM/min in the reaction that took place in the presence of 5µg/ml of the fraction. This type of inhibition is not dependent on the substrate concentration as the inhibitor binds to another site apart from the enzyme's active site but not withstanding it slows down the action of the enzyme.

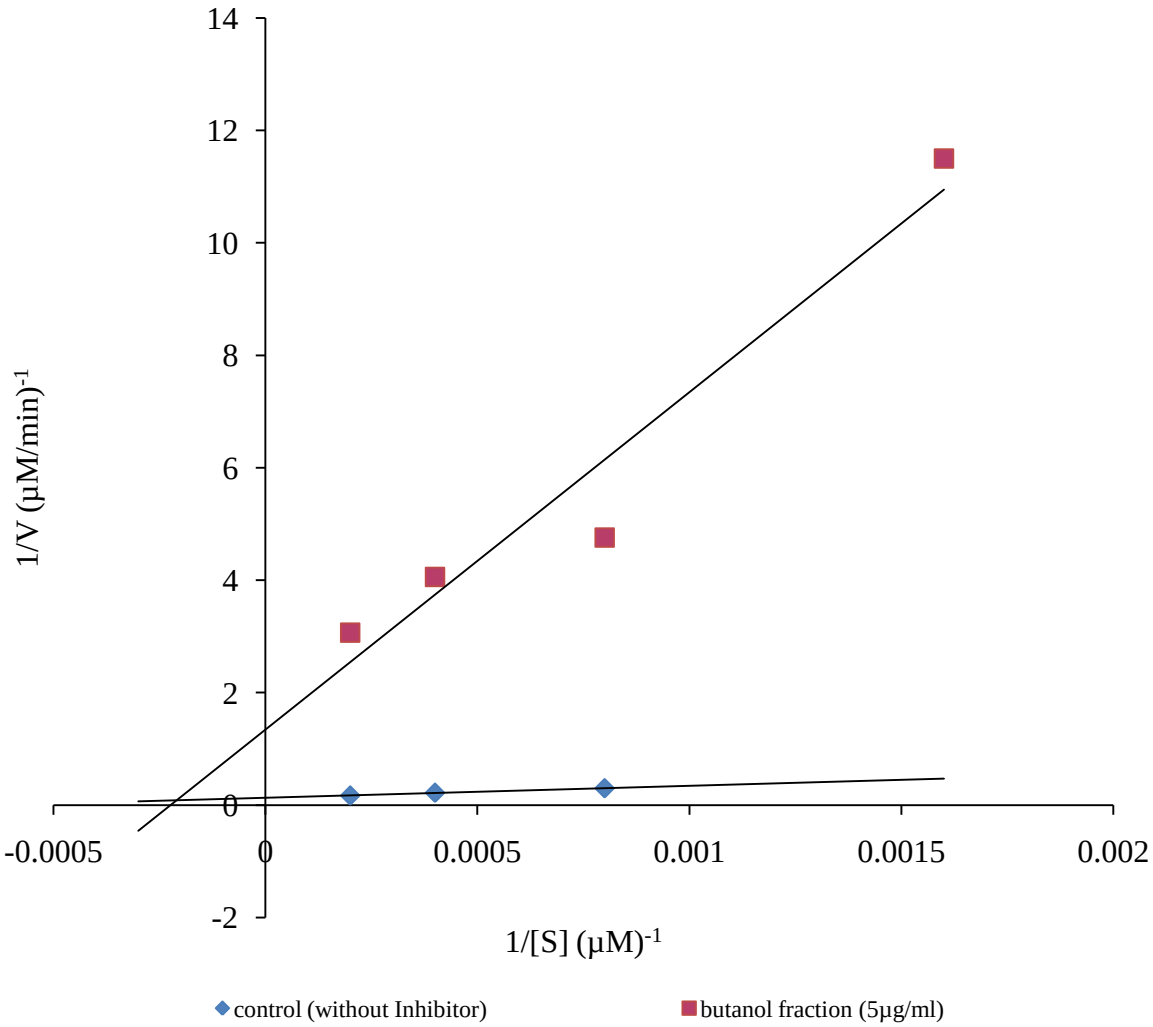


Figure 4.7; The Lineweaver Burk plot for the activity of alpha glucosidase in the presence of different concentration of the butanol fraction of the methanolic extract of *Detarium microcarpum*.

4.6.2 Mode of inhibition of alpha amylase by the butanol fraction of the methanolic extract of *Detarium microcarpum* stem bark

Figure 4.8 also shows that the butanol fraction of the the methanolic extract of *detarium microcarpum* stem bark is a competitive inhibitor of alpha amylase. This stems from the fact that in the presence of the fraction the V_{max} of the enzyme does not change but the K_m is increased from 0.67mg/ml to 5.56mg/ml. In this type of inhibition, increase in substrate concentration will reduce the effect of the inhibitor, since both the inhibitor and the substrate are competing for the same active site.

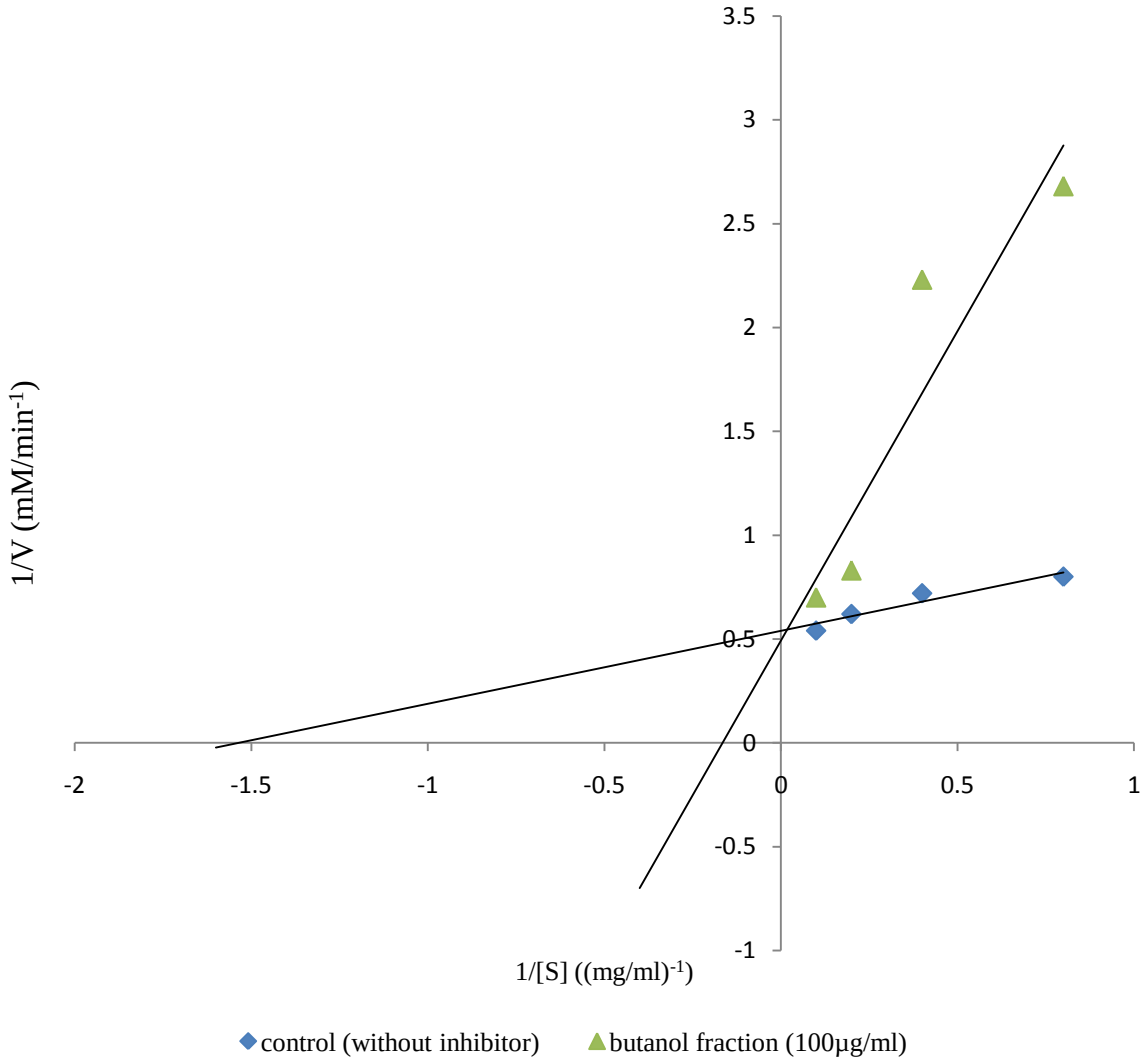


Figure 4.8; The Lineweaver Burk plot for the activity of alpha amylase in the presence of the butanol fraction of the methanolic extract of *Detarium microcarpum*.

CHAPTER FIVE

DISCUSSION

Several researches have reported the use of the extracts of *Vitex doniana* and *Detarium microcarpum* in the management of type II diabetes and its accompanying complications, (Ezekwesili, *et al.,* 2012; Okpe, 2012; Odoh, *et al.,* 2013) but there has been no report on the mechanism of action of these two plants. Since there is no report on the mechanism of action of the plants in the management of type II diabetes, there is also no information on the inhibitory activity of the plants on alpha glucosidase and alpha amylase, therefore making this study necessary.

Phytochemicals are a large group of plant-derived compounds hypothesised to be responsible for much of the disease protection conferred from consumption of plant based diets and concoctions (Arts and Hollman 2005). According to Varadarajan et al., (2008), the phytochemicals (secondary metabolites) and other chemical constituents of medicinal plants account for their medicinal value. This therefore made it necessary to screen the extracts of the stem bark of *Detarium microcaarpum* and the leaves of *Vitex doniana* used in this research to know the kind of phytochemicals present in them. In order to be able to gain access to as many as possible number of compounds in the plants, different solvents with increasing polarity were used to extract the plant constituents successively. This therefore informed the use of hexane, methanol and water to extract the stem bark of *Detarium microcarpum* and the leaves of *Vitex doniana* for the presence of phytochemicals successively. The phytochemical screening of both plants revealed that the hexane extracts of both plants contained the least number of phytochemicals when compared to the methanol and aqueous extracts. This can be attributed to the fact that most of the phytochemicals screened for were more

soluble in polar solvents. The phytochemical screening also revealed that the plants contained polyphenols, flavonoids, tannins, alkaloids, saponins, triterpenes, steroids and carbohydrates. These results are in line with previous reports by Agbafor and Nwachukwu (2011), Rueben and Jada (2013), Sani et al., (2014), Iwueke et al., (2006) and a host of other researchers.

Plants rich in polyphenolic compounds have been reported to cause insulin-like effects in glucose utilisation (Gruenwald *et al.* 2010), act as good inhibitors of key enzymes linked to type 2 diabetes (Ranilla *et al.*, 2010*;* Cheplick *et al.*, 2010). Additionally quercetin derivatives isoquercetin and isorhamnetine-3-O-rutinoside (flavonoids) have been shown to exhibit inhibitory activity on rat intestinal α-glucosidase (Rigelsky and Sweet, 2002). This link between the polyphenolic content of plants and their inhibitory activity against alpha amylase and alpha glucosidase therefore necessitated the quantitative analysis of the extracts of the stem bark of *Detarium microcarpum* and the leaves of *Vitex doniana* for their polyphenol and flavonoid contents. This analysis was also necessary to see if there is a positive correlation between the polyphenol and flavonoid content of the extracts and their inhibitory capacity against alpha amylase and alpha glucosidase. The results from the research revealed that all the extracts contained polyphenols except the hexane extract of the stem bark of *Detarium microcarpum* and the leaves of *Vitex doniana.* The methanolic extract of *Detarium microcarpum* however had the highest quantity of polyphenols when compared to the other extracts. As for the flavonoids on the other hand, all the extracts had some quantity of flavonoids but the hexane extracts had the lowest values while the aqueous extract of *Vitex doniana* had the highest quantity of flavonoids when compared to the other extracts. The results for the polyphenols and flavonoids in the extracts of *Vitex doniana* were lower than those reported by James et al., (2014) and Yakubu et al., (2014). This difference may be due

to the difference in the extraction process and difference in the solvents used for the extraction. In addition, the polyphenol and flavonoid content of all the extracts were considerably lower than that found in other extracts that has shown inhibitory activity against alpha glucosidase and alpha amylase, and example of such an extract is the methanolic and aqueous extract of *Leptadenia hastata* (Bello *et al.*, 2011).

Diabetes mellitus is a metabolic disorder of multiple aetiologies that is characterized by chronic hyperglycaemia with disturbed carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin actions or both (WHO, 1999). One therapeutic approach for treating diabetes is to decrease post-prandial hyperglycaemia. Decreasing postprandial hyperglycemia peak is therefore crucial in the treatment of diabetes (Aguilar-Santamaría, *et al*., 2009). This is done by hindering the absorption of glucose through inhibition of the carbohydrate hydrolysing enzymes, alpha amylase and alpha glucosidase, in the digestive tract. Inhibitors of these enzymes delay carbohydrate digestion, causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial increase of plasma glucose (Rhabasa-Lhoret and Chiasson, 2004). Report from previous research has shown that the fractions of the methanolic extract of the stem bark of *Detarium microcarpum* showed significant anti-diabetic activity in alloxan-induced diabetic rats (Odoh *et al.*, 2013). In addition, the aqueous and ethanolic extracts of the leaves of *Vitex doniana* were reported to significantly reduce the blood glucose in diabetic rats (Ibrahim *et al.*, 2014; Ezekwesili *et al.*, 2012). These reports and others on the anti-diabetic effect of the extracts of the stem bark of *Detarium microcarpum* and the leaves of *Vitex doniana* did not however investigate the inhibitory potentials of these extracts of alpha amylase and alpha glucosidase therefore justifying the present research. The result of this research shows that the hexane, methanolic and aqueous extract of the stem bark of *Detarium microcarpum* and the

leaves of *Vitex doniana* inhibit both alpha amylase and alpha glucosidase at a concentration of at most 1mg/ml. The minimum inhibitory concentration of 50% of the enzyme activity (IC_{50}) of the hexane extract of both plants on alpha glucosidase was the highest but was quite low against alpha amylase, making the hexane extract more potent against alpha amylase than alpha glucosidase. The methanolic and the aqueous extract of the stem bark of *Detarium microcarpum* were the most potent inhibitors against both enzymes as they had significantly ($P<0.05$) lower IC_{50} than the other extracts. The methanolic and the aqueous extract of the stem bark of *Detarium microcarpum* even had a lower IC_{50} against alpha glucosidase than the standard drug acarbose. These results are similar to those reported by Mohamed et al, (2012), Ali et al., (2013). Since the methanolic extract of *Detarium microcarpum* had a lower IC_{50} for both enzymes than the aqueous extract; it was picked for the *in vivo* confirmatory tests and fractionation for further inhibitory tests. In addition, the mode of inhibiton of the methanolic extract on both enzymes was also studied.

The determination of the IC_{50} for the various extracts gave information on the inhibitory potentials of the extracts and this informed the selection of the most potent extract against both enzymes for the *in vivo* confirmatory test. These tests were necessary in order to have an idea of the effect of the extract on alpha glucosidase and alpha amylase *in vivo*. Therefore since the methanolic extract of the stem bark of *Detarium microcarpum* had the lowest IC_{50} for both enzymes, different concentrations of the extract were used to conduct starch tolerance test and sucrose tolerance test on normal wistar rats. The idea was that since the extracts inhibited the enzymes *in vitro*, it was wise to test the effect of the extract *in vivo* on starch and sucrose breakdown and consequent release of glucose into the blood. The potency of the extract *in vivo* was determined by the use of peak blood glucose and the area under the curve of the blood

glucose level that was taken over a period of two hours after the administration of the extract and then the starch or sucrose depending on the test. The results show that all the extract treated groups had significantly ($P<0.05$) reduced peak blood glucose and area under the curve after the sucrose tolerance test and the group treated with 1000mg/kg body weight of the extract showed the least peak blood glucose and area under the curve for the sucrose tolerance test. There was also a significant ($P<0.05$) reduction in the peak blood glucose and the area under the curve in the extract treated groups for the starch tolerance test, though not in a dose dependent manner but the group treated with the highest concentration of the extract showed the most reduction in peak blood glucose and area under the curve. These results go to show that the methanolic extract of *Detarium microcarpum* might also have some level of *in vivo* inhibition against alpha glucosidase and alpha amylase. This result is similar to that reported by Ali et al., (2013) and Subramanian et al., (2008).

In order to have a better insight into the part of the extract that is responsible for the inhibition of the enzymes, the extract was subjected to liquid-liquid chromatography (partitioning) using different solvents of varying polarity to separate the compounds in the extract based on their polarity in these solvents. The solvents used for the partitioning were ethyl acetate, butanol and water. This now resulted in three different fractions of the same extract. The fractions were subjected to quantitative and qualitative phytochemical screening as well and the qualitative phytochemical screening showed that the fractions contained the same phytochemicals as the methanolic extract of *Detarium microcarpum* and they include flavonoids, phenols, alkaloids, tannins, saponins, triterpenes and carbohydrates. There was however, absence of triterpenes and saponins in the ethyl acetate fraction. The quantitative screening for flavonoids and polyphenols showed a significantly ($P<0.05$) higher concentration of polyphenols and

flavonoids in the butanol fraction than in the parent extract. This may be due to the fact that during fractionation, most of the flavonoids and polyphenols were concentrated in the butanol fraction. The ethyl acetate had a lower concentration of polyphenols and flavonoids than the parent extract but the difference was not significant ($p>0.05$) while the aqueous extract on the other hand had the least concentration of both flavonoids and polyphenols when compared to the parent extract than the other two fractions. This may be due to the polyphenols and flavonoid being concentrated in the other two fractions and what is left in the aqueous fraction is actually the residue. This result is similar to that reported by Yakubu et al., (2014), Valyova et al., (2008) and Sanjay et al., (2010). In all these researches, one or more fractions of the plant extract had a higher concentration of polyphenols and flavonoids than the extract.

The ethyl acetate, butanol and aqueous fractions of the methanolic extract of *Detarium microcarpum* was then evaluated for their inhibitory activities on alpha amylase and alpha glucosidase and as was done for the extract, the IC_{50} of the fractions were determined so as to ascertain their relative inhibitory potency against the enzymes. The fractions inhibited alpha glucosidase more than they inhibited alpha amylase as was seen with the extract but unlike the extract, the least concentration of the fractions used inhibited the alpha glucosidase by more than fifty percent, as such the IC_{90} (minimum concentration that inhibited 90% of the enzyme activity) was used to determine the inhibitory potential of the fractions. The ethyl acetate, butanol and aqueous fractions of the methanolic extract of the stem bark of *Detarium microcarpum* had lower IC_{90} than the extract itself although there was no significant difference ($p>0.05$) between the IC_{90} of the fractions and that of the extract. The butanol fraction however had the lowest IC_{90} therefore making it the most potent against alpha glucosidase. The inhibitory action of the fractions on alpha amylase on the other hand was not as strong as it was on alpha

glucosidase, therefore the IC_{50} of the fractions on alpha amylase was determined. The aqueous and butanol fractions had the lowest IC_{50} against alpha amylase with the butanol fraction having the lowest therefore making it the most potent, however there was no significant difference ($p>0.05$) between the IC_{50} of the fractions and that of the extract on alpha amylase. It is worthy of note however to state that the butanol and aqueous fraction had a lower IC_{50} than acarbose on alpha amylase. Generally, the inhibitory potency of all the fractions against both enzymes increased although not significantly ($p>0.05$), except for ethyl acetate which had a higher IC_{50} against alpha amylase than the extract. These findings are similar to those made by Karthic et al., (2008), Ibrahim et al., (2014) and Lee et al.,(2014).

The results from the inhibition studies on the methanolic extract of *Detarium microcarpum* and its ethyl acetate, butanol and aqueous fractions are all potent inhibitors of alpha glucosidase and alpha amylase, further kinetics studies on the enzymes in the presence of the most potent fraction was therefore necessary to have an idea of the type of inhibitor(s) that are potentially present in the stem bark of *Detarium microcarpum*. This information will bring us a step closer to standardising the plant as a potent inhibitor of these enzymes related to the management of type 2 diabetes mellitus.

The Lineweaver Burk plot for the activity of alpha glucosidase in the presence of the butanol fraction showed that the butanol fraction is a non competitive inhibitor of alpha glucosidase. Also, in the presence of the butanol fraction, the Lineweaver-Burk plot for alpha amylase revealed that the fraction is a competitive inhibitor of alpha amylase. These findings are similar to those made in earlier researches on the mode of inhibition of other plants on alpha glucosidase and alpha amylase, (Park *et al*., 2011, Mogale *et al.* 2011, Kazeem *et al*.,2013[a,b,c]).

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMEDATIONS

6.1 Summary

- From the present study, it was revealed that extracts of *Vitex doniana* leaves and *Detarium microcarpum* stem bark contained more phytochemicals than the hexane extract of both plants. In addition, the aqueous extract of *Vitex doniana* had the highest flavonoid content (0.87 ± 0.03 mg quercertin equivalent/g dry sample) in comparison to the other extracts while the methanolic extract of *Detarium microcarpum* had the highest polyphenol content (0.62±0.13 mg gallic acid erquivalent/g dry sample) when compared to the other extracts.

- The hexane, methanol and aqueous extracts of *Vitex doniana* and *Detarium microcarpum* showed a dose dependent pattern of inhibition on both alpha glucosidase and alpha amylase. In addition, the aqueous and methanolic extract of *Detarium microcarpum* showed the most potent inhibitory activity against alpha glucosidase and alpha amylase in comparison to the other extracts as they had the lowest IC_{50} (4.63 ± 1.64µg/ml, 228.16 ± 119.53 µg/ml and 5.88 ± 0.47 µg/ml, 73.98 ± 19.59 µg/ml respectively).

- *In vivo*, the methanolic extract of *Detarium microcarpum* stem bark significantly ($P<0.05$) reduced the blood glucose in the rats that were subjected to starch and sucrose tolerance test after being treated with different doses of the extract. A dose of 1000mg/kg body weight extract reduced the blood glucose of the experimental animals most in the starch tolerance test as the group treated with

1000mg/kg body weight extract reduced the peak blood glucose and area under the curve most (37.77% and 28.60% respectively) while a dose of 250mg/kg body weight reduced the blood glucose most in the sucrose tolerance test as the group had the most reduction in the peak blood glucose and area under the curve (38.46% and27.89% respectively).

- Upon the fractionation of the methanolic extract of *Detarium microcarpum* stem bark, qualitative phytochemical screening of the fractions revealed that the butanol and aqueous fractions contained similar phytochemical as the extract except for the ethyl acetate fraction which didn't contain saponin and triterpenes as compared to the methanolic extract of *Detarium microcarpum* stem bark and the other two fractions. In addition, the butanol fraction of the methanol extract of *Detarium microcarpum* stem bark had the highest polyphenol and flavonoid content as compared to the other fractions and even the methanolic extract of *Detarium microcarpum* stem bark.

- The ethyl acetate, butanol and aqueous fractions of the methanol extract of *Detarium microcarpum* inhibited alpha glucosidase strongly at low concentrations of 5µg/ml with the butanol fraction being the most potent with IC_{90} of 7.79 ± 1.36µg/ml. As for the effect of the fractions on alpha amylase, the three fractions exhibited a dose dependent inhibitory effect on the enzyme with the butanol fraction still being the most potent with an IC_{50} of 26.33 ± 0.19µg/ml.

- ❖ The butanol fraction which showed the best inhibitory potential against alpha glucosidase and alpha amylase was a non competitive inhibitor of alpha glucosidase and a competitive inhibitior of alpha amylase.

6.2 Conclusion

The result of this study shows that the hexane, methanol and aqueous extracts of *Vitex doniana* leaves and *Detarium microcarpum* stem bark possess possible inhibitory potential against alpha glucosidase and alpha amylase with the methanolic extract of *Detarium microcarpum* being the most potent inhibitor of both enzymes in comparison with the other extracts. The ethyl acetate, butanol and aqueous fractions of the methanol extract of *Detarium microcarpum* also showed strong inhibitory potentials against both alpha amylase and alpha glucosidase. Generally, the extract and its fractions inhibited alpha glucosidase more strongly than they inhibited alpha amylase. The mode of inhibiton of the butanol fraction of the extract on both enzymes was different. These findings therefore validate the use of the stem bark of *Detarium microcarpum* by folk medicine in the treatment of type-2 diabetes as the results suggest that the plant is a good candidate for the control of postprandial hyperglycemia.

6.3 Recommendations

1. The butanol fraction of the methanol extract of *Detarium microcarpum* stem bark should be subjected to a bioassay guided fractionation in order to isolate the active compound that is responsible for the inhibition of alpha glucosidase and alpha amylase and the mode of interation of the compound and the enzymes should also be studied.

2. Further studies should be carried on *Vitex doniana* leaves and *Detarium microcarpum* stem bark in order to identify and isolate the compound(s) responsible for their inhibitory activity against alpha amylase and alpha glucosidase.
3. Further *in vivo* studies should be carried out on both extracts so as to establish their *in vivo* inhibitory activity against alpha glucosidase and alpha amylase.

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LIST OF APPENDICES.

APPENDIX I

Table for the substrate concentration and activity of alpha glucosidase in the presence of butanol fraction of the methanolic extract of *Detarium microcarpum* stem bark.

[S](µM)	**1/[S](µM^{-1})**	**v(µM/min) (without inhibiton**	**1/v(µM/min) (without inhibiton**	**v(µM/min)(in the presence of 5µg/ml butanol fraction)**	**1/v(µM/min)(in the presence of 5µg/ml butanol fraction)**
5000	0.0002	5.88	0.17	0.33	3.07
2500	0.0004	4.55	0.22	0.25	4.06
1250	0.0008	3.33	0.30	0.21	4.76

APPENDIX II

Table for the substrate concentration and activity of alpha amylase in the presence of butanol fraction of the methanolic extract of *Detarium microcarpum* stem bark.

[S](mg/ml)	**1/[S](mg/ml)$^{-1}$**	**v(mM/min) (without inhibiton**	**1/v(mM/min) (without inhibiton**	**v(mM/min)(in the presence of 100µg/ml butanol fraction)**	**1/v(mM/min)(in the presence of 100µg/ml butanol fraction)**
10	0.1	1.85	0.54	1.43	0.70
5	0.2	1.61	0.62	1.20	0.83
2.5	0.4	1.39	0.72	0.45	2.23
1.25	0.8	1.25	0.80	0.37	2.68