

**ANTIDIABETIC POTENTIALS OF THE BI HERBAL AQUEOUS ROOT EXTRACT IN  
STZ INDUCED DIABETIC MALE WISTAR RATS**

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**OCTOBER, 2025.**

## CERTIFICATION

This is to certify that this project titled “**ANTIDIABETIC POTENTIALS OF THE BI HERBAL AQUEOUS ROOT EXTRACT IN STZ INDUCED DIABETIC MALE WISTAR RATS**” was carried out by **Peace OMORODION**, with matriculation number **LSC2007340**, of the Department of Science Laboratory Technology (Physiology/Pharmacology), Faculty of Life Sciences, University, Benin City, Edo state, Under the supervision of DR. B. O. Gabriel.

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## **DEDICATION**

This work is dedicated to God almighty

## **ACKNOWLEDGEMENT**

I AM GRATEFUL TO God Almighty for his grace upon my life and strength to complete this project work and his love for me throughout my study.

To my supervisor Dr Benjamin .O. Gabriel , I wish to express my sincere and utmost gratitude for his words of encouragement and guidance for taking out time to ensure that this work was a success.

My gratitude also goes to the Head of Department, Prof. J. O.OSARUMWENSE and my lecturers for their invaluable contribution to my academic journey.

To my parents (MR and MRS Omorodion), Daddy Ferdinand, my lovely siblings and to Isaac Osahon Egbon, may God reward you all for constantly paving the way for me throughout my stay at the University of Benin, I appreciate all your efforts, resources and support.

I sincerely extend my heartfelt gratitude to my amazing friends who contributed immensely to this work: Imadayon Sandra, Annabel, Osato, Uwa, Ivy, Harrietta, Thelma, Mary ,Eugene, Glory,Grace, Onoriode, Steph, Success, Princess, Kosi, Ifeanyi, Genevieve, Frank and Elliot. You are all truly wonderful, and I pray that God blesses you all abundantly.

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## ABSTRACT

This study investigated the antidiabetic potential of a bi-herbal aqueous leaf extract in streptozotocin (STZ)-induced diabetic male Wistar rats. Thirty-two rats were divided into six groups: normal control, diabetic untreated control, a group treated with glibenclamide (10 mg/kg), and three groups treated with the bi-herbal extract at 50, 100, and 200 mg/kg. Treatments were administered orally for 14 days. Blood glucose levels and lipid profiles were monitored. The results showed that the bi-herbal extract significantly ( $p < 0.05$ ) reduced blood glucose levels in a manner comparable to glibenclamide. Furthermore, the extract significantly ameliorated diabetes-induced dyslipidemia, as evidenced by reduced levels of total cholesterol, triglycerides, and Low-Density Lipoprotein (LDL) in the treated groups compared to the untreated diabetic control. The study concludes that the bi-herbal extract possesses significant antihyperglycemic and lipid-lowering properties, validating its traditional use in diabetes management.

## CHAPTER ONE

### INTRODUCTION

#### 1.0 BACKGROUND OF THE STUDY

Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain (Khumalo *et al.*, 2022). The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed. The World Health Organization estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs (Nsagha *et al.*, 2020). Medicinal plants represent a consistent part of the natural biodiversity endowment of many countries in Africa (Khumalo *et al.*, 2022). In addition, modern pharmacopoeia still contains at least 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants. Interest in medicinal plants as a re-emerging health aid has been fueled by the rising costs of prescription drugs in the maintenance of personal health and well-being and the bioprospecting of new plant-derived drugs (Rizvi *et al.*, 2022). Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies. The medicinal properties of plants could be based on the antioxidant, antimicrobial antipyretic effects of the phytochemicals in them (Nwozo *et al.*, 2023).

According to studies, traditional medicines are the main treatment for 80% of people in underdeveloped nations for a variety of illnesses (Ekor, 2014). Traditional remedies made from plants are the most often utilized treatment for a variety of illnesses worldwide. In many underdeveloped nations, these traditional medicines represent a significant part of primary

healthcare. Tanzania is one of the nations where the vast majority of people rely on traditional medicine to treat their illnesses, including diabetes (Valdez-Solana *et al.*, 2015). According to Lunyera *et al.* (2016), 77% of diabetic patients in Northern Tanzania treat their condition with traditional medicines. According to Maregesi (2016), diabetes patients in Northern Tanzania utilize a variety of traditional medications, many of which have not been documented and whose pharmacological characteristics in relation to blood glucose management have not been investigated. The community's sociocultural viewpoints may be responsible for the usage of traditional medicines, which may also exacerbate the consequences of illness. Common foods like fruits, vegetables, flowers, seeds, spices, and herbs are used as traditional medicines. Certain foods are useful in the prevention and treatment of a number of illnesses and offer health advantages beyond their nutritional content (McCrimmon *et al.*, 2012).

Certain medicinal plants are thought to help treat diabetes, and research has shown that some of them actually have antidiabetic qualities, including increased insulin sensitivity and hypoglycemic effects (Di Fabio *et al.*, 2016). This is frequently linked to their high concentration of alkaloids, glycosides, terpenoids, flavonoids, and phenolic chemicals, which can enhance insulin production and regulate blood sugar (Kooti *et al.*, 2016). According to Atanasov *et al.* (2015), quercetin and resveratrol, which are present in grapevine (*Vitis vinifera L.*) and onion (*Allium cepa L.*), respectively, are two substances that are known to have positive effects on the metabolism of carbohydrates. *Vernonia amygdalina* (Bitter leaf plant), is thought to contain antidiabetic properties. Following their artificial destruction by streptozotocin induction, bitter leaf extracts have been shown to aid in the regeneration of pancreatic beta cells (Patel *et al.*, 2012). It was also found that bitter leaf promoted the body's muscle and liver cells' absorption of glucose. Furthermore, it was observed that the aqueous extracts of bitter leaf significantly

reduced the blood sugar levels of both normal and diabetic rats to a level that was similar to the outcomes of the usual medication, chlorpropamide (Onyibe *et al.*, 2021).

A major global health burden, diabetes mellitus (DM) is becoming more and more common. Its numerous consequences, including coronary heart disease, nephropathy, and neurotoxicity, have been linked to steady rises in mortality. In Southeast Asian countries and Sub-Saharan African countries, the lack of resources, specialized services, and the low number of skilled health workers contributes to the steady rise of the diabetes epidemic, especially type 2 DM in adults, even though diagnosis and management of the disease have significantly improved, according to the World Health Organization (WHO) (Cousins, 2017). Therefore, the development of affordable diabetic treatments is necessary, particularly in underprivileged populations across many countries (Cousins, 2017).

#### **1.4 AIM**

To investigate the Antidiabetic potential of the Biherbal Aqueous root extract of Bitter leaf (*Vernonia amygdalina*), and Scent leaf (*Ocimum gratissimum*), in Streptozotocin (STZ) induced Diabetic male Wistar Rats.

#### **1.5 THE SPECIFIC OBJECTIVES ARE TO;**

- i. Access and check the effects on blood glucose level
- ii. Evaluate lipid profile and biochemical parameter
- iii. Investigate pancreatic histopathology

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.0 *Vernonia amygdalina* (Bitter leaf plant)

Researchers are interested in the many types of medicinal plants that are found around the world, especially those that contain a variety of health-repairing qualities (Farombi and Owoeye, 2011). Researchers are interested in these plants because of the unique physiological effects they have on human health, which are explained by the bioactive substances they contain (Ugbogu *et al.*, 2021). Furthermore, over 80% of individuals are thought to use herbal products as nutraceuticals (Thakkar *et al.*, 2020), alternative treatments, or dietary supplements (Hassan *et al.*, 2021). This is mainly due to the fact that these plants' bioactive components are responsible for their anti-inflammatory, anti-bacterial, anti-diabetic, anti-oxidant, anti-cancer, anti-asthmatic, anti-parasitic, and other properties (Tungmunnithum *et al.*, 2018). One such medicinal plant that is currently found in tropical Africa is *Vernonia amygdalina*, a perennial shrub that is also referred to as a bitter leaf plant because of the bitter flavor of its leaves. The plant, which belongs to the Asteraceae (compositae) family, typically grows to a height of 2.5–3 m as a shrub (Achuba, 2018). However, a few number of occurrences have documented tall *V. amygdalina* trees up to 7 meters (Farombi and Owoeye, 2011). The green elliptic leaves of *V. amygdalina* have a distinctive odor and a harsh flavor, and the bark is rough with deep black fissures (Akpogheli *et al.*, 2022). Other African names for *V. amygdalina* include ewuro (Yoruba), etidot (Efik), ityuna (Tiv), Congo Bololo (D. R. Congo), oriwo (Edo), onugbu (Igbo), and grawa (Amharic). According to Adebukola *et al.* (2022), bitter leaf extracts can be used as tonics to treat a wide range of illnesses and maladies, such as emesis, nausea, diabetes, anorexia, diarrhea, dysentery,

and other gastrointestinal tract issues. This is why many herbalists and traditional African physicians advise their patients to take aqueous preparations of bitter leaf to prevent certain health conditions (Usai *et al.*, 2022). Bitter leaves have a long history of culinary and traditional usage. For example, in Nigeria, the leaves are used as a green vegetable in the well-known bitter-leaf soup. To make this soup, the plant's bitter leaves are either cooked or macerated in cold water until the bitterness is manageable. Additional seasonings are then added to finish the soup (Alara *et al.*, 2019). According to Ugbogu *et al.* (2021), bitter leaf is also traditionally used as an appetizer and the aqueous extracts as digestive tonics.

The young leaves are also used in traditional medicine as expectorants, purgatives, and to help infertile women conceive. These days, people can easily use bitter leaves in their everyday meals because they are readily available as supplements. However, before utilizing bitter leaves to cure any illness, it's crucial to speak with a healthcare provider (Sofowora *et al.*, 2013). This article seeks to provide a full analysis of the bioactive components of the stated leaf, describe its biological activities and the benefits that can be acquired from drinking its juice.

## 2.1 SCIENTIFIC CLASSIFICATION OF *Vernonia amygdalina* (BITTER LEAF)

Kingdom	Plantae
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Subclass	Asteridae
Order	Asterales
Family	Asteraceae (Compositae)
Genus	<i>Vernonia</i>
Species	<i>amygdalina</i> (Achuba, 2018)



**Plate 2.1:** Bitter leaf (Edo *et al.*, 2023)

## 2.2 BIOACTIVE COMPOUNDS IN *Vernonia amygdalina*

Complex compounds called tannins (also known as tannic acid) are produced from phenolic acids. They are a class of astringent polyphenolic biomolecules that, when bound to proteins, precipitate proteins and a range of other organic materials, including alkaloids and amino acids (Edo *et al.*, 2024). According to Edo (2024), the term "tannins" itself is typically used to describe any large polyphenolic compound that contains enough hydroxyls and other suitable groups to form strong complexes with macromolecules. Numerous plant species from various climate zones and geographical locations contain tannins (Kumar *et al.*, 2017). Tannins, like many other polyphenols, have antioxidant properties that help prevent and/or repair cellular damage caused by free radical attack (Rudrapal *et al.*, 2022). Condensed tannins and hydrolyzable tannins are the two categories of tannins. An acid or an enzyme can hydrolyze hydrolyzable tannins to produce ester-like molecules, which are polymers of gallic and ellagic acids (Lisak Jakopović *et al.*, 2022). Hydrolyzable tannins have anti-inflammatory, anti-ulcerative, antioxidant, anti-cancer, and anti-angiogenic qualities (Jikah and Edo, 2023). Conversely, catechins and flavan-3,4-diols are the sources of condensed tannins, which are typically resistant to hydrolysis. Instead, when these molecules are treated with acids or enzymes, they re-decompose into phlobaphenes (Edo *et al.*, 2024). Ether or carbon-carbon bonds hold the nuclei in place in this class of tannins as well. Condensed tannins have helped animals by increasing milk and wool production and improving fertility. Condensed tannins can be found in a variety of plants, including amla, cinchona bark, male fern, areca seeds, tea leaves, wild cherry bark, and behera fruit (Edo *et al.*, 2022).

A broad class of chemical substances known as terpenoids contains unsaturated molecules composed of interconnected isoprene units. Terpenes, sesquiterpenes, and diterpene are among them (Masyita *et al.*, 2022). Terpenoids have therapeutic promise against protozoan-caused

disorders such leishmaniasis, malaria, and trypanosomiasis, according to experimental evidence from numerous bioassays (Ugbogu *et al.*, 2021). According to other investigations, terpenoids show these antiparasitic properties through other routes. These includes inhibiting mitochondrial respiration, interacting with important parasite proteins, and altering the parasite cell membrane topology (Egharevba, 2014). The terpenoids; sesquiterpene lactones (vernolide, vernolepin, vernodalinol, hydroxyvernolide, vernolic, vernodalin, vernomenin, vernomydin) are thought to be connected with bitter leaf's ability to regulate blood glucose (Owheru *et al.*, 2023).

A cyclic organic molecule with nitrogen in a condition of negative oxidation is called an alkaloid. Antibacterial, antimitotic, anti-inflammatory, analgesic, local anesthetic, hypnotic, psychotropic, and anticancer properties are just a few of the many physiological effects of alkaloids (Jan *et al.*, 2021). Alkaloids, such as morphine, quinine, ephedrine, and others, are helpful in pharmaceuticals and nutritional supplements. According to Dey *et al.* (2020), they are also an essential part of organic synthesis. Abay and his team's research revealed that bitter leaves contained 20 alkaloids. The range was between 0.03 and 34.0 percent. As a result, they proposed that bitter leaves could be very helpful in treating both human and animal diseases if consumed in large enough amounts.

According to Samje *et al.* (2014), flavonoids are a class of naturally occurring compounds with different phenolic structures. Fruits, vegetables, cereals, bark, roots, stems, flowers, tea, and wine are all sources of dietary flavonoid compounds. Flavonoids have been reported to be produced by the bitter leaf plant in response to microbial infection (Samje *et al.*, 2014). However, the flavonoids are important for human health in addition to being relevant to the plant because of their potent pharmacological effects (Mainka *et al.*, 2021). They have a number of advantageous biochemical and antioxidant properties that help prevent a number of diseases, such as cancer,

Alzheimer's disease (AD), atherosclerosis, and more (Semerdjieva and Zheljazkov, 2019). Other evidences exists which suggest that flavonoids have free radical scavenging, coronary heart disease prevention, hepatoprotective, anti-inflammatory properties and antiviral properties. Luteoin 7- $\beta$ -glucuronoside is an example of a flavonoid in bitter leaf.

Large amounts of naturally occurring compounds called saponins are present in all of the cells of leguminous plants. Their antifungal, antibacterial, and antiparasitic properties enable plants to meet their defensive requirements while fending off microbial and illness attacks. The ability of this intricate and varied group of compounds to form stable, soap-like foams and emulsions in aqueous media gives them their name. This is made feasible by their amphipathic structure, which consists of a hydrophilic glycan component and a hydrophobic aglycone backbone (Ashour *et al.*, 2019). The pharmaceutical industry has used saponins for a variety of purposes. One of these uses is as an initiative precursor for the semi-synthesis of steroidal drugs. Saponins are versatile glycosidic compounds with a number of biological properties, including anti-hyperglycemic, anti-inflammatory, hypocolesterolemic, antioxidant, and anti-tumor properties (Semerdjieva and Zheljazkov, 2019). Additionally, saponins' dioscoresides make it appropriate to utilize them in the prevention and treatment of cardiovascular and cerebrovascular illnesses. As an anti-rheumatic treatment, other saponin compounds like as dioscin, gracillin, and pseudo-protodioscin are employed (Kunnumakkara *et al.*, 2023).

## **2.3 PHARMACOLOGICAL PROPERTIES OF *Vernonia amygdalina***

### **2.3.1 ANTICANCER PROPERTIES**

With breast and prostate cancers being the most common non-skin malignancies in women and men, respectively, the burden of cancer has grown significantly on a global scale. According to Sung *et al.* (2021), breast cancer accounts for 15% of all new cases of cancer in developed nations, while prostate cancer accounts for 15.3% of all cancers in men. As a result, there is an urgent need to develop botanicals or phytotherapeutics that are effective against various cancers (Nwosu *et al.*, 2022). It is believed that bitter leaf's coumarins, edotides, flavonoids, and sesquiterpene lactones contribute to its anticancer properties.

### **2.3.2 HEPATOPROTECTIVE PROPERTIES**

Because the liver is essential to xenobiotic transformations and metabolic equilibrium, liver disorders are acknowledged as major medical difficulties (Casas-Grajales, 2015). According to one study, a diet that included the bitter leaves shielded weanling albino rats from the hepatotoxicity caused by aflatoxin B1 (Iwo *et al.*, 2017). Additionally, another study discovered that bitter leaf extracts reversed the hepatic damages that occurred in the rats in addition to providing protection against tetrachloromethane-induced hepatotoxicity (Achuba, 2018).

### **2.3.3 ANTIOXIDANT PROPERTIES**

Medicinal plants' capacity to scavenge free radicals has been used to temper the body's reactions to electron-deficient compounds like free radicals. Reactive oxygen species, also referred to as free radicals, include the hydroxyl (OH) radical, superoxide (O<sub>2</sub>), nitric oxide (NO), nitrogen

dioxide (NO<sub>2</sub>), and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). These molecules lack electrons and can readily attack body cells in their quest for them, which can result in the development of diseases (Phaniendra *et al.*, 2015). These substances have been linked to a number of pathological diseases, including lipid peroxidation-induced cell damage, DNA damage, and cellular degeneration (Sharifi-Rad *et al.*, 2021). The chemicals known as antioxidants, which are substances with enough electrons available to give to the free radicals, can fight against free radicals. Flavonoids, specifically luteolin, luteolin 7-O-βglucuronoside, and luteolin 7-O-β-glucoside, have been found to have antioxidant properties against free radicals in bitter leaves. Owoeye and Farombi (2011). When the antioxidant activity of the three flavones was compared, luteolin performed better than the other two. Moreover, bitter leaf aqueous extracts were used to investigate oxidative stress in diabetic rats. Malondialdehyde serum levels were seen to drop, indicating antioxidant properties (Owolabi *et al.*, 2011).

#### **2.3.4 CHOLESTEROL LOWERING PROPERTIES**

It is commonly recognized that dyslipidemia increases the risk of cardiovascular illnesses. Lethal adverse effects are frequently associated with synthetic pharmaceutical therapies intended to alter lipid levels (Berberich and Hegele, 2022). It became crucial to look for less hazardous substitutes that could nevertheless accomplish the same functions as their synthetic equivalents (Maertens *et al.*, 2021). Studies using bitter leaves in meals showed that they increased HDL cholesterol while decreasing blood triacylglycerol and LDL cholesterol (Abdulmalik *et al.*, 2016). Onyibe *et al.* (2021) found that bitter leaf aqueous extracts decreased triacylglycerol levels and stabilized serum cholesterol concentrations in an experiment with diabetic rats as well. With dosages ranging from 100 to 1000 mg/kg body weight, the ethanol extracts of bitter leaf were

also seen to maintain the rats' lipid profiles within the typical range. These results, along with studies of bitter leaf's antidiabetic and antioxidant qualities, imply that bitter leaf may eventually play a significant role in the treatment of chronic illnesses.

### **2.3.5 ANTIDIABETIC PROPERTIES OF *Vernonia amygdalina***

It is estimated, that about 200 million people worldwide suffer from diabetes mellitus, a metabolic disease. 366 million people are predicted to be impacted by it by 2030 (Saeedi *et al.*, 2019). The primary energy source for the body is blood glucose, which is obtained from our diet (Wang and Wang, 2017). When the body's cells resist insulin or the pancreas becomes incapable of producing adequate insulin, the cells' ability to absorb glucose is restricted, which raises blood glucose levels (Drzewoski and Hanefeld, 2021). An estimated 1.5 million deaths annually are directly related to diabetes, while an additional 2.2 million deaths are ascribed to elevated blood sugar levels (Al-Jawaldeh and Abbass, 2022). Weight gain, hypoglycemia, and a higher risk of cardiovascular death are some adverse consequences of using medications to treat diabetes mellitus. Due to the limitations of medication pharmacological therapy, complementary therapies made from plant extracts, such as bitter leaf as an anti-diabetic remedy, have become more popular. After pancreatic beta cells were intentionally damaged by streptozotocin induction, bitter leaf extracts were found to aid in their regeneration (Patel *et al.*, 2012). Bitter leaf was also found to promote the body's muscle and liver cells' absorption of glucose. Furthermore, it was shown that the bitter leaf aqueous extracts significantly reduced the blood sugar levels of both normal and diabetic rats to a level that was equivalent to that of the usual medication, chlorpropamide (Onyibe *et al.*, 2021). Rats' blood glucose levels were shown to drop when the

leaves' ethanol extracts were administered. Inform them that both polar and non-polar solvents can be used to extract the active substances that lower blood glucose (Edo *et al.*, 2023)

#### **2.4 PHYTOCHEMICAL COMPOSITION OF *Vernonia amygdalina* ROOTS**

The roots contains Steroids, Oxalate, Tannins, Flavonoids, Saponins, Phenols, Anthraquinone, Alkaloids, Polyphenols (Edo *et al.*, 2023).

#### **2.5 *Astonia boonei***

Many plants, including *Alstonia boonei*, are said to possess exceptional pharmacological qualities. Nigeria's lowlands and rainforest regions, as well as other regions of Angola, the Central African Republic, Ghana, the Democratic Republic of the Congo, and Cote d'Ivoire, are home to the huge, common deciduous medicinal tree *Alstonia boonei* De Wild (Apocynaceae) (Adotey *et al.*, 2012). It can reach a height of 40 m and a branchless height of 27 m. With high, narrow buttresses, its cylindrical bole can reach a diameter of up to 100 cm (Burkil, 1985).

In Ghana, *Alstonia boonei* is referred to as Onyame-dua in Ashanti and Emian in Cote d'Ivoire (Malan and Neuba, 2011), Ekouk in Fang, Ahun in Yoruba, Egbu or Egbu-ora in Igbo, Ukhu in Edo, and Ukpukunu in Urhobo (Majekodunmia *et al.*, 2008). It is referred known as stool wood, pattern wood, or cheese wood in English. Emien is the trade name for it. In Africa, *A. boonei*'s leaves, stem bark, root bark, and occasionally stem latex are used to cure a range of illnesses. In some regions of West Africa, its stem bark is used as an antimalarial treatment. In Ghana, it is grown because of its great ethnobotanical importance including its use as a spice crop (Opoku and Akoto, 2014).



**Plate 2.2:** *Alstonia boonei* (Edo et al., 2023)

### 2.5.1 ETHNOMEDICINAL USES

It has been demonstrated that *A. boonei* De Wild, in conjunction with the various plant parts of several other plants of pharmaceutical significance, can be used to treat a variety of illnesses (Opoku and Akoto, 2014). In many regions of West Africa, where it is found, decoctions of the leaves, stem bark, root bark, and/or latex are traditionally used either alone or in combination with other plant parts in herbal mixes as a treatment for a wide range of illnesses.

*A. boonei* leaves are mashed and applied topically in several regions of West and Central Africa to heal ulcers, rheumatic pains, muscle aches, and hypertension as well as to lessen swellings. Additionally, a leaf decoction is utilized to treat resistant malaria (Omoya and Oyebola, 2019). The bark of the stem Boonei is also used as an anti-venom for snake bites (Olanlokun and Olorunsogo, 2018; Osuntokun and Ajiga, 2020), arrow poison (Akinloye *et al.*, 2013), and to treat malaria, dizziness, impotence, breast pain, rheumatic pain, and toothache (Akinmoladun *et al.*, 2007). There have also been reports of its application in the management of asthma (Akinmoladun *et al.*, 2007), rheumatic disorders, and painful micturition. *A. boonei* stem bark preparations are used to treat postpartum hemorrhage, induce labor, and remove retained placenta. The root bark of *Alstonia boonei* has been used, over the years, in the treatment of rheumatic and breast pain (Osadebe, 2003). Numerous herbal remedies that contain either the plant parts of *A. boonei* or parts of other plants have been used to treat a variety of conditions, including cancer (Languon *et al.*, 2018), hypertension (Turkson *et al.*, 2019), gastritis, pelvic and chest pains, skin infections, and malaria and typhoid fever (Etame *et al.*, 2019). However, by evaluating the mixture, it is impossible to link either of the plant components to the observed pharmacological activity of the mixture. Additionally, some of the herbal mixes' reported

therapeutic benefits can be due to the synergy between several ingredients and cannot be directly linked to *A. boonei*

## **2.6 PHYTOCHEMICAL PROPERTIES**

Tannins, alkaloids, saponins, steroids, triterpenes, cardiac glycosides, cyanogenetic glycosides, carbohydrates, and reducing sugars are abundant in different amounts in the leaves, stem bark, and root bark of *Altonia boonei* De Wild (Osadebe, 2003; Ojo *et al*, 2014; Opoku and Akoto, 2014; Akinlawo *et al*, 2017; Omoya and Oyebola, 2019; Ajose *et al*, 2019). Certain plant sections have also been found to have notable concentrations of calcium, phosphorus, iron, salt, potassium, and magnesium (Akinmoladun *et al*, 2007). It has been demonstrated that the secondary metabolites found in root bark are comparable to those found in stem bark (Opoku and Akoto, 2014; Klu *et al*, 2016; Omoya and Oyebola, 2019). From various plant components, a number of bioactive secondary metabolites have been identified and/or extracted.

## **2.7 PHARMACOLOGICAL ACTIVITY**

### **2.7.1 ANTIOXIDANT ACTIVITY**

According to several models, *A. boonei* leaf extracts and fractions exhibit dose-dependent antioxidant activity (Omoriegie *et al.*, 2014). Two caffeic acid derivatives, 5-caffeoylquinic acid (Chlorogenic acid) and 4,5-dicaffeoylquinic acid, as well as a number of flavonoid glycosides, were isolated from the leaves and further linked to the antioxidant potentials of the leaves in certain investigations employing the DPPH free radical scavenging model (Okoye and Okoye, 2016). Similarly, Akinmoladun *et al.* (2007) found that the stem bark extracts of *A. boonei* had a low antioxidant activity when examined using the DPPH radical scavenging activity model. This suggests that the various pharmacological activities seen in *A. boonei* stem bark may be due to

synergy and the presence of other phytoconstituents, such as minerals. However, this study goes against Nkono *et al.* (2014)'s finding that the stem bark extract exhibits antioxidant activity.

In the DPPH free radical scavenging model, the root bark extract of *A. boonei* and a few isolated components also demonstrated strong antioxidant activity (Obiagwu *et al.*, 2014). Therefore, it has been proposed (Obiagwu *et al.*, 2014) that the consistent antioxidant activity of extracts of various plant sections of *A. boonei* is what gives the plant its many beneficial medicinal qualities and justifies its widespread use in the treatment of a variety of illnesses.

### **2.7.2 ANTICANCER ACTIVITY**

According to Ohiagu *et al.* (2020), methanol extract of *A. boonei* stem bark is cytotoxic to human colon cancer. Numerous studies have linked the observed cytotoxic effects to the presence of 1, 2-benzenedicarboxylic acid in the root bark extracts (Ohiagu *et al.*, 2020), echitamine, eugenol in the leaf, ursolic acid and quecertin (AlQathama *et al.*, 2020), and lupeol in the stem bark. According to a different study, extracts of *A. boonei*'s stem and root barks in CH<sub>2</sub>Cl<sub>2</sub>/MOH shown exceptional cytotoxic properties against a few human cancer cell lines. However, it was also discovered that the extract was harmful to healthy human cells, indicating that it was not selective (AlQathama *et al.*, 2020).

### **2.7.3 ANTI INFLAMMATORY ACTIVITY**

When Wister albino rats were given rat paw oedema, separate tests of the anti-inflammatory properties of the methanol extract (Iniaghe *et al.*, 2012), as well as the aqueous and ethyl acetate (Akinawo *et al.*, 2017) fractions of its leaves, revealed significant dose-dependent effectiveness. Its methanol stem bark preparations yielded similar findings (Olajide *et al.*, 2000). Furthermore,

the solvent fractions exhibit notable anti-inflammatory activity, including the n-hexane fraction (Olanlokun *et al.*, 2021) and compounds (Okoye *et al.*, 2014). From its methanol extract, the root bark's activity was also documented. These separate results might support the traditional usage of *A. boonei* De Wild to treat rheumatic, muscular, breast, and toothaches. Analysis of *A. boonei*'s methanol leaf (Iniaghe *et al.*, 2012), stem bark (Olajide *et al.*, 2000), and ethanol root bark extracts revealed a notable and dose-dependent analgesic effect in previous investigations.

#### **2.7.4 ANTIMALARIAL ACTIVITY**

Numerous studies on the antimalarial activity of *A. boonei* leaf aqueous and methanol extract showed that the antiplasmodial effect of chloroquine (Imam *et al.*, 2017) was similar to the dose-dependent chemo-suppression and cure of parasitaemia in rodents infected with *P. berghei* (Dibua *et al.*, 2013; Omoya and Oyebola, 2019). Other models were also used to confirm this activity (Dibua *et al.*, 2013). However, it was pointed out that the reported anti-plasmodial efficacy might be mostly due to synergy. Ethanol (Iyiola *et al.*, 2011); methanol (Omoya and Oyebola, 2019); and aqueous (Ebiloma *et al.*, 2012; Omoya and Oyebola, 2019) stem bark extracts produced comparable outcomes.

#### **2.7.5 ANTI HELMINTHS ACTIVITY**

Using a closely comparable adult Indian earthworm (*Pheretima posthuma*) as a surrogate model and human intestinal roundworms, the anti-helminthic activity of *A. boonei*'s roots and stem bark was examined (Klu *et al.*, 2016). The findings demonstrated that the extracts' efficacy against the examined worms varied with dose, with the stem bark extract having the highest activity. The

presence of alkaloids may potentially be the cause of the observed activity, according to the paper.

### **2.7.6 ANTIMICROBIAL ACTIVITY**

According to reports, *A. boonei* leaves and stem bark methanol (Irulandi *et al.*, 2017) and ethanol extracts exhibit mild to moderate antibacterial activity (Ajose *et al.*, 2019). An ethanol fraction of a benzene extract of *A. boonei* leaves demonstrated MIC values of 12.5 mg/mL against common pathogens, including *Proteus mirabilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus pneumoniae*, in a related investigation (Okwu and Ighodaro, 2010). The ethanol fractions, in contrast to the chloroform fractions, showed the lowest MIC values, according to findings on the optimization of the antibacterial solvent fractions of *A. boonei* stem bark extracts (Amole and Ilori, 2010; Ogueke *et al.*, 2014). According to studies on the antibacterial activity of *A. boonei*'s aqueous and ethanol root extracts, common bacterial and fungal species, including *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*, were significantly inhibited. However, compared to the aqueous root extracts, the ethanol extract was shown to have lower MIC values. .

### **2.7.7 ANTIDIABETIC PROPERTY**

Results of antidiabetic research revealed that *A. boonei*'s leaf, stem bark, and root extracts (Akinloye *et al.*, 2013; Nkono *et al.*, 2014) all exhibit a good hypoglycemic effect on rat models, with the stem bark extract exhibiting the highest activity (Osadolor *et al.*, 2015; Owolabi *et al.*, 2014).

### **2.7.8 CENTRAL NERVOUS SYSTEM EFFECT**

In a number of recent investigations, test mice were made drowsier by both water and methanol extracts of *A. boonei* leaves and stem bark (Omoya and Oyebola, 2019, Dibua *et al.*, 2013a, Idowu *et al.*, 2010). The traditional usage of *A. boonei* stem bark in the treatment of mental health issues may be explained by this, which may be connected to a central depressive action.

## **2.8 DIABETES**

Defects in the secretion and function of the pancreatic hormone insulin cause diabetes, a chronic noncommunicable disease, that is typified by persistent hyperglycemia (Kharroubi and Darwish, 2015). This collection of metabolic illnesses is regarded as one of the most important worldwide health issues. Generally speaking, diabetes mellitus is divided into three types according to its genesis and clinical characteristics: type 1, type 2, and gestational diabetes (Kumar *et al.*, 2020). Type 1 diabetes, also known as insulin-subordinate mellitus diabetes or teenage diabetes (Barnett, 2018), is a disorder where T lymphocytes destroy pancreatic  $\beta$ -cells, leaving the patient completely insulin deficient (Tomita, 2017). Five to ten percent of diabetic people have type 1 disease (Atkinson, 2012). Type 2 diabetes, sometimes referred to as adult onset diabetes or non-insulin dependent diabetes, is the most common type of the disease, accounting for at least 90% of all cases (Wu *et al.*, 2014). It is characterized by insulin resistance and relative insulin deficiency. It is thought that a combination of genetic predisposition and other environmental variables is the primary cause of type 1 diabetes (Rewers and Ludvigsson, 2016). Insulin resistance is the primary cause of type 2 diabetes (Ndisiang *et al.*, 2017). One of the main causes of diabetes is obesity (Al-Goblan *et al.*, 2014). Obesity affects over 90% of persons with type 2 diabetes. Additionally, pancreatic injury (Ewald and Hardt, 2013), and certain uncommon

hereditary types (Prasad and Groop, 2015), can result in diabetes. The consequences linked to diabetes can be avoided or postponed by early diagnosis and blood pressure, cholesterol, and sugar control. In Asian nations, diabetes is more common, especially in China and India (Ramachandran *et al.*, 2012)

Diabetes mellitus has a significant financial impact on countries, healthcare systems, and patients and their families, particularly when out-of-pocket (OOP) costs are used to pay for care (Zargar *et al.*, 2022). Low- and middle-income nations are home to about 79% of DM sufferers (Zargar *et al.*, 2022). A major medical and economic challenge to economies already hampered by communicable diseases is the sharp increase in diabetes mellitus among people living in underdeveloped countries. Natural and less intrusive products are becoming more and more popular as preventative and therapeutic treatments due to the rising global diabetes costs of over USD 700 billion (10%) (Zhang and Gregg, 2017) and the negative effects of long-term use of traditional medications. Certain natural products have been demonstrated to be reasonably priced, have few or no adverse effects, and efficiently regulate the aberration in carbohydrate metabolism, hence decreasing the decline in the general health of those who are impacted (Tabatabaei-Malazy *et al.*, 2016).

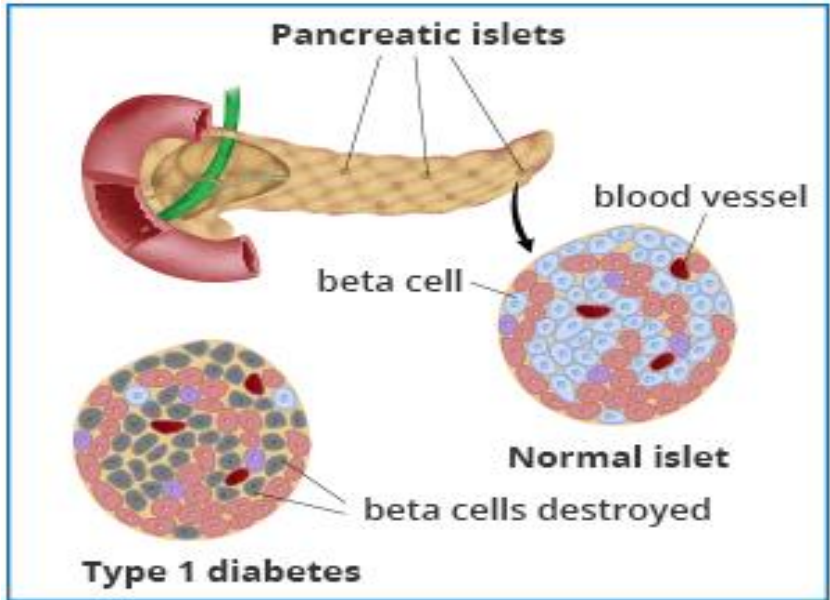


Plate 2.3: Diabetes (Tomita, 2017).

## **2.8.1 TYPES OF DIABETES**

### **2.8.1.1 TYPE 1 DIABETES**

Insulin insufficiency results from an autoimmune disease in which the body's immune system unintentionally targets and kills the beta cells in the pancreas that produce insulin. Although it can strike at any age, children, teenagers, and young adults are the most commonly diagnosed with this kind of diabetes. To control their disease, people with type 1 diabetes need to take insulin for the rest of their lives (American Diabetes Association, 2011).

### **2.8.1.2 TYPE 2 DIABETES**

The most prevalent form of diabetes, accounting for approximately 90% of all diabetes cases. T2DM is characterized by insulin resistance, where the body's cells do not respond effectively to insulin. Over time, the pancreas may also lose its ability to produce sufficient insulin to meet the body's needs. T2DM is typically associated with aging, obesity, physical inactivity, and unhealthy lifestyle factors, such as poor dietary habits (Carter *et al.*, 2024).

### **2.8.1.3 GESTATIONAL DIABETES**

A type of diabetes that usually goes away after giving delivery and appears during pregnancy. Women with a history of GDM have an increased chance of developing type 2 diabetes later in life. Both the mother and the growing fetus are at higher risk of problems when GDM is present (Carter *et al.*, 2024).

### **2.8.1.4 OTHER TYPES OF DIABETES**

These include genetic defects in beta-cell function, genetic defects in insulin action, diseases of the exocrine pancreas (e.g., cystic fibrosis), and drug- or chemical-induced diabetes (e.g., Steroid Induced Diabetes Mellitus). These less common forms of diabetes may have varying degrees of severity and treatment requirements (Carter *et al.*, 2024).

## **2.8.2 EPIDEMIOLOGY OF DIABETES**

With a sharp rise in frequency over the last few decades, diabetes mellitus poses a serious threat to global health. Around 537 million individuals (20–79 years old) worldwide had diabetes as of 2021, making up 10.5% of the adult population, according to the International Diabetes Federation (IDF). If present trends continue, this number is expected to increase to 643 million by 2030 and 783 million by 2045. About 90% of persons with diabetes who live in low- and middle-income countries go undiagnosed, indicating that the burden of diabetes is not uniformly distributed throughout the world (Aneed *et al.*, 2024).

## **2.8.3 RISK FACTORS**

Having a close family member with diabetes significantly increases an individual's susceptibility to developing the disease. Excess body fat, particularly around the abdominal region, is a major contributor to insulin resistance and the development of Type 2 diabetes. Lack of regular physical activity is linked to an increased risk of Type 2 diabetes. The risk of developing type 2 diabetes increases with age, particularly after 45 years. A diet high in processed foods, sugary beverages, and low in fiber is associated with a higher risk of developing diabetes. Certain ethnic groups, such as African Americans, Hispanics, Native Americans, and Asian Americans, have a disproportionately higher risk of developing diabetes. Women who have had gestational diabetes during pregnancy have an increased likelihood of developing type 2 diabetes later in life. Chronic stress and mental health issues can affect blood sugar levels and contribute to unhealthy lifestyle choices, increasing diabetes risk (Ahmed and Ali, 2022).

#### **2.8.4 DIAGNOSIS OF DIABETES**

Fasting Plasma Glucose (FPG) to measure blood glucose after fasting for at least 8 hours. A result  $\geq 126$  mg/dL (7.0 mmol/L) indicates diabetes, oral Glucose Tolerance Test (OGTT) where a person consumes a glucose-rich drink after fasting, and blood glucose levels are measured after 2 hours. A result  $\geq 200$  mg/dL (11.1 mmol/L) indicates diabetes. Hemoglobin A1c (HbA1c) reflects the average blood glucose levels over the past 2-3 months. A result  $\geq 6.5\%$  (48 mmol/mol) indicates diabetes. Random Plasma Glucose, a blood glucose level  $\geq 200$  mg/dL (11.1 mmol/L) at any time of the day, along with symptoms of hyperglycemia (e.g., excessive thirst, frequent urination), also indicates diabetes (Ahmed and Ali, 2022).

#### **2.8.5 TREATMENT OF DIABETES**

Effective diabetes management requires a multifaceted approach that integrates lifestyle adjustments, pharmaceutical interventions, and consistent blood glucose surveillance. Central to this is adopting a healthy lifestyle, which includes a balanced diet designed to regulate carbohydrate consumption through an emphasis on fruits, vegetables, lean proteins, and whole grains. Regular physical activity is also crucial, as it enhances the body's response to insulin and supports weight maintenance. For those who are overweight, achieving a healthier weight can lead to substantial improvements in glycemic control. Pharmacologically, treatment is tailored to the individual's needs; insulin replacement is indispensable for Type 1 diabetes and often required for advanced Type 2 diabetes. A range of oral hypoglycemic agents, such as metformin, sulfonylureas, DPP-4 inhibitors, GLP-1 agonists, and SGLT2 inhibitors, are used to manage Type 2 diabetes. Additionally, some patients may benefit from injectable therapies like GLP-1 receptor agonists to achieve optimal blood sugar levels (Nathan *et al.*, 2009).

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 APPARATUS AND EQUIPMENT

The equipment and materials used for this study include the following:

An analytical weighing balance (Ohaus Corp, Pine Brook, NJ, USA, China), mortar and pestle, rat cages, water bath, industrial blender (MODEL: KCB2239K), glucometer, automatic vernier caliper, microliter pipette, conical flask (500 ml), beaker, measuring cylinder, plain bottles, universal bottles (10 ml), syringes and needles (1, 2, 5 ml), oral gastric tube, hand gloves, cotton wool, masking tape, marker, strainer, stirrer, knives and chopping board.

#### 3.2 CHEMICALS AND REAGENTS

The following chemicals and reagents were used in this study:

Streptozotocin, sildenafil citrate, chloroform, formalin and distilled water. All chemicals and reagents used were of analytical grade.

#### 3.3 COLLECTION OF PLANT MATERIAL

Fresh leaves of *Yermonia amygdalina* and *Alstonia boonei* were obtained from Ikpoba-Okha, Benin city, Edo state from the wild in the month of June and was identified and authenticated by Prof T. Odaro in the Department of plant and Biotechnology.

#### 3.4 PREPARATION OF PLANT

The collected leaves were washed and air-dried for 14 days, after which, was pulverized into fine powder using a mechanical grinder. Equal weight of the powdered leaves was 177 g with ratio 1:1 and was subjected to cold maceration techniques with aqueous extraction process. The

mixture was soaked in a jar, 2500 ml of water was added, shaken and stored for 72 hours. The mixture was filtered, and the filtrate was concentrated into semi-acid. The extract was stored at 4°C until use.

### **3.5 EXPERIMENTAL ANIMALS**

Thirty-two (32) healthy male Wistar rats were used for this experiment. They were housed in Phytomedicine Research animal house, Department of Plant biology and Biotechnology, University of Benin, Benin City, in a well-ventilated plastic cage, maintained under controlled environmental conditions (12 hours' light/dark cycle:  $23\pm 2^{\circ}\text{C}$ / $23\pm 2^{\circ}\text{C}$ ) and fed with standard diet. All selected animals were acclimatized for 14 days.

### **3.6 EXPERIMENTAL DESIGN**

Male albino rats were obtained and randomly divided into six (6) groups. Treatment groups received 50, 100, 200 mg/kg of the bi-herbal extract orally, 10 mg/kg of glibenclamide, normal control (0.5 ml/kg of distilled water) and negative control (50 mg/kg of STZ).

### **3.7 GLUCOSE ASSAY**

The blood glucose assay procedure begins with pre-procedure preparation, where rats are fasted for 6-12 hours if required for the experimental design, while ensuring water remains available. The glucometer must be calibrated using control solution to guarantee accuracy. For the sampling itself, the rat is placed in a suitable restrainer and its tail is gently warmed to vasodilate the veins and increase blood flow. A sterile lancet is then used to make a shallow puncture in a lateral tail vein.

The first drop of blood is carefully wiped away to prevent contamination from tissue fluid, ensuring measurement accuracy. The subsequent, fresh drop of blood is applied to the test strip

inserted in the activated glucometer, which provides a digital readout of the glucose concentration. After the reading is complete, gentle pressure is applied to the puncture site to halt bleeding. The result must be recorded immediately alongside the corresponding animal identification to maintain data integrity throughout the study. Consistency in using the same glucometer and handling techniques for all animals is paramount for obtaining reliable and comparable results.

### **3.9 DETERMINATION OF BODY WEIGHT**

The weights of the rat were determined using My Weight 70001DX Multi-Purpose Digital Scale on day zero (0) and at the end of a ten (10) days period of the experiment. The Net change in the body weight (difference between final body weight and initial body weight) was calculated using the formula: Net change in Body Weight = Final Body Weight – Initial Body weight.

### **3.10 STATISTICAL ANALYSIS**

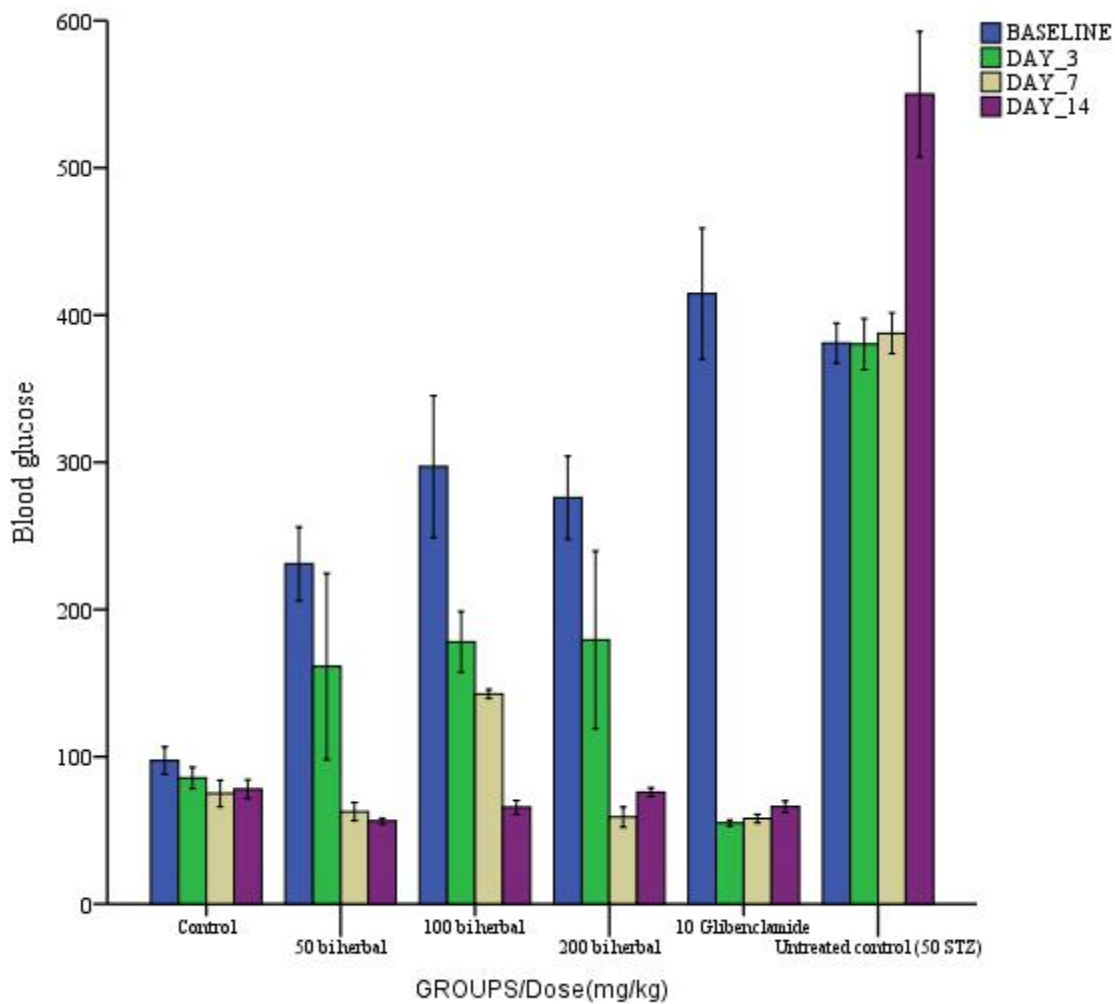
The results are expressed as mean  $\pm$  SEM (standard error of the mean) using SPSS version 19. Data for the groups were compared using one-way analysis of variance (ANOVA) and the significant difference as p -value < 0.05.

## CHAPTER FOUR

### 4.0 RESULTS

**Figure 4.1:** The groups administered the bi herbal extracts showed significant decrease in blood sugar level from baseline to day 14 ( $p < 0.05$ ). Similar to the standard drug which decreased blood sugar level.

**Table 4.2:** Cholesterol was higher in the untreated group compared to the treated groups ( $p < 0.05$ ). There was a dose dependent increase in cholesterol amongst the extract group, with the 200mg/kg extract group showing the highest cholesterol amongst the groups treated with the bi-herbal extract. Similar result was shown in the Triglyceride and High density Lipoprotein parameters, where the untreated groups showed a significantly higher Triglyceride level than the treated groups ( $P < 0.05$ ). Treated groups comprises the groups administered 50, 100 and 200mg/kg of the bi-herbal extract, and the group administered the standard drug Glibenclamide.



**Figure 4.1:** Effect of bi herbal extract on the blood glucose level of Wistar rats on various days. Mean  $\pm$  SEM was used. n = 3

**Table 4.2:** Effect of bi-herbal on the lipid profiles amongst the groups

<b>GROUPS/Dose(mg/kg)</b>	<b>CHOL (mg/dl)</b>	<b>HDL (mg/dl)</b>	<b>LDL (mg/dl)</b>	<b>TRIG (mg/dl)</b>
<b>50 bi-herbal</b>	94.00 ± 1.73 <sup>b</sup>	30.00 ± 1.73 <sup>b</sup>	64.00 ± 2.89 <sup>b</sup>	60.00 ± 5.17 <sup>b</sup>
<b>100 bi-herbal</b>	95.00 ± 3.46 <sup>b</sup>	28.00 ± 1.16 <sup>b</sup>	53.00 ± 4.04 <sup>b</sup>	81.00 ± 2.31 <sup>b</sup>
<b>200 bi-herbal</b>	98.00 ± 4.04 <sup>b</sup>	30.00 ± 2.31 <sup>b</sup>	60.00 ± 4.04 <sup>b</sup>	70.00 ± 5.20 <sup>b</sup>
<b>10 Gibenclamide</b>	106.00 ± 2.89 <sup>b</sup>	32.00 ± 3.46 <sup>b</sup>	66.00 ± 3.46 <sup>b</sup>	40.00 ± 4.04 <sup>b</sup>
<b>Untreated control</b>	143.00 ± 3.46 <sup>a</sup>	43.00 ± 1.16 <sup>a</sup>	72.00 ± 4.04 <sup>a</sup>	106.00 ± 3.46 <sup>a</sup>
<b>Normal Control</b>	110 ± 10.97 <sup>c</sup>	24.33 ± 4.37 <sup>c</sup>	67.00 ± 3.22 <sup>c</sup>	60.33 ± 4.37 <sup>c</sup>

Key: P < 0.05 was considered statistically significant Key: P < 0.05 was considered statistically significant *values were expressed as Mean ± SEM*; values with same alphabetical superscript are non-significant across the column. n=3 (total number of animals per group). The data were analyzed using Mean±SEM and analysis of variance; where *p-value* > <sup>a</sup> = 0.05.

CHOL: Cholesterol, HDL: High density Lipoprotein, LDL: Low density Lipoprotein, TRIG: Triglyceride

## CHAPTER FIVE

### 5.0 DISCUSSION

The aim of this research is to investigate the antidiabetic potential of the bi-herbal aqueous root extract in streptozotocin (STZ)-induced diabetic male Wistar rats concerning blood glucose regulation and lipid metabolism. The results demonstrate that the bi-herbal extract possesses hypoglycemic and lipid-lowering properties.

The results of this study demonstrate that the bi-herbal aqueous extract possesses significant antihyperglycemic activity. As illustrated in Figure 4.1, all groups administered the bi-herbal extract (50, 100, and 200 mg/kg) decrease in blood glucose levels from baseline to day 14 ( $p < 0.05$ ). This hypoglycemic effect was comparable to that achieved by the standard drug, glibenclamide, a known anti-diabetic medication. The progressive reduction in blood sugar across the treatment period suggests that the extract acts effectively to restore glycemic control in diabetic conditions. This antidiabetic effect can be explained by the rich phytochemical composition of both plants. *Vernonia amygdalina* is reported for its ability to regenerate pancreatic beta cells following artificial destruction by streptozotocin induction (Patel *et al.*, 2012). Given that STZ-induced diabetes is characterized by the selective destruction of pancreatic  $\beta$ -cells, leading to insulin deficiency, the regenerative potential of bitter leaf is an important mechanism for its efficacy (Wickramasinghe *et al.*, 2021). Furthermore, extracts from bitter leaf have been shown to promote the absorption of glucose by the body's muscle and liver cells, thereby enhancing peripheral glucose utilization and reducing circulating blood sugar levels (Onyibe *et al.*, 2021). The presence of sesquiterpene lactones such as vernolide and vernodalin is thought to be instrumental in this blood glucose regulatory activity (Owheruo *et al.*,

2023). Studies have confirmed that extracts from the stem bark and leaves of *A. boonei* exhibit a potent hypoglycemic effect in rat models, with the stem bark extract showing the highest activity (Osadolor *et al.*, 2015; Owolabi *et al.*, 2014). The antidiabetic property is due to its diverse phytochemicals, including alkaloids, flavonoids, and tannins, which may work individually or together to improve insulin sensitivity or stimulate insulin secretion (Alam *et al.*, 2022). The combined action of the bi-herbal extract likely produces a more multifaceted attack on hyperglycemia, potentially through beta-cell regeneration, enhanced insulin secretion, improved insulin sensitivity, and increased peripheral glucose uptake.

The untreated control group exhibited significantly higher levels of total cholesterol, triglycerides, and Low-Density Lipoprotein (LDL), along with an elevated High-Density Lipoprotein (HDL), compared to the treated groups. This dyslipidemic profile is a classic marker of diabetes and a major risk factor for cardiovascular complications. In contrast, all groups treated with the bi-herbal extract showed a significant protective effect of these parameters ( $p < 0.05$ ). The extract induced a dose-dependent relationship of the lipid profile. Total cholesterol and triglyceride levels were lower in the treated groups compared to the untreated control. The group receiving the highest dose of the extract (200 mg/kg) showed cholesterol and triglyceride levels closest to those of the normal control group, showing a moderating effect. Studies have shown that bitter leaf in diets can increase HDL cholesterol while decreasing blood triacylglycerol and LDL cholesterol (Abdulmalik *et al.*, 2016). Furthermore, Onyibe *et al.* (2021) reported that bitter leaf aqueous extracts decreased triacylglycerol levels and stabilized serum cholesterol concentrations in diabetic rats. The ethanol extracts of bitter leaf have also been observed to maintain lipid profiles within the typical range (He *et al.*, 2018).

## **CONCLUSION**

This research demonstrate that the bi-herbal aqueous extract possesses significant antidiabetic activity. The extract effectively lowered elevated blood glucose levels in STZ-induced diabetic rats, with an efficacy comparable to the standard drug glibenclamide. Additionally, it exhibited a beneficial modulatory effect on the lipid profile, counteracting the dyslipidemia commonly associated with diabetes. These observed pharmacological effects are likely attributable to the rich and synergistic blend of bioactive phytochemicals present in both plants, such as alkaloids, flavonoids, and saponins, which may work through mechanisms like pancreatic beta-cell regeneration and enhanced peripheral glucose uptake. Therefore, this study provides scientific validation for the traditional use of this herbal combination in the management of diabetes mellitus and its associated complications.

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