CERTIFICATION

I certify that this research project **CHEMICAL CONSTITUENTS AND BIOCHEMICAL EFFECTS OF *VERNONIA AMYGDALINA* METHANOL LEAF EXTRACTON THE PLASMA OF STREPTOZOTOCIN-INDUCED DIABETIC RATS** was carried out, compiled and written by **OBAYEMI, TITILAYO PRECIOUS** with Matriculation number **15010102009,** Department of Biological Sciences, College of Basic and Applied Sciences, Mountain Top University, has been carefully supervised and approved as adequate for the partial fulfillment of the ward of Bachelor of Science (B.Sc.) Biochemistry of the Mountain Top University.

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DEDICATION

I dedicate my project to GOD Almighty for his grace, mercy, strength, favor and for being my support through this period of my life and I also dedicate this project to my ever supportive parents MR & MRS OBAYEMI and my uncle MR OBEY SAMSON.

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ACKNOWLEDGMENT

I would like to express my profound gratitude to GOD Almighty for his infinite mercy upon my life as I started and completed my project successfully.

I want to specially acknowledge my parents MR & MRS A.O.OBAYEMI and my siblings for their care, love, financial support and emotional support during this period.

I want to specially acknowledge and appreciate my supervisor, Dr. G.O. Ajayi for his patience, precise, constructive criticism and made it possible for the successful completion of this project.

I want to acknowledge DR. A.A. Adeiga, the H.O.D of biological sciences for his contribution towards the success and the completion of this project. I will also like to acknowledge all my biochemistry lecturers and the laboratory technicians for all their contributions and assistances toward the successful completion of my project.

Finally, my gratitude goes out to my ever supportive friends; AbindeDamilola, Achebumere kindness, Elizabeth Elegbeleye, OwoyemiIfeoluwa, IdowuFolashade, OsaghaeOsayande, Ajayi Ayooluwa, Chuks Emmanuel, Judah Fisayo and the last but not the least my biochemistry colleagues.

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ABSTRACT

This study was designed to evaluate the chemical constituents and also the anti-diabetic effects of the methanol leaf extract of *Vernonia amygda*lina on some biochemical parameters such as creatinine, triglycerides, total protein, total bilirubin, and cholesterol, in the plasma of a streptozotocin-induced diabetic rats. The methanol leaf extract was first screened for the presence of phtyochemicals using standard methods and the result of the phytochemical screening showed the presence of Alkaloid, Carbohydrate, Protein, Fat and oil, Terpenoids, Phenol, Flavonoid, Tannin, Glycoside, Glycoside, Polyphenol, and Saponin. The methanol leaf extract was also analyzed for the chemical constituents present in it, using gas chromatography-mass spectrometry (GC-MS), the major chemical constituents identified in the methanol leaf extract of *Vernonia amygdalina* were; hexadecanoic acid, methyl ester (16.2%), 9,12-Octadecadienoic acid (Z,Z), methyl ester (11.8%), cis-13-Octadecenoic acid, methyl ester (14.1%), phyto (10.5%), 9,12-Octadecadienoic acid (Z,Z) (11.9%). In the biochemical study, 25 male albino rats were divided into 5 groups of 5 rats per group. Group 1 was the control, group 2-5 was first treated orally with streptozotocin (STZ) only and observed for 3 days for inducement of diabetes. Thereafter, groups 3, 4, and 5 were orally treated with metformin, 150 mg/kg b.wt. VAM and 300 mg/kg b.wt. VAM respectively for 7 days. The blood glucose levels were determined on days 1, 4, and 11 with Glucometer (Accu-Chek, Manheim, Germany). The extract and metformin significantly reduced ($P<0.05$) the fasting blood glucose levels in the diabetic treated rats when compared to the diabetic control. In total protein a significant increase was observed on group IV and V. In Triglycerides a significant increase was observed in group IV and V. In cholesterola significant increase was observed in group IV and V. In Creatinine a significant decrease was observed in all the group expect group V.In bilirubin a significant

decrease was observed in group II, III, IV and V ($P<0.05$). In conclusion, the results from this study show that *Vernonia amygdalina* can be used in the management and treatment of diabetes, hypertension, and some other diseases as stated in previous researches.

CHAPTER ONE

1.0 INTRODUCTION

Diabetes mellitus (DM) is a common disorder that is associated with the increase in the mortality and morbidity, and it can also be described as a group of metabolic diseases that is characterized by chronic hyperglycemia, which is because of the defect in insulin action, insulin secretion, or both, which results in impaired carbohydrate, lipid, and protein metabolism (Lebovitz, 1994; Andreoli *et al.*, 1990). Pharmacological treatment of DM is based on oral hypoglycemic agents and insulin which have so many side effects (Andreoli *et al.*, 1990). DM has been reckoned as one of the most important leading health problems in Africa, which has contributed significantly to the morbidity and mortality (Garcia *et al*., 1974) and adversely affecting both the quality and length of life (Akah *et al*., 2009). The assessment of medicinal plants that is used traditionally in the treatment of diabetes is of a growing concern (Holman and Turner, 1991; Williams and pickup, 1991; Kameswara Rao *et al*., 1997). The World Health Organisation (WHO), has also recommended and encouraged the practice, particularly in countries where there is inadequate access to standard diabetes treatment (WHO, 1980). *Vernonia amygdalina* is widely used in the treatment of DM and some studies have led to this being credential (Akah and Okafor, 1992; Akah *et al*., 2002, 2004; Gyang *et al*., 2004).

Vernonia amygdalina is a member of the *Asteraceae family*. It is commonly referred to as "Bitter leaf" in English language, as "Shuwaka" in Hausa language, as "Onugbu" in Igbo language and as "Ewuro" in Yoruba language. The leaves are commonly used for fever medicine and are known as quinine replacements (Challand and Willcox, 2009). It is used in preparing cough medicine in Ghana (Akinpelu, 1991) and the root infusion is taken as an antihelminthic in Nigeria as well as for enteritis and rheumatism (Ainslie, 1937). The leaves have found relevance

in traditional folk medicine as anthelmintics, antimalarial, antimicrobial, anticancer and as a laxative herb (Akah *et al.,* 2009).

Pharmacological trials have shown that the leaf extract of *V.amygdalina* has both hypoglycaemic and hypolipidemic characteristics in experimental animals and can therefore be used to treat diabetes, hypertension and other conditions (Akah and Okafor, 1992).The leaves of the plant can either be eaten as a vegetable (macerated leaves in soup) after rounds of washing to remove the bitter taste, or aqueous extract as tonics for the treatment of various ailments (Arhoghro *et al.,* 2009.). *V.amygdalina* is rich in minerals sources such as K^{+}, Ca^{2+}, Na^{+}, P^{3-}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+} (Asaolu *et al.,* 2012). It also contains saponins, tannins, ascorbic acid, amino acids (glycine, cysteine and nicotinamide), iodine, hydrochloric acid, sugar and oxalate (Erasto *et al.,* 2007; Egedigwe, 2010). *V.amygdalina* extract is traditionally used to heal a number of diseases, including anti-inflammatory, wound healing, immune modulation, anti-tumour, anti-bacteria, antiviral laxative and purgative properties (Adaramoye, *et al.,* 2009; Ijeh *et al.,* 2011;Erasto, *et al.,* 2007; and Gresham, *et al.,* 2008.). The extracts of *V.amygdalina* have been recorded to have analgesic and antipyretic impacts (Tekoba *et al.,* 2002). It was also note that the aqueous extract of *V.amygdalina* has anti-oxidant properties (Nwajo and Nwokoro, 2004).

1.1 STATEMENT OF PROBLEM

This research work studied or investigated the effect of the aqueous leaf extract of *Vernonia amygdalina* on some biochemical parameters in a streptozotocin (STZ)-induced diabetes in male wistar rats. The study also evaluated phytochemicals present in *Vernonia amygdalina.* The overall investigation of this study is to explore the potentials and the benefits of *V.amygdalina,* for its uses in the pharmaceutical industry in the treatment of diabetes in the near future.

1.2 AIM AND OBJECTIVES OF THE STUDY

1.2.1 AIM OF THE STUDY

The aim of this study is to evaluate the chemicals constituents and the impacts of *Vernonia amygdalina* methanol leaf extract on certain biochemical parameters in astreptozotocin-induced diabetic rats.

1.2.2 OBJECTIVES OF THE STUDY

This study carried out the following:

1. To determine the phytochemical components of *Vernonia amygdalina* leaf extract.
2. To determine some biochemical parameters; creatinine, total protein, total bilirubin, cholesterol, and triglycerides.
3. To determine the blood glucose levels.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 MEDICINAL PLANTS

A plant is said to be medicinal when one or more of its parts have been claimed or used to cure disorders or ailments.Medicinal plants constitute as effective source of traditional as well as modern medicine. Plants have been used as sources of remedies to treat ailments since ancient times, and people of all continents have this ancient tradition, especially in Africa. Despite the remarkable advances in twentieth-century synthetic organic medicinal products, more than 25% of medicinal goods prescribed in industrialized countries are obtained directly or indirectly from plants (Newman *et al.,* 2000). Medicinal plants from ancient time have been used for preventive and curative measures for different ailments and a disease due to their readily availability and low cost of preparation.Large population of humans still relies on plants as a source of medicine (Ogunrinola *et al*., 2019).

However, there is still research into medicinal plants used in traditional medicine (Kirby, 1996). The production of new medicines is not often affordable in developing countries, such as West Africa. Therefore, about up to 80% of the population uses medicinal plants as remedies for various types' diseases (Kirby, 1996; Hostellmann and Marston, 2002). Plants used in the tropical and subtropical regions are diverse and most of the medicinal uses are used as medicine, source of food, clothing and shelter. Several ethnobotanical studies focusing on medicinal plants have been recorded around the globe (Ekpendu *et al*., 1998; Balansard and Timon, 2000; Singh and Singh, 2001; Wang *et al.*, 2002; Cox, 2005; Kumar *et al.*, 2005; Pei, 2005). Documentation of African plants medicinal uses is becoming increasingly important because of the fast loss of natural habitat for some of these plants as a result of anthropogenic activities *(pollution).* It has

been proved from previous researches that medical plants has a great impact in reduction of blood glucose level as well as other diabetes-related compounds (V.Raks *et al*., 2017). A lot of plants are rich source of antioxidants such as flavonoids, tannins and lignin, as well as example of phenolic compounds, vitamins A, C, E are all found in plants (Plants Basal, 2017).

Medicinal plants with antioxidant activity can reduce oxidative stress and improve the functions of various hyperglycemia-affected organs. *Vernonia amygdalina* contains natural antioxidants that can act as antioxidants to aqueous radicals and reactive ions (Adeoye *et al*., 2018). However, the rise in population, insufficient drug supply, excessive therapy costs and side effects of several standard drugs have increased reliance on medicinal plants as a medicinal source for multitude of medicinal products for a variety of ailments. Medicinal plants are efficiently and effectively being used in most parts of the world as: hypolipidemic, contraceptive, or cytotoxic, antihypertensive, treatment for skin diseases, wound healers, and hypoglycaemic. The hypoglycemic agents have been used in the management of diabetes mellitus (Mustafa *et al*., 2011), that imputes to its pharmacological functions,as anti-diabetic, antimalarial, anti-helminth, antibiotic, treatment of diarrhoea, dysentery, fertility inducer, kidney problems, stomach discomfort, hypolipidaemic, and other several uses (Ogunrinola *et al*., 2019).

Table 1: Examples of some medicinal plants.

SCIENTIFIC NAME	TRADITIONAL USES
Vernonia amygdalina	Treatment of diabetes
Allium sativum	Treatment of hypoglycaemia
Heteromorphica arborescens	Treatment of diabetes

Cannabis sativa	It has *invivo* anti-diabetic properties
Albuca setosa	Prevention of oxidative stress
Albuca bracteata	It has anti-diabetic properties
Strychno shenningsii	Induces hypoglycemia
Ruta graveolens	Decreases hepatic glucose output and ameliorating oxidative stress
Solanum aculeastrum	It has anti-diabetic properties
Leonotis leonorus	Reduces blood glucose level.

2.2 VERNONIA AMYGDALINA

Vernonia amygdalina belongs to the family Asteraceae (Alhassan *et al, 2008*). *Vernonia amygdalina's* leaves are green with a distinctive scent and bitter taste. Due to its distinctive bitter taste and flavor, it is known as a bitter leaf (Ugochukwu *et al,* 2003).It is a shrub of 2 - 5m tall with abundant bitter principle in every part of the plant (Alhassan *et al,* 2008). *Vernonia amygdalina* is a small, tropical African-growing shrub. The leaves are elliptical with a length of up to 20cm. Its bark is rough. The cooked leaves are major vegetables throughout Equatorial Africa in soups and stews of different cultures (Iloh *et al,* 2011). It produces no seeds and must be distributed or propagated through cutting. It is mainly used for human consumption and has to be washed to remove the bitter taste. People consume the leaves of this herb but only after rinsing them thoroughly to end their bitter taste (Iloh *et al,* 2011*).*

The leaves are widely used medicinally for fever and are known as quinine replacements (Challand and Willcox, 2009). It is used in Ghana to prepare cough medicine (Akinpelu, 1991) and root infusion is taken as antihelminthic in Nigeria as well as for enteritis and rheumatism

(Ainslie, 1937).The leaves have found relevance in traditional folk medicine as anthelmintics, antimalarial, antimicrobial, anticancer and as a laxative herb (Akah *et al.,* 2009). Pharmacological research has shown that *V.amygdalina* leaf extract in experimental animals has both hypoglycaemic and hypolipidemic characteristics and can therefore be used for diabetes management, hypertension and other diseases (Akah and Okafor, 1992).The leaves of the plant can be consumed either as a vegetable after rounds of washing to remove the bitter taste, or as a tonics aqueous extract for the treatment of multiple ailments (Arhoghro *et al.,* 2009.). *V.amygdalina* is wealthy in sources of minerals such as K^{+}, Ca^{2+}, Na^{+}, P^{3-}, Mg^{2+}, Mn^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+} (Asaolu *et al.,* 2012). It also contains saponins, tannins, ascorbic acid, amino acids (glycine, cysteine and nicotinamide), iodine, hydrochloric acid, sugar and oxalate (Erasto,*et al.*,2007; Egedigwe, 2010). Extract of *V.amygdalina* is traditionally used in Anti-inflammatory treatment, wound healing, immune modulation, anti-tumour treatment, anti-bacteria, antiviral laxative and purgative properties (Adaramoye, *et al.,* 2009; Ijeh *et al.,* 2011; Erasto *et al.,* 2007; and Gresham *et al.,* 2008.). it has been recorded that the extracts of *V.amygdalina* have analgesic and antipyretic impacts (Tekoba *et al.,* 2002). The aqueous extract of *V.amygdalina* has also been revealed to have anti-oxidant properties (Nwajo and Nwokoro, 2004).

2.2.1 TAXONOMY OF *V. amygdalina*

Table 2: The taxonomy of *Vernonia amygdalina*

Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Magnoliopsida
Order	Asterales

Family	Asteraceae(Compositae)
Genus	*Vernonia*
Specie	*Amygdalina*

2.2.2 COMMON NAMES OF *V. amygdalina*

Table 3; the common names of *Vernonia amgydalina.*

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

2.2.3 USES OF *V.amgydalina*

This plant contains complex active components that are useful in pharmacologically applications. It can be used as an effective anticancer, antibacterial, antimalarial and anti-parastic agent (Udochukwu *et al*, 2015). The roots and the leaves are used in ethno medicine to treat fever, hiccups, kidney issues and stomach pain (Udochukwu *et al, 2015*). It has also been documented that *V.amygdalina* has traditionally been used in the treatment of blood clotting and has elicited a significant decrease in the blood glucose levels (Udochukwu, *et al, 2015*). It had been reported that *V.amygdalina* has an activity of hypoglycaemic. They observed a closely-dependent decrease in the level of fasting blood sugar in streptozotocin-induced diabetic rats after therapy with various aqueous leaves extract concentration levels. *V.amygdalina* leaf extracts were also shown to be a DNA-damaging of anticancer agent in the management of breast cancer (Udochukwu *et al, 2015*). It is a widely used local plant in Nigeria for both therapeutic and nutritional purpose, where it serves as the main ingredient in 'bitter leaf soap'. Some authors noted that *Vernonia amygdalina* leaf extract could safeguard the heart from impairment and total destruction due to diabetes. *Vernonia amygdalina* protects against and also reversed the hepatic damage caused by tetrachloromethane-induced hepatotoxicity in albino rats (Akpanyung *et al, 1995*). The methanol leaf extract of V. amygdalina was found to have a wide-spectrum growth inhibitory activity against beta-lactamase producing bacteria such as S. aureus, K. pneumonia, P.aeruginosa and E. coli in vitro (Oseni *et al*, 2009).

Figure 1; picture of *V.amygdalina plant.*

2.3 DIABETES MELLITUS (DM)

Diabetes mellitus is group of metabolic disorders characterised with hyperglycemia, glycosuria and hyperlipaemia. Diabetes is not a single disease it is a group of heterogeneous syndromes such as heart attack, stroke and peripheral vascular illness (Tripathi and Verma, 2014). Diabetes mellitus is described as metabolic disorders defined by enhanced blood sugar (hyperglycemia) due to insulin secretion failure, insulin action or both (olokoba *et al*, 2012). Beta cells produce insulin. Insulin is produced by beta cells of the pancreas which use glucose from digested food as an energy source.

If either the pancreas cannot produce insulin or the insulin it produces is not sufficient and cannot operate correctly, glucose builds up in the blood at a high level and often secrete urine which is classical symptom of diabetes mellitus (Qais *et al*, 1995). Diabetes mellitus is a common and highly prevalent disease that affects both developed and developing country people.

It either results from insufficient hormone insulin secretion, insufficient target cell report to

insulin, or a mixture of these variables (Malviya *et al.,* 2010). Diabetes mellitus (DM) is a metabolic disorder characterized by insulin deficiency hyperglycemia, insulin secretion or both (Tchimene *et al, 2016).* Diabetes mellitus (DM) is the most common disease which is prevalent and epidemic disease that affects both emerging and advanced individuals (Jeeva and Anlin, 2014). DM is triggered by carbohydrate metabolism abnormality or dysfunction. The food we consume is broken into simple sugar called glucose. Glucose is the primary energy source of the body. Insulin is very essential for the glucose uptake into the cells. Insulin is a hormone secreted by the pancreas. If insulin is not produced by the pancreas, glucose enters the cells of the body so that glucose remains in the blood. This makes the body hyperglycemic (Jeeva and Anlin, 2014). Glucose is the cell's major source of energy. To join the cells, glucose requires insulin from the endocrine pancreas. If the pancreas does not generate enough insulin the cells are resistant to the activity of blood glucose uptake.

Insulin releases is affected by hyperglycaemia, increased beta adrenergic stimulation and by intestinal hormone, secretion, among others (Adejuwon *et al,* 2009).Diabetes mellitus is a chronic disease that occurs when the pancreas does not produce enough insulin (a hormone that regulates blood sugar) or when the body is unable to use the insulin it produces effectively (WHO, 2010). Diabetes mellitus is a condition in which the body does not correctly process food for use as energy. Diabetes mellitus is a prevalent disease linked to enhanced morbidity and mortality and can be described as a group of metabolic diseases associated by acute hyperglycemia due to insulin secretion deficiency, and insulin deficiency or both, arising in carbohydrate, lipid, and protein deficiencies metabolism (Lebovitz, 1994; Andreoli *et al.,* 1990).

Chronic diabetes hyperglycemia is correlated with comparatively particular long-term micro-vascular complications affecting the eyes, kidneys and nerves and an enhanced risk of cardiac illness (Ronald, 2013).

2.3.1 BRIEF HISTORY ON DIABETES MELLITUS

Diabetes is Greek, meaning a "siphon." The disease of ***diabainein*** was named by "**Aretus the Cappadocian**", a second century A.D. Greek physician. He defined patients who passed too much water like a siphon (polyuria). The term became "diabetes" after Medieval Latin diabetes was adopted in English. In 1675, though frequently referred to merely as diabetes, Thomas Willis added mellitus to the word.

2.3.2 CLASSICFICATION OF DIABETES MELLITUS

There are three (3) main types of diabetes mellitus. They are as follows:

- Type 1 Diabetes mellitus.
- Type 2 Diabetes mellitus.
- Gestational diabetes.

Type 1 Diabetes Mellitus:

It has earlier been called insulin-dependent diabetes mellitus (IDDM) or juvenile-onset diabetes, which can account for 5% to 10% of all diagnosed diabetes instances. Risk variables for type 1 diabetes are less well defined than for type 2 diabetes, but the growth of this type of diabetes involves autoimmune, genetic, and environmental variables.

Type 2 Diabetes Mellitus:

Non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes was earlier referred to. Type 2 diabetes can account for approximately 90% to 95% of all diagnosed diabetes instances. Older age, obesity, diabetes family history, preceding gestational diabetes history, impaired tolerance of glucose, physical inactivity, and race / ethnicity include risk factors for type 2 diabetes. For type 2 diabetes in specific, African Americans, Hispanic / Latin Americans, American Indians, and some Asian Americans and Pacific Islanders are in high risk.

Gestational Diabetes Mellitus:

It develops in 2% to 5% of all pregnancies, but usually disappears after a pregnancy has ended. Gestational diabetes occurs more frequently in African Americans, Hispanic / Latino Americans, American Indians, and people with a diabetes family history than in other organisations. Obesity is also correlated with enhanced risk. Women with gestational diabetes are subsequently at increased risk of type 2 diabetes. In some studies, nearly 40 percent of women with a history of gestational diabetes developed diabetes in the future.

2.3.3 SYMPTOMS OF DIABETES

- Fatigue or severe weakness.
- Abnormal thirst.
- Irritability.
- Unexplained weight loss
- Increased hunger.
- Recurrent infections.
- Blurred vision.

- Increased urination and nocturia (Jeeva and Anlin, 2014).

The symptoms are almost the same in the two major types of diabetes, but they differ in their intensity. Elevated blood-glucose concentrations are ascribed to the original symptoms of untreated diabetic patients. As a result, glucose loss in urine improves urine production, resulting in dehydration accompanied by thirst and increased water consumption. Insulin deficiency ultimately leads to weight loss despite increased appetite and food consumption. Untreated diabetic patients also suffer from fatigue, nausea and vomiting. They are susceptible to develop bladder, skin, and vaginal infections.

2.3.4 TREATMENT OF DIABETES MELLITUS

- Insulin and oral hypoglycemic drugs.
- Herbal treatment of diabetes (Swaroopa *et al, 2012*).

The presently used oral hypoglycaemic agents in clinical practice have distinctive profiles of severe side effects. The adverse effect of ongoing use of synthetic drugs has promoted the use of plant-based medicine that could deliver maximum cure with minimal or no side impact (Prabakaran et al, 2005). Plants and their extracts have been used to fight against diabetes since ancient times. The World Health Organization (WHO) has listed 21,000 plants that are used around the world for medicinal purposes. Among them, 150 species are used commercially on a relatively big scale. Some medicinal plants have recently been revealed to be helpful in diabetes around the world and have been used empirically inantidiabetic and anti-hyperlipidemic remedies. that are frequently implicated as having antidiabetic effect (Prabakaran, et al.,2005).

Although, pharmaceutical oral hypoglycaemic drugs and insulin Diabetes therapy has prominent side effects and does not change or change the course of diabetic complications considerably. The prevalent side effects of hypoglycaemic oral pharmaceutical agents are hypoglycaemia, weight gain, gastrointestinal disorders, peripheral oedema and impaired liver function, besides the cost of treatment. Since natural remedies are somewhat safer and more efficient than pharmaceutically derived remedies, the practice or study of medicinal herbs has become the mainstream worldwide. The use of crude extracts of medicinal plants in the management and treatment of diabetes mellitus is widely practiced in Nigeria. Plant drugs and herbal formulations are frequently considered to be less toxic and free from side effects than synthetic drugs. Anti-hyperglycaemic effects of some of these traditional plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin production or restore the functions of insulin receptors. Some inhibit the intestinal absorption of glucose through the inhibition of digestive enzymes of carbohydrates, mainly alpha-amylases and alpha-glucosidases thereby affecting the glycaemic index of foods. The anti-diabetic properties of these plants could be attributed to their constituents which include; Glycosides, alkaloids, terpenoids, flavonoids, carotenoids, and so on, all of which are often referred to as having anti-diabetic effect. These herbal drugs protect the ß-cells during the diabetic condition and reduce the amount of glucose level in the blood. The medicinal plants used on anti-diabetic treatments possess pancreatic ß-cells Insulin-regenerating activity and also combating the insulin resistance issue. These herbs also increased insulin secretion, increased glucose uptake from adipose tissue, and inhibited glucose uptake from the intestine (Jeeva and Anlin, 2014).

2.4 STREPTOZOTOCIN (STZ)

Streptozotocin (STZ) is a naturally occurring antineoplastic alkylating agent that is particularly toxic to pancreatic beta cells producing insulin in mammals. A big dose animal model for hyperglycemia, as well as type 2 diabetes or type 1 diabetes with various low doses, is used in medical research. Streptozotocin As an antibiotic and anticancer agent, pancreatic b-cells have been commonly used to induce type I diabetes in a variety of livestock. (Eidi *et al* 2006). Streptozotocin2-deoxy-2-[3-[methyl-3-nitrosoureidp]-d-glucopyranose]] is synthesized by steptomycetes achromogenes and is used to induce both type-1 and type-2. Streptozotocin causes diabetes in nearly all species and the amount of diabetes differs with the species and the ideal dose needed for diabetes in rats has been found to be (50-60mg/kg i.p. or i.v), in mice (175-200mg/kg i.p. or i.v) and in the dogs (15 mg/kg for 3 days).Due to its low solubility the rapid i.v; injection appears to be best route of administration. Streptozotocin is naturally occurring chemical; used to produce Type- 1 diabetes in animal model and Type- 2 diabetes with multiple low doses. (Swaroopa *et al, 2012*).

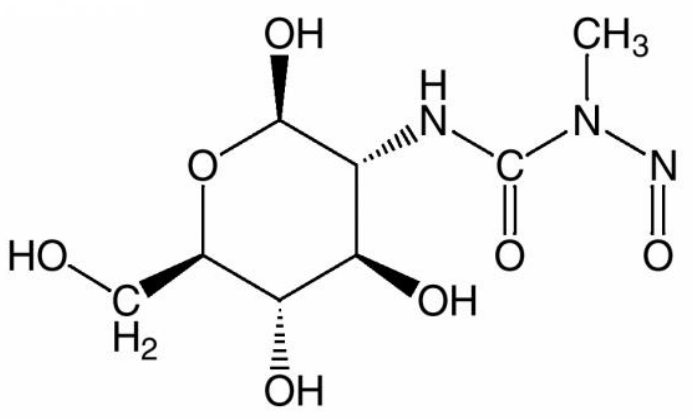

Figure 2; chemical structure of streptozotocin.

2.4.1 STZ ADMINISTRATION

STZ is commonly injected either intraperitoneally (IP) or intravenously (IV), although other methods including subcutaneous, intracardiac, and intramuscular have been used in medical research works (Deeds *et al.*, 2011).

2.5 METFORMIN

Metformin is generally recommended as a first line treatment for type 2diabetes, as there is good evidence that it decreases mortality (Ripson *et al*, 1987). Biguanides reduce the production of glucose by the liver. Metformin is usually administered orally. Biguanides improve diabetic control, despite reducing circulating insulin level, in obese patients with NIDDM.

Several studies have shown that metformin improves both peripheral and hepatic insulin sensitivity in patients with NIDDM (Tripathi and Srivastava, 2015). Metformin is the only established antidiabetic drug that deals with insulin resistance (Tripathi and Srivastava, 2015). Biguanides, of which metformin is the most frequently used in overweight and obese patients, suppresses the production of hepatic glucose, reduces insulin sensitivity, reduces glucose intake by the phosphorylated GLUT enhancer factor, reduces fatty acid oxidation and reduces glucose absorption from the gastrointestinal tract (Olokoba *et al*., 2010). It has a low incidence of hypoglycemia compared to sulfonylureas (Olokoba *et al.*, 2010).

Although it must be used with caution in patients with impaired liver or kidney function, in kids and adolescents, metformin, a biguanide, has become the most frequently used agent for type 2 diabetes. Metformin is the only commonly used oral drug that does not cause weight gain among prevalent diabetic medicines (Eurich*et al*., 2012). By reducing hepatic glucose production and gastrointestinal glucose absorption and enhancing insulin sensitivity, metformin deteriorates hyperglycemia and is often the first drug used to treat freshly diagnosed T2D.

However, the glycemic response to metformin is variable and roughly 35-40 per cent of patients

receiving the drug do not attain acceptable fasting glucose control. Metformin is not metabolized, but is rapidly removed in proximal tubules (DiStefano and Watanbe) through glomerulus filtration and net secretion. Metformin has become one of the most widely used drugs in the treatment of type 2 diabetes mellitus (T2DM). According to the American Diabetes Association / European Association for Diabetes Study Guidelines, it is the first-line therapy for patients with T2DM (Wang *et al*, 2009).

2.6 GAS CHROMATOGRAPHY- MASS SPECTROMETRY (GC-MS)

Gas Chromatography-mass spectrometry is an analytical method combining the characteristics of gas-chromatography and mass spectrometry to recognize various substances in the test sample. GC-MS applications Includes detection of drugs, fire investigation, environmental assessment, explosive investigation and identification of unidentified samples, including material samples acquired from planet Mars during rehearsal missions as early as the 1970s. In airport security, GC-MS can also be used to detect drugs in luggage or on people. Additionally, in products earlier believed to have disintegrated beyond identification, it can recognize trace components. GC-MS has been considered a "gold standard" for the identification of forensic substances because it is used to conduct a 100% specific test that positively recognizes the existence of a particular substance. A non-specific test simply shows that there is one of several in a substance category. Although a nonspecific test could statistically suggest the identity of the substance, this could lead to false positive identification.

2.6.1PRINCIPLE OF GC-MS

GC / MS-a two distinct analytical methods, Gas Chromatography (GC) and Mass Spectrometry (MS), are combined to evaluate complexity, biochemical and organic mixtures. Two primary parts are the GC-MS tool. The gas chromatography portion separates different compounds in the

sample into pulses of pure chemicals based on their volatility by Inert gas (mobile phase) flowing through the sample Through a stationary phase fixed in the Spectra column, compounds are gathered as they leave a chromatographic column using a mass spectrometer to identify and quantify chemicals by their mass-to-charge ratio (m / z). Then you can store these spectra on the computer and analyzed (Hussain and Maqbool, 2005).

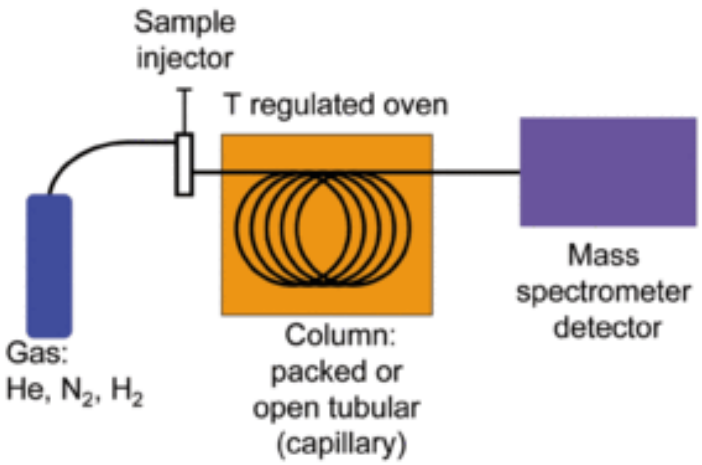

Figure 3; GC-MS schematic

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

Plant sample, Methanol, a muslin cloth, Beakers, Micro pipette, Spectrophotometer, Water bath, Funnel, Rotatory evaporator, Oven, Homogenizer, Formalin, Phosphate buffer, Distilled water, Syringes, Cannula, Glass jar, Weighing balance, Mechanical blender, Streptozotocin, Citric buffer, Hydrochloride, Mayer's reagent, Molisch reagent, Concentrated sulphuric (H_2SO_4) acid, Chloroform, Ammonia solution, Ferric chloride ($FeCl_3$), Sodium hydroxide (NaOH), Ninhydrin reagent, Potassium ferricyanide, Metformin.

3.2 METHODS

3.2.1 PLANT MATERIAL

Vernonia amygdalina (bitter leaf) plant was used for this study. The bitter leaf sample (*V. amygdalina*) was collected in Feburary, 2019 from an individual local farm at Imushin, Ijebu-ode, Ogun state, Nigeria through Mr. Ojo Opeyemi, the laboratory technician of the department of Chemistry, Mountain Top University, Ibafo, Ogun state.

3.2.2 PREPARATION OF EXTRACT

The leaves were separated from the stalk, it was air-dried for two weeks, after which it was oven dried for 30minutes at 100^{O}C and blended to a powdery form with the use of a mechanical blender. The blended sample was weighed and stored in a glass air-tight jar. Eighty four grams (84 g) of the blended powder form of the plant was weighed into three (3) jars and was macerated in 70% of methanol and with frequent shaking at room temperature for 72hours twice.

Filtration was done by using muslin cloth. The filtrate was concentrated in a vacuum at 60°C to about one-tenth the original volume using a rotary evaporator. The concentrates gotten from the above were kept in the oven (40°C) for complete dryness of the methanol leaf extracts and was then stored in a refrigerator at −4°C and the percentage yield of the extracts was then calculated.

3.3 PHYTOCHEMICAL SCREENING

The methanol leaf extract was tested for the presence of bioactive compounds using standard methods as described by Sofowora (1993), Trease and Evans (1989) and Harbone (1973) with slight modification made to it.

Test for polyphenol

0.5g of the methanol leaf extract of *V.amygdalina* was dissolved in 5ml of distilled water. 1ml of 2% $FeCl_3$ solution and 1ml 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 methanol leaf extract of *V.amygdalina* was pressed between two filter papers. The appearance of oil stain on the paper indicated the presence of fixed oil.

Test for alkaloids (Mayer's test)

0.5g of the methanol leaf extract of *V.amygadlina* was dissolved in 5ml of distilled water. 2ml of 1% hydrochloride (HCl) was added and heated gently. 3ml 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)

0.5g of methanol leaf extract of *V.amygdalina* was dissolved in 5ml of distilled water. 2ml of Molisch reagent was added and the mixture was shaken properly. 2ml of conc. sulphuric (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)

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

Test for saponin (Froth test)

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

Test for terpenoids (Salkowski's test)

0.5g of methanol leaf extract of *V.amygdalina* was dissolved in 5ml of distilled water. 2ml of chloroform was added and 3ml 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)

0.5g of methanol leaf extract of *V.amygdalina* was dissolved in 5ml 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)

0.5g of methanol leaf extract of *V.amygdalina* was dissolved in 5ml 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

0.5g of methanol leaf extract of *V.amygdalina* was dissolved in 5ml 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)

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

Test for phytosterol (Libermann-Burchard's test)

0.5g of methanol leaf extract of *V.amygdalina* was dissolved in 5ml 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 anthraquinone

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

3.4 GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS (GC-MS)

The GCMS analysis of the methanol leaf extract of the plant sample was carried using standard methods as described Ajayi *et al.*, 2011.

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 1.0µm. 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.

3.5 EXPERIMENTAL ANIMALS

Twenty-five (25) albino male rats with body weight range (115-230) were used for this study and kept in well ventilated animal cages under controlled surroundings for 12 hours light/dark rotation at the animal house of the Department of Biological Sciences, College of Basic and Applied Sciences, Mountain Top University, Nigeria. All the rats were left to acclimatize for seven (7) days. They were kept on standard feed and water *ad libitum*. All experiments on rats were carried out in absolute compliance with the ethical guide for care and use of laboratory animals based on the guidelines of the Institutional Animal Ethics Committee (IAEC) (Giles, 1987).

3.5.1 EXPERIMENTAL DESIGN

The twenty-five (25) rats were arbitrarily distributed into five (5) groups (I-V) of 5 animals each:

Group I: Normal control; water and feed only.

Group II: Negative control; Streptozotocin (STZ) induced diabetic rats.

Group III: STZ (45mg/kg) induced diabetic rat treated with Metformin

Group IV: STZ induced rats treated with 150mg of *V.amygdalina* methanol leaf extract.

Group V: STZ induced rats treated with 300mg of *V.amygdalina* methanol leaf extract

3.6 INDUCTION OF DIABETES

The animals of groups II-V were weighed and their fasting blood glucose level was determined before induction. Diabetes was induced in the rats by intraperitoneally injecting freshly prepared streptozotocin (STZ) dissolved in citrate buffer (0.1M, pH 4.5) to overnight fasted rats at a dose based on their individual body weights after which they were given standard feed and 0.5%

glucose as water. The animals were checked after 48 hours of induction and 72 hours post induction. Blood was obtained from the rat's tail to confirm the fasting blood glucose (FBG) using a glucometer (Accu-check). Hyperglycemia was confirmed after the induction, and animals with blood glucose > 200 mg/dl were said to be diabetic.

3.7 DRUG ADMINISTRATION

Metformin was administered to experimental rats of group III and *V.amygdalina* methanol leaf extract was suspended in distilled water and administered via oral gavage at doses based on body weight to the experimental rats of group IV (150mg of VAM) and V (300mg of VAM). The Drug administration was done for 7days.

3.8 COLLECTION AND PREPARATION OF BLOOD PLASMA

After the seven (7) days administration of the methanol leaf extract of the plant and the metformin, the animals were sacrificed by cervical dislocation under anesthesia. The blood samples were collected using ocular method. Blood samples were transferred to Lithium heparin bottle. The blood samples were centrifuged at 4000 rpm for 10 minutes at $37^{O}C$ and the plasmawas obtained.

3.9 BIOCHEMICAL ASSAY

The plasma obtained was used to evaluate the changes in the following biochemical assay; creatinine, total protein, total cholesterol, total bilirubin, and triglycerides serum level using standard laboratory kit from Randox laboratories, UK.

3.9.1 TOTAL PROTEIN (TTP)

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 content of R2 was diluted with 400ml of distilled water.

a. Procedure for reagent blank

20μl 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

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

c. Procedure for sample

20μl of blood plasma was added to 1000μl 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 546nm.

3.9.2 TRIGLYCERIDE (TRIG)

Reagent Composition

Contents Initial Concentrations

R1a buffer

R1b Enzyme Reagent

Reconstitute one vial of enzyme reagent R1b with 15ml of buffer R1a. Stable for 21days at +2 to +8oC or 3days at +15 to +25oC stored protected from light.

Pipes buffer	38.7 mmol/L, pH 7.5
4-chloro-phenol	3.4 mmol/L
Magnesium ions	16.9 mmol/L
4-aminophenazone	0.25 mmol/L
ATP	1.2 mmol/L
Lipases	≥ 10 u/mL
Glycerol kinase	≥ 0.4 u/mL
Glycerol-3-phosphate oxidase	≥ 1.5 u/mL

Peroxidase	≥ 0.5 u/mL
Sodium azide	0.05

Procedures

Using fresh distilled water in test tube to carry out a water blank.

	Reagent blank(µl)	standard(µl)	sample(µl)
Sample	-	-	10
Standard	-	10	-
Reagent	1000	1000	1000

Solution was incubated for 5mins at 37^{O}C. Absorbance of the sample and standard against the reagent blank was read at 500nm within 60minutes.

3.9.3 CHOLESTEROL (CHOL)

Reagents

1. Reagent (R1)

Pipes buffer	pH=6.90 50 mmol/l
Phenol	24 mmol/l
Sodium cholate	0.5 mmol/l
4-Aminoantipyrine	0.5 mmol/l
Cholesterol esterase	180 U/l
Cholesterol oxidase	200 U/l
Peroxidase	1000 U/l

PROCEDURE

	Blank	Standard	Sample
Reagent	1 ml	1 ml	1 ml
Distilled water	10µl		
Standard		10µl	
Sample			10µl

The solution was incubated for 5 minutes at 37°C. The absorbance of the sample (Asample) and standard (Astandard) was read at 500nm against the reagent blank within 60 minutes.

3.9.4 CREATININE (CREA)

Reagent composition

CONTENTS	INITIAL CONCENTRATION OF SOLUTION
CAL. Standard	See lot specific insert
R1a. Picric Acid	35 mmol/l
R1b. Sodium Hydroxide	200 mmol/l

50μl of centrifuged organs supernatants was added to 500μl of the reagent at 25℃. The solution was mixed and the absorbance was read at 492nm against blank (air blank). The initial absorbance was read after 0.5min and the absorbance was re-read after 1, 2, and 3 mins.

3.9.5 BILIRUBIN

REAGENT COMPOSITION

Contents Initial Concentration of Solutions

R1. Sulphanilic acid29 mmol/l
Hydrochloric acid 0.17 N

R2. Sodium Nitrite -38.5 mmol/l

R3. Caffeine - 0.26 mol/l
Sodium benzoate - 0.52 mol/l

R4. Tartrate -0.93 mol/l
Sodium Hydroxide - 1.9

Total Bilirubin (TB)

Pipette into cuvette:
Sample Blank (ml) Sample (ml)
Reagent 1 0.20 0.20
Reagent 2 -- 1 drop (0.05 ml)
Reagent 3 1.00 1.00
Sample 0.20 0.20

Mix, and allow to stand for 10 min at 20-25°C.

Reagent 4 1.00 1.00

Mix, and allow to it stand for 5-30mins at 20-25°C and thenread the absorbance of the sample against the sampleblank.

3.10 STATISTICAL ANALYSIS

The statistical analysis for this study was done using Graph pad prism 8.2. 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

RESULTS

4.1 PHYTOCHEMICAL SCREENING

The table below show the result of the phytochemical screening analysis carried out on the methanol leaf extract of the plant.

Table 4: Phytochemical Screening of methanol leaf extract of *V.amygdalina*

	PHYTOCHEMICAL	**RESULTS**
1.	Alkaloid	Positive
2.	Carbohydrate	Positive
3.	Protein	Positive
4.	Fat and oil	Positive
5.	Terpenoids	Positive
6.	Phenol	Positive
7.	Flavonoid	Positive
8.	Tannin	Positive
9.	Glycoside	Positive
10.	Phytosterol	Positive
11.	Polyphenol	Positive
12.	Saponin	Positive
13.	Anthraquinone	Negative

4.2 GAS CHROMATOGRAPHY-MASS SPECTROSCOPY (GC-MS) ANALYSIS

The figure 1; below shows the result of the GC-MS chromatogram analysis done on the methanol leaf extract to identify the compounds present.

4.2.1 GC-MS chromatogram of methanol leaf extract of VAM

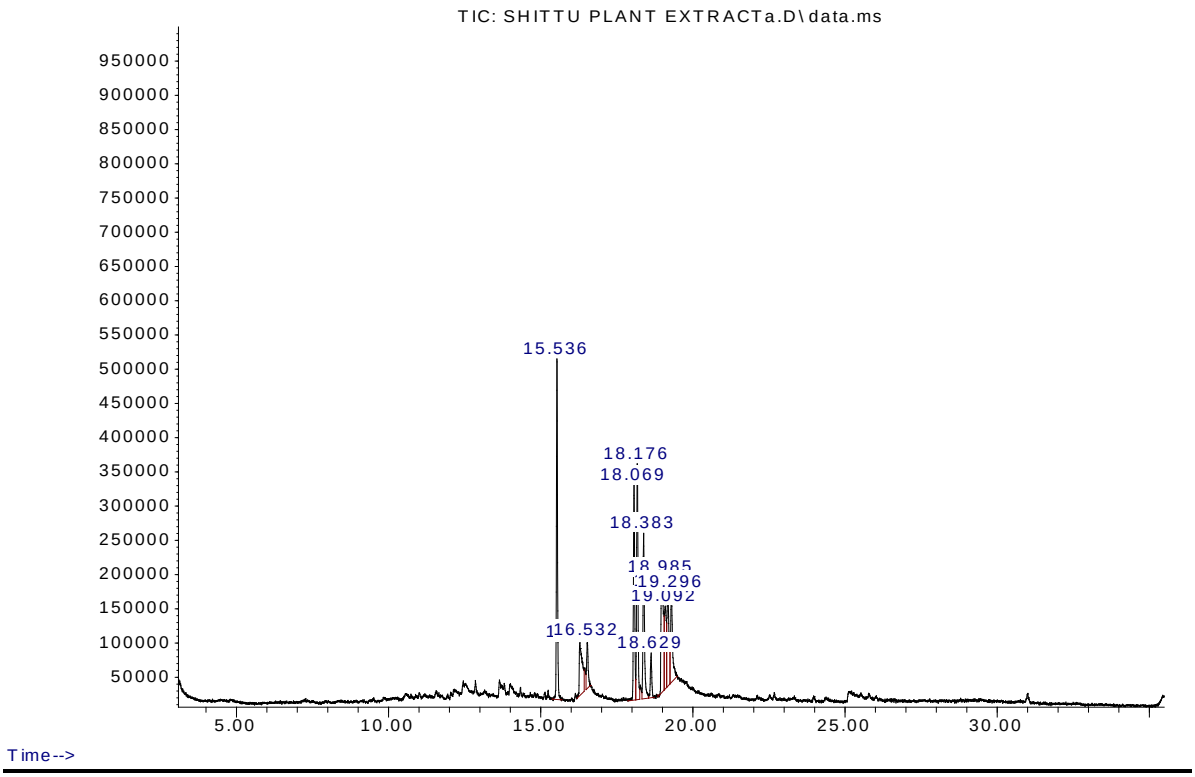

Figure 4; GC-MS chromatogram of methanol extract of VAM.

The table below shows the compounds that have been identified to be present in the methanol leaf extract, with the peak, R.T, the % of total, the library ID and the chemical formular of the compound.

Table 5; GC-MS analysis result of VAM.

PEAK	**R.T**	**% OF TOTAL**	**LIBRARY ID**	**CHEMICAL FOMULAR**
1	15.536	16.262	Hexadecanoic acid, methyl ester.	$C_{17}H_{34}O_2$
2	16.288	6.841	n-Hexadecanoic acid.	$C_{16}H_{32}O_2$
3	16.532	3.197	Hexadecanoic acid, ethyl ester.	$C_{18}H_{36}O_2$
4	18.069	11.832	9,12-Octadecadienoic acid (Z,Z), methyl ester	$C_{19}H_{34}O_2$
5	18.176	14.075	cis-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$
6	18.383	10.528	Phytol	$C_{20}H_{40}O$
7	18.629	2.611	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$
8	18.985	11.968	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$
9	19.180	8.203	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$
10	19.296	7.828	9, 12, 15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$

4.2.2 Structures of each chemical constituents found in VAM using GC-MS

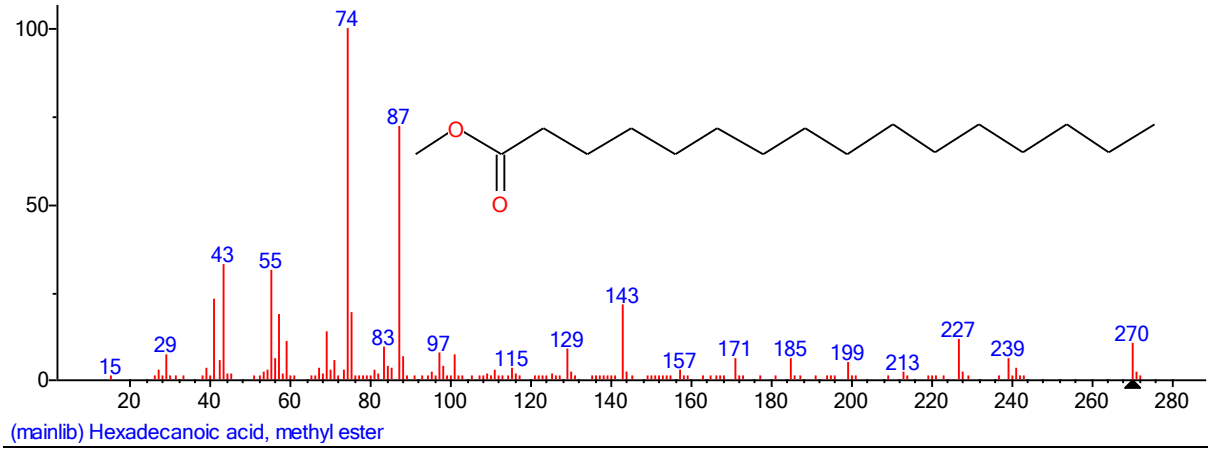

Figure 5; Structure of hexadecanoic acid, methyl ester

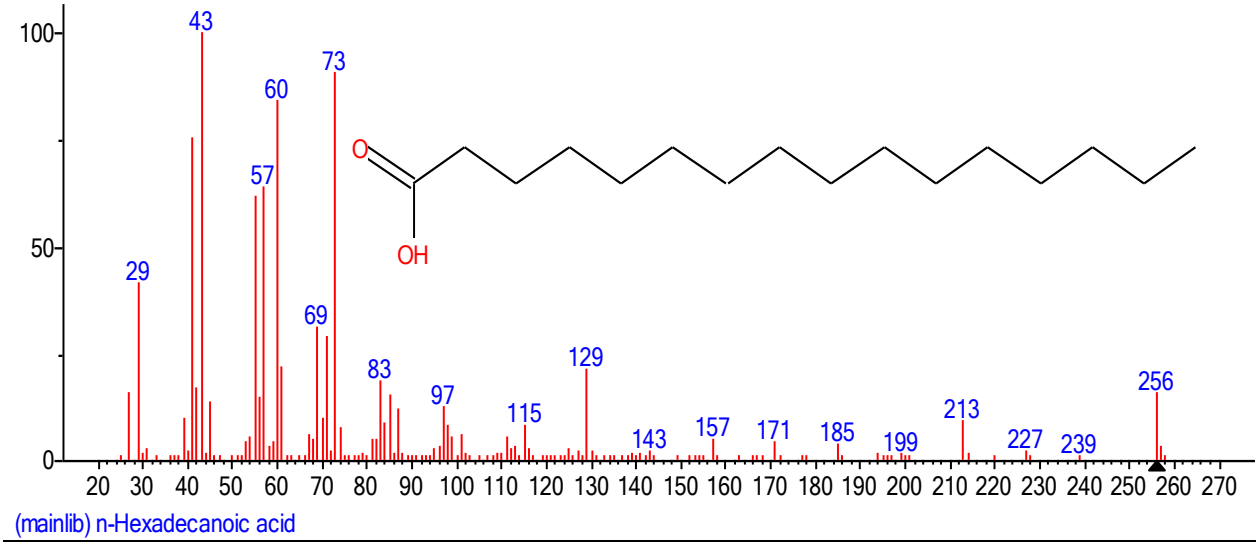

Figure 6; Structure of n-Hexadecanoic acid

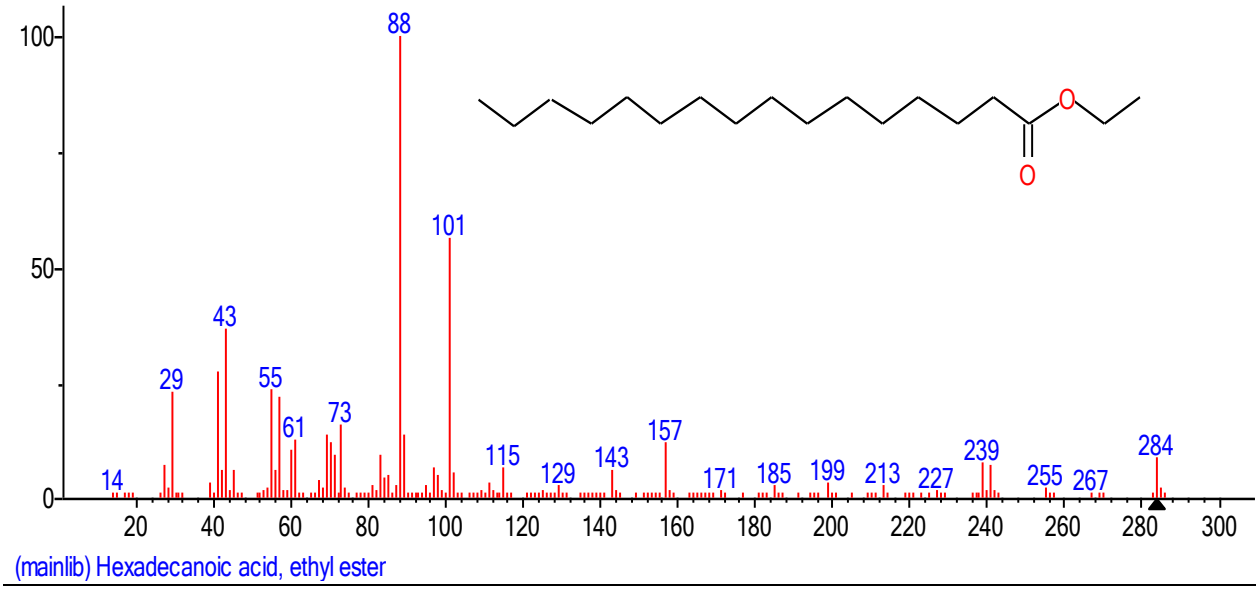

Figure 7; Structure of hexadecanoic acid, ethyl ester

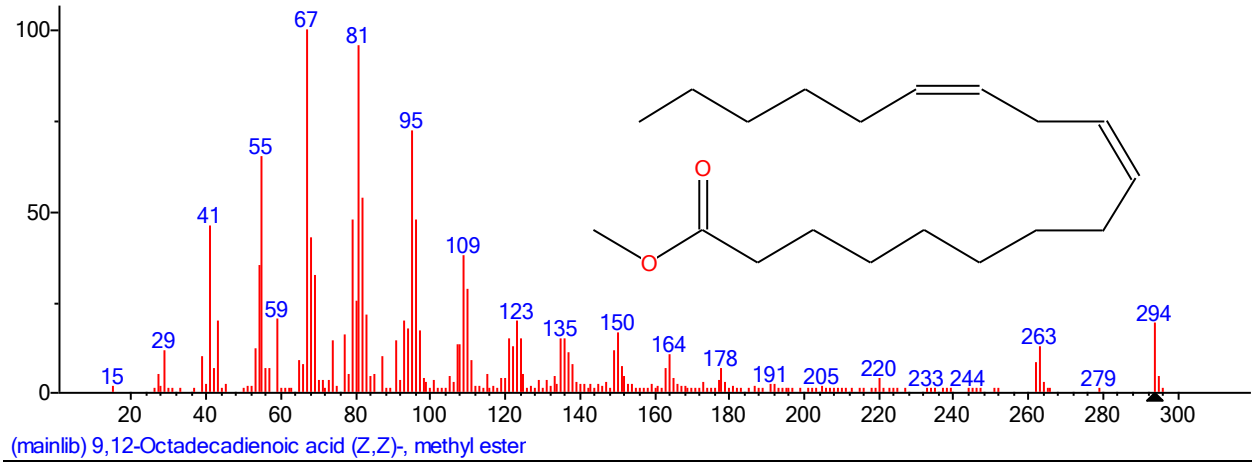

Figure 8; Structure of 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester

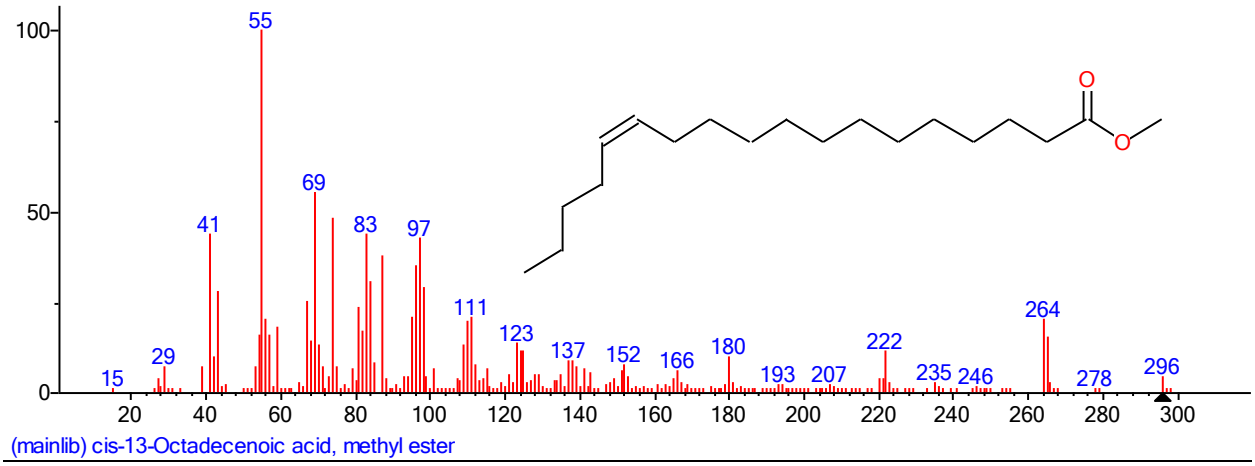

Figure 9; Structure of cis-13-Octadecenoic acid, methyl ester

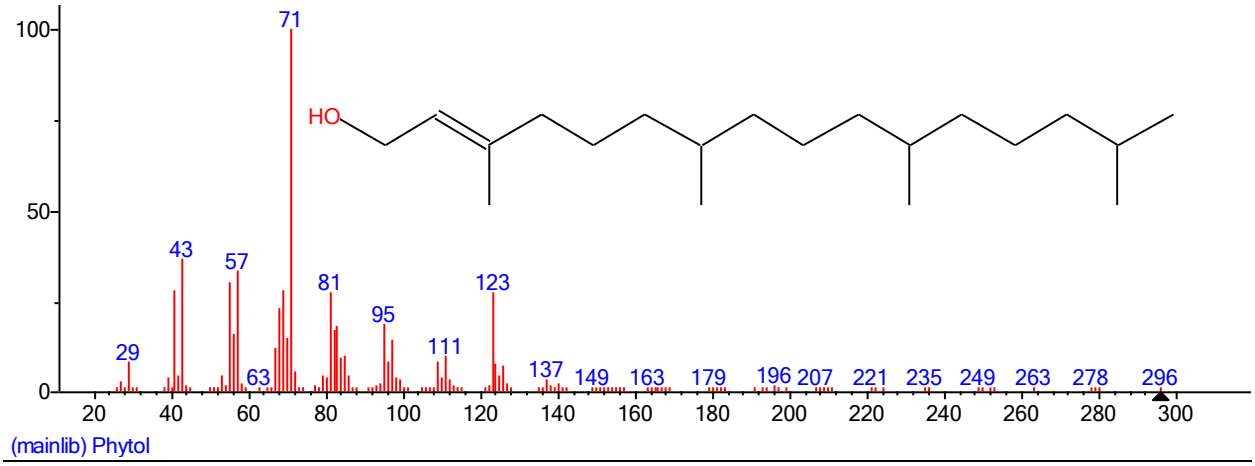

Figure 10; Structure of phytol

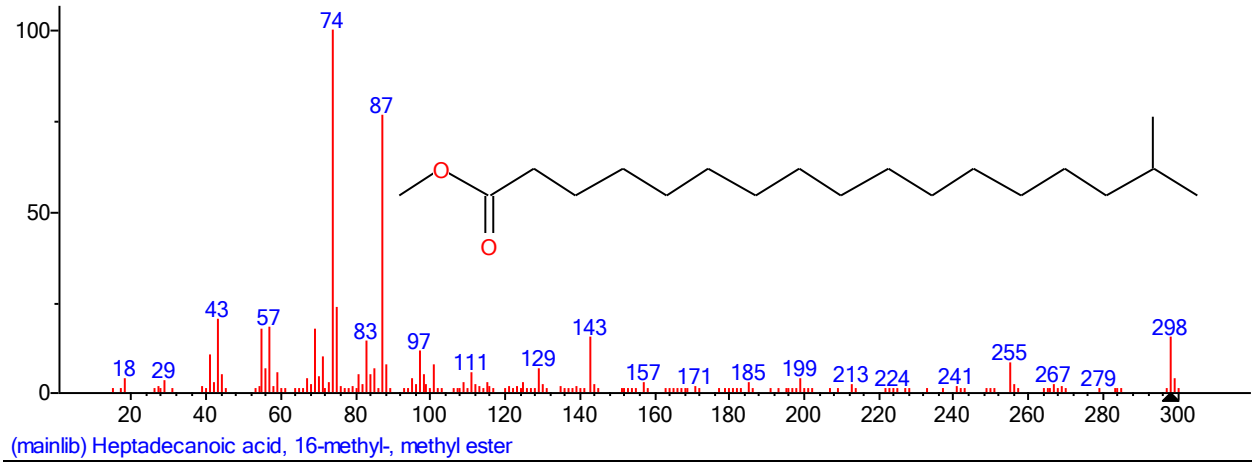

Figure 11; Structure of heptadecanoic acid, 16-methyl-, methyl ester

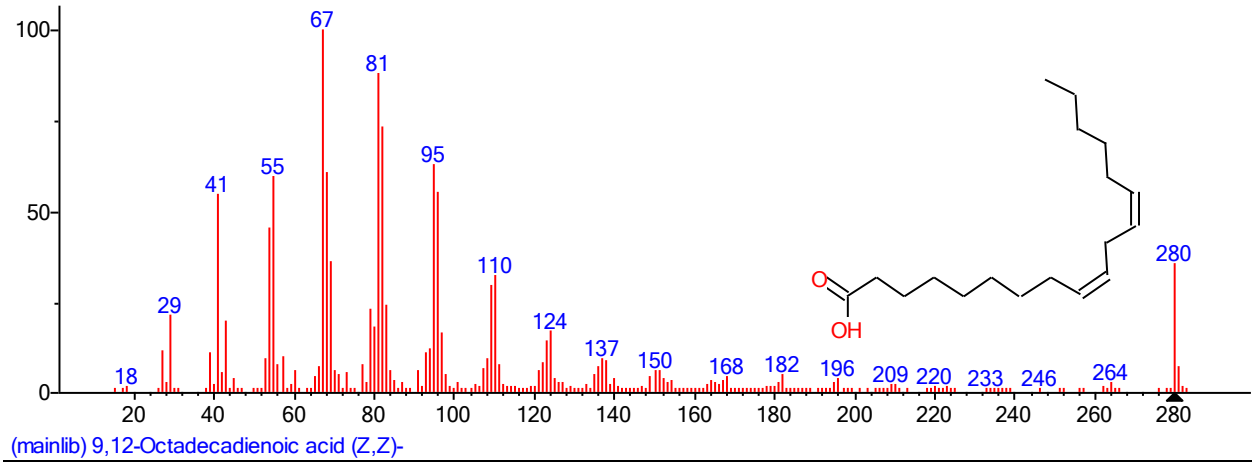

Figure 12; Structure of 9, 12-Octadecadienoic acid

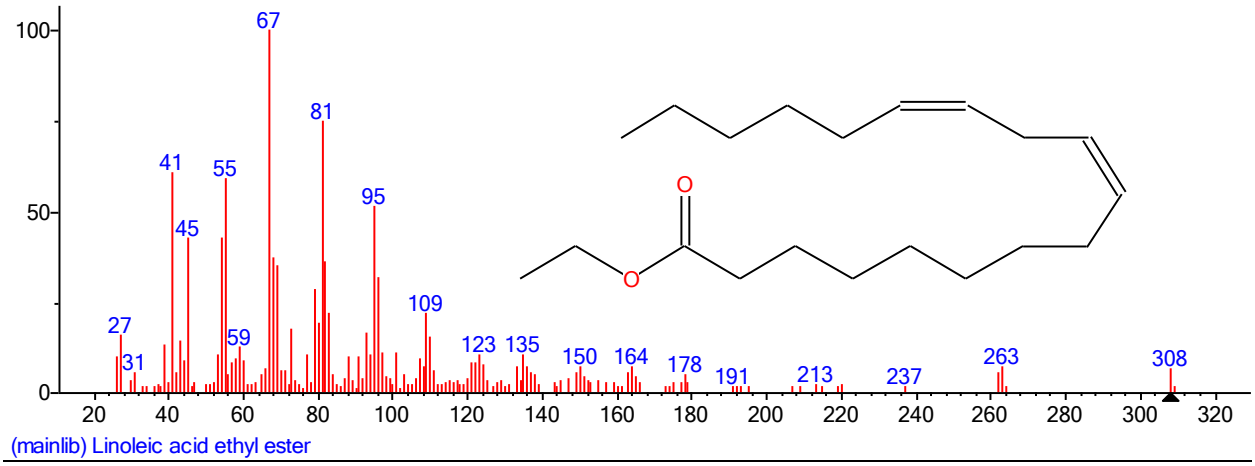

Figure 13; Structure of Linoleic acid ethyl ester

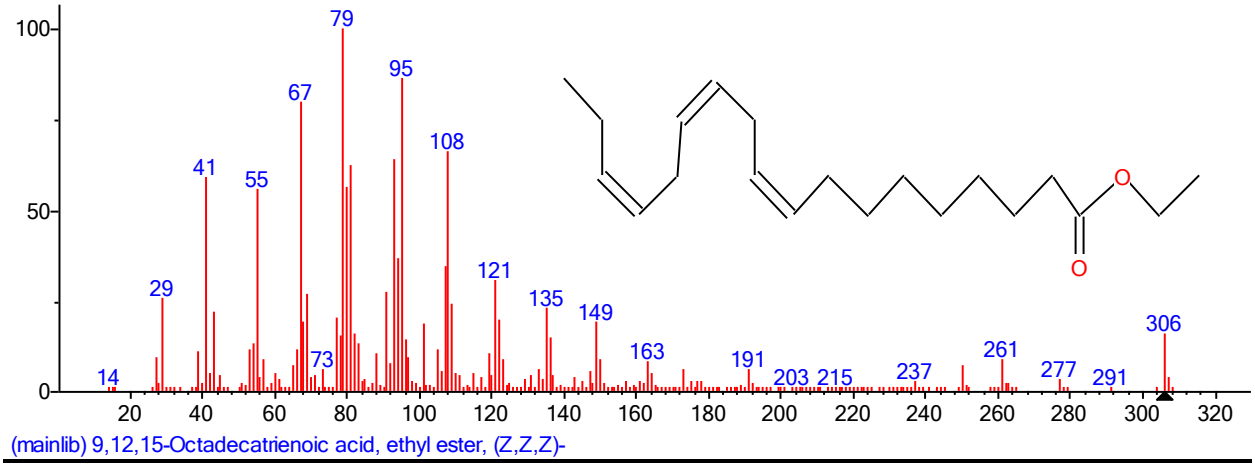

Figure 14; Structure of 9, 12, 15-Octadecatrienoic acid, ethyl ester (Z, Z, Z)

4.3 RESULTS FOR THE BODY WEIGHT OF THE RATS

As seen in Figure 15 below there were significant changes in the body weights of the rats, in day 4, there was a significant decrease in the body weight of the rats in group 2, 3,4 and 5 when compared to the day 1, after the oral administration of the extract and metformin, in day11 there was a significant increase in the body weight of the rats.

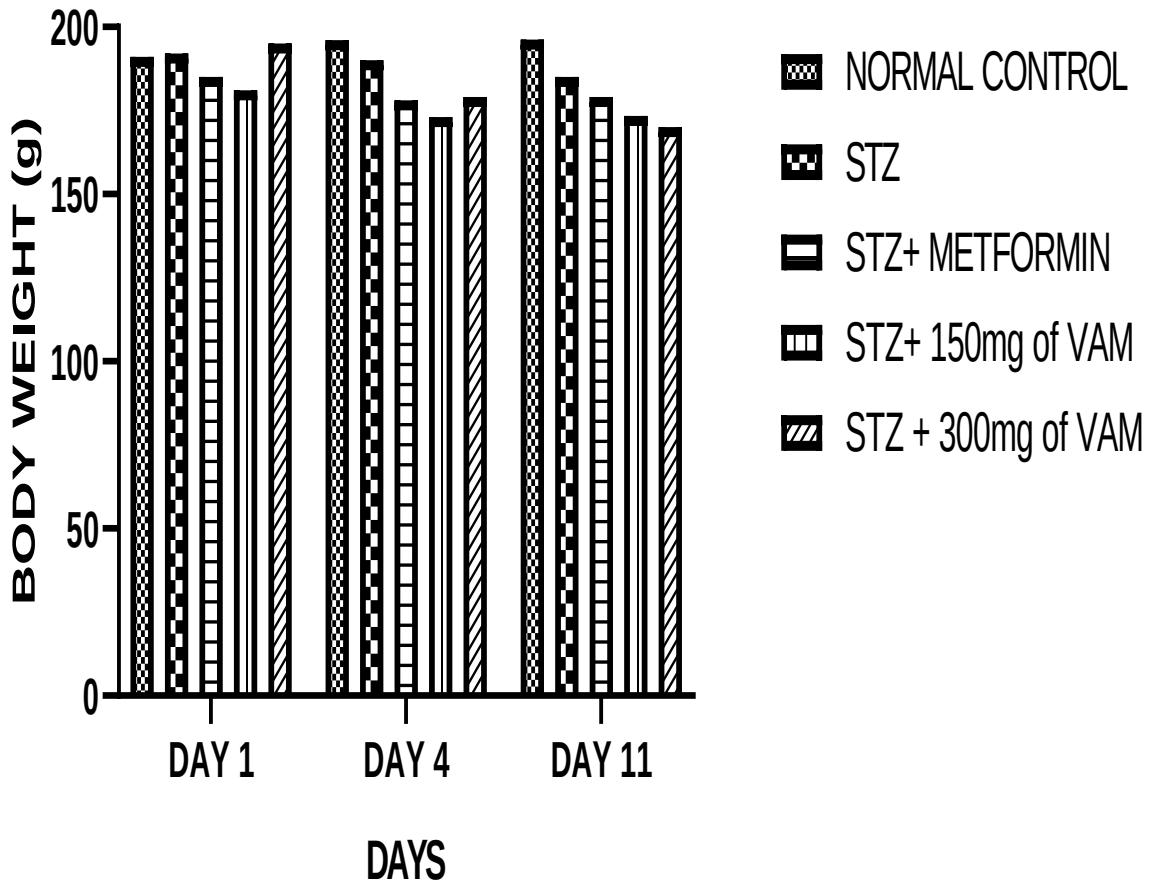

Figure 15; The body weight of the rats in grams.

The values represent mean± S.E.M of the 5 animals per group. There was a significant parameter ($P<0.05$) in column factor and insignificantly different ($P>0.05$) in row factor.

4.4 FASTING GLUCOSE CONCENTRATION

As seen in Figure 16; below, it show that there was a significant increase in the fasting blood glucose concentration of the rats on day 4, after streptozotocin was inducted into the rats and was not treated. There was a significant decrease in the group 3, 4 and 5, when compared to the diabetic control ($P<0.05$) on day 11 after the oral administration of the treatment with metformin and I50mg dose and 300mg doses of the methanol leaf extract.

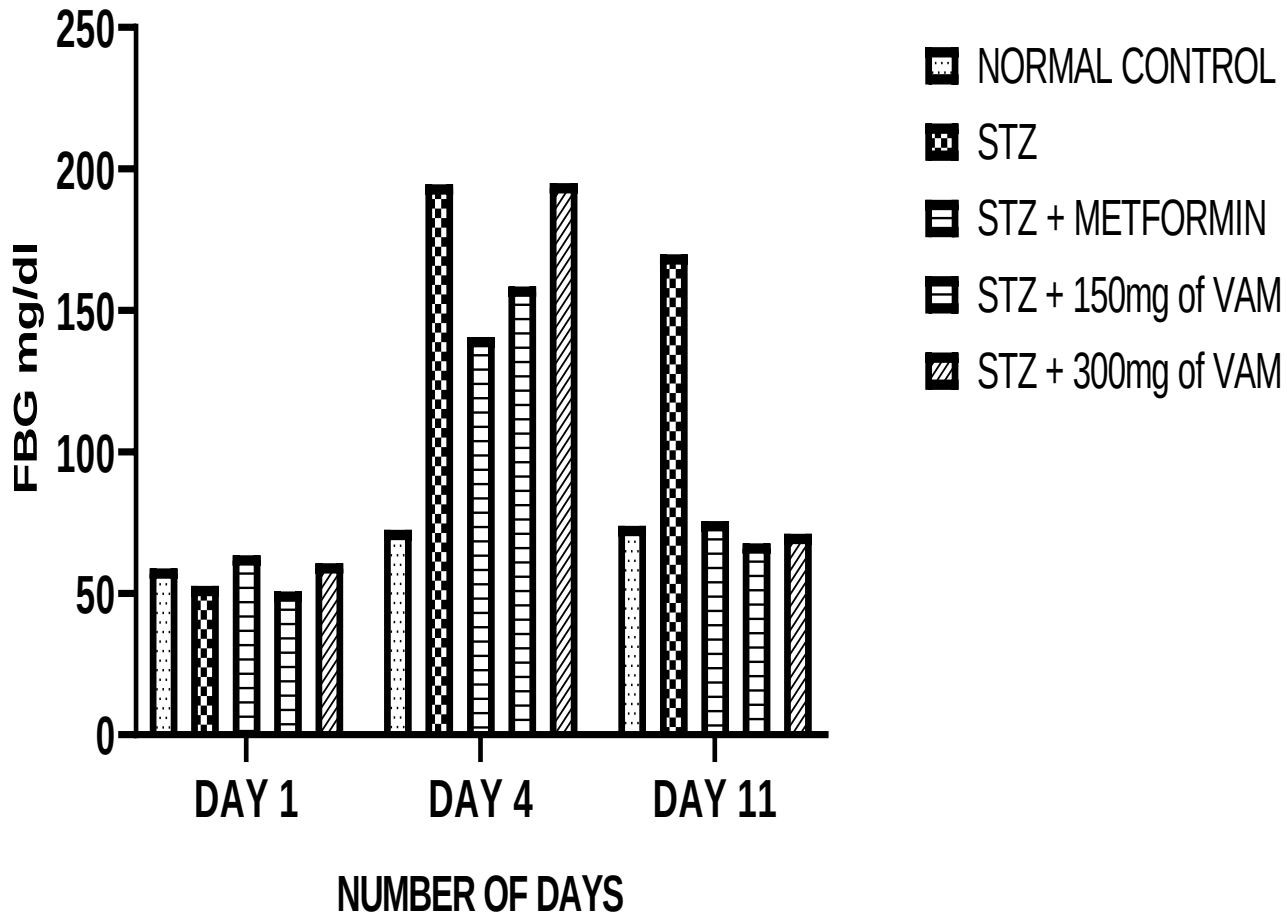

Figure 16; Fasting Blood Glucose concentration of the rats in mg/dl.

The Values represent mean± S.E.M of 5 animals per group. There was a significant difference of ($P<0.05$).

4.5 RESULTS FOR BIOCHEMICAL ASSAY

The Figures below shows the results and the significant increase or decrease in the various biochemical parameters analysed, the figures represent the mean and standard error of the mean ($P>0.05$).

In Figure 17; below has seen that there was a significant decrease in the total protein level in the STZ control group, while there was a significant increase in the total protein level of the STZ groups that were treated with metformin and the methanol leaf extract (group III, IV and V) a observed, the group V diabetic rats treated with 300mg of the methanol extract of VAM had the highest total protein level, when compared to the normal control and the STZ control.

4.5.1 TOTAL PROTEIN (TTP)

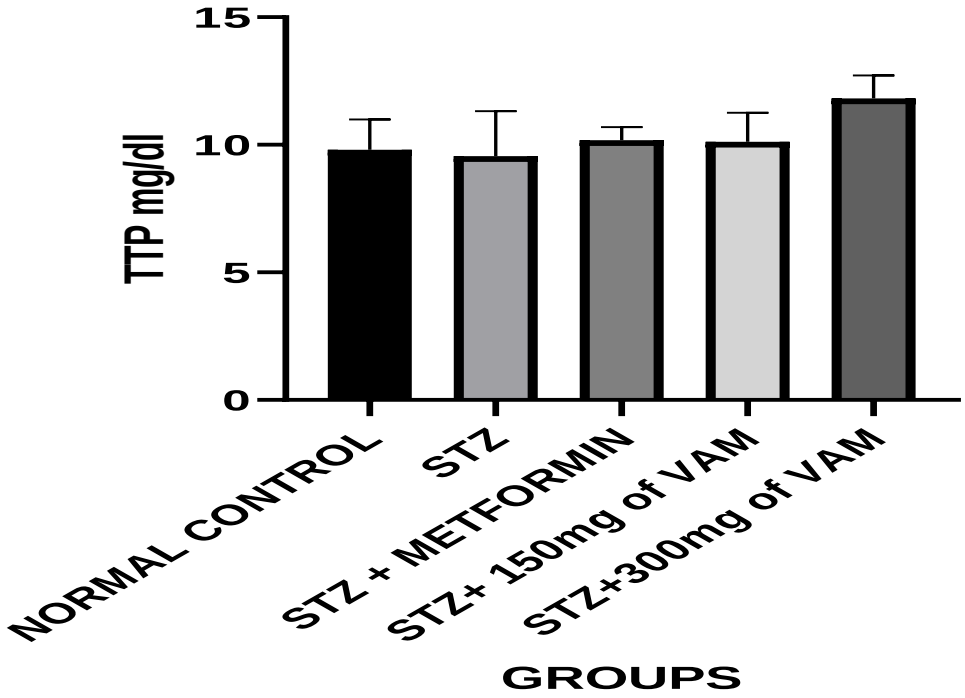

Figure 17; Concentration level of total protein in mg/dl.

The values represent mean± S.E.M of 5 animals per group. There was a significant difference of ($p>0.05$).

4.5.2 CREATININE (CREA)

Figure 18; below express the mean and standard error mean of the creatinine level in the various groups of the rats. It can be observed from the figure below that was a significant decrease in the creatinine serum level of group II, III and IV rats, when compared to the normal control. However, there was a significant increase in the creatinine level when compared to the normal control.

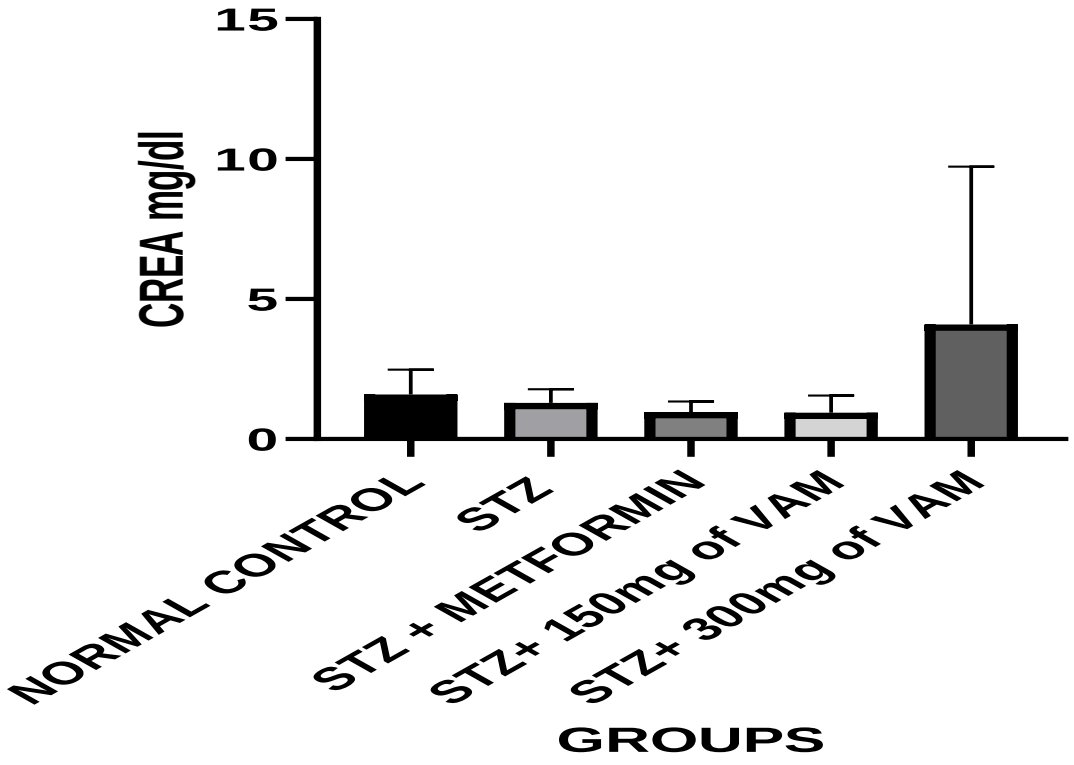

Figure 18; Concentration level of creatinine in mg/dl.

The values represent mean± S.E.M of 5 animals per group. There was a significant difference of ($p>0.05$).

4.5.3 TOTAL BILIRUBIN (BIL)

In the Figure 19; the total bilirubin concentration level expressed, represents the mean and standard error mean

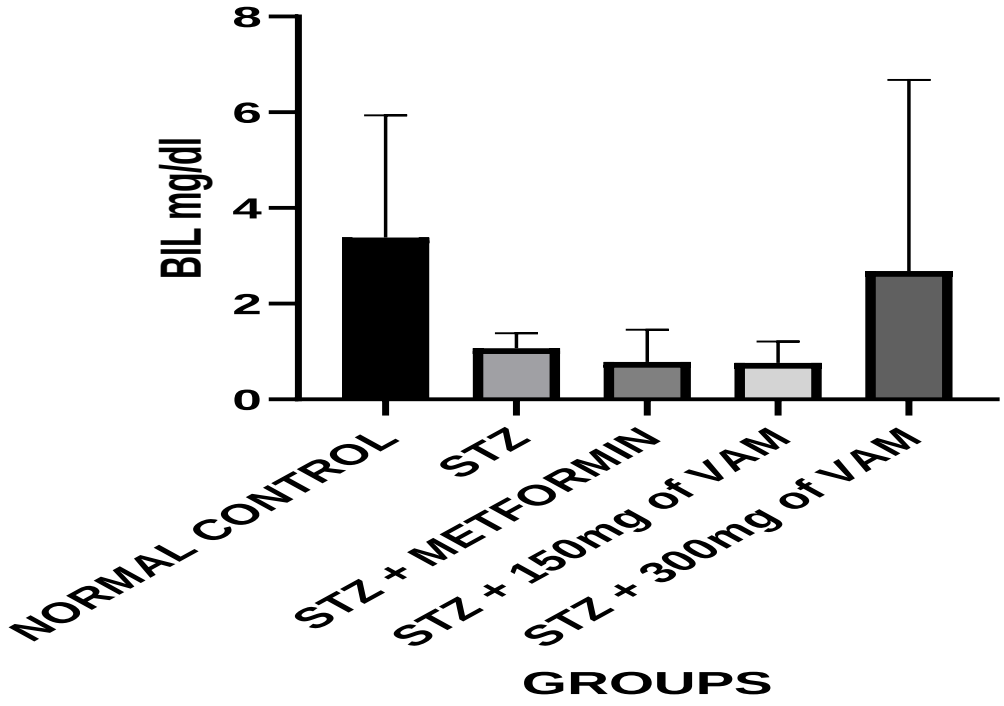

Figure 19; Concentration level of total bilirubin in mg/dl.

The values represent mean± S.E.M of 5 animals per group. There was a significant difference of ($p>0.05$).

4.5.4 TRIGLYCERIDES (TRIG)

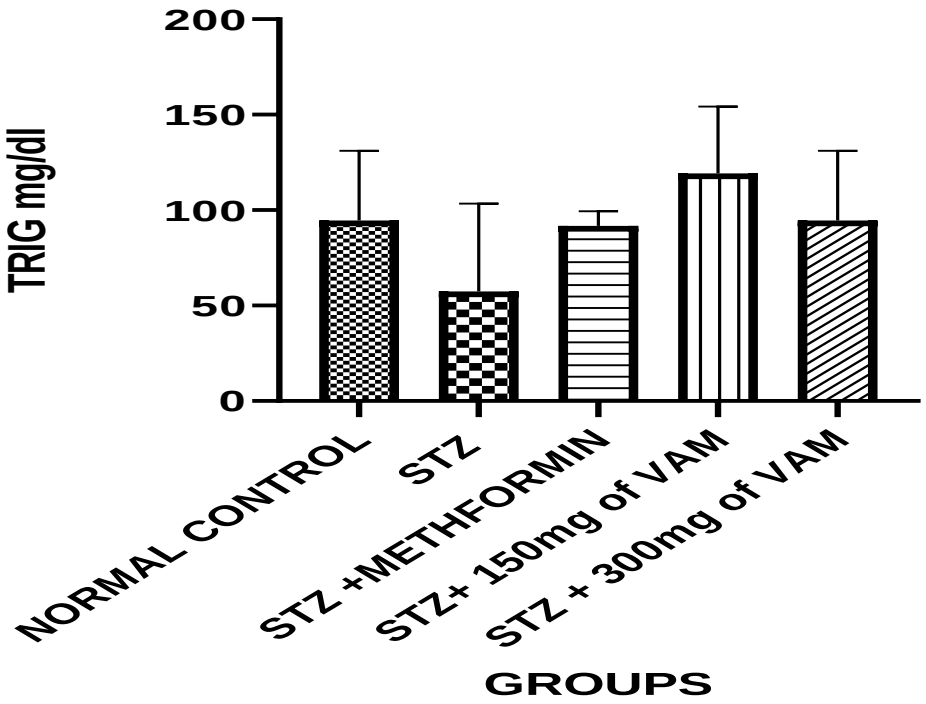

Figure 20; Concentration level of triglycerides in mg/dl.

The values represent mean± S.E.M of 5 animals per group. There was a significant difference of ($p>0.05$).

4.5.5 CHOLESTEROL (CHOL)

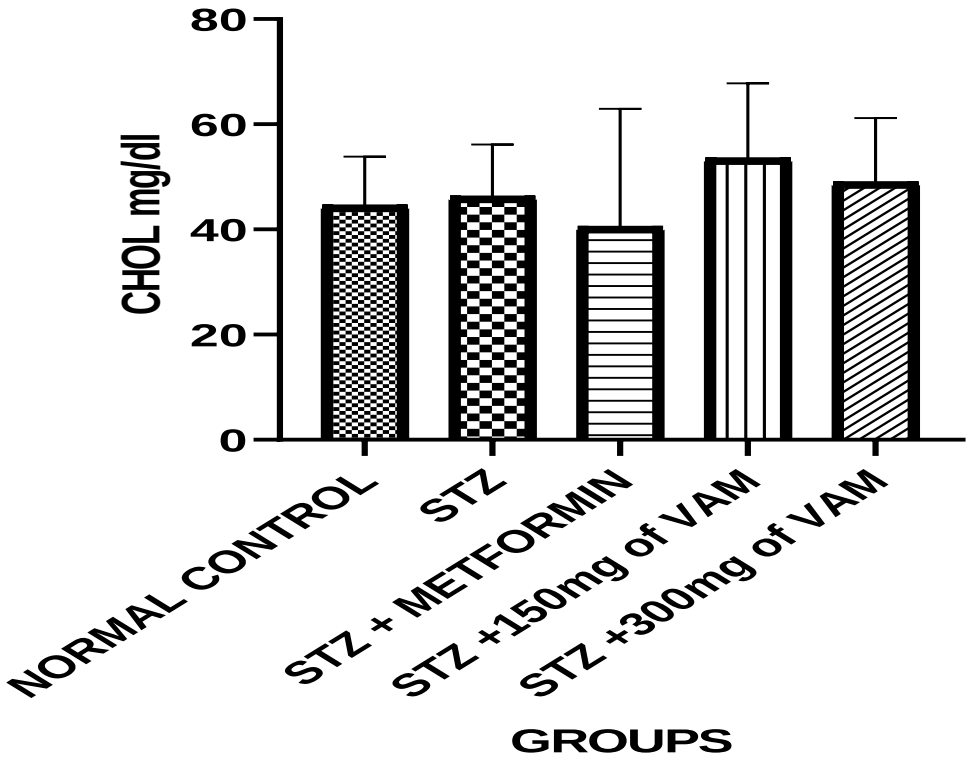

Figure 21; Concentration level of cholesterol in mg/dl.

The values represent mean± S.E.M of 5 animals per group. There was a significant difference of ($p>0.05$).

CHAPTER FIVE

5.0 DISCUSSION

In this study, the chemical constituents and the biochemical effect of *Vernoniaamygdalina* was determined using the plasma serum of the rats. The table 4 above shows the result of the phytochemicals present in the methanol leaf extract. The compounds present are Alkaloid, Carbohydrate, Protein, Fat and oil, Terpenoids, Phenol,Flavonoid, Tannin, Glycoside, Phytosterol, Polyphenol, Saponin, expect for Anthraquinone as described by sofowora (1993), Trease and Evans (1989), and Harbone (1973). Following STZ administration for four days, a loss of weight was observed in the groups that were induced with STZ, when compared to the normal control. A significant increase was also observed in the blood glucose level in the STZ groups when compared to the normal control. On administration of metformin and *Vernoniaamygdalina*extract, the fasting blood glucose level was observed to have significantly decreased in groups III, IV and V. Also, significant increases in the body weights of the rats were observed on administration of metformin and *Vernoniaamygdalina*extract in group III, IV and V. This implies that the plant contains anti-diabetic and hypoglycemic properties has it been reported in previous research works (Adarmoye*et al.*, 2008).

The following biochemical parameters were also analyzed; triglyceride, cholesterol, creatinine, total protein and bilirubin. It was observed that there was a significant increase in the total protein level of groups IV and V, the diabetic rats treated with doses of 150mg and 300mg of VAM, which indicates that the methanol leaf extract contains protein, as earlier detected in the phytochemical screening result of the leaf extract. It was be observed from the figure below that was a significant decrease in the creatinine serum level of group II, III and IV rats, when compared to the normal control. However, there was a significant increase in the creatinine level

when compared to the normal control.A significant increase was observed in the cholesterol and triglycerides concentration level of group IV and V rats when compared to the normal control and STZ control. A significant decrease was observed in the concentration level of group II, III, IV and V when compared to the normal control.

CONCLUSION

The effect of the methanol leaf extract of *V.amygdalina* as it has been seen from the research carried out above. The results from the research work carried out showed that the methanol leaf extract of *V.amygdalina*possesseshypoglycaemic characteristics which are of significance to the treatment and management of diabetes mellitus.

RECOMMENDATION

I would recommend use of*V.amygdalina* because of its anti-diabetic property, it has great medicinal benefits in the treatment of Diabetes mellitus and also doesn't cause side effects compared to synthetic drugs used to treat diabetes mellitus and it can also be used in the treatment of other ailments and diseases.

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