BIOACTIVE COMPONENTS AND THE BIOCHEMICAL EFFECTS OF METHANOL LEAF EXTRACT OF VERNONIA AMYGDALINA ON THE LIVER AND KIDNEY IN STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

BY

OBI ELVIS UCHECHUKWU
(15010102011)

A DISCENTATION SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, MOUNTAIN TOP UNIVERSITY, OGUN STATE, NIGERIA. IN PARTIAL FULFILMENT OF THE REQUIREMENTS, FOR THE AWARD OF THE BACHELOR OF SCIENCE DEGREE (BSC) IN BIOCHEMISTRY.

JULY 2019

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CERTIFICATION

I certify that this project **BIOACTIVE COMPONENTS AND BIOCHEMICAL EFFECTS OF METHANOLIC LEAF EXTRACT OF VERNONIA AMAGDALINA ON THE LIVER AND KIDNEY IN STREPTOZOTOCIN-INDUCED DIABETIC RATS**has been carefully supervised and approved as adequate for the partial fulfillment of the award of Bachelor of science, B.Sc.(BIOCHEMISTRY) of Mountain Top University.

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OBI ELVIS UCHECHUKWU DATE

STUDENT

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DR. G.O. AJAYI DATE

SUPERVISOR

....

DR. A. A. ADEIGA DATE

HOD

DEDICATION

I dedicate my project to God Almighty for His grace, strength and favour. He who was my support through this period of my life and to my amazing parents; Mr and Mrs S. Obi.

ACKNOWLEDGEMENT

I would like to express my immense gratitude to Almighty God for his infinite mercy as I started and completed my project successfully.

I want to show gratitude to the Dean of College of Basic and Applied Sciences (Prof. A. I. Akinwande) and to the Head of Department, Biological Sciences (Dr. A. A. Adeiga) for their fatherly support through-out my stay in the university. I want to specially acknowledge my supervisor; Dr. G.O Ajayi, who made it possible for me to embark on a project dear to my heart and to all the lecturers and the technical staff of the Department of Biological sciences, College of basic and applied sciences. I am truly grateful for the help throughout my stay in the university. I also want to express my utmost gratitude to my parents, Mr and Mrs S. Obi and my brothers and sister for showing their undying support and encouragement. Finally, my gratitude goes to my ever supportive friends; Obi Collins, Okunbi Favour, David Ukegbu, Emeka Aka and Ntuk Anderson.

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ABSTRACT

This study was designed to investigate the effect of methanol leaf extract of *Vernonia amygdalina* on the kidney and liver functions of Streptozotocin induced diabetic wistar rats. Twenty-five healthy, young adult male rats (115-220g) were divided into five groups of five rats each. Group 1 (Normal control) received water and feed, groups 2, 3, 4, and 5 received Streptozotocin (45mg/kg) by inter-peritoneal injection to induce diabetes. Group 3 rats were administered with metformin for seven (7) days, and the rats in group 4 were administered with 150mg/kg of *Vernonia amygdalina* methanol leaf extract seven (7) days, while the rats in group 5 were administered with 300mg/kg of *Vernonia amygdalina* methanol leaf extract for seven (7) days. The body weight and fasting blood glucose of animals were taken at Day 1, day 4 and Day 11 before they were sacrificed and the organs were weighed and obtained for biochemical assay analysis. The results suggested that *Vernonia amygdalina* methanol leaf extract caused a significant decrease in FBG levels of Groups 4 and 5 animals, and Metformin caused a low amount of decrease in Group 3 animals. *Vernonia amygdalina* methanol leaf extract also caused a significant increase in weight of Groups 4 and 5 animals as compared to Groups 3 and 2. Relative liver and kidney weights were decreased as compared to that of the control.

In conclusion, the effect of *Vernonia amygdalina* methanol leaf extract shown in the present study implies a pharmaco-therapeutic and protective role in liver and kidney functions in Streptozotocin induced diabetic wistar rats. The results of the phyto-chemical screening showed the presence of some basic phyto-chemical components in *Vernonia amygdalina.*

The GCMS analysis displayed hexa-decanoic acid, methyl ester, cis-13Octadecenoic acid, methyl ester, 9, 12 Octadecadienoic acid (Z, Z), and Phytol as the organic components with the highest percentages.

CHAPTER ONE

1.0 INTRODUCTION

Diabetes mellitus (DM) is a metabolic syndrome defined by hyperglycaemic effects caused by the lack of insulin or disruption of insulin signalling as a result of the lack of hypoglycaemic agent or insensitiveness of insulin hormone. DM is related to aberrant metabolism of macromolecules (Kumar, *et al.,* 2011). The occurrence of this syndrome worldwide is at a growing rate, which led to the use of several therapeutic methods (currently available) for the management of this chronic metabolic disorder, including the stimulation of endogenous insulin secretion, improvement of insulin action at the target sites, inhibition of dietary starch and lipid degradation, and treatment with oral hypoglycaemic agents (Aloulou, *et al.,* 2012). In Africa, there has been vigorous growth of phyto-pharmaceuticals with proven efficacy in a variety of medicinal problems. Among other things, demand for the use of plant natural products with antidiabetic activity due to low cost, easily available and lesser side effects has been on the rise. Therefore, plant elements are constantly explored and examined for their effect as hypoglycaemic agents. One of such plant is *Vernonia amygdalina*. *Vernonia amygdalina* commonly called Bitter leaf is a medicinal plant of the family Asteraceae. It is a small perennial shrub that grows in tropical Africa. It is found in Angola, Burundi, Cameroun, Central Africa Republic, Nigeria and in many other countries. It is widely called Ewuro in Yoruba land and Onugbu in Igbo land. The leaves of the plant may be consumed either as a vegetable (softened leaves in soup) after rounds of washing to remove the bitter taste, or aqueous extract as tonics for the treatment of various illnesses (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). Extract of *V. amygdalina* is used traditionally to cure a number of ailments and its actions include, anti-inflammatory, wound healing, immune modulation, anti-tumour, anti-bacteria, antiviral laxative and purgative properties (Ijeh and, Ejike., 2011). *V. amygdalina* can also be used as food supplements when prepared with melon (egusi soup) a nutritious African food (Erasto *et al.,* 2006).

This study seeks to investigate the evaluation of the bioactive component and biochemical effects of methanol extract of *V. amygdalina* leaves on liver and kidney parameters of streptozotocin-induced diabetic wistar rats.

1.1 STATEMENT OF THE PROBLEM

This study aims to discover better ways to tackle diabetes and improvements of diabetic treatment measures. The research work is focused on the study of the use of plants in disease treatments and the use of *V. amygdalina* in diabetic treatment by administering it to rats and compares its anti-diabetic effects to that of drugs used in diabetes treatment. It also helps to reduce the use of drugs in disease treatment to help reduce anti-biotic resistance.

1.2 SIGNIFICANCE OF STUDY

Bitter leaf consumption has a lot of benefits to the health and since the bitter leaf has constituents that are highly anti-diabetic and can be applied in drug therapy, they provide essential contents for diabetic treatments, anti-oxidation, anti-inflammation, anti-cancer and anti-malarial. Besides being edible, they are now increasingly being used in industrial applications such anti-malarial production, anti-oxidant production and anti-diabetic medical research.

1.3 JUSTIFICATION OF STUDY

Several studies were observed that *Vernonia amygdalina* has phytochemical properties that have an effect on diabetes. These phytochemical properties were used in this study by administering the methanol leaf extract of *Vernonia amygdalina* to the rats to check for its biological effect on the biochemical components of the liver and kidney of these rats and it has proven successful through results that there was a decrease in the diabetic level of these STZ-diabetic induced wistar rats after *Vernonia amygdalina* administration.

1.4.0 AIMS AND OBJECTIVES

1.4.1 AIM OF THE STUDY

The aim of this study is to evaluate the bioactive components and effect of the methanol leaf extract of *Vernonia amygdalina* on the biochemical parameters of the liver and kidney of a steptozotocin-induced diabetic wistar rats.

1.4.2 OBJECTIVES OF THE STUDY

This project includes;

1. To evaluate the phytochemical properties of *V. amygdalina* leaf extract.
2. To determine some biochemical parameters; creatinine (CREA), total bilirubin (TB), total protein (TP), lactate dehydrogenase (LDH), alanine transferase (ALT), aspartate transferase (AST), and Gamma-glutamyl transferase (GGT).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 DIABETES

Diabetes mellitus (DM) commonly referred to as diabetesis a medical condition or a group of metabolic disorders characterized by hyperglycemia, glycosuria and hyperlipidemia that damages the body's ability to process blood glucose, otherwise known as blood sugar. Diabetes is not a single disease it’s group of heterogeneous syndromes such as heart attack, stroke and peripheral vascular disease (Tripathi and Verma., 2014).Recent research into the pathophysiology of type 2 DM has led to the introduction of new medications like glucagon-like peptide 1 analogues: dipeptidyl peptidase-IV inhibitors, inhibitors of the sodium-glucose cotransporter 2 and 11ß-hydroxysteroid dehydrogenase 1, insulin-releasing glucokinase activators and Pancreatic-G-protein-coupled fatty-acid-receptor agonists, glucagon-receptor antagonists, metabolic inhibitors of hepatic glucose output and quick-release bromocriptine. (Olokoba *et al*., 2012). Diabetes mellitus is characterized as metabolic disorders defined by increased glucose levels (hyperglycemia) of blood because of failings in insulin secretion, insulin action or both. Insulin is produced by beta cells of the pancreas which use glucose from digested food as an energy source. When the pancreas either cannot make insulin or the insulin it does make is not enough and cannot function properly, glucose builds up in the blood at a high level and frequently secrete through urine which is classical symptom of diabetes mellitus (Qais *et al*., 1991). Diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger).

Diabetes mellitus is a common and very prevalent disease affecting the citizens of both developed and developing countries. Diabetes mellitus is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin (Arumugam *et al*., 2010). Diabetes mellitus is a group of metabolic disorders with one common manifestation in hyperglycaemia (Malviya *et al*., 2010). It results either from inadequate secretion of hormone insulin, an inadequate response of target cells to insulin, or a combination of these factors (Malviya *et al*., 2010). Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycaemia due to defective insulin action, insulin secretion or both (Tchimene *et al*., 2016).

Diabetes mellitus is caused by the abnormality or dysfunction of carbohydrate metabolism. The food we eat is broken into simple sugar called glucose. Glucose is the main source to get energy for the body. After digestion, it reaches our blood stream and the body cells utilize it for energy. Insulin is very essential for the glucose uptake into the cells. Insulin is a hormone secreted by the pancreas. If the pancreas does not produce enough insulin, glucose get into the body cells so, glucose stays in the blood. This makes the blood as hyperglycaemic condition (Jeeva and Anlin. 2014). The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat protein metabolism resulting from defects in insulin secretion, insulin action, or both (Adejuwon *et al*., 2012). Glucose is the major source of energy for the cell. For glucose to enter the cells it needs insulin that comes from endocrine pancreas. If the pancreas does not make sufficient insulin or cells are resistant to its activity of promoting glucose uptake, the blood glucose levels become elevated. Insulin release is effected by hyperglycaemia, increased beta adrenergic stimulation and by intestinal hormone, secretin, among others.(Adejuwon *et al*., 2012).Diabetes mellitus is a chronic metabolic disorder

which is characterized by hyperglycaemia and long term complications such as retinopathy, nephropathy, neuropathy, and angiopathy (Osadebe *et al*., 2016).

Diabetes mellitus is a group of metabolic disorder associated with the endocrine system that resulted in hyperglycaemic condition(Alhassan *et al*., 2008).Diabetes mellitus According to WHO, the term diabetes mellitus is defined as a metabolic disorder of multiple etiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Swaroop*et al*., 2012).Diabetes is a metabolic disorder characterized by chronic hyperglycaemia and impaired insulin signalling which generates metabolic changes and an inflammatory status that will eventually affect all body tissues (Prabhakaran *et al*., 2005). Diabetes mellitus is characterized as metabolic disorders defined by increased glucose levels (hyperglycaemia) of blood because of failings in insulin secretion, insulin action or both (Qais *et al*., 1991). Diabetes mellitus (DM) is a chronic disease characterized by a deficiency in insulin production and its action or both. That leads to prolonged hyperglycaemia with disturbances in most metabolic processes inside the human body (Singab *et al*., 2005).

2.2 TYPES OF DIABETES

There are three main types of diabetes mellitus (WHO 2013):

- TYPE 1 Diabetes Mellitus results from the pancreas' failure to produce enough insulin due to loss of beta cells. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or ‘juvenile diabetes’. The cause is unknown.
- Type 2 Diabetes Mellitus begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the disease progresses, a lack of insulin may also develop. This form was previously referred to as "non-insulin dependent diabetes

mellitus" (NIDDM) or "adult-onset diabetes" The most common cause is a combination of excessive body weight and insufficient exercise.

- Gestational diabetes is the third main form, and occurs when pregnant women without a previous history of diabetes develop high blood sugar levels. Gestational diabetes usually resolves after the birth of the baby.

2.3 MEDICINAL PLANTS

A plant is said to be medicinal when one or more of its fragments have been claimed or used to cure disorders or ailments. Medicinal plants constitute as an effective source of both traditional and modern medicine. Plants have been used as sources of remedies for the treatment of many diseases since the ancient times and people of all continents especially Africa have this old tradition. Despite the remarkable progress in synthetic organic medicinal products of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants (Calixto, 2005). Medicinal plants from ancient time have been used for preventive and curative measures for different ailments and diseases due to their readily availability and low cost of preparation. Large population of humans still rely on plants as a source of medicine (Ogunrinola *et al*., 2018).

However, medicinal plants used in traditional medicine are still under investigation (Kirby, 1996). In developing countries, such as West Africa, the production of new drugs is not often affordable. Therefore, about up to 80% of the population uses medicinal plants as remedies for various types of ailments (Kirby, 1996; Hostellmann and Marston, 2002). The use of plants in the tropical and subtropical regions is diversified and most of the uses are for medicine, source of food, clothing and shelter. All over the world, several ethnobotanical studies focusing on medicinal plants have been documented (Ekpendu *et al*., 1998; Balansard and Timon, 2000; Singh and Singh, 2001; Kang *et al*., 2004; Cox, 2005; Kumar *et al*., 2005; Pei, 2005). Documentation of medicinal uses of African plants is becoming increasingly

urgent because of the rapid loss of the natural habitat for some of these plants due to anthropogenic activities *(pollution).* It has been proved from previous researches that medical plants have 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 possessing antioxidant activity may reduce oxidative stress and improve the functions of various organs affected by hyperglycaemia. *Vernonia amygdalina* contain natural antioxidants which have the potential to act as antioxidants against aqueous radicals and reactive species ions (Adeoye *et al*., 2018). However, population increase, inadequate drug supply, excessive cost of treatment and side-effects of several conventional drugs have increased the dependence on medicinal plant as source of medicine for a variety of ailments.

Medicinal plants are efficiently and effectively being used in most parts of the world as: hypo-lipidaemic, contraceptive, or cytotoxic, antihypertensive, treatment for skin diseases, wound healers, and hypoglycaemic. The hypoglycaemic agents have been used in the management of diabetes mellitus (Mustafa *et al*., 2016), that imputes to its pharmacological functions as an anti-diabetic, antimalarial, anti-helminth, antibiotic, treatment of diarrhoea, dysentery, fertility inducer, kidney problems, stomach discomfort, hypo-lipidaemic, and other several uses (Ogunrinola *et al*., 2019).

2.4 SOME ANTI-DIABETIC PLANTS

Acacia Arabica: This is a tree from the family *Vachellia nilotica* of the western Arab community (commonly known as gum Arabic tree, babul, thorn mimosa, Egyptian acacia or thorny acacia) is a tree in the family Fabaceae. It is native to Africa, the Middle East and the Indian subcontinent. It is also a Weed of National Significance and is an invasive species of significant concern in Australia. It can be used for medicinal purposes which can aid in diabetic research.

***Acosmium panamense*:** Native to the tropical regions of America, *Acosmium panamense* is naturally distributed in southern Mexico and all of Central America. The species is part of the evergreen tropical forests. *Acosmium panamense* is an evergreen tree that grows up to 40 m in height and 95 cm d.b.h. Its rate of growth is unknown. The trunk is straight and may have small spurs. Composed of rising branches; the crown is spread out, pyramidal, and sometimes flattened. Also applied in the research of diabetes because of its Anti-diabetic potentials (Stanley and Steyermark, 1946).

Table 1: Examples of some medicinal plants.

S/N	LOCAL NAME	SCIENTIFIC NAME	TRADITIONAL USES
1	Bitter leaf	*Vernonia amygdalina*	Treatment of diabetes
2	Garlic	*Allium sativum*	Treatment of hypoglycaemia
3	Parsley tree	*Heteromorphica arborescens*	Treatment of diabetes
4	Marijuana	*Cannabis sativa*	It has in vivo anti-diabetic properties
5	Small white albuca	*Albuca setosa*	Prevention of oxidative stress
6	Sea onion	*Albuca bracteata*	It has anti-diabetic properties
7	Muchimbi	*Strychnos henningsii*	Induces hypoglycemia
8	Herb-of-grace	*Ruta graveolens*	Decreases hepatic glucose output and ameliorating oxidative stress
9	Soda apple nightshade	*Solanum aculeastrum*	It has anti-diabetic properties
10	Wild dagga	*Leonotis leonorus*	Reduces blood glucose level.

2.5.0 VERNONIA AMYGDALINA

Vernonia amygdalina is a member of the Asteraceae family (Alhassan *et al*., 2008).The leaves of *Vernonia amygdalina* are green with a characteristic odour and bitter taste. It is known as bitter leaf due to its characteristic bitter taste and flavour (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 shrub that grows in the tropical Africa. The leaves are elliptical and up to 20 cm long. Its bark is rough. The cooked leaves are a staple vegetable in soups and stews of various cultures throughout equatorial Africa (Iloh *et al*., 2011). It does not produce seeds and has to be distributed or propagated through cutting. It is majorly used for human consumption and has to be washed to remove the bitter taste. The leaves of this herb are consumed by people but only after rinsing them thoroughly to do away with their bitter taste (Iloh *et al*., 2011).

Medicinally, the leaves are widely used for fevers and are known as quinine substitute (Challand and Willcox, 2009). It is used to prepare cough medicine in Ghana (Akinpelu, 1991) and the root infusion is taken in Nigeria as an antihelminthic as well as for enteritis and rheumatism (Ainslie, 1937).The leaves have found relevance in traditional folk medicine as anti helmintics, antimalarial, antimicrobial, anticancer and as a laxative herb (Akah*et al.*, 2009). Pharmacological studies have shown that the leaf extract of *V. amygdalin* has both hypoglycaemic and hypolipidemic properties in experimental animals and so could be used in the management of diabetes, hypertension and other diseases (Akah and Okafor, 1992).The leaves of the plant may be consumed either 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 illnesses (Arhoghro *et al.*, 2009). *V. Amygdalina* is rich in minerals sources such as K+, Ca2+, Na+, P3-, Mg2+, Mn2+, Fe2+, Cu2+ and Zn2+ (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 used traditionally to cure a number of ailments and its actions include, 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 reported to have analgesic and antipyretic effects (Tekoba*et al.*, 2002). The aqueous extract of *V. Amygdalina* has also been reported to have anti-oxidant property (Nwajo and Nwokoro, 2004).

2.5.1 TAXONOMY OF VERNONIA 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*

2.5.2 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

2.5.3 USES OF VERNONIA AMGYDALINA

This plant contains complex active components that are useful in pharmacologically areas. It can be used as an active anticancer, antibacterial, antimalarial and anti-parasitic agent (Ugochukwu *et al*., 2003). In ethno medicine, the roots and the leaves are used to treat fever, hiccups, kidney problems and stomach discomfort (Kadiri, and Olawoye, 2016). It has also been documented that *V. amygdalina* has been used traditionally in the treatment of blood clotting and has elicited a substantial reduction in the level of glucose in the blood (Ugochukwu. *et al* 2003). It had been reported that *V. amygdalina* has hypoglycaemic activity. They observed a close-dependent reduction in fasting blood sugar level in streptozotocin-induced diabetic rats after treatment with different concentrations of the aqueous leaf extracts. It had also been demonstrated that *V. amygdalina* leaf extracts as a DNA-damaging of anticancer agent in the management of breast cancer (Ugochukwu., *et al* 2003). 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 soup'. Some authors observed that the leaf extract of *Vernonia amygdalina* could protect the heart against impairment and complete 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 possess broad-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).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

Plant sample, Methanol, muslin cloth, Beakers, Micro pipette, Spectrophotometer, Water bath, Funnel, Rotary evaporator, Oven, Homogenizer, Formalin, Phosphate buffer, distilled water, Syringes, Cannula, 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.

3.2.0 METHODS

3.2.1 COLLECTION OF PLANT MATERIAL

V. amygdalina (bitter leaf) plant was used for this study. The bitter leaf sample (*V. amygdalina*) was collected in February, 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 stem, air-dried for two weeks, oven dried for 30minutes at 100^{O}C and blended into a powdery form with the use of a mechanical blender. The blended sample was weighed and stored in an air-tight jar. Eighty-four grams (84 g) of the blended powder was weighed into three (3) jars and was macerated in methanol with occasional shaking at room temperature for 72hours twice. Filtration was done using a 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 were kept in the oven (40°C) for complete dryness of the

methanol extracts and were then stored in a refrigerator at −4°C and the percentage yield of the extracts was calculated.

3.2.3 QUALITATIVE PHYTOCHEMICAL ANALYSIS

The methanol extract was tested and tried 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)

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

0.5g of crude 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 0f 10% ammonia solution was added. The appearance of pink colour indicated the presence of glycosides

Test for saponin (Froth test)

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

Test for flavonoid (Alkaline test)

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

Test for tannin

0.5g of crude extract 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 crude extract 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 colour indicated the presence of amino acids

Test for phytosterol (Libermann-Burchard's test)

0.5g of crude extract 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 colour (violet to blue) indicated the presence of steroids.

Test for polyphenol

0.5g of crude extract was dissolved in 5ml of distilled water. 1ml of 2% $FeCl_3$ solution and 1ml of 1% potassium ferri-cyanide solution were added. The formation of green-blue colour 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

0.5g of extract was boiled with 10ml of H2SO4 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 colour indicated the presence of anthraquinone.

3.3 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 were 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).

3.4 ANIMAL EXPERIMENTATION

Twenty-five (25) male wistar rats weighing (115-230g) were randomly arranged in well ventilated cages under controlled conditions of 12hours light/dark cycle at the Animal house of the Department of Biological Sciences in 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).

3.5 EXPERIMENTAL DESIGN

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

Group I: Normal control; water and feed

Group II: positive control; STZ (streptozotocin) induced diabetic wistar rats

Group III: a single dose of STZ (45mg/kg)induced diabetic wistar rats were treated with Metformin

Group IV: a single dose of STZ (45mg/kg) induced rats were treated with 150mg of *V. amygdalina* plant extract

Group V: STZ and 300mg of *V. amygdalina* plant extract

3.6 INDUCTION OF DIABETES

Animals of Groups II-IV were weighed and their fasting blood glucose levels were determined before induction. Diabetes was induced in the rats by intraperitoneal injection of freshly prepared streptozotocin (STZ) dissolved in citrate buffer (0.1 M pH 4.5) to overnight fasted rats at a dose based on their body weights. Animals were checked for successful induction of diabetes after 48 hours 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). Hyper-glycaemia was confirmed four days after injection and animals with blood glucose > 200 mg/dL were classified as diabetic.

3.7 DRUG ADMINISTRATION

Metformin was administered to experimental rats of group 3 and *V. amygdalina* methanol leaf extract was dissolved in distilled water and administered orally on doses based on body weight to the experimental rats of group 4 and 5. Drug administration was done for 7days. Groups 1 and 2 were not administered any drugs because they acted as control measures, with group 1 being the normal control and group 2 being the negative control.

3.8 COLLECTION AND HOMOGENIZATION OF ANIMAL ORGANS

After the seven day-administration of the methanol leaf extract of the plant and the metformin, the animals were sacrificed by cervical dislocation under anaesthesia using 10% chloroform. The organs (liver and kidney) were removed and homogenized using a laboratory mortar and pestle. 5ml of buffer solution (In molarity and pH) was added to the homogenate to mimic a favourable pH condition for the organs in order to avoid cell lysis before homogenization. After homogenization, the homogenized organs were collected in plain bottles(liver in red covers, kidney in blue covers) and centrifuged at 4000 rpm for 10 minutes at 37^{O}C and the supernatants were obtained.

3.9.0 BIOCHEMICAL ASSAY

Kidney and Liver supernatants were used for the assay of creatinine, total protein, total bilirubin, Aspartate amino transferase (AST), Alanine transferase (ALT), Gamma-Glutamyl transferase (GGT), and Lactate dehydrogenase (LDH) levels using standard laboratory kit from Randox laboratories, UK.

3.9.1 Aspartate amino transferase (AST)

Table 4: 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

250μl of reagent 1 was added to 50μl 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.

b. Procedure for sample

50 μl of centrifuged organs supernatants were added to 250μl 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 20mins 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 5minutes.

3.9.2 Alanine amino transferase (ALT)

Table 5: 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

250μl of reagent 1 was added to 50μl 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.

b. Procedure for sample

50 μl of blood plasma was added to 250μl 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 20mins 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 5minutes.

3.9.3 Gamma Glutamyl Transferase (GGT)

Table 6: 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.

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

3.9.4 Lactate Dehydrogenase (LDH)

Table 7: 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.

20μl of centrifuged organs supernatants were added to 500μl of the reagent at 25℃. The solution was mixed and the absorbance was read at 340nm 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 Total Protein (TP)

Table 8: 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

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 centrifuged organs supernatant were 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.

3.9.6 Creatinine (CREA)

Table 9: 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 were 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.

3.10 STATISTICAL ANALYSIS

The statistical analysis was done using Graph pad prism 6.0. 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 Table 10: PHYTOCHEMICAL SCREENING

	PHYTOCHEMICALS	**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

4.2.1 GC-MS chromatogram of methanol leaf extract of *V. amygdalina*

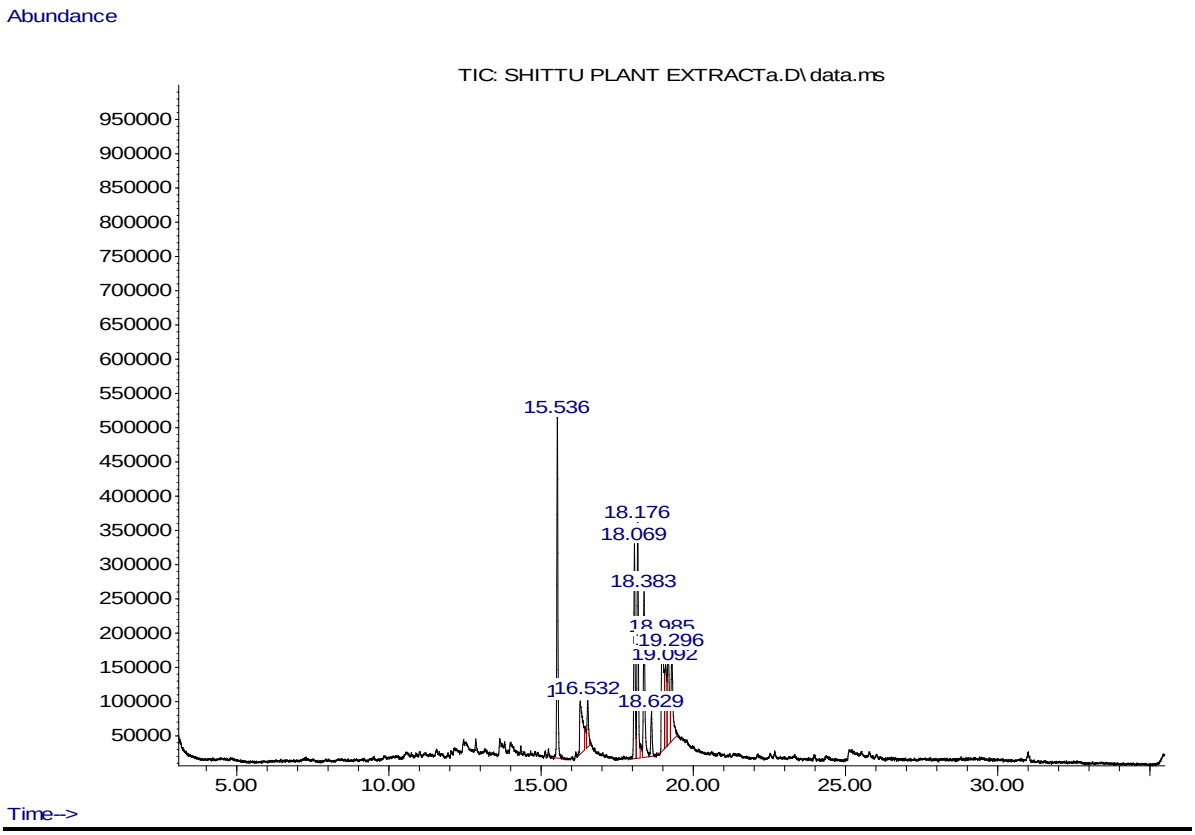

Figure 1: chromatogram of methanol leaf extract of *V. amygdalina*

Table 11: List of bioactive components in methanol leaf extract of *V. amygdalina* identified by GC-MS analysis.

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	Hepta-decanoic 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 CHROMATOGRAM AND STRUCTURES OF BIOACTIVE COMPONENTS

IN*V. AMYGDALINA*

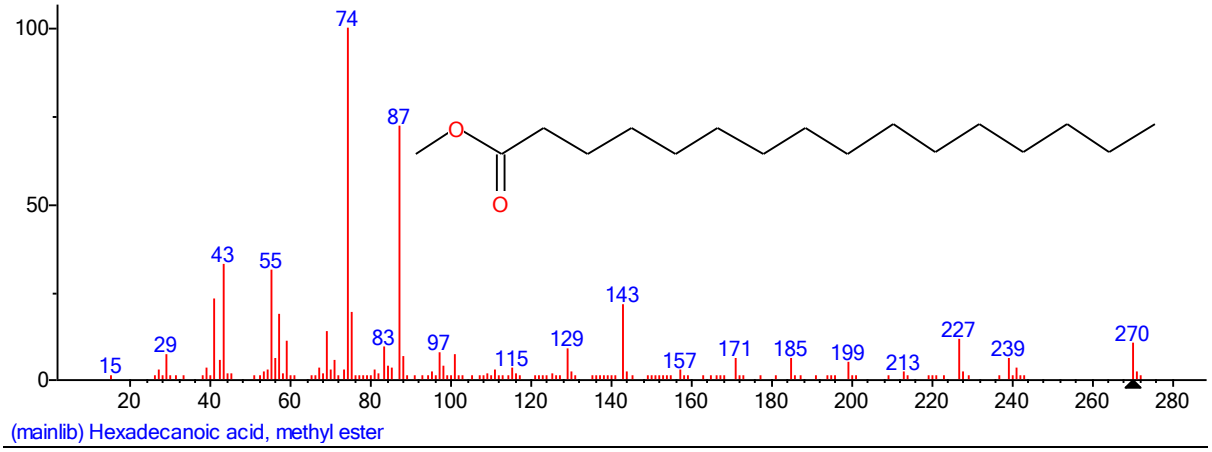

Figure 2: Chromatogram and structureof hexa-decanoic acid, methyl ester

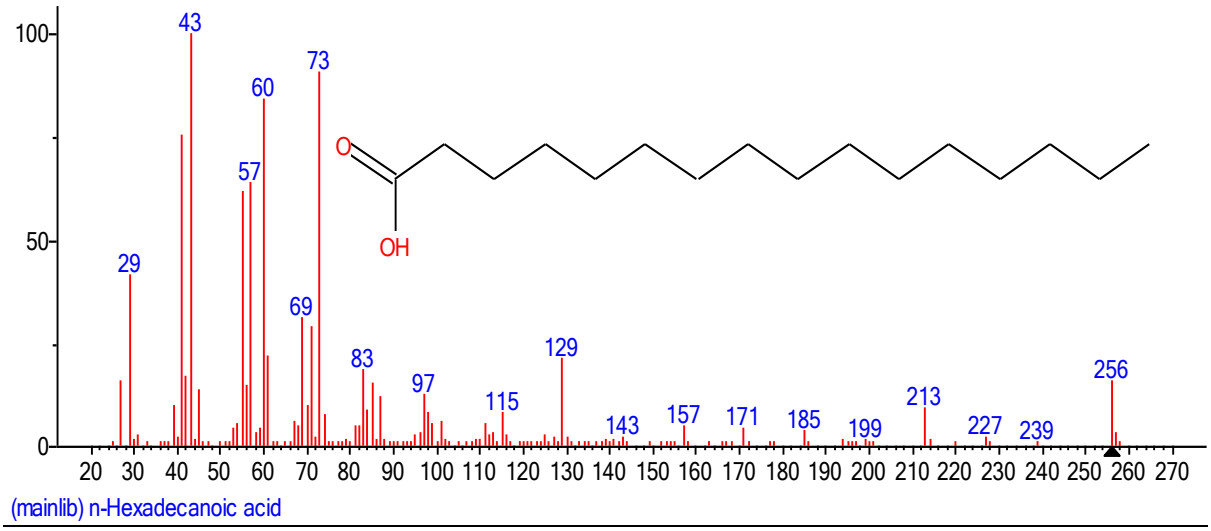

Figure 3: Chromatogram and structure of n-Hexadecanoic acid

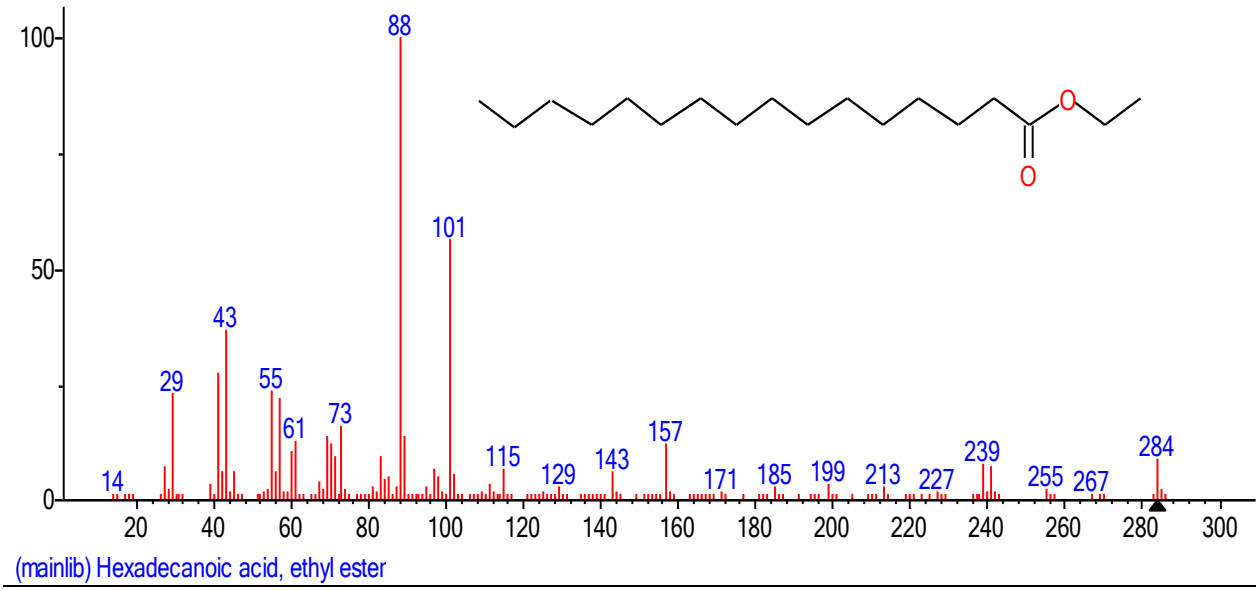

Figure 4: Chromatogram and structure of hexadecanoic acid, ethyl ester

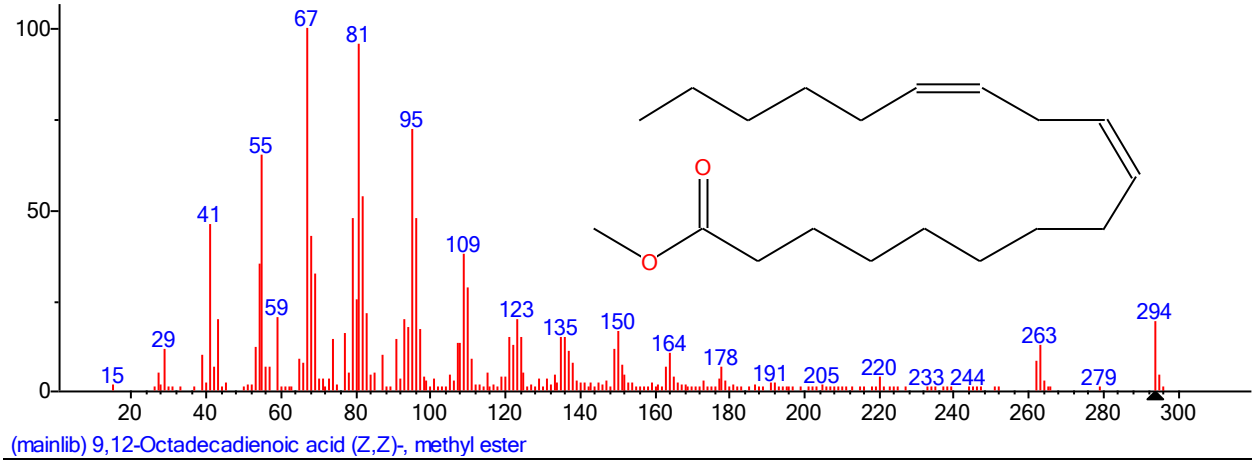

Figure 5: Chromatogram and structure of 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester

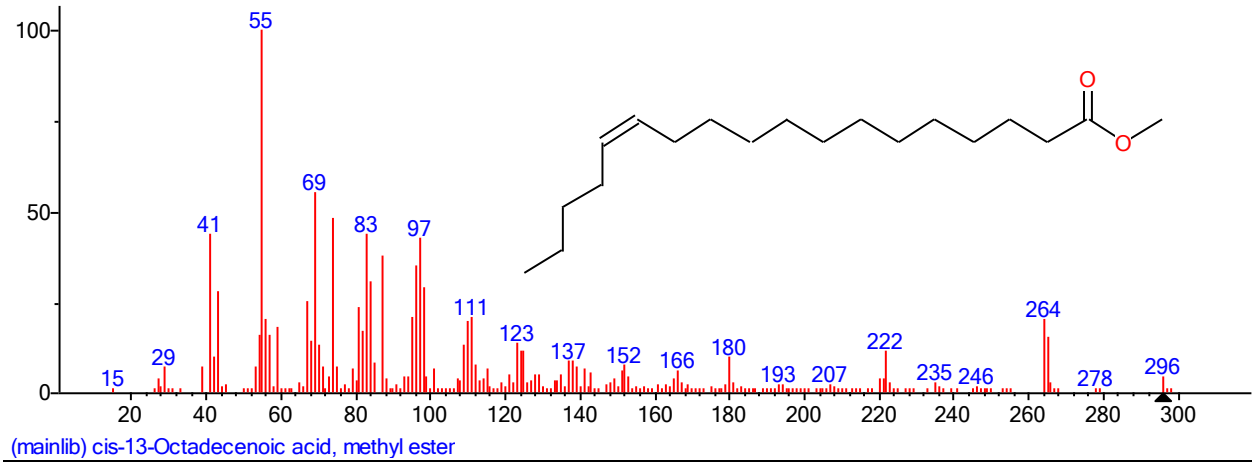

Figure 6: Chromatogram and structure of cis-13-Octadecenoic acid, methyl ester

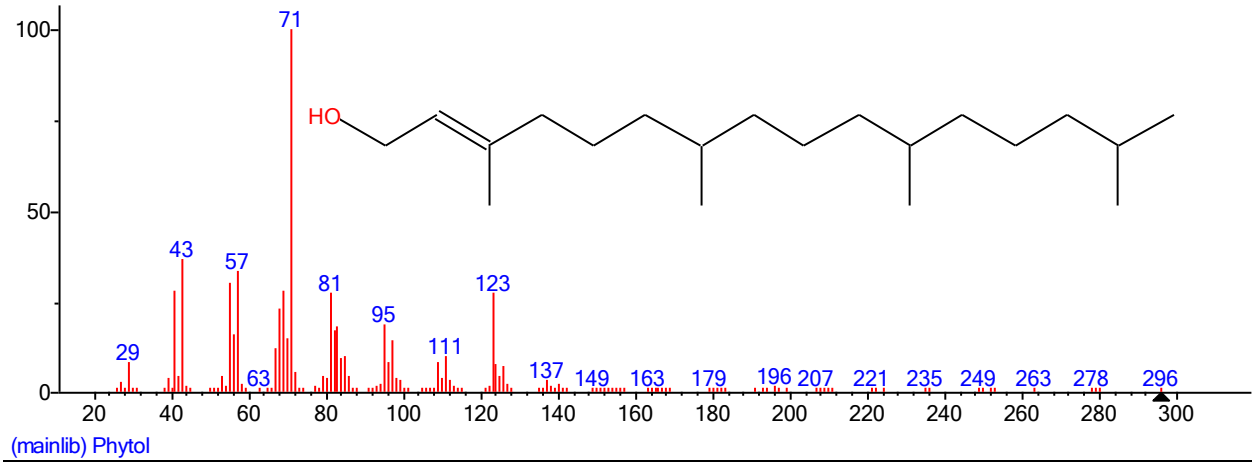

Figure 7: Chromatogram and structure of phytol

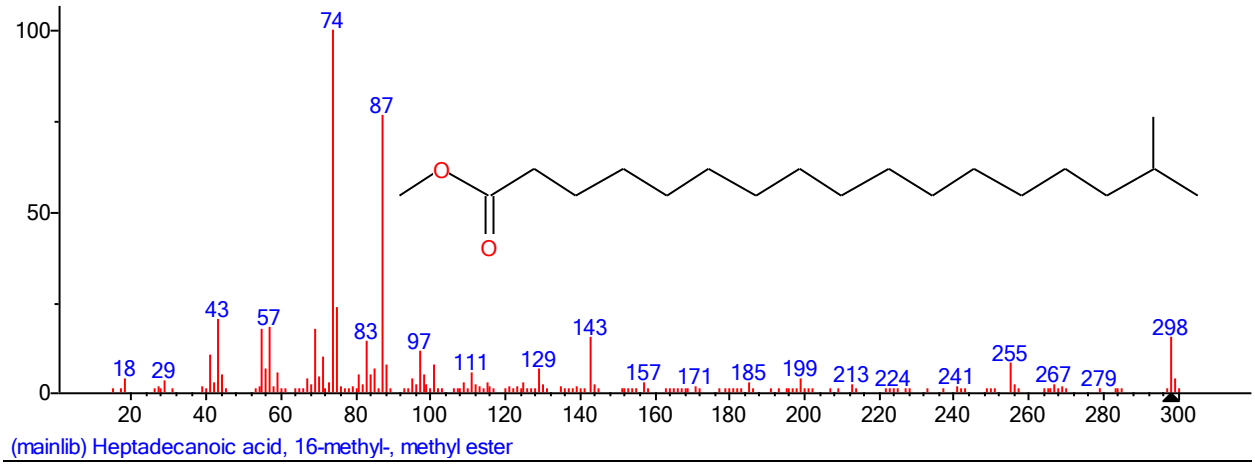

Figure 8: Chromatogram and structure of heptadecanoic acid, 16-methyl-, and methyl ester

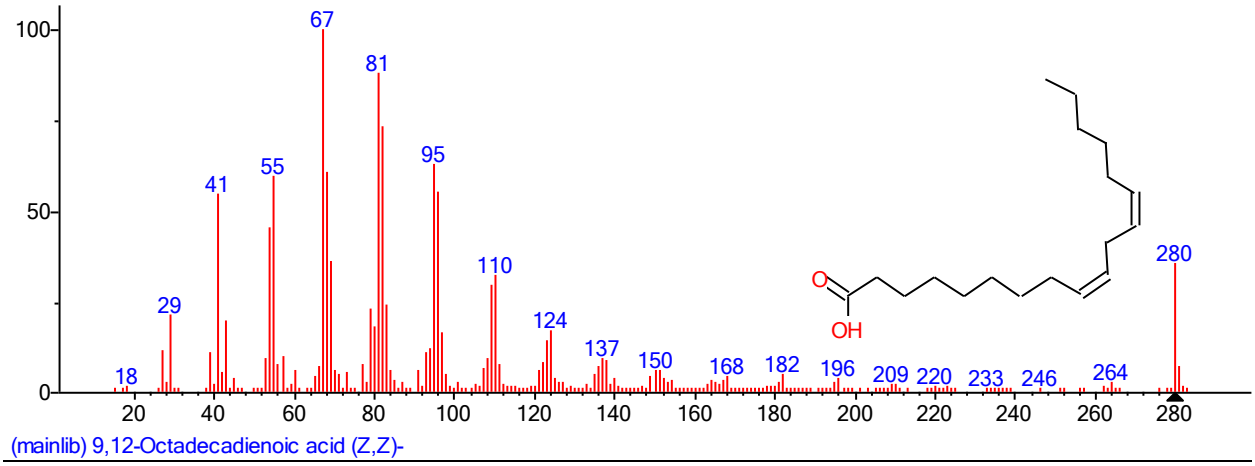

Figure 9: Chromatogram and structure of 9, 12-Octadecadienoic acid

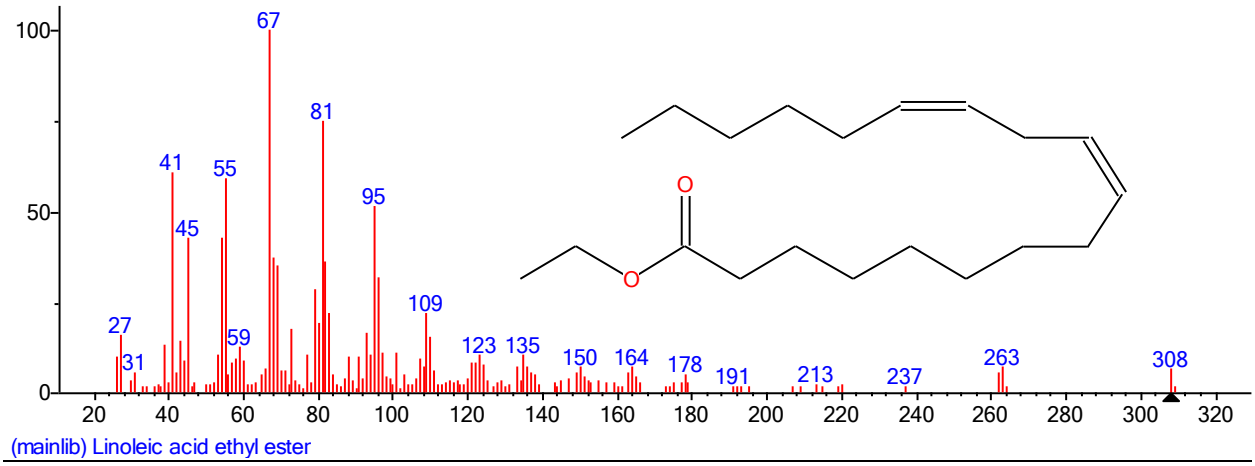

Figure 10: Chromatogram and structure of Linoleic acid ethyl ester

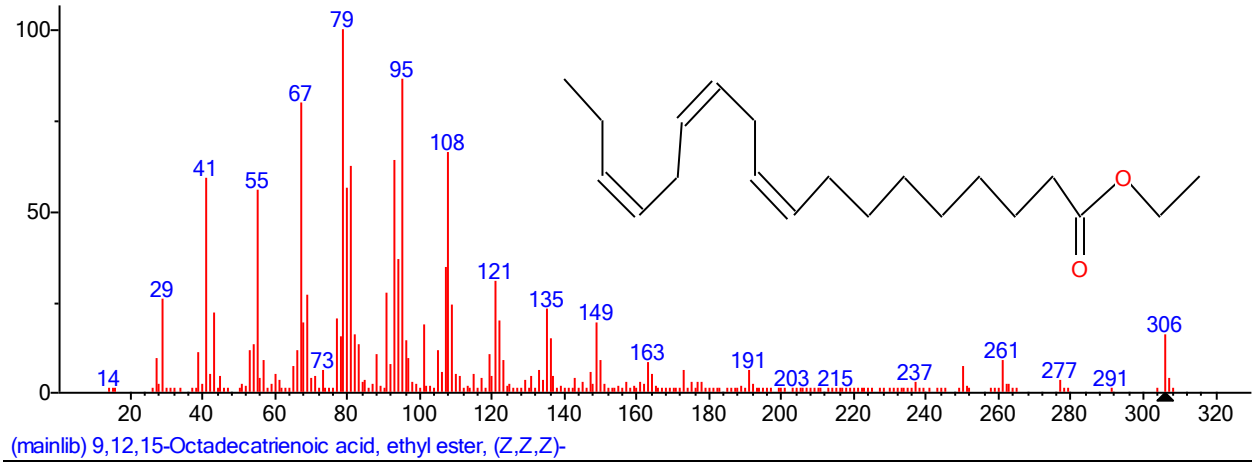

Figure 11: Chromatogram and structure of 9, 12, 15-Octadecatrienoic acid, ethyl ester (Z, Z, Z)

4.3 BODY WEIGHT OF ANIMALS

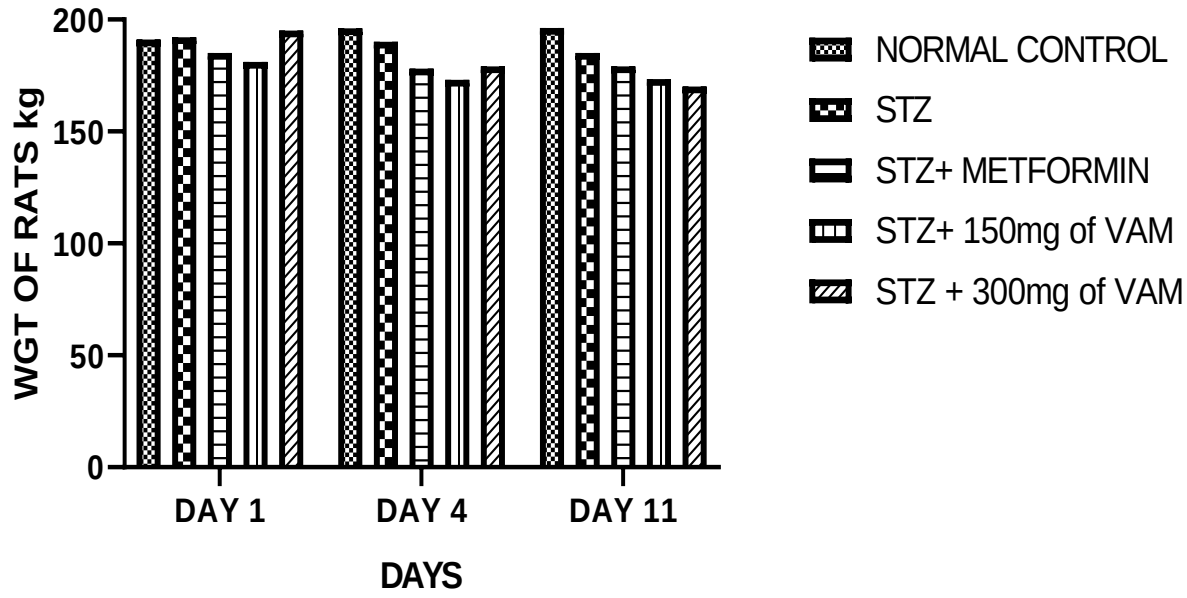

Figure 12: Effect of methanol leaf extract of *V. amygdalina* on the average body weight of streptozotocin-induced diabetic rats. Values represent mean ± SD of 5 animals per group. There was a significant parameter ($P<0.05$) in column factor and insignificantly different ($P>0.05$) in row factor.

4.4 GLUCOSE CONCENTRATION

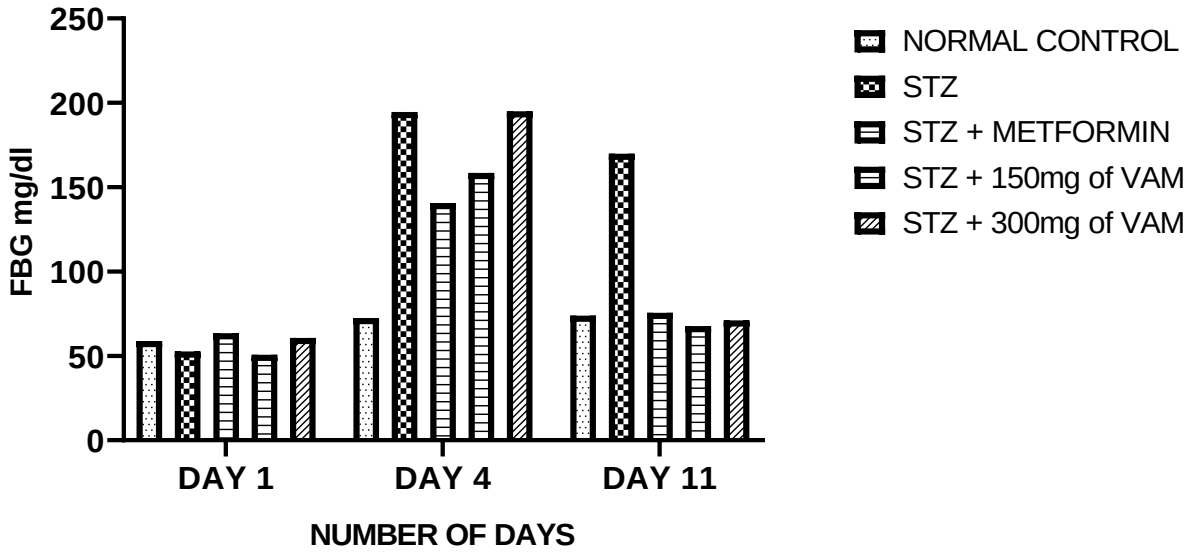

Figure 13: Effect of methanol leaf extract of *V. amygdalina* on fasting blood glucose level of streptozotocin-induced diabetic rats. Values represent mean ± SD of 5 animals per group. There was a significant difference of ($P<0.05$).

4.5 RESULT OF BIOCHEMICAL ASSAYS

4.5.1 ENZYME ASSAYS FOR LIVER

TABLE 12: Effect of methanol leaf extract of *V. amygdalina* on liver enzymes of streptozotocin-induced diabetic rats

GROUPS	**AST (U/L)**	**ALT (U/L)**	**GGT (U/L)**	**LDH (U/L)**
NORMAL CONTROL	44.25 ± 15.78	42.00 ± 2.92	9.84 ± 2.57	12.38 ± 5.05
STZ 45mg/kg	43.25 ± 17.45	36.25 ± 3.04	9.55 ± 6.08	10.32 ± 2.66
STZ + METFORMIN	12.25 ± 0.75	25.50 ± 8.47	11.58 ± 0.47	10.32 ± 1.19
STZ + 150mg/kg of VAM	15.80 ± 5.08	48.60 ± 1.66	11.58 ± 3.17	15.68 ±2.02
STZ + 300mg/kg of VAM	13.60 ± 1.99	44.00 ± 5.46	7.87 ± 4.15	15.68 ± 4.78

Values represent mean ± SEM of 5 animals per group. There was a significant parameter ($P<0.05$) in column factor and insignificantly different ($P>0.05$) in row factor.

4.5.2 ENZYME ASSAYS FOR KIDNEY

TABLE 13: Effect of methanol leaf extract of *V. amygdalina* on kidney enzymes of streptozotocin-induced diabetic rats

GROUPS	AST (U/L)	ALT (U/L)	GGT (U/L)	LDH (U/L)
NORMAL CONTROL	13.00 ± 0.95	6.40 ± 1.60	10.89 ± 7.86	17.33 ±2.74
STZ 45mg/kg	13.75 ± 3.01	5.00 ± 1.00	140.12 ± 135.11	25.79 ± 7.02
STZ + METFORMIN	13.75 ± 0.75	7.00 ± 1.00	8.68 ± 1.67	22.70 ± 10.85
STZ + 150mg/kg OF VAM	15.40 ± 1.47	14.60 ± 2.60	9.26 ±3.17	18.16 ± 5.92
STZ + 300mg/kg OF VAM	22.80 ± 6.15	7.20 ± 1.96	15.52 ± 1.82	22.29 ± 7.79

Values represent mean ± SEM of 5 animals per group. There was a significant parameter ($P<0.05$) in column factor and insignificantly different ($P>0.05$) in row factor.

4.5.3 RESULTS OF OTHER BIOCHEMICAL ASSAYS FOR KIDNEY

TABLE 14: Effect of methanol leaf extract of *V. amygdalina* on biochemical parameters of the kidney of streptozotocin-induced diabetic rats

GROUPS	CREATININE (G/DL)	TOTAL BILIRUBIN (G/DL)	TOTAL PROTEIN (G/DL)
NORMAL CONTROL	0.46 ± 0.10	0.55 ± 0.88	2.73 ± 0.63
STZ 45mg/kg	2.30 ± 1.08	1.98 ± 0.90	2.70 ± 0.64
STZ + METFORMIN	2.67 ± 0.68	1.42 ± 0.79	2.66 ± 0.28
STZ + 150mg/kg OF VAM	2.74 ± 1.15	0.28 ± 0.78	2.61 ± 0.79
STZ + 300mg/kg OF VAM	0.72 ± 0.14	0.28 ± 0.14	3.31 ± 0.38

Values represent mean ± SEM of 5 animals per group. There was a significant parameter ($P<0.05$) in column factor and insignificantly different ($P>0.05$) in row factor.

4.5.4 RESULTS OF OTHER BIOCHEMICAL ASSAYS FOR LIVER

TABLE 15: Effect of methanol leaf extract of *V. amygdalina* on the biochemical parameters of the liver of streptozotocin-induced diabetic rats

GROUPS	**CREATININE (G/DL)**	**TOTAL BILIRUBIN (G/DL)**	**TOTAL PROTEIN (G/DL)**
NORMAL CONTROL	1.07 ± 0.33	4.39 ± 1.35	19.26 ± 1.33
STZ 45mg/kg	1.92 ± 0.32	5.43 ± 1.32	17.30 ± 1.15
STZ + METFORMIN	3.38 ± 0.90	2.90 ± 1.00	15.20 ± 1.14
STZ + 150mg/kg OF VAM	0.92 ± 0.29	9.63 ± 2.34	15.55 ± 0.90
STZ + 300mg/kg OF VAM	2.21 ± 1.16	4.38 ± 2.61	13.27 ± 1.31

Values represent mean ± SEM of 5 animals per group. There was a significant parameter ($P<0.05$) in column factor and insignificantly different ($P>0.05$) in row factor.

4.5.5 WEIGHT OF ANIMAL ORGANS

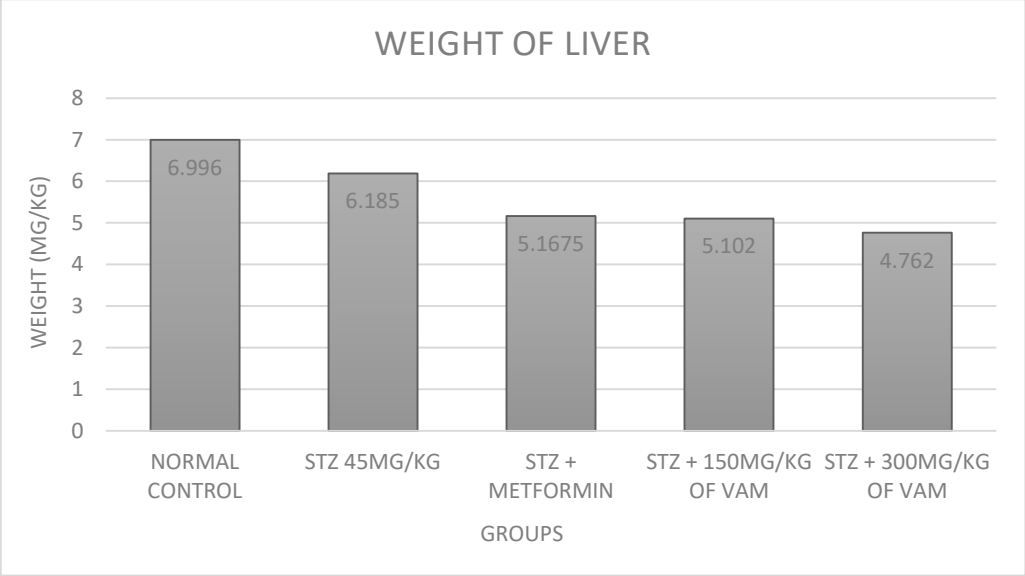

Fig 14. Effect of methanol leaf extract of *V. amygdalina* on the average weight of liver of streptozotocin-induced diabetic rats

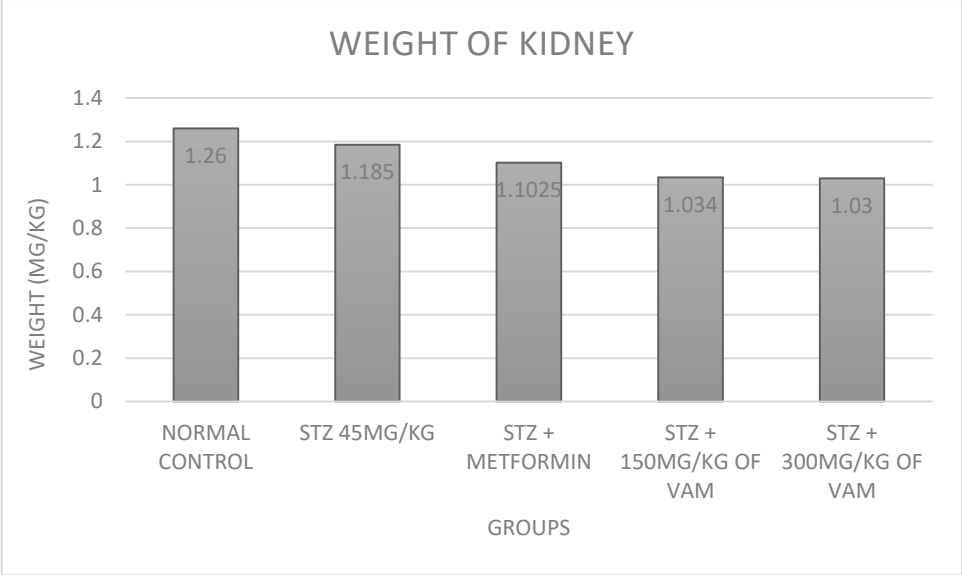

Fig 15. Effect of methanol leaf extract of *V. amygdalina* on the average weight of kidney of streptozotocin-induced diabetic rats

CHAPTER FIVE

5.0 DISCUSSION

The effects of methanol *V. amygdalina* leaf extract on the liver and kidney of STZ induced diabetic wistar rats was investigated in this study. Diabetic-induced condition led to hyperglycaemia. Twenty-four hours after STZ administration, all the rats treated with STZ displayed hyperglycaemia, weakness, hyperlipidaemia (Adewole *et al*., 2009) and significant ($p<0.05$) loss of body weight.

At the beginning of this study, the baseline weights were similar and glucose level of all the rats was normal in all groups. At day four after the administration of STZ the glucose level of each rat in the groups was high compared to the normal control group. There was also a decrease in the body weight of animals due to hyperglycaemia. After the administration of VAM for 7days the glucose level of the experimental rats dropped drastically to the normal glucose level. However, VAM treatment significantly reduced the blood glucose concentration of the VAM treated groups D and E. Creatinine, Bilirubin (BIL), and Total protein (TP) were significantly ($P<0.05$) reduced in metformin and STZ treated groups compared to the normal control groups. ALT, AST, GGT and LDH improved toward normal level after treatment with VAM in rats. There was a decrease in weight of organs of groups 3, 4 and 5 compared to that of the normal control.

The results suggested that *Vernonia amygdalina* methanol leaf extract caused a significant decrease in FBG levels of Groups 4 and 5 animals, and Metformin caused a low amount of decrease in Group 3 animals. *Vernonia amygdalina* methanol leaf extract also caused a significant increase in weight of Groups 4 and 5 animals as compared to Groups 3 and 2. Relative liver and kidney weights were decreased as compared to that of the control.

In conclusion, the effect of *Vernonia amygdalina* methanol leaf extract shown in the present study implies a pharmaco-therapeutic and protective role in liver and kidney functions in

Streptozotocin induced diabetic wistar rats. The results of the phytochemical screening showed the presence of some basic phytochemical components in *Vernonia amygdalina.*

The GCMS analysis displayed hexa-decanoic acid, methyl ester, cis-13Octadecenoic acid, methyl ester, 9,12Octadecadienoic acid (Z, Z), and Phytol as the organic components with the highest percentages. The phytochemical screening displayed all components as positive excluding Anthraquinone which was tested negative, indicating the absence of the component in the methanol leaf extract of *Vernonia amygdalina*.

5.1 CONCLUSION

In conclusion, the effect of the methanol leaf extract of V. amygdalina shown in the present study indicates a chemotherapeutic effect on the organs of streptozotocin induced diabetic wistar rats. Results proposed that *V. Amygdalina* possesses hypoglycaemic properties which have a significant effect on the rat liver and kidney functions. *V. amygdalina* is also capable of stabilizing other biochemical and haematological abnormalities associated with diabetes mellitus and thus could be prescribed as main therapy for diabetes mellitus. The mechanism of action of *V. amygdalina* methanol leaf extract on excretory functions of the kidney and liver function is not fully understood. It may therefore be necessary to investigate further on the effect of the plant on kidney and liver functions of animals.

5.2 RECOMMENDATION

Vernonia amygdalina is to be recommended because of its high anti-diabetic potential, its great medicinal relevance in the treatment of Diabetes mellitus and some oxidative stress-related ailments. And its ability to significantly reduce fasting blood glucose (FBG) levels by a large percentage compared to other anti-diabetic plants. *Vernonia amygdalina* also helps to improve plasma and pancreatic insulin contents respectively and for this reasons, this plant is of very high medicinal purpose and importance and should be recommended in Diabetic researches to help improve the standard of human life.

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