CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Diabetes mellitus is the third leading cause of death, after heart attack and cancer. It is one of the most common endocrine dysfunction in the world resulting from a defect in insulin dynamics and has caused significant morbidity and mortality due to microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications with no known cure (Abdirahman *et al*.,2015; Patel *et al*.,2011). Diabetes mellitus affects millions of people worldwide and its prevalence increases and is projected to reach 500 million by the year 2030 (IDF, 2014). Reasons for this rise include increase in sedentary lifestyle, consumption of energy rich diet, obesity, higher life span, etc. (Yajnik, 2001). Regions with greatest potential are Asia and Africa; diabetes mellitus rates could rise to 2–3folds than the present rates (ADA, 1997).

Diabetes mellitus (DM) is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin (Maiti *et al*., 2004). Currently, available therapies for management of DM include insulin and various oral anti-diabetic agents such as sulfonylureas, biguanides, metformin, alpha-glucosidase inhibitors, thiazolidinediones and meglitinides. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have severe limitations (Maiti *et al*., 2004).

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Treatment of illness and maintenance of health using herbal medicines is the oldest and most popular form of healthcare practice known

to humanity that has been practiced by all cultures in all ages throughout the history of civilization (Chikezie *et al*., 2015). WHO has recommended the evaluation of traditional plant treatments for diabetes because they are readily available, have low side effect and are considered to be excellent aspirants for oral therapy (Shokeen *et al*., 2008).

In the developed countries, the rate of dependence on herbal medicine decreased based on the availability of synthetic drugs during the early part of the twentieth century. The resurgence of interest in anti-diabetic medicinal plant in developed countries is believed to be motivated by several factors that include: adverse reactions, high secondary failure rates and cost of conventional synthetic anti-diabetic remedies (Gurib-Fakim, 2006).

Many herbal medicines have been recommended for the treatment of diabetes; the ethno-botanical information reports about 800 plants that may possess antidiabetic potential (Alarcon-Aguilara *et al*., 1998). Most antidiabetic plants belong to the family Leguminoseae, Curubitaceae, Liliaceae, Laminaceae, Asteraceae, Rosaceae, Euphorbiaceae, Moraceae, and Araliaceae (Saravanamuttu and Sudarsanam, 2012). The herbal drugs with antidiabetic activity are yet to be commercially formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine (Wadkar *et al*., 2008).

The biological effects of the plants or herbal products used as alternative medicines to treat diabetes are related to their chemical composition. Most of plants containing phytochemicals like glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc. are frequently implicated as having antidiabetic effect. Many of such plants known to be used primitively to alleviate symptoms of illnesses have been screened to have medicinal importance, some of which include:

Azadirachta indica, *Vernonia amygdalina*, *Allium sativum*, *Allium cepa*, *Ocimum gratissimum*, *Zingiber officinale*, *Momordica charantia*, *Carica papaya*, *Aloe vera*, *Ocimum sanctum* (Udochukwu *et al*., 2015).

Vernonia amygdalina is one of the most popular antidiabetic traditional herbal remedy in Nigeria (Gbolade, 2009). *V. amygdalina*, also known as ‘‘African bitter leaf’’, is a plant vegetable used for both food and traditional treatment of diseases that is, the leaves are macerated and used in cooking, while the extracts are used as tonic for prevention of certain illness (Farombi and owoeye, 2011). *V. amygdalina* is a valuable medicinal plant that is widespread in West Africa, it is known as bitter leaf due to its characteristic bitter taste and flavour, and can be used as an active anticancer, antibacterial, antimalarial, anti-diabetic and anti-parasitic agent (Udochukwu *et al*., 2015).

V. amygdalina contains complex active components (phytochemicals) that are useful pharmacologically (Udochukwu *et al*., 2015). Phytochemicals are natural occurring bioactive compounds known for their health benefits. They are majorly responsible for the colour, flavour and aroma of fruits and notably vegetables (Kadiri and Olawoye, 2016). The phytochemical studies of *V. amygdalina* reveals the presence of saponins, flavonoids, alkaloids, terpenes, steroids, coumarins, phenolic acids, lignans, xanthones, anthraquinones, edotides and sesquiterpenes (Owoeye *et al*., 2010).

Liver enzymes such as Aspartate amino transferase (AST), Alanine amino transferase (ALT), Gamma glutamyl transferase (GGT), and Alkaline phosphatase (ALP) is the most common laboratory test for the detection of liver disease and are regarded as liver marker enzymes. Elevation of liver marker enzymes is strongly related to obesity, diabetes and dyslipidemia, and

their measurement may act as a surrogate marker for determining the presence non-alcoholic fatty liver disease (NAFLD) (Angulo, 2002; Trombetta *et al*., 2005).

Diabetes mellitus (DM) is associated with non-alcoholic fatty liver disease (NAFLD) including its severe form, non-alcoholic steatohepatitis (NASH) (Matteoni *et al*., 1999; Marchesini *et al*., 1999). Oral administration of the aqueous extract from *V. amygdalina* leaves could accelerate the reversion of liver damage through reduction of liver marker enzymes, including aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), glutamate-oxaloacetate amino transferase, glutamate pyruvate amino transferase, lactate dehydrogenase (LDH) and bilirubin indices in liver biochemical tests (Arhoghro *et al*., 2009; Iwalokun *et al*., 2006).

1.2 STATEMENT OF RESEARCH PROBLEM

The research project is based on the effect of *V. amygdalina* (Bitter leaf) on diabetes mellitus as a result of the increase or decrease of the activities of the following enzymes; aspartate amino transferase (AST), alanine amino transferase (ALT), lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) in blood serum.

1.3 JUSTIFICATION OF STUDY

Studies in metabolism of biomolecules have established a relationship between carbohydrate and lipid metabolism which aids comprehension of the clinical significance; diabetes mellitus and non-alcoholic fatty liver disease (NAFLD). Diabetes Mellitus may be at higher risk for developing non-alcoholic fatty liver disease. There is need for future studies that would integrate more precise and proximal measures of liver health.

Increased level of liver enzymes like aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) characterize the non-alcoholic fatty liver disease (NAFLD), indicating hepatocellular injury (dysfunction) which could result to metabolic diseases and further leading to death.

Adequate insight into the phytochemical properties and bio-molecular activities of *V. amygdalina* (Bitter leaf) and its interaction with the cells will give a better understanding in relation to its effects on Diabetes Mellitus.

1.4 AIM AND OBJECTIVES OF THE STUDY

The aim and objective of the study is:

1. To determine the phytochemical components of *V. amygdalina* (Bitter leaf)
2. To determine the bio-active compounds in the *V. amygdalina* (Bitter leaf) with GC-MS
3. To administer the methanol leaf extract and determine the effects of *V. amygdalina* on some plasma enzymes (AST, ALT, LDH and GGT) activities in streptozotocin induced diabetic rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 DIABETES MELLITUS

Diabetes mellitus (DM) is a group of metabolic disease characterized by high glucose (sugar) level in the blood and urine due to inability to produce, metabolize and regulate the activity of the hormone insulin. It is a prevalent and lethal disease caused by dysfunction of carbohydrate metabolism and inadequate response of target cells to hormone insulin which affects citizens of under developed, developing and developed countries (Arumugam *et al*., 2013). DM is a metabolic disorder associated with the endocrine system that affects most metabolic process (carbohydrate, fat and protein metabolism) in human irrespective of their age (Khalid *et al*., 2014; Sidhu and Sharma, 2013). The food eaten (carbohydrate) is broken down to simple sugar called glucose which is the body main source of energy. Glucose enters the blood stream and gets utilized by the body cells for energy. The hormone insulin produced by the beta cells of the endocrine pancreas is needed for the utilization of glucose by the body cells. When the pancreas cannot make insulin or there is impairment in the insulin produced (over or under secretion of insulin), glucose stay and builds up in the blood and subsequently get secreted out through the urine causing hyperglycemia and hyperglycosuria (Jeeva and Anlin, 2014; Qais *et al*., 2018).

DM is a progressive illness that is initially symptomless but gradually leads to tissue damage and reduction in the mass of pancreatic beta cells. Its complications include retinopathy (eyes damage), nephropathy (kidney damage), neuropathy (peripheral nervous system damage), cardiovascular complications (ischaemia, myocardial infarction, hypertension), ulceration, stroke and erectile dysfunction which lead to higher cost of medical care, diminished quality of life and increased death rate (Oputa and Chineye, 2015). Symptoms of DM include weight loss, fatigue,

headache, polydipsia (increased thirst), nausea, irritability, polyphagia (increased hunger), polyuria (frequent urination), recurrent infection, slow healing of cut, blurred vision, nocturia, and elevated blood glucose level (Jeeva and Anlin, 2014).

DM is one of the leading causes of mortality and its prevalence is increasing globally (Uzor and Osabede, 2016). It is caused by genetic, nutritional and environmental factors which include consumption of energy rich diet, obesity, excess glucocorticoids, pregnancy (pregnancy induced diabetes), mutation of insulin receptor and Autoantibodies to the insulin receptor (Eidi *et al*., 2006; Guyton and Hall, 2006). Nigeria has the highest population of diabetes in Africa followed by South Africa, Ethiopia, and Tanzania. The mortality rate of DM increases due to a great number of undiagnosed diabetes. Most death due to DM occurs in individuals less than 60 years old (Oputa and Chineye, 2015).

Experimental and clinical studies have shown the effect of oxidative stress in the development and advancement of diabetes which is accompanied by increase in the production of free radicals and diminished effect of antioxidant defenses. Free radical formation occurs in diabetes as a result of oxidation of glucose, non-enzymatic glycation of protein and oxidative degradation of glycated proteins. The reduction in antioxidant effect is accompanied with high level of free radicals, increased peroxidation and development of insulin resistance. As a result of this, one of the therapeutic attempts for treating diabetes is decreasing post-prandial high blood sugar (Oridupa and Saba, 2017).

2.1.1 Classification of Diabetes

Diabetes have been identified and classified into three types:

1. Type 1 diabetes

2. Type 2 diabetes
3. Gestational diabetes

Type 1 diabetes

It was formally known as insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes. It is prevalent in 5-10 % of all diagnosed cases of diabetes (Qais *et al*., 2018). It is characterized by inability of islet cells to produce insulin which leads to the accumulation of glucose in the blood serum (Alhassan *et al*., 2017). Type 1 diabetes is inherited, it usually occurs early in life and it often occur in children and young adults. There is an association between IDDM and other endocrine autoimmunity (for example, Addison disease) and an increased incidence of autoimmune diseases are seen in family members of IDDM patients. The factors responsible for type 1 diabetes include: autoimmune disorders, environmental and genetic factors (Qais *et al*., 2018). Type 1 diabetes is controlled with insulin injection.

Type 2 diabetes

It was formerly known as non-insulin dependent diabetes mellitus (NIDDM) or adult-onset diabetes. It is the most common form of diabetes accounting for 90 -95% of all diagnosed cases of diabetes. It is characterized by hyperglycemia, insulin resistance, and relative insulin deficiency. It results from interaction between genetic, environmental and behavioral risk factors. It is predominant among elder people. Type 2 DM is associated with different risk factors which include; physical inactivity, hypertension, gestational diabetes, aging, smoking, consumption of alcohol and Obesity which has been found to contribute to approximately 55% of cases of type 2 DM (Olokoba *et al*., 2012; Qais *et al*., 2018). The management of type 2 diabetes is by the use of oral agents (such as biguanides, sulfonylureas etc.) or insulin.

Gestational diabetes

It refers to the occurrence of glucose intolerance during the period of pregnancy. (Singab *et al*., 2014). About 2-5 % of all pregnant women suffers from gestational diabetes mellitus but usually disappears when a pregnancy is over (Qais *et al*., 2018). It develops during the second trimester of the pregnancy due to placental hormones which promotes insulin resistance (Tripathi and Verma, 2014). It is similar to type 2 diabetes because it involves inadequate insulin secretion and response. 5-10% of women with gestational diabetes are found to have diabetes mellitus after pregnancy, most commonly type 2 diabetes. Gestational diabetes is treatable, but it requires careful medical supervision throughout the pregnancy. Management of gestational diabetes include dietary changes, blood glucose monitoring, and in some cases insulin administration.

2.1.2 Measurement of diabetes

The most popular method for diagnosis of diabetes is the measuring of fasting plasma glucose level (FPG), which is done in the early morning. Patients with FPG below 100 mg/dl are considered normal; those between 100 and 125 mg/dl indicate pre-diabetic while those individuals with glucose levels above 125 mg/dl are considered diabetic (Singab *et al*., 2014)

2.1.3 Treatment of diabetes

Diabetes can be controlled through pharmacological method (use of anti-diabetic agents) and non-pharmacological method (food diet and body exercise). Minor food components (secondary metabolites) have been shown to alter biological process thereby reducing the risk of diseases in humans (Nwanjo, 2005). Pharmacological treatment of diabetes is composed of both insulin and oral glucose lowering drugs and in some instances complementary and alternative medicine.

The mechanisms adopted in treating diabetes in both traditional and western medicine involve decreasing blood sugar by stimulating pancreatic β-cells; inhibiting hormone elevating hyperglycemia; increasing the affinity and sensitivity of insulin receptor; reducing glycogen degradation; raising glucose utilization in tissues and organs; eliminating free radicals and improving blood circulation. The oral anti-diabetic agents include sulfonylurea, biguanides (metformin), alpha-glucosidase inhibitors (starch blockers), acarbose, thiazolidinediones (TZDs) and non-sulfonylurea (Sigab *et al*., 2014).

2.2 PLANTS AS ANTI-DIABETIC AGENTS

Due to the side effects and less efficiency of many anti-diabetic agents, plants have been explored for treating diabetes because of their ease of availability, low cost, better compatibility with the human body and least side effect (Singab *et al*., 2014). The anti-hyperglycemic effect of different plant drugs is a result of several mechanisms, including: inhibition of intestinal absorption of glucose through inhibition of digestive enzymes (alpha-amylases and alpha-glucosidases) of carbohydrates, and hepatic glucose metabolising enzymes; inhibition of glycosylation of haemoglobin; modulation of glucose absorption from the gut; and plant antioxidant effects (Oridupa and Saba, 2017). A broad number of plant species have been reported for their antidiabetic effect. Some antidiabetic agents are derived from plant sources, for example Glucophage (metformin) is gotten from *Galega officinalis.* The antidiabetic effect of plants have been attributed to the presence of phytochemicals which include; alkaloids, terpenoids, glycosides, flavonoids, carotenoids, phytosterol, polyphenol etc.

2.2.1 *VERNONIA AMYGDALINA* (VAM)

V. amygdalina popularly called bitter leaf is a green leafy plant with a characteristic odor and bitter taste (Udochukwu *et al*., 2015). It was named after William Vernon a 17th century botanist

and the complete binomial name is *Vernonia amygdalina Del* (Farombi and Owoeye, 2011). Vernonia is a genus of about 1,000 species of forbs and shrubs of which VAM is the most prominent specie (Clement *et al*., 2014). There are about 200 species of Vernonia. VAM is a small shrub whose leaves is elliptical in shape and grows up to 20cm lo ng with height of about 2-5m). It is a seedless plant that is propagated by cutting and vegetative cultivated by stem cutting. It grows under a range of ecological zones in Africa and it is drought resistant. VAM thrives on all types of soil but grows better on humus-rich zones soils (Emmanuel *et al*., 2015). VAM is a short cycle crop which can be harvested twice per month and its bitter characteristics prevents it from animals, insects and microbial attack (Yeap *et al*., 2010).

It is a known medicinal plant used in the treatment of diabetes and fever (Amodu *et al*., 2013). VAM leaf is broadly consumed as vegetable because of its sufficient nutritional constituents and phytochemicals that are responsible for its medicinal activities (Kadiri and Olawoye, 2016). The bitterness of VAM can be reduced by boiling or by soaking the leaves in several changes of water (Farombi and Owoeye, 2011) and the bitter taste is known to have an after taste of sweetness (Anibijuwon *et al*., 2012)

Figure 1: Picture of *Vernonia amygdalina* (bitter leaf)

Scientific classification of VAM

Table 1: The scientific classification of VAM

Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Magnoliopsida
Order	Asterales
Family	Asteraceae (Compositae)
Genus	Vernonia
Specie	*Vernonia amygdalina*

VAM is a popular leafy vegetables among the Africans and it is has different common names based on the country language. It is popular in East Africa and West Africa countries including Nigeria, Cameroon, Zimbabwe, Gabon, Congo democratic republic (Oladosu-Ajayi *et* al., 2017)

Table 2: The common names of *V. amygdalina*

English	Bitter leaf
Yoruba	Ewuro
Efik/Ibibio	Etidot
Ebira	Uzi
Igbo	Onugbu
Hausa	Chusar-doki or Shiwaka
Cameroon	Muop or Ndole
Tanzania	Tuntwano
Uganda	Mululuza
Edo	Oriwo
Tiv	Ityuna
Luo	Olusia
Igala	Ilo
Amharic	Grawa

2.2.2 Phytochemical constituent of VAM

The therapeutic potentials of VAM are attributed to the presence of secondary metabolites (Emmanuel *et al*., 2015). It have been reported that VAM contains bioactive components (phytochemicals) like alkaloids, saponins, flavonoids, tannins, anthraquinones, oxalate, glycosides, phlobatannins, steroids, terpenoids, poly phenol and phenol. Some phytochemicals exerts pharmacological and antagonistic effect while others protect the active components from degrading (Alhassan *et al*., 2017; Emmanuel *et al*., 2015). Research showed that VAM leaves contain stigmastane-type saponins such as vernoniosides A1, B1, A2, A3, B2, D2, A4 and C.

VAM bitter taste is attributed to the presence of some of these bioactive components. The bitterness is caused by sesquiterpene lactones (vernodalin, vernolepin and vernomygdin) and steroid glucosides (vernoniosides) (Okore *et al*., 2014).

The proximate analysis of VAM revealed that it possesses significant quantities of lipids, carbohydrate, proteins with high essential amino acid score. VAM also contains some macroelements like ascorbic acid, carotenoids, calcium, iron, potassium, phosphorous, manganese, copper and cobalt. Research also revealed that VAM contains coumarins, phenolic acids, edotides, lignans, and xanthones (Akpanyung *et al*., 2018). Bioactive compounds prevent the advent of many diseases such as cancer, diabetes, heart and Alzheimer's disease (Kadiri and Olawoye, 2016). Recently, some researchers isolated and characterized a sesquiterpene lactone, epivernodalol, another elemanolide from the dichloromethane fraction of Vernonia amygdalina (Farombi and Owoeye, 2011).

2.2.3 Uses of VAM

Various uses of VAM have been reported. It is used to prepare the Nigerian bitter leaf soup, and as also used as spice in the Cameroon dish called Ndole (Kadiri and Olawoye, 2016). It is used as both therapeutic and nutritional agents. The roots and leaves of VAM have been used in the treatment of kidney disease, fever and stomach discomfort. The roots and stems of VAM can also be used as chewing sticks which help in the treatment of dental caries. It had been reported that VAM has antidiabetic, anti-inflammatory, anti-cancer and blood clotting effect (Udochukwu *et al*., 2015). The roots of VAM have been used in treating gingivitis and toothache as a result of its antimicrobial activity. It can be used as an anti-helmint, a laxative and an amti-malarial agent due to the presence of quinine substitute in the leave (Ekam *et al*., 2010). Parasitic infestation can be treated with the juice extract of VAM (Emmanuel *et al*., 2015).

VAM had been employed in the traditional treatment of diseases such as infertility, gastrointestinal problems and sexually transmitted disease. VAM can be added to horse food to prepare a strengthening and fattening tonic called Chusan Dokan in the northern part of Nigeria. VAM act as blood purifier and it helps prevent atherosclerosis. It can also be used for treating anemia, nausea and dysentery (Kadiri and Olawoye, 2016). The bitter juice extract of VAM is used by nursing mothers to wean their babies by rubbing it on their breast (Anibijuwon *et al*., 2012). It is used as a bittering agent, a hop substitute and an agent for the control of microbial contamination in beer brewing without affecting the quality of malt for instance; it is used in Ethiopia for the preparation of Tela beer (Yeap *et al*., 2010). VAM may also provide anti-oxidant benefits, enhancement of immune system as well as bacteriostatic and bactericidal effect (Kadiri and Olawoye, 2016).

Different solvents extraction has varying abilities to liberate the bioactive compounds in a plant which determine the effect of the extracted component. Research showed that the aqueous extract of the leaves has inhibitory effect on the growth of gram Positive bacterium; *Staphylococcus aureus* and the gram negative bacterium; *Escherichia coli* and *Pseudomonas aeruginosa* (Kadiri and Olawoye, 2016). Ethanolic extract of VAM improves glucose tolerance in streptozotocin-induced diabetic and normal wistar rats at a high dose (Clement *et al*., 2014). The ethanolic extract was proved to reduce glucose, urea, Creatinine, potassium, sodium, chloride and aminotransferase activity in serum and regenerated glomerular tuft, endothelial cells, glomerular capsule and juxtaglomerular of kidney when administered to alloxan induced diabetic rat. V. amygdalina reduced the plasma glucose level to normal in streptozotocin induced diabetic rats (Yeap *et al*., 2010).

2.3 STREPTOZOTOCIN (STZ)

Streptozotocin is an antibiotic and anticancer agent that is used for inducing type I diabetes in a variety of animals by affecting degeneration and necrosis of pancreatic b-cells (Eidi *et al*., 2006). Streptozotocin is a monofunctional nitrosourea derivative that is isolated from *Steptomycetes achromogenes* and is used to induce both type 1 diabetes and type 2 diabetes. STZ induces diabetes in almost all the species and diabetes dose varies with the species and the optimal dose required to produce diabetes in rat was found to be (40-60mg/kg intraperitoneal or intravascular administration). Due to the low solubility of STZ, intravascular injection is the best route of administration (Swaroopa *et al*., 2017; Tripathi and Verma, 2014). The administration of STZ leads to the damage of the β-cells after three days (Khalid *et al*., 2014).

Mechanism of Action

Streptozotocin enters the pancreatic cell via a glucose transporter-GLUT2 (Glucose transporter 2) and causes alkylation of DNA. It induces activation of poly adenosine diphoshate ribosylation and nitric oxide release which damage pancrestic cells by necrosis thereby inducing insulin dependent diabetes (Tripathi and Verma, 2014).

2.4 METFORMIN

Biguanides is an anti-diabetic agent, of which metformin is the most commonly used. It suppresses hepatic glucose production, increases insulin sensitivity, enhances glucose uptake by phosphorylating glucose transporter (GLUT) enhancer factor, elevating fatty acid oxidation, and reducing the absorption of glucose from the gastrointestinal tract (Olokoba *et al*., 2012). Metformin is the first line of treatment for type 2 diabetes. It is an oral antidiabetic agent that is used in the treatment of type 2 diabetes and it deals with insulin resistance (Tripathi and

Srivastava, 2006). Metformin has few side effects which include lactic acidiosis, gastrointestinal symptoms (nauseas and vomiting) (Wang *et al*., 2017).

MECHANISM OF ACTION

Metformin works by decreasing intestinal glucose absorption, improving peripheral glucose uptake, lowering fasting plasma insulin levels and increasing insulin sensitivity, which result in a reduction of blood glucose concentrations without causing hypoglycemia (Wang *et al*., 2017) Metformin action is initiated by the activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK), which leads to termination of glucose production through gluconeogenesis and elevated level of peripheral glucose uptake. Its glucose reducing effect is as a result of decreased hepatic glucose output (gluconeogenesis and glycogenolysis) and increased insulin induced glucose uptake and glycogenesis in skeletal muscle (Tripathi and Srivastava, 2006).

2.5 GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

GC-MS is an analytical technique that combines the separation properties of gas-liquid chromatography with the detection characteristics of mass spectrometry to identify different substances within a test sample (Chauhan *et al*., 2014). GC can separate volatile and semi-volatile compounds with great resolution, but it cannot identify them while mass spectrometry can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them (Hussain and Maqbool, 2014). It is used in the determination of drugs and metabolites in the pharmaceutical area, and molecular weights and elemental composition in complex mixtures. It is used in the determination of volatile and semi-volatile organic compounds in mixture (Sneddon *et al*., 2007).

Principle of GC-MS

GC/MS combines of two different analytical techniques, Gas Chromatography (GC) and Mass Spectrometry (MS) to analyze complex organic and biochemical mixtures. The GC-MS instrument consists of two main components. The gas chromatography portion separates different compounds in the sample into pulses of pure chemicals based on their volatility by flowing an inert gas (mobile phase), which carries the sample, through a stationary phase fixed in the column. Spectra of compounds are collected as they exit a chromatographic column by the mass spectrometer. The separation of the phase ions is achieved within the mass spectrometer using electrical and/or magnetic fields to differentiate ions. The mass spectrometer identifies and quantifies the chemicals according to their mass-to-charge ratio (m/z). These spectra can then be stored on the computer and analyzed (Hussain and Maqbool, 2014). A plot of this signal as a function of time generates a series of symmetrical peaks in a chromatogram, which provides some information on the sample composition. The retention time of the peaks may help identify the sample components by comparing them to the retention time of some standard, while the heights of the peaks or the area under the peaks provide a quantitative measure of the amount of each component (Hussain and Maqbool, 2014).

2.6 ASPARTATE AMINO TRANSFERASE (AST)

AST is one of the groups of enzymes that catalyze the inter conversion of amino acids and α-keto acids by transfer of amino groups. It exist in two different isoenzyme forms which are genetically distinct; the mitochondrial and cytoplasmic form. Normal serum level of AST is between 0 to 35U/L. The highest concentration of AST is found in the heart, liver, skeletal muscle and kidney tissue and elevated level of AST occurs as a result of damage to theses tissues. Increased level of AST is found in myocardial infarction, hepatitis, chronic liver disease,

and musculoskeletal disease. Reduced level of AST occurs in uremia and vitamin B deficiency (Gowda *et al*., 2009).

2.7 ALANINE AMINO TRANSFERASE (ALT)

ALT is one of the groups of enzymes that catalyze the inter conversion of amino acids and α-keto acids by transfer of amino groups. It is a cytoplasmic enzyme that is primarily found in hepatocytes and can also be found in other tissues. Alt is released into the blood during cell damage. Increased level of AST is found in hepatitis, cirrhosis, obstructive jaundice and other hepatocellular diseases. Increase level is also seen in myocardia infraction. The determination of ALT activity in serum is used mainly to assess the liver damage. Normal serum level of ALT is 30 U/L (Luke *et al*., 2013).

2.8 GAMMA GLUTAMYL TRANSFERASE (GGT)

GGT is a metabolic enzyme expressed primarily in the liver, kidneys, pancreas, intestine and prostate. It is located on the external surface of most cells and mediates the uptake of glutathione, a vital constituent of intracellular antioxidant defenses. The normal range is 0-30U/L. Increase in GGT concentration has been regarded as a marker of alcohol consumption or liver disease. (Thapa and Walia, 2007).

2.9 LACTATE DEHYDROGENASE (LDH)

LDH is an enzyme which can be found in most major tissues such as heart muscle. LDH catalyzes the conversion of lactate to pyruvate, the forward reaction and the conversion of pyruvate to lactate, the reverse reaction. It is present in every cell and it is a tetramer molecule. There are five different isoenzymes: LDH-1; LDH-2; LDH-3; LDH-4 and LDH-5. It is released during tissue damage and it is a marker of diseases such as heart failure.

2.10 TOTAL PROTEIN (TP)

The liver is the major source of serum protein. Total Protein measures the amount of protein in your blood. The two main proteins found in the blood are globulins and albumin. Globulin is a protein made in the liver which helps the immune system fight infections. Low globulin levels can be a sign of liver damage or other conditions. Albumin is another protein made in the liver. Albumin test measures how well the liver makes protein needed by the body. Low albumin levels can be a sign of liver damage. (Thapa and Walia, 2007)

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 MATERIALS

Fresh bitter leaf (*V. amygdalina*) plant was used for this research. The reagent used were of analytical grade product of Butch Drug House (BDH) and distilled water

3.2 METHODOLOGY

3.2.1 Sample Collection and Preparation

The bitter leaf sample (*V. amygdalina*) was collected from an individual farm at Imushin, Ijebu-Ode, Ogun state in February 2019. The leaves were separated from the stem and air-dried for two weeks. They were then pulverized by means of blender and stored in air-tight jar. Eighty four grams (84 g) of the powder was weighed into three (3) jars and was macerated in methanol with occasional shaking at room temperature for 72 hours twice. Filtration was done using muslin cloth. The filtrate was concentrated at 60°C to about one-tenth the original volume using a rotary evaporator. The concentrates were kept in the oven (40°C) for complete dryness of the methanol extracts and were then stored in a refrigerator at −4°C.

3.2.2 Qualitative Phytochemical analysis

The methanol leaf extract was tested for the presence of bioactive compounds using standard methods as described by Trease and Evans (1989) with slight modification.

Test for alkaloids (Mayer's test)

0.5 g of methanol leaf extract of *V. amygdalina* (crude extract) was dissolved in 5mls of distilled water. 2 ml of 1% hydrochloride (HCl) was added and heated gently. 3 ml of Mayer's reagent

was added to the mixture. Turbidity of the resulting precipitate indicated the presence of alkaloids.

Test for carbohydrates (Molisch's test)

0.5 g of crude extract was dissolved in 5 ml of distilled water. 2mls of Molisch reagent was added and the mixture was shaken properly. 2 ml of conc. sulphuric acid (H_2SO_4) was poured carefully along the side of the test tube. Appearance of a violet ring at the interphase indicated the presence of carbohydrate.

Test for glycosides (Borntrager's test)

0.5 g of crude extract was dissolved in 5 ml of distilled water. 3 ml of chloroform was added and the mixture was shaken. The chloroform layer was separated and 2 ml of 10% ammonia solution was added. The appearance of pink color indicated the presence of glycosides.

Test for saponin (Froth test)

0.5 g of crude extract was diluted with distilled water to 20 ml and was shaken in a graduated cylinder for 5 mins. Formation of foam indicated the presence of saponin.

Test for terpenoids (Salkowski's test)

0.5 g of crude extract was dissolved in 5 ml of distilled water. 2 ml of chloroform was added and 3 ml of conc. H_2SO_4 was carefully added to form a layer. The appearance of reddish brown coloration at the interphase indicated the presence of terpernoids.

Test for phenol (Ferric chloride test)

0.5 g of crude extract was dissolved in 5 ml of distilled water and 4 drops of ferric chloride ($FeCl_3$) solution was added. The formation of bluish black color indicated the presence of phenol.

Test for flavonoid (Alkaline test)

0.5 g of crude extract was dissolved in 5 ml of distilled water and few drops of 10% sodium hydroxide (NaOH) solution were added. The formation of intense yellow color indicated the presence of flavonoid.

Test for tannin

0.5 g of crude extract was dissolved in 5 ml of distilled water and 2ml of 2% $FeCl_3$ solution was added. The formation of blue-green coloration indicated the presence of tannin.

Test for protein (Ninhydrin test)

0.5 g of crude extract was dissolved in 5 ml of distilled water. 2 ml of 0.2% Ninhydrin reagent was added and the mixture was boiled for 5 mins. The formation of violet/blue color indicated the presence of amino acids.

Test for phytosterol (Libermann-Burchard's test)

0.5 g of crude extract was dissolved in 5 ml of distilled water. 2 drops of conc. H_2SO_4 was added slowly along the side of the test tube. Change in color (violet to blue) indicated the presence of steroids.

Test for polyphenol

0.5 g of crude extract was dissolved in 5 ml of distilled water. 1 ml of 2% $FeCl_3$ solution and 1 ml of 1% potassium ferricyanide solution were added. The formation of green-blue color indicated the presence of polyphenol.

Test for fat and oil (Spot test)

Small quantity of the crude extract was pressed between two filter papers. The appearance of oil stain on the paper indicated the presence of fixed oil.

Test for anthraquinone

0.5 g of extract was boiled with 10 ml of H_2SO_4 and filtered hot. The filtrate was shook with 5 ml of chloroform. The chloroform layer was pipetted into another test tube and 1 ml of ammonia was added. The formation of a violet color indicated the presence of anthraquinone.

3.2.3 GC-MS ANALYSIS

The GC-MS analysis was carried out using a Hewlett Packard Gas Chromatograph (Model 6890 series) equipped with a flame ionization detector and Hewlett Packard 7683 series injector, MS transfer line temperature of 250°C. The GC was equipped with a fused silica capillary column-HP-5MS (30 x 0.25 mm), film thickness 1.0 μm. The oven temperature was held at 50°C for 5 min holding time and raised from 50 to 250°C at a rate of 2°C /min, employing helium gas (99.999%) as a carrier gas at a constant flow rate of 22 cm/s. 1.0 micron of extract (1 mg dissolved in 1 ml absolute alcohol), at a split ratio of 1:30 was injected. MS analysis was carried out on Agilent Technology Network Mass Spectrometer (Model 5973 series) coupled to Hewlett Packard Gas Chromatograph (Model 6890 series) equipped with NIST08 Library software database. Mass spectra were taken at 70 eV/200°C, scanning rate of 1 scan/s. Identification of compounds was conducted using the database of NIST08 Library. Mass spectrum of individual unknown compound was compared with the known compounds stored in the software database Library (Ajayi *et al.*, 2011).

3.3 Experimental animals

Twenty-five (25) male albino Wistar rats weighing (137-223g) was selected and used for this study were obtained and kept at the animal house, Department of Biological sciences, College of Basic and Applied Sciences, Mountain Top University, Nigeria. The animals were allowed free

access to feed and water *ad libitum* and were allowed to acclimatize for 7 days before the commencement of the experiment. The ethical regulations in accordance with National and Institutional guidelines for the protection of animals' welfare were strictly adhered to during the experiments.

3.4 Experimental design

The twenty-five (25) rats were randomly distributed into five (5) groups (I-V) of five (5) animals each in well ventilated cages. The experimental animals received the following treatments with stipulated feed and water:

Group I (Normal control): No treatment was administered.

Group II (Negative control): Received a single dose of streptozotocin (STZ) (45 mg/kg).

Group III (Test group): Received a single dose of STZ (45 mg/kg) before treatment with 45mg/kg of metformin for seven (7) consecutive days.

Group IV (Test group): Received a single dose of STZ (45 mg/kg) before treatment with 150mg/kg of methanol extract of *V. amygdalina* for seven (7) consecutive days.

Group V (Test group): Received a single dose of STZ (45 mg/kg) before treatment with 300mg/kg of methanol extract of *V. amygdalina* for seven (7) consecutive days.

3.4.1 Induction of Diabetes

Diabetes was induced experimentally by intraperitoneal administration of STZ (45 mg/kg body weight) dissolved in citrate buffer (0.01 M, pH 4.5) to overnight fasted rat (12 hours after last feeding). Injected rats were returned to their cages and provided with 5% glucose solution for 12 hours to overcome STZ-induced hypoglycemia. After 72 hours of STZ administration, fasting blood glucose (FBG) level was measured using a glucometer.

3.4.2 Drug administration

The administration of methanol extract of *V. amygdalina* and metformin started after 72 hours of STZ administration; the extract and metformin was suspended in distilled water and administered orally. The volume of administrated extract was 1 ml for each animal. The normal group and diabetic control group were administered only with vehicle.

After seven (7) days of administration of plant extract and metformin, the body weight and fasting blood glucose of the animals were again determined.

3.4.3 Collection and preparation of Blood plasma

After seven (7) days of administration of plant extract and metformin, the animals were sacrificed by cervical dislocation under anesthesia using 1% chloroform. The blood sample was collected from eyes (ocular). Blood samples were transferred to heparin bottles and centrifuged at 2500 rpm for 10 mins to obtain plasma.

3.5 Biochemical assay

Blood plasma was used for the assay of aspartate amino transferase (AST), alanine amino transferase (ALT), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH) and total protein (TP) level using standard laboratory kit from Randox laboratories, UK.

3.5.1 Aspartate amino transferase (AST)

Principle

$$\alpha \text{ Ketoglutarate} + \text{L-aspartate} \xrightarrow{\text{AST}} \text{L-glutamate} + \text{oxaloacetate}$$

$$\text{Oxaloacetate} + \text{2,4-dinitrophenyl hydrazine} \xrightarrow{\text{NaOH}} \text{2,4-dinitrophenyl hydrazone}$$

AST present in the sample catalyzes the conversion of L-Aspartate and α ketoglutarate to oxaloacetate and glutamate. The oxaloacetate formed reacts with 2,4-dinitrophenyl hydrazine to

produce a hydrazine derivative which in alkaline medium (addition of NaOH) produces a colored complex whose intensity is measured.

Table 3: AST reagent composition

Contents	Initial concentration of solutions
R1. Buffer	
Phosphate buffer	100 mmol/l, pH 7.4
L-aspartate	100 mmol/l
∝-oxoglutarate	2 mmol/l
R2. 2,4-dinitrophenylhydrazine	2 mmol/l

a. Procedure for reagent blank

250 μl of reagent 1 was added to 50 μl of distilled water. The solution was mixed and allowed to stand for 30mins at 37℃. Reagent 2 was added and the solution was allowed to stand for 20mins at 25℃. 2500 μl of 0.4mol NaOH was added. The solution was mixed and the absorbance was taken at 546nm.

b. Procedure for sample

50 μl of blood plasma was added to 250 μl of reagent 1. The solution was mixed and allowed to stand for 30 mins at 37℃. Reagent 2 was added and the solution was allowed to stand for 20 mins at 25℃. 2500 μl of 0.4mol NaOH was added. The solution was mixed and the absorbance of the sample was read at 546nm against the reagent blank after 5 minutes.

3.5.2 Alanine Transaminase (ALT)

Principle

α Ketoglutarate + L-alanine $\xrightarrow{ALT}$ Pyruvate + oxaloacetate

Pyruvate + 2,4-dinitrophenyl hydrazine $\xrightarrow{NaOH}$ 2,4-dinitrophenyl hydrazone

ALT present in the sample catalyzes the conversion of L-Alanine and α ketoglutarate to oxaloacetate and pyruvate. The pyruvate formed reacts with 2,4-dinitrophenyl hydrazine to produce a hydrazine derivative which in alkaline medium (addition of NaOH) produces a colored complex whose intensity is measured.

Table 4: ALT reagent composition

Contents	Initial concentration of solutions
R1. Buffer	
Phosphate buffer	100 mmol/l, pH 7.4
L-alanine	200 mmol/l
∝-oxoglutarate	2 mmol/l
R2. 2,4-dinitrophenylhydrazine	2 mmol/l

a. Procedure for reagent blank

250 μl of reagent 1 was added to 50 μl of distilled water. The solution was mixed and allowed to stand for 30mins at 37 ℃. Reagent 2 was added and the solution was allowed to stand for 20 mins at 25℃. 2500 μl of 0.4mol NaOH was added. The solution was mixed and the absorbance was taken at 546nm.

b. Procedure for sample

50 μl of blood plasma was added to 250 μl of reagent 1. The solution was mixed and allowed to stand for 30mins at 37 Reagent 2 was added and the solution was allowed to stand for 20 mins at 25 ℃. 2500 μl of 0.4 mol NaOH was added. The solution was mixed and the absorbance of the sample was read at 546nm against the reagent blank after 5 minutes.

3.5.3 Gamma Glutamyl Transferase (GGT)

Principle

L-γ-glutamyl-3-carboxy-4-nitroanilide + glycylglycine $\xrightarrow{\gamma\text{-GT}}$ L-γ-glutamylglycylglycine + 5-amino-2-nitrobenzoate

The substrate L- γ -glutamyl-3-carboxy-4-nitroanilide, in the presence of glycylglycine is converted by γ-GT in the sample to 5-amino-2-nitrobenzoate. The intensity of the formed complex is measured.

Table 5: GGT reagent composition

Contents	Concentration in the test
R1a. Buffer/Glycylglycine	
Tris buffer	100 mmol/l. pH 8.25
Glycylglycine	100 mmol/l
R1b. substrate	
L-γ-glutamyl-3-carboxy-4-nitroanilide	2.9 mmol/l

One vial of substrate Rib was reconstituted with 3ml of buffer/glycylglycine R1a.

50 μl of blood plasma was added to 500 μl of the reagent at 25 ℃. The solution was mixed and the absorbance was read at 405 nm against blank (air blank). The initial absorbance was read and the absorbance was re-read after 1, 2, and 3 mins.

3.5.4 Lactate Dehydrogenase (LDH)

Principle

L-Lactate + NAD^+ $\xrightarrow{LDH}$ Pyruvate + NADH + H^+

Lactate and NAD+ are converted to pyruvate and NADH by the action of LDH. NADH strongly absorbs light at 340 nm, whereas NAD+ does not. The rate of increase in absorbance at 340 nm is directly proportional to the LDH activity in the sample.

Table 6: LDH reagent composition

Contents	Concentration in the test
R1a. Buffer/substrate	
Phosphate buffer	50 mmol/l, pH 7.5
pyruvate	0.6 mmol/l
R1b. NADH	0.18 mmol/l

One vial of NADH R1b was reconstituted with 3ml 0f buffer/substrate R1a.

20 μl of blood plasma was added to 500 μl of the reagent at 25 ℃. The solution was mixed and the absorbance was read at 340 nm against blank (air blank). The initial absorbance was read after 0.5min and the absorbance was re-read after 1, 2, and 3 mins.

3.5.5 Total Protein (TP)

Principle

Protein + Cu^{++} —^{NaOH}—> Colored Complex

Total protein determination is based on the principle of biuret reaction (copper salt in an alkaline medium). Protein in plasma forms a blue colored complex when treated with cupric icons in alkaline solution. The intensity of the blue color is proportional to the protein concentration when compared to a solution with known protein concentration.

Table 7: TP reagent composition

Contents	Concentration of solutions
R1. Biuret reagent	
Sodium hydroxide	100 mmol/l
Na-K-tartrate	16 mmol/l
Potassium iodide	15 mmol/l
Cupric sulphate	6 mmol/l
R2. Blank reagent	
Sodium hydroxide	100 mmol/l
Na-K-tartrate	16 mmol/l
CAL. Standard	
Protein	
Sodium Azide	<0.1% w/v

R1 was diluted with 400ml of distilled water. The contents of R2 were diluted with 400ml of distilled water.

a. Procedure for reagent blank

20 μl of distilled water was added to 1000μl of R1. The solution was mixed and incubated for 30mins in the water bath at 25 ℃.

b. Procedure for standard

20μ l of standard (CAL) was added to 1000 μl of R1. The solution was mixed and incubated at 25 ℃.

c. Procedure for sample

20 μl of blood plasma was added to 1000 μl of R1. The solution was mixed and incubated at 25℃. The absorbance of the sample and of the standard was measured against the reagent blank at 546 nm.

3.6 Statistical analysis

The statistical analysis was done using Graph pad prism 7.0. The results were reported as mean ± SEM (standard error of mean). The data collected were subjected to Analysis of Variance (ANOVA) to test for variations of the different parameters observed in the study. Test of significance was at 0.05% probability ($p<0.05$).

CHAPTER FOUR

4.0 RESULTS

4.1 PHYTOCHEMICAL ANALYSIS

The result of the phytochemical analysis carried out on the methanol leaf extract of *V. amygdalina* revealed the presence of some important bioactive components which is shown in Table 8.

Table 8: Results of phytochemical analysis of methanol leaf extract of *V. amygdalina*

S/N	PARAMETERS	RESULT
1.	Alkaloid	Positive
2.	Carbohydrate	Positive
3.	Protein	Positive
4.	Fat and oil	Positive
5.	Anthraquinone	Negative
6.	Phenol	Positive
7.	Flavonoid	Positive
8.	Tannin	Positive
9.	Glycoside	Positive
10.	Phytosterol	Positive
11.	Polyphenol	Positive
12.	Saponin	Positive
13.	Terpenoids	Positive

4.2 Gas chromatography-Mass spectrometry (GC-MS) analysis

4.2.1 Chromatogram of methanol leaf extract of *V. amygdalina*

Figure 2 shows the GC-MS chromatogram of methanol leaf extract of *V. amygdalina.* Peak 1 with the retention time of 15.536 was identified as Hexadecanoic acid, methyl ester and as the major phyto-component of *V. amygdalina* while the other peaks were of other phyto-components present in the plant.

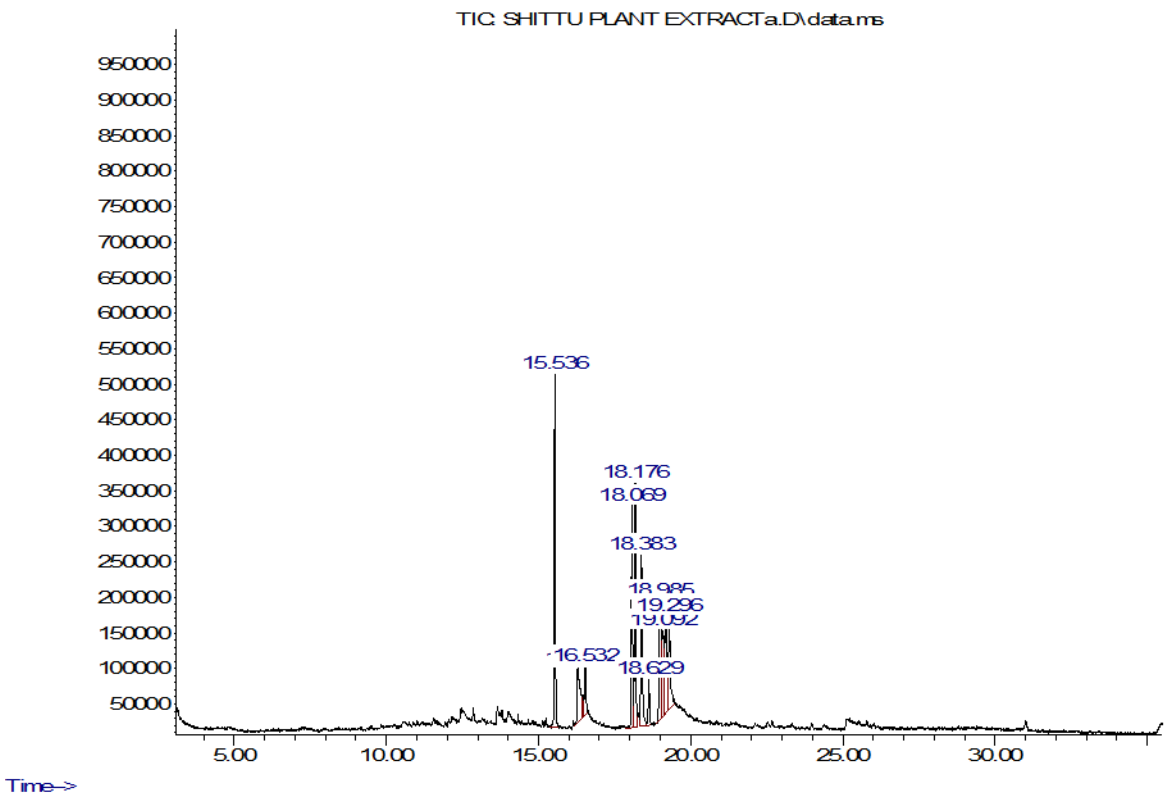

Figure 2: GC-MS chromatogram of methanol leaf extract of *V. amygdalina*

4.2.2 Phyto-component of methanol leaf extract of *V. amygdalina*

Other phyto-components of methanol leaf extract of *V.amygdalina* other than Hexadecanoic acid, methyl ester were identified. The phytochemical components identified in the methanol leaf extract of *V. amygdalina* by GC-MS showing their peak, retention time, library ID, % of total and chemical formula is shown in Table 9 and Figures 3-12.

Table 9: Phytochemical components identified by GC-MS in methanol leaf extract of *V. amygdalina* showing the peak, retention time, library ID, % of total and chemical formula

Peak	Retention time	Library ID	% of total	Chemical formula
1	15.536	Hexadecanoic acid, methyl ester	16.262%	$C_{17}H_{34}O_2$
2	16.288	n-Hexadecanoic acid	6.841%	$C_{16}H_{32}O_2$
3	16.532	Hexadecanoic acid, ethyl ester	3.197%	$C_{18}H_{36}O_2$
4	18.069	9, 12-Octadecadienoic acid (Z, Z)-, methyl ester	11.832%	$C_{19}H_{34}O_2$
5	18.176	cis-13-Octadecenoic acid, methyl ester	14.075%	$C_{19}H_{36}O_2$
6	18.383	Phytol	10.528%	$C_{20}H_{40}O$
7	18.629	heptadecanoic acid, 16-methyl-, methyl ester	2.611%	$C_{19}H_{38}O_2$
8	18.985	9, 12-Octadecadienoic acid	11.968%	$C_{18}H_{32}O_2$
9	19.180	Linoleic acid ethyl ester	8.203%	$C_{20}H_{36}O_2$
10	19.296	9, 12, 15-Octadecatrienoic acid, ethyl ester (Z, Z, Z)	7.828%	$C_{20}H_{34}O_2$

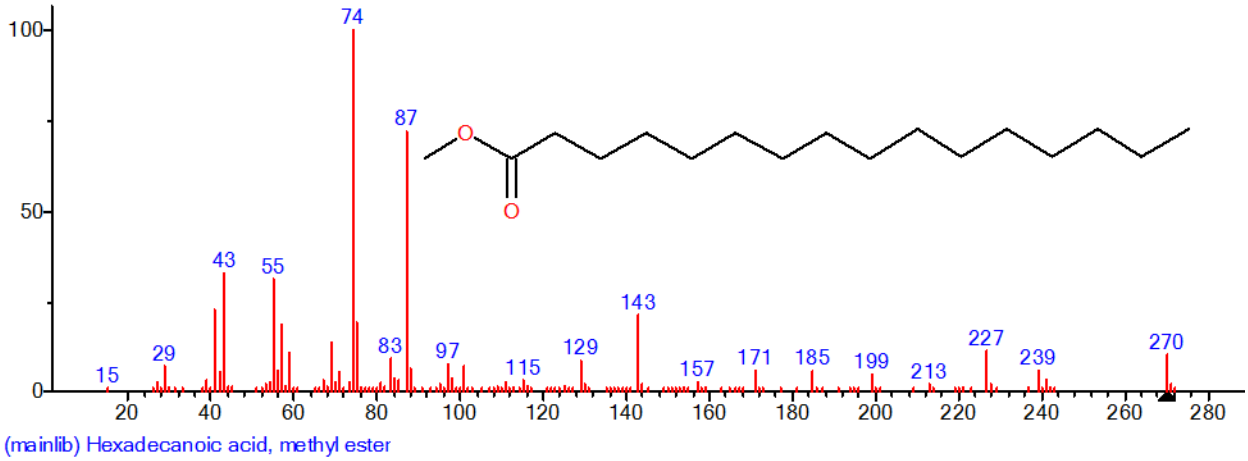

Figure 3: Structure of hexadecanoic acid, methyl ester

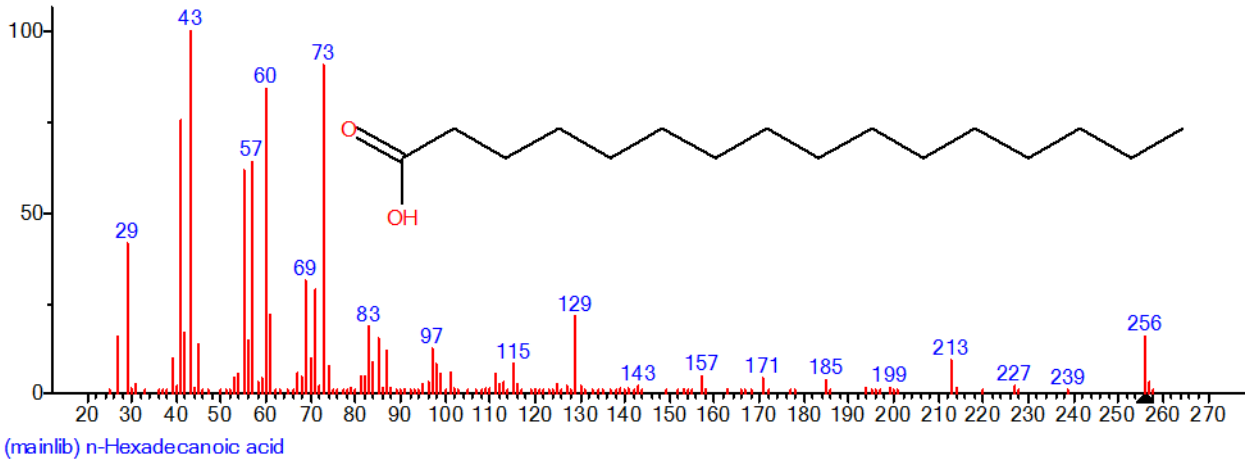

Figure 4: Structure of n-Hexadecanoic acid

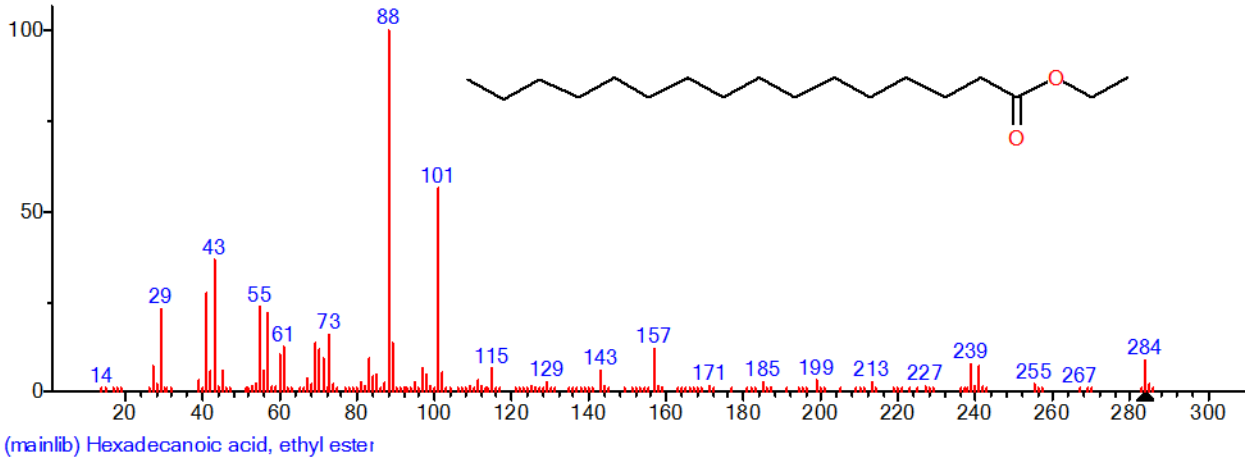

Figure 5: Structure of hexadecanoic acid, ethyl ester

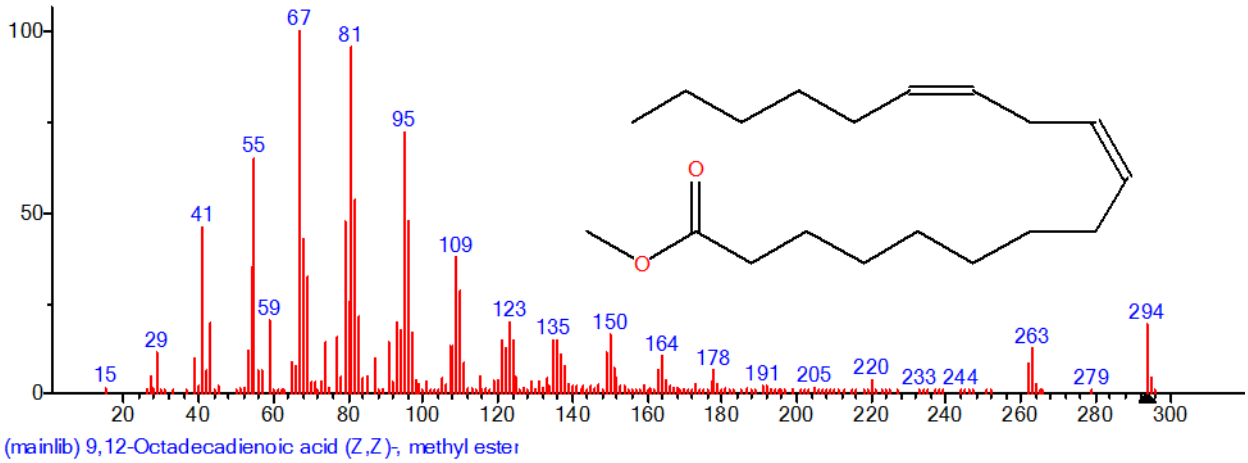

Figure 6: Structure of 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester

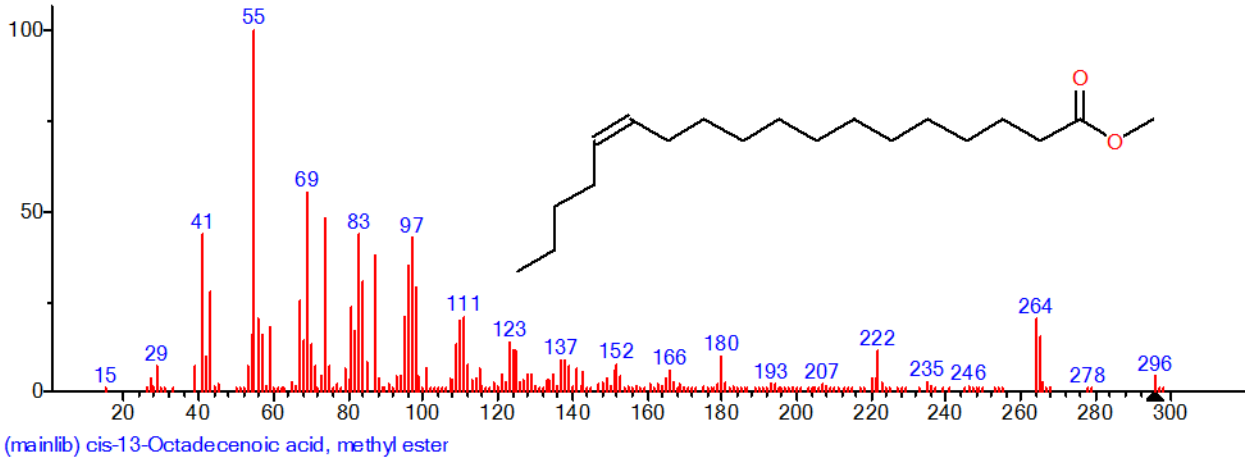

Figure 7: Structure of cis-13-Octadecenoic acid, methyl ester

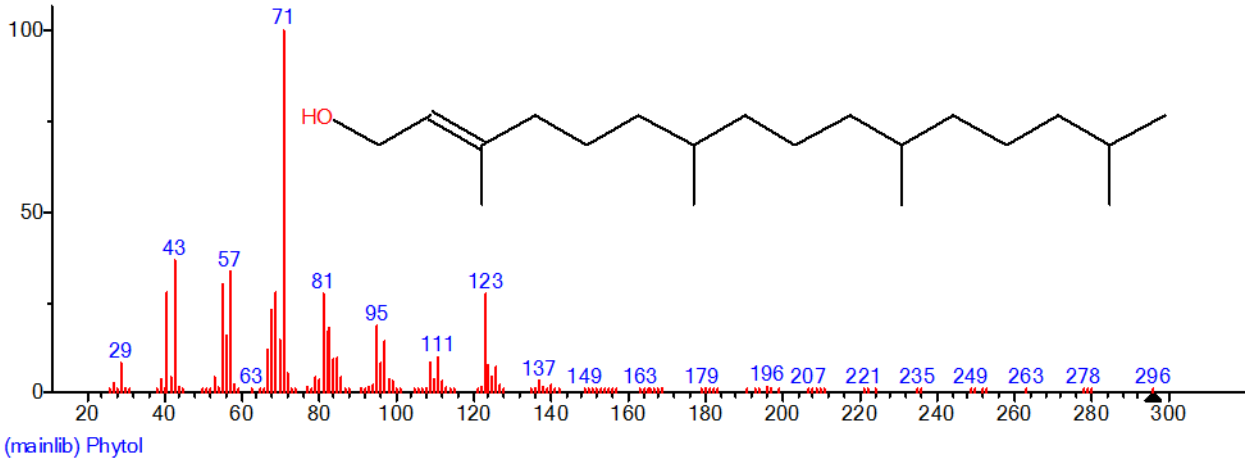

Figure 8: Structure of phytol

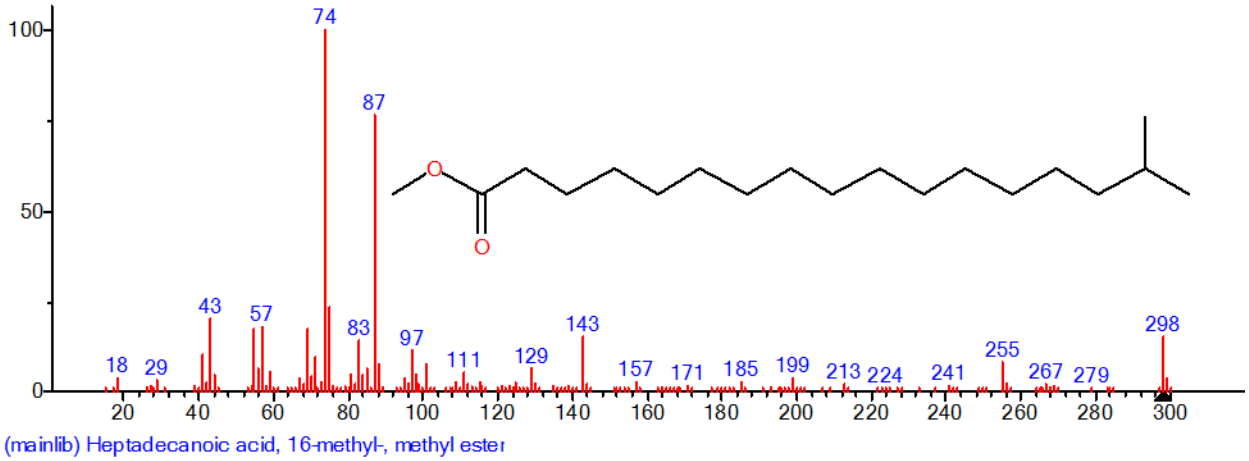

Figure 9: Structure of heptadecanoic acid, 16-methyl-, methyl ester

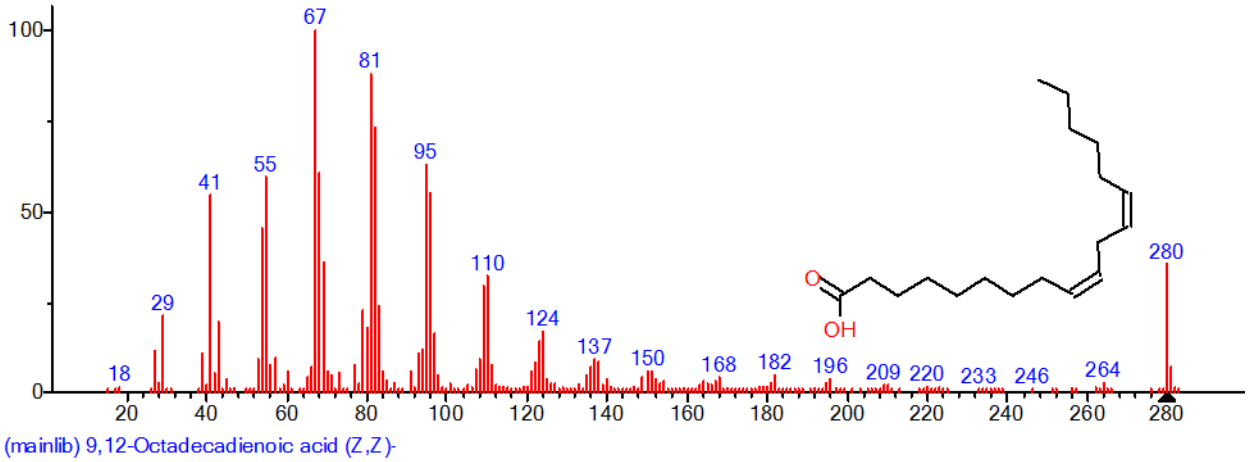

Figure 10: Structure of 9, 12-Octadecadienoic acid

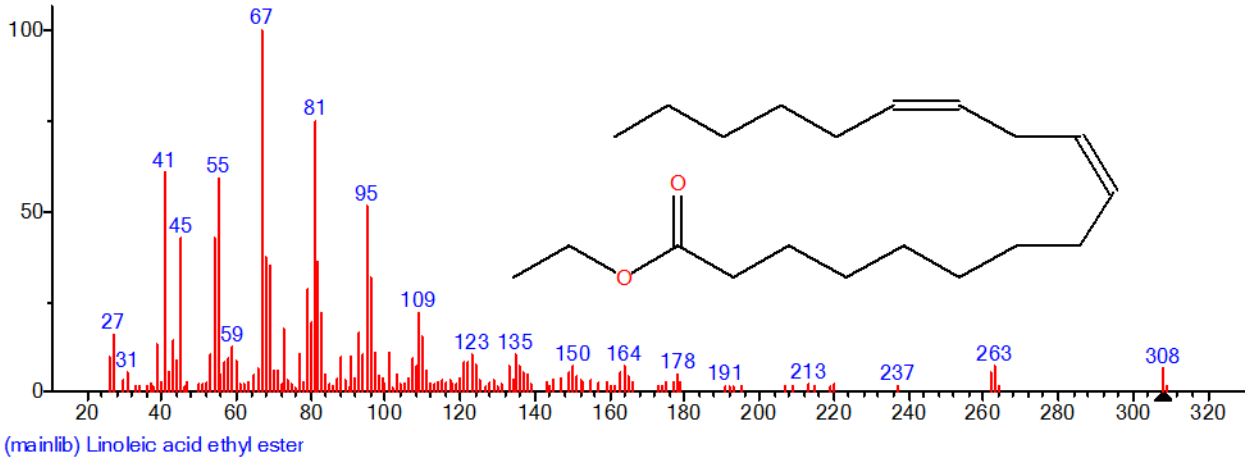

Figure 11: Structure of Linoleic acid ethyl ester

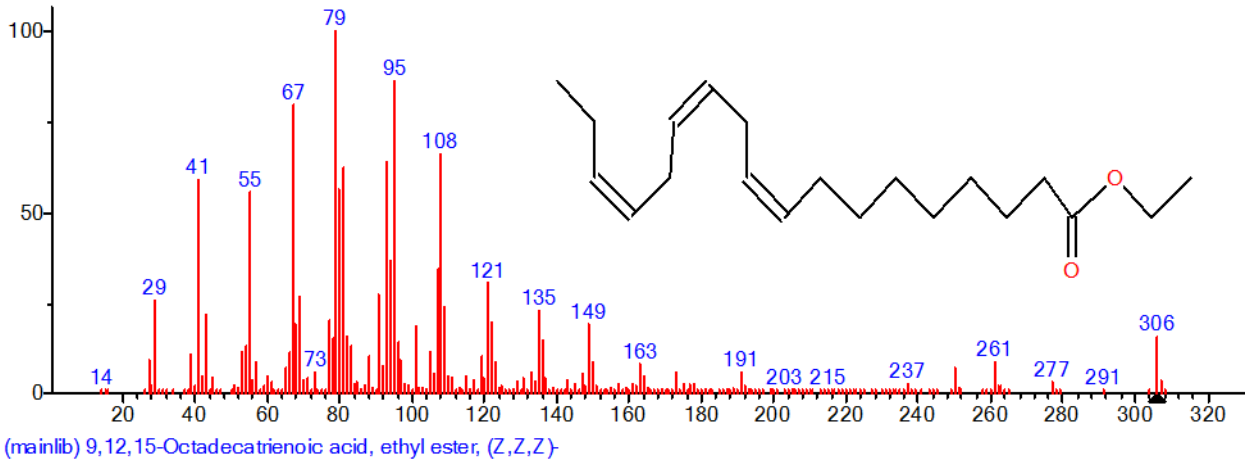

Figure 12: Structure of 9, 12, 15-Octadecatrienoic acid, ethyl ester (Z, Z, Z)

4.3 Change in body weight

Figure 13 shows the difference in weight before the induction of diabetes (Day 1), after the induction of diabetes using streptozotocin (Day 4) and after the administration of methanol leaf extract of *V. amygdalina* (Day 11). There was no significant difference in the body weight of the experimental animals in Day 4 and Day 11 when compared to Day 1 at $p \leq 0.05$.

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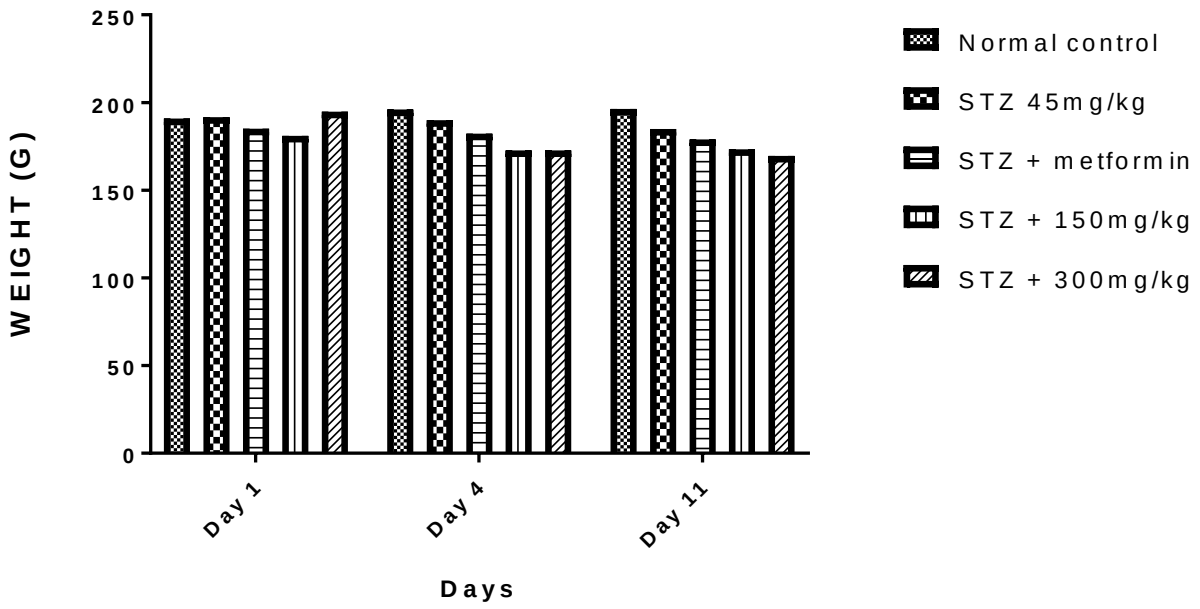

Figure 13: Change in body weight of streptozotocin induced diabetic rats

Values are expressed as mean ± standard error of mean of 5 animals in each group (n=5), Significant at $p \leq 0.05$

4.4 Change in fasting blood sugar (FBS)

Figure 14 shows the difference in fasting blood sugar level before the induction of diabetes (Day 1), after the induction of diabetes using streptozotocin (Day 4) and after the administration of methanol leaf extract of *V. amygdalina* (Day 11). There was significant increase in FBS level in Day 4 of rats treated with STZ (STZ 45mg/kg, STZ + Metformin, STZ + 150mg/kg VAM, STZ + 300mg/kg VAM) when compared to control rats at $p \leq 0.05$.

There was significant decrease in FBS level of STZ + metformin treated rats, STZ + 300mg/kg VAM treated rats and STZ + 150mg/kg VAM treated rats in Day 11 when compared to control rats at $p \leq 0.05$. However, FBS level of STZ 45mg/kg treated rats remained high in Day 11 when compared to control rats at $p \leq 0.05$.

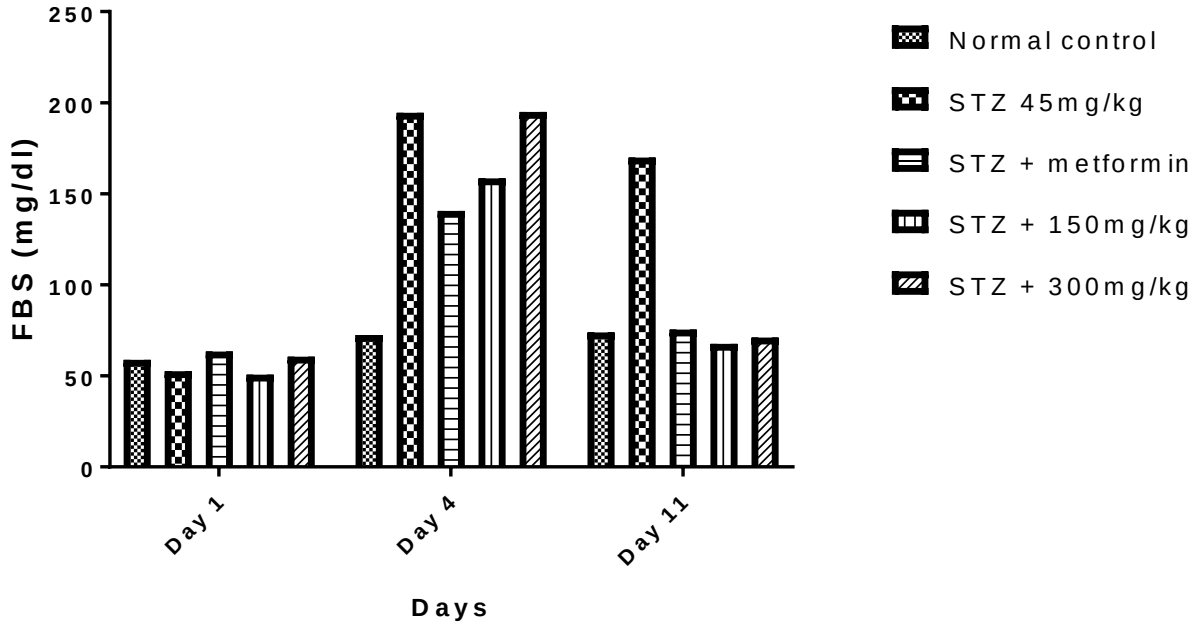

Figure 14: Change in Fasting blood sugar of streptozotocin induced diabetic rats

Values are expressed as mean ± standard error of mean of 5 animals in each group (n=5), Significant at $p \leq 0.05$

4.5 Effect of Methanol leaf extract of *V. amygdalina* on plasma total protein (TP)

As shown in Table 10, there was no significant difference in TP level of all treated groups when compared to control rats at $p \leq 0.05$.

Table 10: Effects of methanol leaf extract of *V. amygdalina* on plasma total protein in streptozotocin induced diabetic rats

GROUPS	**TP (g/dl)**
Normal Control	9.80±0.53
STZ 45mg/kg	9.56±0.88
STZ+Metformin	10.18±0.26
STZ+150mg/kg VAM	10.11±0.51
STZ+300mg/kg VAM	11.82±0.40

Values are expressed as mean ± standard error of mean of 5 animals in each group (n=5), significant at $p \leq 0.05$

4.6 Effect of methanol leaf extract of *V. amygdalina* on plasma enzymes

The mean values of plasma enzymes are shown in Table 11. There was significant decrease in AST level of STZ + 150mg/kg VAM treated rats when compared to the control rats, but no significant difference in STZ 45mg/kg treated rats, STZ + metformin treated rats and STZ + 300mg/kg VAM at $p \leq 0.05$.

There was no significant difference in ALT level of all treated groups when compared to control rats at $p \leq 0.05$.

There was significant decrease in GGT level of STZ 45mg/kg treated rats, STZ + 150mg/kg VAM treated rats and STZ + 300mg/kg VAM treated rats when compared to control rats. However, there was significant increase in GGT level of STZ + metformin treated rats when compared to controlled rats at $p \leq 0.05$.

There was significant decrease in LDH level of STZ 45mg/kg treated rats and STZ + metformin treated rats when compared to control rats but no significance difference in STZ + 150mg/kg treated rats and STZ + 300mg/kg VAM treated rat when compared to control rats at $p \leq 0.05$.

Table 11: Effect of methanol leaf extract of *V.amygdalina* on plasma enzymes in streptozotocin induced diabetic rats

GROUPS	**AST (U/L)**	**ALT (U/L)**	**GGT (U/L)**	**LDH (U/L)**
Normal Control	28.60±0.98	4.00±0.00	14.36±1.35	33.84±0.83
STZ 45mg/kg	21.25±3.33	5.00±1.00	5.21±0.75*	16.51±4.13*
STZ+Metformin	19.75±3.95	5.00±1.00	30.69±1.53*	20.64±3.77*
STZ+150mg/kgVAM	15.40±2.21*	4.00±0.00	4.17±1.58*	26.41±2.10
STZ+300mg/kgVAM	23.60±1.40	5.6±0.98	3.94±0.69*	26.41±2.10

Values are expressed as mean ± standard error of mean of 5 animals in each group (n=5)

* = Significantly different when compared to normal control at $p \leq 0.05$

CHAPTER FIVE

5.0 DISCUSSION

Many Nigerian medicinal plants have been reported to have antidiabetic activities. *V. amygdalina* is cultivated mainly for its nutritional value and it is used traditionally in the treatment of fever, gastrointestinal disease, infertility and sexually transmitted disease. (Kadiri and Olawoye, 2016). Phytochemicals are naturally occurring substances found in plant; they are also known as secondary metabolite. The phytochemical analysis of methanol leaf extract of *V. amygdalina* revealed the presence of alkaloid, carbohydrate, protein, fat and oil, phenol, flavonoid, tannin, glycoside, phytosterol, polyphenol, saponin and terpenoids. The GC-Ms analysis done on the extract revealed a complex mixture of compounds. The compounds identified in the extracts are characterized by the abundance of fatty acids and their esters. Phytol occurs in all the *Vernonia* species but vary in their percentage abundance (Ilondu, 2013).

The administration of methanol leaf extract of *V. amygdalina* demonstrates significant anti-diabetic activity in streptozotocin induced diabetic rats. The leaf extract of *V. amygdalina* has been reported to exhibit hypoglycemic (anti-diabetic) activity (Clement *et al*., 2014). The presence of some phytochemical compounds such as flavonoid, alkaloid, steroid, tannin and terpenoids has been related to the anti-diabetic potential of *V. amygdalina* (Alhassan *et al*., 2017; Emmanuel *et al*., 2015).

Liver is the central metabolic organ in the body responsible for glucose and lipid homeostasis, diabetes causes hepatic dysfunction. Streptozotocin induced diabetes in rats causes increase in the activities of liver marker enzymes (AST and ALT) due to the destruction of hepatocytes (Khalid *et al*., 2014). Rats treated orally with 150mg/kg methanol leaf extract of *V. amygdalina* were found to have significantly reduced level of AST which indicate less damage to

hepatocytes. There was no significant difference in the ALT level of rats treated orally with methanol leaf extract of *V. amygdalina* when compared to the control which also indicates no damage to hepatocyte.

Elevated level of GGT is observed in diabetes mellitus and it is a marker of alcoholic consumption and liver disease (Thapa and Walia, 2007). There was significant decrease in GGT level of streptozotocin induced diabetic rats treated with methanol extract of *V. amygdalina* (150mg/kg and 300mg/kg) when compared to controlled rats which also indicates no damage to liver cells. However, there was significant increase in GGT level of streptozotocin induced diabetic rats treated with metformin which indicate damage to hepatocyte.

Total protein measures the amount of protein in the body and it is composed of both albumin and globulin. Tissue wasting is a typical characteristic of poor glycemic control in diabetes and it leads to protein and fat utilization (Luke *et al*., 2013). There was no significant difference in TP level of all treated groups when compared to control rats.

Lactate dehydrogenase is an enzyme that catalyzes the transfer of pyruvate to lactate and lactate to pyruvate. It is expressed in body tissues and it is released during tissue damage that is, it is a marker of tissue damage. There was no significant difference in the LDH level of rats treated orally with methanolic leaf extract of *V. amygdalina* when compared to the control. There was significant decrease in the LDH level of diabetic control rats and rats treated with metformin.

CONCLUSION

In conclusion, the result of this study showed that methanol leaf extract of *V. amygdalina* possesses anti-diabetic and hepato-protective activity in streptozotocin induced diabetic rats. The result obtained showed the effect of the extract on blood glucose level and some plasma enzymes of importance in liver function test and that the activity may be as a result of its phytochemical constituents.

RECOMMENDATION

Further studies should be done on the effect of various extracts of *V. amygdalina* (ethanol, aqueous, methanol extract) on plasma enzymes and hepatocytes.

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