NEPHROPROTECTIVE EFFECT OF BIHERBAL LEAF AQUEOUS EXTRACT (*Vernonia amygdalina and Alstonia boonei*) ON STREPTOZOTOCIN-INDUCED NEPHROTOXICITY IN WISTAR RATS

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FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY. IN PARTIAL FULFILMENT FOR THE REQUIREMENTS FOR THE AWARD OF BACHELORS OF SCIENCE DEGREE (B.Sc.)

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CERTIFICATION

This is to certify that this project titled Streptozotocin-Induced Diabetic Nephrotoxicity: Effect of Biherbal Leaf Aqueous Extract on Male Wistar Rats" was carried out by IZEVBIZUA Abigail Iyobosa (Miss) with matriculation number LSC2009931, in the Department of Science Laboratory Technology, (Physiology/Pharmacology Techniques) Faculty of Life Sciences, University of Benin, Benin City, Nigeria, under the supervision of DR B.O Gabriel.

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DEDICATION

I dedicate this work to Almighty God, whose infinite wisdom, strength, and grace have guided me throughout the course of this study. I also dedicate it to my beloved family and friends for their unwavering support, encouragement, and prayers.

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ABSTRACT

Diabetes is a chronic metabolic disorder affecting how the body processes fats, proteins, and carbohydrates, stemming from insulin deficiency or dysfunction. The disease burden continues to grow, bringing complications that damage multiple organ systems. Diabetic nephropathy, one of the most serious complications, remains a leading cause of kidney failure. Traditional herbal remedies have shown promise in managing diabetes and its complications, with several plants demonstrating protective effects on kidney function. This study evaluated the nephroprotective effects of a bi-herbal leaf aqueous extract combining *Vernonia amygdalina* and *Alstonia boonei* against streptozotocin-induced kidney damage in Wistar rats. The leaves were washed, air-dried for two weeks, ground into powder, and soaked in distilled water for three days to create an extract, which was then concentrated and refrigerated for later use. The rats were grouped into 6 groups and kept in comfortable conditions with proper temperature control, regular feeding schedules, and care that followed all ethical guidelines for animal research. The rats were induced with STZ (streptozotocin), and a renal function test was carried out to assess kidney health. The results showed that the bi-herbal extract provided maximum protective effects at the lowest dose tested, significantly outperforming higher doses ($P<0.05$). The extract demonstrated measurable nephroprotective activity, suggesting potential therapeutic value in preventing diabetes-related kidney damage. These findings indicate that lower concentrations of the bi-herbal combination may be more effective than higher doses in protecting kidney function. Additional research is needed to establish optimal dosing protocols and confirm long-term safety before clinical application.

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Herbal medicine has been used for centuries as a primary form of treatment in many cultures and traditions across the world. Certain constituents are obtained from plants and plant-derived materials, providing a rich source of biological activity with significant therapeutic benefits (Wang *et al*., 2023). According to the World Health Organization (WHO): herbal medicine is defined as the body of knowledge, skills, and practices based on theories, beliefs, and experiences that are indigenous to different cultures and are used to maintain health as well as to prevent, diagnose, and treat physical and mental illnesses (Che *et al.,* 2017). Herbal medicine is classified under complementary and alternative medicine (CAM) (Aljawahrah *et al.,* 2020). Plants have long been a major source of drugs, with about 20 percent of drugs currently in use worldwide derived from plants. Plant parts used in the production of herbal medicine include roots, stems, leaves, flowers, fungi, and algae (Aljawahrah *et al.,* 2020). The manufacturing of herbal medicines from plants is often based on folkloric or traditional knowledge, which is later subjected to scientific validation. The major reason for the continuous use of herbal medicine is its perceived natural origin and historical significance. Traditional knowledge of herbal remedies is largely transmitted through generations and cultural beliefs, offering valuable insights into the healing properties of the same or different plant species (Wang *et al*., 2023).

Herbal medicines are widely applied in the treatment of various ailments and diseases, and they have shown excellent results in patients, thereby raising questions about their scientific validity. Issues such as quality control, identification, standardization of chemical compounds, and

formulation into dosage forms have become increasingly important (Juntra *et al*., 2019). Natural drugs, as alternatives to synthetic chemical drugs, play a crucial role in human health and development. However, differences in genetic background, geographical origin, planting environment, cultivation techniques, harvesting time, processing methods, and the presence of exogenous impurities all influence the quality of herbal medicines. These factors highlight the key distinction between natural medicines and synthetic drugs, making quality consistency a fundamental requirement for herbal products (Xi-chuan *et al.,* 2020). Quality control in herbal medicine involves a systematic approach to monitoring and managing the various stages of production, including development, manufacturing, and distribution, in order to ensure consistent product quality. This is essential in the pharmaceutical industry, as it guarantees that the final product meets established standards and expectations. Methods used in quality control may include the standardization and identification of herbs, processing and manufacturing controls, and quality assessment during production (Wang *et al.,* 2023). Furthermore, the application of modern technologies and methodologies in advancing herbal medicine can significantly enhance its scientific validity, improve quality, and promote standardization (Juntra *et al.,* 2019).

Diabetes is a chronic metabolic disorder affecting the metabolism of fats, proteins, and carbohydrates. It results primarily from insulin deficiency or malfunction. Globally, an estimated 2.8% of the population currently suffers from the disease, and this figure is expected to rise to over 5.4% by 2025. Diabetes requires early diagnosis, appropriate treatment, and lifestyle modifications to manage and prevent its complications effectively. Traditional plants that have been scientifically screened and have shown anti-diabetic properties include *Allium sativum* (garlic): *Hibiscus sabdariffa L.* (Roselle): *Momordica charantia* (bitter melon): *Zingiber officinale* (ginger): *Acacia arabica*, *Achyranthes aspera*, Acosmium panamense, *Aegle marmelose*, *Andrographis paniculata*,

Annona squamosa, *Argyreia nervosa, Securimegra virosa, Salacia reticulate, Rhus coriaria* (sumac seed): *Phyllanthus amarus, Origanium vulgare, Ocimum santum, Nigella sativa, Myrcia Bella, Margifera indica, Lepidium sativum, Hypoxia hemerocavidea, Helicteres isora, Fraxinus excersior, Eclipta alba, Dorema auchen, Coriandium sativum, Citruius colocyrithis, Cichorium intybus, Chamaemium mobile, Casearia esculenta, Carum carvi, Cajanus cajan, Caesalpinia bonducella, Bryonia alba, Brassica nigra, Biophytum sensitivum, Barleria pronitis, Azadweacha indica* among others (Wasam *et al.,* 2016; Clement *et al.,* 2023).

Vernonia amygdalina, commonly known as bitter leaf, is a perennial shrub of the Asteraceae family that grows widely in tropical Africa. It is well known for its intensely bitter taste, which is due to compounds such as sesquiterpene lactones (vernodalin, vernolide, and hydroxyvernolide) (Erasto *et al.,* 2006). Beyond its culinary role as a leafy vegetable, bitter leaf is one of the most important medicinal plants in African traditional medicine. It is widely used to manage malaria, diabetes, gastrointestinal problems, fever, and general weakness (Ogunyemi *et al.,* 2020). Thus, *Vernonia amygdalina* represents both a nutritional vegetable and a medicinal resource whose bioactive compounds make it a versatile plant in promoting health and managing disease.

Alstonia boonei (De Wild.) is a large tropical tree belonging to the genus Alstonia, family Apocynaceae, order Gentianales, and class Asterids. It is native to West and Central Africa, where it thrives in rainforests, riverine habitats, and moderately disturbed areas up to 1,000 meters in altitude (Wikipedia, 2023; World Agroforestry, 2023). The tree can grow up to 45 meters tall with a fluted trunk and whorled oblanceolate leaves arranged in groups of five to eight. Its bark is greyish-green and rough, exuding a characteristic white latex, while its flowers are yellowish-white and borne in terminal cymes, producing pendulous follicles containing silky seeds (World Agroforestry, 2023). *Alstonia boonei* has been widely applied in African medicine. Its bark, leaves,

and roots are used for the treatment of malaria, fever, rheumatism, hypertension, gastrointestinal disorders, and painful conditions such as toothache and arthritis. The plant also plays a role in wound healing, ulcer management, and as an anti-snakebite remedy (Akinmurele *et al*., 2023). The medicinal properties are attributed to diverse phytochemicals, including alkaloids such as echitamine, triterpenoids like lupeol and α-amyrin, flavonoids, tannins, and saponins, which collectively exhibit antimalarial, anti-inflammatory, analgesic, antimicrobial, antidiabetic, and antioxidant activities.

1.2 AIM

The aim of this study was to evaluate the nephrotoxic effect of the biherbal leaf aqueous extract on Streptozocin induced toxicity in male Wistar rats

1.3 OBJECTIVES

The following are the objectives of the study which includes to:

1. evaluate the nephrotoxic effect of the plant extract on diabetic induced toxicity
2. explore the safety profile of biherbal leaf aqueous extract by assessing any adverse effect on other organ system or biochemical parameters

CHAPTER TWO
LITERATURE REVIEW

2.0 *Vernonia amygdalina*

Vernonia amygdalina, commonly known as bitter leaf, is a perennial shrub that belongs to the family Asteraceae. It is widely distributed across tropical Africa and has become one of the most culturally and medicinally significant plants on the continent. The plant is well recognized for its characteristic bitter taste, which is attributed to the presence of bioactive phytochemicals such as sesquiterpene lactones, alkaloids, saponins, and flavonoids (Farombi and Owoeye, 2011). Due to its strong ethnomedicinal reputation, *Vernonia amygdalina* has been extensively studied both in its traditional context and through modern pharmacological research. Bitter leaf is often cultivated in homesteads across West and Central Africa, where it is used both as a vegetable and as a medicinal plant. In Nigeria, it is a popular culinary ingredient, especially in soups such as "ofe onugbu" among the Igbo people and other vegetable-based dishes in different ethnic groups. Beyond its nutritional value, its medicinal role has been deeply integrated into African traditional healthcare practices for centuries. The leaves, stems, and roots are employed for treating a variety of ailments, ranging from malaria and gastrointestinal disturbances to diabetes and microbial infections (Erasto *et al.,* 2006). This dual role of *Vernonia amygdalina* serving as both food and medicine, has made it a critical plant for the health and sustenance of local communities.

Nutritionally, *Vernonia amygdalina* is a source of vitamins (notably vitamin C, vitamin E, and folate): essential minerals such as potassium, calcium, and iron, and dietary fiber. The leaves are often consumed after washing or boiling to reduce their bitterness, a process that removes some phytochemicals but retains important nutrients (Atangwho *et al.,* 2009). This integration of nutritional and medicinal values underscores its role as a functional food, bridging the gap between

diet and therapy. In recent years, there has been a growing interest in studying bitter leaf as a nutraceutical, especially in managing chronic diseases such as diabetes, hypertension, and cancer. In addition to its chemical and nutritional profile, *Vernonia amygdalina* is valued for its ecological adaptability. It thrives in diverse soil types and can withstand harsh tropical conditions, making it readily available in many rural communities where access to conventional healthcare is limited. This accessibility strengthens its role as a "first line of defense" in traditional medicine systems. Moreover, the plant has been integrated into modern pharmacological studies that confirm and sometimes extend the claims made in traditional contexts. For instance, aqueous extracts of *Vernonia amygdalina* have been shown to lower blood glucose levels in diabetic rat models, validating its folkloric use as an antidiabetic agent (Okolie and Akpa, 2017). Similarly, studies have demonstrated hepatoprotective, anti-inflammatory, and immunomodulatory effects, supporting its relevance in disease prevention and management. Despite its wide applications, *Vernonia amygdalina* is not without limitations. The intense bitterness can limit its acceptance as food, particularly among younger populations unaccustomed to its taste. In addition, variations in phytochemical composition due to geographical location, cultivation methods, and preparation techniques can affect its efficacy. Nonetheless, continuous research into standardizing extracts and isolating bioactive compounds is helping to overcome these challenges, paving the way for its integration into modern drug formulations. (Dibie and Dibie, 2022).

Figure 2.1: Picture of *Vernonia amygdalina*

2.1.2 Taxonomy of *Vernonia amygdalina*

The accepted taxonomic breakdown of Vernonia amygdalina is as follows:

Kingdom: Plantae – Plants

Subkingdom: Tracheobionta – Vascular plants

Division (Phylum): Magnoliophyta – Angiosperms (flowering plants)

Class: Magnoliopsida – Dicotyledons

Order: Asterales

Family: Asteraceae – Sunflower family

Genus: *Vernonia*

Species: *Vernonia amygdalina* Delile

2.2 Botanical Description of *Vernonia amygdalina*

Vernonia amygdalina, commonly called bitter leaf, is a perennial shrub or small tree that can grow up to 2–5 meters in height, although in favorable environments it may reach even greater heights (Burkill, 1997). The plant is characterized by a rough bark that is brown or grey in color and branches that are often brittle. One of its most distinguishing features is the production of a characteristically bitter taste from its leaves, a trait that has given rise to its common name and has long been associated with its medicinal importance. The leaves of *Vernonia amygdalina* are simple, opposite, and elliptic-lanceolate in shape, typically measuring between 6–40 cm in length and 2–12 cm in width. They are green and leathery in texture, with a slightly rough surface and prominent veins. The margins are usually finely serrated. When crushed, the leaves emit a strong bitter aroma, and their taste is intensely bitter due to the presence of bioactive compounds such as sesquiterpene lactones (Erasto *et al*., 2006). This bitterness is often reduced by washing or boiling, especially when the leaves are used in food preparation. The flowers of *Vernonia amygdalina* are small, white to yellowish, and borne in dense clusters forming terminal inflorescences called capitula, which are typical of members of the Asteraceae family. Each flower head is composed of tiny florets, usually hermaphroditic, that are arranged together in composite structures. The plant produces fruits in the form of small, narrow, and hairy achenes, which facilitate seed dispersal by wind. These morphological traits are consistent with the general characteristics of the genus *Vernonia* and are useful in its taxonomic identification (Judd *et al.,* 2016). The stems of *Vernonia amygdalina* are woody and have a soft pith. They exude a watery sap when cut, unlike the milky latex seen in plants from families such as Apocynaceae. The bark has been reported to contain medicinal compounds, though the leaves remain the primary part of the plant utilized in both culinary and medicinal contexts. *Vernonia amygdalina* thrives in tropical environments and is

commonly found in lowland forests, grasslands, and farmlands across Africa. It grows well in well-drained soils and can tolerate both cultivated and wild conditions. Farmers often cultivate it in backyard gardens for easy access as both a food source and a medicine. The plant is hardy and capable of withstanding pruning, which makes it suitable for repeated harvest of leaves for domestic use. Ecologically, the plant plays an important role as a source of food and shelter for insects and small animals. Its ability to grow rapidly and adapt to different soils makes it useful in agroforestry systems and for erosion control in certain regions (Atangwho *et al.,* 2009). In terms of organoleptic properties, the intense bitterness of the leaves serves a protective function in nature, deterring herbivores from consuming them excessively. However, this same trait has been harnessed by humans, with the bitterness often considered a therapeutic marker, particularly for digestive and metabolic health.

2.2.1 Ethnomedicinal Uses of *Vernonia amygdalina*

One of the most common ethnomedicinal applications of *Vernonia amygdalina* is in the management of malaria and fever. Decoctions of the leaves are prepared and consumed as herbal teas, either alone or in combination with other plants such as *Azadirachta indica* (neem). The bitter principle of the plant, largely due to sesquiterpene lactones, is believed to be responsible for its antimalarial effects. In many Nigerian households, bitter leaf tea is a first-line remedy for malarial symptoms, especially in rural areas where access to modern healthcare may be limited (Farombi and Owoeye, 2011). The plant also plays an important role in the treatment of **gastrointestinal disorders**. Aqueous leaf extracts or leaf infusions are used to relieve stomach upset, diarrhea, dysentery, and intestinal worms. The bitterness of the leaves is thought to stimulate bile flow and improve digestion, which explains its use as a digestive aid and appetite stimulant (Atangwho *et al.,* 2009). Traditional healers often recommend chewing the fresh leaves or drinking the juice

expressed from them to expel intestinal parasites. In the management of diabetes **mellitus,** bitter leaf is highly valued. Ethnomedical practices often prescribe the aqueous extract of the leaves for lowering blood sugar levels. This practice has been validated by scientific studies demonstrating its hypoglycemic properties in experimental animals (Okolie and Akpa, 2017). Among the Igbo of southeastern Nigeria, drinking the squeezed juice of the leaves daily is a common practice among diabetics, while others boil the leaves and drink the decoction as part of their routine therapy. *Vernonia amygdalina* is also traditionally employed for female reproductive health. Women consume leaf decoctions after childbirth to cleanse the womb, restore appetite, and promote lactation. In some cultures, the leaves are also used in treating infertility and menstrual disorders, although these uses are largely anecdotal and require further scientific validation (Ogunyemi *et al*., 2020) The plant is additionally used for **liver-related conditions**. In traditional practice, the leaves are prepared as tonics to support liver health, especially in cases of jaundice or suspected hepatotoxicity due to food or alcohol. This use has been reinforced by experimental studies showing hepatoprotective effects of the plant's extracts against chemically induced liver injury (Farombi and Owoeye, 2011). Beyond these major uses, *Vernonia amygdalina* has been employed in treating **skin infections and wounds,** where the crushed leaves are applied topically to promote healing. Its juice is also gargled for sore throat and mouth infections. In some communities, the plant is considered a general "immune booster," and people drink the leaf decoction regularly as a preventive tonic against diseases (Ezeonu *et al.,* 2023). Ethnomedicinally, the plant is rarely used in isolation. Traditional medicine often employs it in polyherbal preparations, combining it with other local species to enhance therapeutic effectiveness. This synergistic use demonstrates the deep empirical knowledge embedded within African traditional healing systems (Ijeh and Ejike, 2011).

2.2.2 Phytochemical Screening of *Vernonia amygdalina*

Phytochemical screening of *Vernonia amygdalina* has consistently revealed the presence of several classes of secondary metabolites that account for its medicinal importance. Standard qualitative tests such as Mayer's and Dragendorff's reagents confirm the presence of alkaloids**,** while frothing tests detect saponins, and ferric chloride tests show the presence of tannins and other phenolic compounds (Atangwho *et al.,* 2009). Flavonoids are identified through Shinoda and alkaline reagent tests, while Liebermann–Burchard and Salkowski reactions detect steroids and terpenoids (Ogunyemi *et al.,* 2020). Screening studies generally report that the leaves contain abundant alkaloids, flavonoids, saponins, tannins, phenolics, and terpenoids, alongside glycosides. These phytochemical groups are found in both aqueous and methanolic extracts, although concentration levels may differ depending on the solvent used. Sesquiterpene lactones such as vernodalin and vernolide, which give the plant its characteristic bitterness, are consistently detected. Importantly, phytochemical screening has demonstrated that the **aqueous extract**, the most commonly used form in ethnomedicine, contains nearly all the major groups of bioactive compounds. This validates its traditional preparation method by boiling or squeezing the leaves. The presence of both polar (flavonoids, phenolics) and nonpolar (terpenoids, steroids) constituents suggests a broad pharmacological spectrum.

2.4 Pharmacological Study of *Vernonia amygdalina*

2.4.1 Antimalarial / Antiplasmodial Activity

Recent studies have confirmed that *Vernonia amygdalina* (bitter leaf) has real antimalarial benefits. According to Tona *et al.* (2013): ethanolic extracts of the leaves reduced malaria parasites (*Plasmodium berghei*) in infected mice and improved survival. In another study, Ogbeche *et al.* (2014) showed that both water and methanol extracts of the plant were able to stop the growth of *Plasmodium falciparum*, the parasite responsible for most malaria deaths. More recently, Oyeyemi *et al.* (2019) found that bitter leaf fractions not only lowered parasite levels in rodents but also helped restore blood balance and reduced malaria-related damage.The active compounds believed to be responsible are sesquiterpene lactones such as vernodalin and vernolide. These chemicals interfere with the parasite's ability to survive and multiply. At the same time, *Vernonia amygdalina* has antipyretic (fever-reducing) and anti-inflammatory effects, which help patients feel better during malaria attacks (Ogunyemi *et al*., 2020). These findings explain why bitter leaf is still widely used in African homes to manage malaria. Traditional healers usually prepare it as a decoction or squeezed juice, and modern research shows that these simple water-based methods still carry strong antimalarial activity. (Sha'a *et al.,* 2021)

2.4.2 Antidiabetic / Hypoglycemic Activity

Recent research has strongly supported the traditional use of *Vernonia amygdalina* (bitter leaf) in the management of diabetes. According to Okolie and Akpa (2017): aqueous extracts of the leaves significantly reduced fasting blood glucose levels in alloxan-induced diabetic rats, while also improving liver enzyme activity and antioxidant status. In another experiment, Nwanjo (2015) reported that ethanol leaf extract improved glucose tolerance and restored pancreatic β-cell function in streptozotocin-induced diabetic models. These results suggest that bitter leaf can act

both by lowering blood sugar directly and by protecting insulin-producing cells from damage. Beyond blood sugar control, *Vernonia amygdalina* also improves lipid metabolism. Atangwho *et al*. (2010) observed that rats treated with this leaf extracts showed lower levels of cholesterol and triglycerides, which are often elevated in diabetic conditions. This effect makes the plant useful not only in controlling hyperglycemia but also in reducing the risk of cardiovascular complications linked to diabetes. (Ogbuagu *et al.,* 2019) The hypoglycemic effect is linked to the plant's phytochemicals, especially flavonoids, alkaloids, and saponins. Flavonoids act as antioxidants, reducing oxidative stress associated with diabetes, while saponins may enhance insulin sensitivity and glucose uptake (Ogunyemi *et al.,* 2020). Importantly, studies have shown that the aqueous preparations commonly used in ethnomedicine retain strong hypoglycemic effects, validating traditional methods such as leaf squeezing and decoction. In summary, both experimental and ethnomedicinal evidence show that *Vernonia amygdalina* is effective in reducing blood sugar, improving lipid profiles, and protecting vital organs. These findings highlight its potential as a safe, affordable adjunct in diabetes management.

2.4.3 Antioxidant Activity

According to Adedapo *et al.* (2014): aqueous and ethanol extracts of the leaves significantly reduced oxidative stress markers in laboratory animals by increasing antioxidant enzymes such as superoxide dismutase and catalase. In another study, Oboh *et al.* (2012) demonstrated that the leaf extracts effectively scavenged free radicals in vitro, confirming their role in protecting cells from oxidative damage. Oyeyemi *et al*. (2019) further reported that bioactive fractions of bitter leaf not only improved antioxidant status in malaria-infected rodents but also helped restore hematological balance. This highlights its dual role in reducing parasite load and protecting body tissues from damage caused by free radicals. The antioxidant effect of *Vernonia amygdalina* is largely linked

to its flavonoids, phenolic compounds, and vitamins (A, C, and E). These compounds act by neutralizing harmful free radicals, preventing lipid peroxidation, and protecting proteins and DNA from oxidative injury (Ogunyemi *et al*., 2020). Since oxidative stress plays a major role in diabetes, cancer, liver diseases, and kidney damage, the plant's antioxidant properties explain its wide use in ethnomedicine.

2.4.4 Antimicrobial (Antibacterial, Antifungal) Activity

Vernonia amygdalina has been widely studied for its antimicrobial activity, supporting its traditional use for treating infections. According to Ezeigbo *et al*. (2016): ethanol and aqueous extracts of the leaves showed strong antibacterial effects against common pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*. These are organisms often linked to diarrhea, food poisoning, and wound infections. More recent work by Ekor *et al.* (2020) demonstrated that methanolic extracts of bitter leaf inhibited the growth of *Candida albicans* and *Aspergillus niger*, two fungi associated with candidiasis and respiratory infections. This supports the ethnomedicinal use of bitter leaf for skin infections and gastrointestinal disturbances. The antimicrobial activity is mainly attributed to **alkaloids, saponins, tannins, and flavonoids,** which damage microbial cell walls, disrupt protein synthesis, and interfere with DNA replication. Oboh *et al.* (2012) further noted that the antioxidant properties of bitter leaf may complement its antimicrobial role by reducing oxidative stress in infected tissues, thereby speeding up recovery. Importantly, crude aqueous preparations similar to those used by traditional healers retain significant antibacterial activity, showing that simple household methods of preparation are effective (Ogunyemi *et al.,* 2020).

2.4.5 Anti-inflammatory Activity

Inflammation is a natural defense mechanism, but when it becomes chronic, it contributes to diseases such as arthritis, diabetes, and cardiovascular disorders. *Vernonia amygdalina* has been reported to possess strong anti-inflammatory properties, which support its wide use in African traditional medicine for treating fever, pain, and inflammatory conditions. According to Omoregie and Pal (2016): aqueous extracts of *Vernonia amygdalina* significantly reduced paw edema in rats induced with carrageenan, showing dose-dependent anti-inflammatory effects similar to standard drugs. More recently, Adeoye *et al*. (2018) reported that methanol leaf extracts inhibited the production of pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) in laboratory models. This suggests that the plant works by suppressing pathways that drive inflammation. The activity is linked to its flavonoids, saponins, alkaloids, and sesquiterpene lactones**,** which act by reducing oxidative stress, inhibiting enzymes like cyclooxygenase, and blocking the release of inflammatory cytokines. Ogunyemi *et al.* (2020) also noted that its antioxidant compounds complement its anti-inflammatory effects by preventing tissue damage associated with oxidative stress. Importantly, these effects have been observed in both aqueous and ethanol extracts, supporting the effectiveness of traditional methods such as boiling or squeezing the leaves. This validates its long history of use for treating inflammatory conditions like rheumatism, fever, and gastrointestinal pain.

2.4.6 Analgesic Activity

Pain management is one of the oldest applications of medicinal plants, and *Vernonia amygdalina* has been traditionally used to relieve headaches, stomach pain, and body aches. Modern studies now provide evidence for its analgesic properties. According to Owolabi *et al.* (2011): ethanolic leaf extracts of *Vernonia amygdalina* significantly reduced pain responses in mice using both the

tail-flick and hot plate tests. The analgesic effect was dose-dependent and comparable to standard drugs such as aspirin. More recently, Akinmoladun *et al*. (2015) reported that methanol extracts of the plant inhibited writhing in mice induced with acetic acid, further confirming its pain-relieving ability. The analgesic effect is largely associated with alkaloids, flavonoids, and saponins present in the plant. These compounds are believed to act by suppressing prostaglandin synthesis and inhibiting pain mediators in the central and peripheral nervous systems (Ogunyemi *et al*., 2020). The anti-inflammatory properties of the plant also complement its analgesic role, since inflammation often underlies pain.

2.4.7 Anticancer / Cytotoxic Activity

Cancer remains a leading cause of death worldwide, and medicinal plants are being explored for safer, natural alternatives to chemotherapy. *Vernonia amygdalina* has shown promising anticancer and cytotoxic properties in both laboratory and animal studies. According to Izevbigie (2013): aqueous extracts of *Vernonia amygdalina* inhibited the growth of breast cancer (MCF-7) cells in vitro by inducing apoptosis and reducing cell proliferation. More recent work by Oyeyemi *et al*. (2019) demonstrated that bioactive fractions of the plant suppressed abnormal cell growth while protecting normal blood parameters in rodent models, suggesting selective cytotoxicity. Similarly, Njan *et al.* (2017) reported that methanolic extracts of bitter leaf reduced tumor progression in chemically induced cancer models, attributing this to the presence of sesquiterpene lactones. The anticancer effects are linked to phytochemicals such as vernodalin, vernolide, flavonoids, and phenolic compounds**.** These compounds act by triggering programmed cell death (apoptosis): halting the cell cycle, reducing oxidative stress, and inhibiting angiogenesis, which is essential for tumor growth (Ogunyemi *et al.,* 2020). Importantly, studies show that while *Vernonia amygdalina* extracts are toxic to cancer cells, they are less harmful to normal healthy cells, making it a safer

alternative compared to conventional chemotherapy. In ethnomedicine, bitter leaf has long been used as a "blood purifier" and general health tonic. These traditional claims align with modern findings that its extracts may help prevent or slow down cancer development.

2.4.8 Hepatoprotective Activity

The liver plays a central role in detoxification, metabolism, and overall body homeostasis. When damaged by toxins, alcohol, drugs, or infections, liver function can be severely impaired. *Vernonia amygdalina* (bitter leaf) has been shown to protect the liver from such damage, supporting its ethnomedicinal use as a "blood purifier" and tonic.According to Adedapo *et al.* (2014): aqueous and ethanolic extracts of *Vernonia amygdalina* significantly reduced liver enzyme levels (ALT, AST, and ALP) in rats exposed to toxic substances, suggesting protection against liver injury. More recently, Falodun *et al.* (2016) reported that methanolic extracts of the plant prevented acetaminophen-induced hepatotoxicity in rats, with histological examination confirming reduced liver damage. Similarly, Okolie and Akpa (2017) showed that bitter leaf extract not only lowered blood glucose but also improved liver function markers in diabetic rats, highlighting its dual antidiabetic and hepatoprotective roles. The hepatoprotective activity is attributed to flavonoids, phenolic compounds, and sesquiterpene lactones**,** which act as antioxidants to neutralize free radicals and prevent lipid peroxidation. These compounds also stabilize liver cell membranes and enhance the regeneration of hepatocytes (Ogunyemi *et al.,* 2020). Importantly, both water-based and alcohol-based extracts show activity, meaning traditional preparations like decoctions remain effective. This aligns with indigenous practices where bitter leaf juice or teas are used to restore liver health after illness or alcohol consumption.

2.4.9 Immunomodulatory Activity

The immune system plays a vital role in defending the body against infections and maintaining overall health. *Vernonia amygdalina* has been reported to modulate immune function, supporting its widespread use as a general health tonic. According to Ijeh and Ejike (2011): aqueous extracts of bitter leaf enhanced immune responses by stimulating lymphocyte proliferation and increasing antibody production in experimental models. More recently, Oyeyemi *et al*. (2019) found that bioactive fractions of the plant improved white blood cell counts and restored hematological balance in malaria-infected rodents, suggesting its role in boosting host defense. The immunomodulatory effects are attributed to flavonoids, alkaloids, and phenolic compounds**,** which act by regulating cytokine release and enhancing antioxidant defense mechanisms (Ogunyemi *et al.,* 2020). This activity helps explain why bitter leaf is often taken as a preventive "immune booster" in African homes, especially during illness recovery.

2.4.10 Antihypertensive / Cardioprotective Activity

Hypertension and cardiovascular diseases are growing health concerns globally, and bitter leaf has shown potential in managing these conditions.According to Nwanjo (2015): ethanol extracts of *Vernonia amygdalina* lowered blood pressure and improved lipid profiles in hypertensive rat models. Adedapo *et al.* (2014) also reported reductions in serum cholesterol and triglycerides following administration of aqueous extracts, suggesting cardioprotective effects. More recently, Oboh *et al.* (2012) showed that bitter leaf inhibited angiotensin-converting enzyme (ACE) activity in vitro, indicating a possible mechanism for its blood pressure-lowering effect. These effects are linked to the presence of flavonoids, saponins, and terpenoids, which act as antioxidants, vasodilators, and lipid regulators. The improvement in cardiovascular markers makes *Vernonia*

amygdalina relevant in preventing complications like stroke and heart disease (Ogunyemi *et al*., 2020).

2.4.11 Aphrodisiac Activity

Bitter leaf is also traditionally used to enhance sexual health and vitality. In several African communities, decoctions of the leaves are taken to improve stamina, treat erectile dysfunction, and promote fertility. According to Yakubu and Bukoye (2009): ethanolic extracts of *Vernonia amygdalina* increased mounting frequency and mating behavior in male rats, suggesting aphrodisiac activity. A more recent study by Akinmoladun *et al.* (2016) found that the extract improved sperm quality, motility, and testosterone levels in experimental animals. These results provide evidence that bitter leaf can positively influence reproductive health. The aphrodisiac effects are linked to alkaloids, saponins, and antioxidants, which may enhance blood flow, increase testosterone levels, and protect sperm from oxidative damage. This supports the traditional belief that bitter leaf restores vitality and sexual energy.

2.4.12 Appetite Stimulant / Digestive Aid Activity

One of the most common household uses of *Vernonia amygdalina* (bitter leaf) in Africa is as a digestive aid and appetite stimulant. The bitter taste of the leaves, due to sesquiterpene lactones like vernodalin and vernolide, plays a key role in its gastrointestinal benefits. According to Agbo *et al*. (2015): administration of aqueous extracts of bitter leaf in animal models enhanced gastric juice secretion and improved digestion. This finding supports its traditional use in managing loss of appetite and indigestion. Similarly, Nwaichi and Igbinobaro (2012) reported that regular consumption of bitter leaf decoction improved appetite and reduced symptoms of gastrointestinal discomfort such as bloating and constipation in human subjects. The activity is believed to work

by stimulating the secretion of bile and digestive enzymes, which enhance nutrient breakdown and absorption (Ijeh and Ejike, 2011). In addition, tannins and saponins in the plant provide protective effects on the gastrointestinal tract by reducing irritation and supporting gut flora balance. In ethnomedicine, bitter leaf juice is often prescribed to people recovering from illness to "restore appetite and strength." Its dual role as a digestive aid and general tonic aligns with the modern pharmacological evidence showing it supports gastrointestinal health. (Koul *et al.,* 2011).

2.5 *Alstonia boonei*

Alstonia boonei is a large tropical tree belonging to the family Apocynaceae. It is widely distributed across West Africa and parts of Central Africa, where it is commonly referred to as the "pattern wood" or "cheese wood." In traditional medicine, it is highly revered and has been described as one of the most important medicinal trees in African ethnopharmacology (Akinmoladun *et al.,* 2019). The plant, particularly its bark, leaves, and roots, has been employed extensively to treat a wide range of diseases, and its use remains prominent in rural and urban communities alike. Ethnomedicinally, *Alstonia boonei* holds a special position in African traditional healing systems. The bark decoction is widely used as an antimalarial and antipyretic agent, particularly in Nigeria and Ghana where malaria is endemic (Abbah *et al.,* 2010). Traditional healers often prescribe the bark as a first-line remedy for fever, body pain, and other inflammatory conditions. The plant is also used to manage hypertension, rheumatism, diabetes, and gastrointestinal disturbances. In some communities, the latex is used externally for wound healing and skin infections. This breadth of application highlights the tree's role as a "multipurpose healer" addressing both infectious and chronic diseases (Fonkua *et al*., 2021). From a phytochemical standpoint, *Alstonia boonei* is rich in alkaloids, tannins, saponins, flavonoids, and terpenoids, which account for its pharmacological versatility. The alkaloid fraction, in particular, has been identified as the primary contributor to its antimalarial and analgesic activities (Oluwole *et al.,* 2013). For instance, studies have isolated echitamine, a well-characterized indole alkaloid, which has shown significant antipyretic and anti-inflammatory effects (Ogunyemi *et al.,* 2020). The presence of flavonoids and phenolic compounds also endows the plant with antioxidant activity, which is relevant in mitigating oxidative stress commonly associated with chronic diseases like diabetes, cardiovascular disorders, and renal impairment. The medicinal significance of *Alstonia*

boonei has been supported by modern pharmacological studies, which validate its traditional uses. Abbah *et al.* (2010) demonstrated that methanol extracts of the stem bark produced dose-dependent antipyretic and anti-inflammatory effects in animal models. Similarly, Akinmoladun *et al.* (2019) reported that extracts from the plant exhibited hypoglycemic activity in streptozotocin-induced diabetic rats, suggesting its role in glucose regulation and potential benefit in preventing diabetic complications. These findings provide scientific backing to its use in traditional practice and highlight its potential for drug development.

Figure 2: Picture of *Alstonia Boonie*

2.5.1 Taxonomy of *Alstonia boonei*

Kingdom: Plantae – Plants

Subkingdom: Tracheobionta – Vascular plants

Division (Phylum): Magnoliophyta – Flowering plants (Angiosperms)

Class: Magnoliopsida – Dicotyledons

Order: Gentianales

Family: Apocynaceae – Dogbane family

Genus: Alstonia

Species**:** *Alstonia boonei* De Wild

2.5.2 Botanical Description of *Alstonia boonei*

Alstonia boonei, often referred to as pattern wood or stool wood, is a large deciduous tree that belongs to the family Apocynaceae. It is one of the most prominent forest trees in tropical West and Central Africa and is valued for both its medicinal and timber uses. Morphologically, *Alstonia boonei* is a tall tree, capable of reaching heights of 35–45 meters under natural conditions, with a straight cylindrical bole that can measure up to 3 meters in diameter (Burkill, 1997). The trunk is usually buttressed at the base, giving it stability in forest environments. The outer bark is grey to whitish-grey and rough, while the inner bark is pale, soft, and fibrous. When cut, the bark exudes a copious milky latex, which is characteristic of plants within the Apocynaceae family (Ogunmoyole *et al.,* 2018). The leaves of *Alstonia boonei* are simple, leathery, and arranged in whorls of four to eight around the stem nodes. Each leaf is oblanceolate to elliptic in shape, measuring between 10–30 cm in length and 3–10 cm in width. The leaves have an entire margin and are dark green above with a lighter green underside. The venation is prominent, with lateral veins radiating from the midrib toward the margin, a feature that is typical of the genus *Alstonia*. The whorled leaf arrangement is one of the most distinctive identifying features of this tree in the wild (Abbah *et al.,* 2010). The flowers of *Alstonia boonei* are small, white to greenish-white, and fragrant, occurring in terminal cymose panicles. Each flower is actinomorphic and bisexual, with a tubular corolla and five lobes, which is consistent with the floral morphology of the Apocynaceae family. Flowering usually occurs during the rainy season, and the pollination is believed to be mediated by insects attracted to the fragrance and nectar of the flowers. The fruits are long, thin follicles that occur in pairs, each measuring up to 50 cm in length. Inside the follicles are numerous seeds, each with a tuft of silky hairs that facilitate wind dispersal. This fruit and seed morphology is a distinctive reproductive strategy in *Alstonia* species, aiding in the spread of the plant across

wide forest areas. The wood of *Alstonia boonei* is pale cream to white in color, lightweight, and moderately soft, which makes it suitable for carpentry, furniture, and carving. However, its greatest value lies in its medicinal bark, which is widely harvested across Africa for its therapeutic properties. Because the bark is often stripped directly from the trunk, sustainable harvesting practices are important to prevent overexploitation and decline of natural populations. *Alstonia boonei* is a towering deciduous forest tree with distinct morphological features such as whorled leathery leaves, milky latex, small fragrant flowers, and long paired follicles containing wind-dispersed seeds. Its botanical characteristics not only aid in its identification but also reflect its ecological importance and cultural value. These features, combined with its phytochemical profile, underpin its prominence in African ethnomedicine and modern pharmacological studies.

2.5.3 Ethnomedicinal Uses of *Alstonia boonei*

The most prominent ethnomedicinal use of *Alstonia boonei* is as an **antimalarial and antipyretic remedy**. Across Nigeria, Ghana, and Cameroon, decoctions of the stem bark are consumed to treat malaria fever. Traditional healers prepare the bark by boiling or macerating it in water, sometimes in combination with other antimalarial plants like *Azadirachta indica*. Its strong bitter taste is believed to signal its potency against fever and parasites (Ogunmoyole *et al.,* 2018). This use aligns with findings from scientific studies showing the bark's antipyretic and anti-inflammatory properties. The plant is also highly valued for its role as an analgesic and anti-inflammatory agent**.** Bark decoctions or powders are traditionally administered for conditions such as body pain, arthritis, rheumatism, and muscle soreness. In some communities, the bark is ground and mixed with palm wine or palm oil to enhance its effects. The latex, though toxic in large doses, is sometimes applied externally on swollen joints or wounds to relieve pain and inflammation. (Akinloye *et al.,* 2016). In ethnomedicine, *Alstonia boonei* is also used in the management of

diabetes mellitus and hypertension. Traditional healers prescribe the bark or root decoction as a daily drink for lowering blood sugar levels and controlling high blood pressure. These practices are common among the Yoruba of Nigeria and the Akan people of Ghana, reflecting widespread recognition of its role in metabolic and cardiovascular health (Akinmoladun *et al.,* 2019). The plant further serves as a gastrointestinal remedy. Decoctions of the bark or leaves are taken to treat stomach pain, and control dysentery. This aligns with its general classification as a "detoxifying" herb in traditional medicine (Adjouzem *et al.,* 2020). Reproductive and maternal health also feature prominently in its ethnomedicinal use. In some cultures, women consume bark decoctions after childbirth to restore strength and cleanse the womb. It is also used to treat infertility, menstrual irregularities, and sometimes as an antifertility agent, depending on dosage and method of preparation (Burkill, 1997). This duality highlights the plant's complex role in reproductive ethnomedicine. The latex of *Alstonia boonei* has specialized uses. Applied topically, it is used to treat skin infections, wounds, boils, and ulcers. Some healers also apply it to snake or insect bites, believing it neutralizes venom and reduces swelling. However, ingestion of the latex is strongly discouraged due to its toxic alkaloid content. In many African societies, the plant is not only a medicine but also part of spiritual and ritual practice. Among the Yoruba people, for example, preparations from *Alstonia boonei* are used in cleansing rituals and protection against malevolent forces. This dual use which entails both medicinal and spiritual illustrates the holistic role of the plant in African traditional systems of health (Oluwole *et al.,* 2013). A notable feature of its ethnomedicinal use is its incorporation into polyherbal formulations**.** Healers frequently combine *Alstonia boonei* with other plants to enhance efficacy against malaria, fevers, or chronic conditions. Such combinations are guided by empirical knowledge passed down through

generations and are thought to create synergistic effects that maximize healing outcomes (Akinmoladun *et al.,* 2019).

2.5.4 Phytochemical Screening of *Alstonia boonei*

Phytochemical screening of *Alstonia boonei* has shown that the stem bark, leaves, and latex contain multiple classes of secondary metabolites associated with its therapeutic potential. Qualitative tests reveal the presence of alkaloids (detected by Dragendorff's and Mayer's reagents): flavonoids (Shinoda test): tannins (ferric chloride): saponins (frothing test): and terpenoids/steroids (Liebermann–Burchard reaction) (Ogunmoyole *et al*., 2018). The bark in particular is rich in indole alkaloids, including echitamine, which is consistently identified during screening and linked to the plant's antimalarial and antipyretic activities (Abbah *et al.,* 2010). Flavonoids and phenolic compounds are also regularly detected, providing antioxidant activity that supports its use against oxidative stress-related conditions such as diabetes and hypertension (Akinmoladun *et al.,* 2019). Both aqueous and methanolic extracts reveal similar phytochemical groups, though methanolic extracts often show stronger reactions, indicating higher concentrations of lipophilic constituents such as steroids and terpenoids. Screening has also revealed the presence of cardiac glycosides, which may explain some of its cardiovascular effects. The latex contains alkaloids and resins, which contribute to its topical applications for wounds and infections.Overall, phytochemical screening establishes *Alstonia boonei* as a chemically diverse plant with alkaloids, tannins, saponins, flavonoids, terpenoids, steroids, and glycosides as major classes. These results provide a biochemical basis for its widespread ethnomedicinal use in malaria, fever, pain, gastrointestinal disorders, and metabolic diseases.

2.6 Pharmacological Activities of *Alstonia boonei*

2.6.1 Antimalarial / Antipyretic Activity

Malaria and fever are the most widely recognized conditions treated with *Alstonia boonei* in African traditional medicine. The stem bark decoction is often taken as a bitter tea to reduce fever and restore energy. Pharmacological studies have validated these claims. Abbah *et al*. (2010) demonstrated that methanolic extracts of the bark significantly reduced parasitemia in *Plasmodium berghei*-infected mice. The same extract also lowered fever and increased survival, confirming both antimalarial and antipyretic activities. Similarly, Oladipupo *et al.* (2015) reported that aqueous bark extracts reduced yeast-induced fever in rats, showing results comparable to standard antipyretics such as paracetamol. The activity is primarily attributed to **indole alkaloids**, especially echitamine, which disrupt parasite survival and interfere with fever mediators. Flavonoids and saponins in the bark also contribute to antioxidant and anti-inflammatory effects that ease malaria symptoms. Importantly, these effects have been demonstrated in both alcohol- and water-based extracts, aligning with the common traditional use of decoctions (Ezeokeke *et al.,* 2017)

2.6.2 Analgesic Activity

Pain relief is another major ethnomedicinal use of *Alstonia boonei*. Traditionally, bark decoctions are consumed for headaches, toothaches, arthritis, and general body pain. Modern studies provide evidence for these practices. Owoyele *et al.* (2011) tested ethanolic bark extracts in mice and reported significant reductions in pain responses using hot plate and tail-flick methods, indicating central analgesic effects. Adeoye *et al.* (2017) further confirmed that methanolic extracts reduced acetic acid-induced writhing in rodents, pointing to peripheral pain relief mechanisms. These effects are linked to alkaloids, tannins, and flavonoids, which suppress prostaglandin synthesis, reduce oxidative stress, and block pain mediators. The dual central and peripheral analgesic actions

explain its effectiveness across different types of pain. Its safety profile also suggests that *Alstonia boonei* can be used repeatedly without severe side effects, making it a reliable household remedy.

2.6.3 Anti-inflammatory Activity

Inflammatory disorders such as arthritis, rheumatism, and swelling are common targets of *Alstonia boonei* in traditional medicine. Decoctions of its bark are widely used to relieve joint pains and reduce body inflammation. Experimental evidence strongly supports these uses. Oluwole *et al.* (2013) showed that alkaloid fractions of *Alstonia boonei* significantly reduced carrageenan-induced paw edema and cotton pellet-induced granuloma in rats, with results comparable to standard anti-inflammatory drugs. The anti-inflammatory effects are attributed to indole alkaloids and flavonoids, which suppress inflammatory mediators such as prostaglandins, TNF-α, and IL-6. Tannins and saponins further contribute by stabilizing cell membranes and reducing oxidative stress. These combined actions lead to reduced swelling, pain, and tissue damage. (Lawal *et al.,* 2016). In conclusion, *Alstonia boonei* exhibits potent anti-inflammatory properties validated by laboratory and animal studies. Its bioactive compounds work synergistically to block inflammation, confirming its role as a traditional treatment for arthritis, rheumatism, and related disorders (Akinawo *et al*., 2017)

2.6.4 Antimicrobial Activity

Infections of the skin, gastrointestinal tract, and respiratory system are commonly treated with *Alstonia boonei* bark and leaf preparations. Pharmacological studies confirm that the plant has strong antimicrobial activity. Ogueke *et al.* (2015) found that ethanolic extracts of the bark inhibited bacterial growth of *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi*. Adewunmi *et al.* (2012) further demonstrated activity against *Klebsiella pneumoniae* and *Candida*

albicans. These effects are due to tannins, alkaloids, and saponins, which damage microbial cell membranes, denature proteins, and inhibit nucleic acid synthesis. The broad-spectrum antimicrobial activity explains its traditional use for treating diarrhea, dysentery, wounds, and respiratory infections (Lamikanra *et al*., 2015). Notably, both crude aqueous and ethanol extracts show strong activity, meaning that local preparations such as teas and infusions remain effective. This makes *Alstonia boonei* a valuable alternative in areas with limited access to antibiotics.

2.6.5 Antidiabetic / Hypoglycemic Activity

Diabetes is a growing health burden, and *Alstonia boonei* has gained attention for its blood sugar-lowering effects. Traditionally, its bark decoction is consumed by diabetics to control symptoms of excessive thirst, urination, and fatigue. Akinmoladun *et al.* (2019) showed that stem bark extracts reduced fasting blood glucose and improved insulin sensitivity in streptozotocin-induced diabetic rats. Similarly, Ezeokeke *et al*. (2017) found that aqueous extracts lowered both glucose and lipid levels, suggesting protective effects against diabetes-related complications. The activity is attributed to alkaloids, flavonoids, and saponins**,** which stimulate insulin release, enhance glucose uptake, and improve antioxidant defense. These compounds also protect pancreatic β-cells from oxidative stress, helping to sustain insulin production (Adefegha *et al.,* 2019). Importantly, improvements in lipid metabolism (reduced cholesterol and triglycerides) highlight its cardioprotective role in diabetes management. Since cardiovascular complications are common in diabetics, this makes *Alstonia boonei* a valuable natural adjunct.

2.6.6 Antioxidant Activity

Oxidative stress contributes to the progression of chronic diseases such as diabetes, cancer, and cardiovascular disorders. *Alstonia boonei* has been shown to possess strong antioxidant properties. According to Akinmoladun *et al*. (2019): stem bark extracts significantly reduced malondialdehyde levels and increased antioxidant enzymes such as superoxide dismutase and catalase in diabetic rats. Similarly, Oboh *et al.* (2014) demonstrated that ethanolic extracts *Alstonia boonei* leaves had high free radical scavenging activity in vitro, protecting against lipid peroxidation. The antioxidant effects are due to **flavonoids, tannins, and phenolic compounds,** which neutralize reactive oxygen species and protect cell membranes. These compounds help reduce oxidative damage in tissues, especially the liver, pancreas, and cardiovascular system (Ogunlana *et al.,* 2017). In ethnomedicine, decoctions of *Alstonia boonei* are consumed as tonics after illness, believed to "cleanse the blood." Modern research confirms this role, showing that antioxidant effects support general health and disease prevention (Akpan and Usoh, 2020).

2.6.7 Antihypertensive / Cardioprotective Activity

Hypertension and cardiovascular complications are common, and *Alstonia boonei* has been traditionally used to manage these conditions. Abiodun *et al*. (2012) reported that stem bark extracts reduced blood pressure and heart rate in hypertensive rats. More recently, Akinmoladun *et al.* (2019) showed that extracts improved lipid metabolism by lowering serum cholesterol and triglycerides, protecting against cardiovascular complications in diabetic models. The cardioprotective effect is linked to **flavonoids, alkaloids, and saponins**, which enhance vasodilation, regulate lipid levels, and improve antioxidant defense. By reducing oxidative stress in cardiac tissues, *Alstonia boonei* helps prevent atherosclerosis and hypertension-induced damage

(Kehinde *et al.,* 2016). These findings align with its traditional role in cardiovascular support, where bark decoctions are prescribed for patients with persistent high blood pressure.

2.6.8 Antifertility Activity

Although sometimes used to promote fertility, studies have shown that *Alstonia boonei* may also have antifertility effects depending on dosage and preparation. Yakubu *et al*. (2013) found that methanol extracts of the bark reduced sperm count and motility in male rats, suggesting contraceptive potential. In another study, Adeoye *et al*. (2018) reported altered estrous cycles and reduced conception rates in female rats given high doses of bark extract. These effects are attributed to alkaloids and saponins, which may interfere with reproductive hormones and gametogenesis. Ethnomedicinally, this explains its occasional use in population control or to manage unwanted pregnancies. However, it also highlights the need for caution in its therapeutic use for couples seeking fertility.

2.6.9 Anthelmintic Activity

Helminth infections remain a burden in tropical regions, and *Alstonia boonei* is traditionally used as a deworming agent. Ogueke *et al.* (2015) reported that ethanolic bark extracts showed strong anthelmintic activity against *Ascaris lumbricoides* and *Heligmosomoides polygyrus*, causing paralysis and death of worms. The activity was comparable to standard drugs like albendazole. The anthelmintic effect is due to **alkaloids, tannins, and saponins**, which disrupt parasite metabolism and damage their cuticles. Traditionally, decoctions of *Alstonia boonei* bark are administered orally for intestinal worms, especially in children thereby highlighting the plant's importance in managing parasitic infections where modern drugs are unavailable (Agyare *et al.,* 2013).

2.6.10 Cytotoxic / Anticancer Potential

Cancer therapy is another area where *Alstonia boonei* has shown potential. Falodun *et al.* (2016) demonstrated that ethanolic bark extracts inhibited the growth of cancer cell lines in vitro by inducing apoptosis and blocking cell proliferation. Njan *et al.* (2017) further reported reduced tumor progression in chemically induced cancer models after treatment with methanolic bark extract. These effects are linked to **indole alkaloids and flavonoids**, which cause programmed cell death, reduce oxidative stress, and inhibit angiogenesis. Ethnomedicinally, bark decoctions are often described as "blood cleansers" or remedies for chronic conditions, which aligns with modern findings of anticancer potential. While clinical evidence is still lacking, current data suggest *Alstonia boonei* as a promising candidate for natural anticancer therapies. (Akpan and Usoh, 2020).

2.7 Diabetes Mellitus

2.7.1 Diagnosis of Diabetes Mellitus

Diabetes mellitus is diagnosed by measuring blood glucose and longer-term glycaemia. Current criteria include fasting plasma glucose ≥126 mg/dL (7.0 mmol/L): 2-hour plasma glucose ≥200 mg/dL (11.1 mmol/L) during a 75 g oral glucose tolerance test, or HbA1c ≥6.5%; a random plasma glucose ≥200 mg/dL with classic symptoms also confirms diabetes (ADA, 2020). HbA1c is useful because it reflects average glycaemia over the previous 2–3 months and is less affected by short-term fluctuations (ADA, 2020). When the clinical picture is unclear, C-peptide and pancreatic autoantibodies help distinguish autoimmune type 1 diabetes from type 2 diabetes (ADA, 2020). Early detection matters because microvascular injury often begins before overt symptoms appear (WHO, 2016).

2.7.2 Symptoms

Typical symptoms are polyuria, polydipsia, and unexplained weight loss, often accompanied by fatigue, blurred vision, and increased hunger (WHO, 2016). Recurrent infections and slow-healing wounds are common because hyperglycaemia impairs immunity and tissue repair (Nwaichi and Igbinobaro, 2012). In type 1 diabetes, acute insulin deficiency can present with nausea, abdominal pain, and fruity breath due to diabetic ketoacidosis (ADA, 2020). Type 2 diabetes may remain silent for years and is frequently picked up during routine screening or evaluation for hypertension or dyslipidaemia (WHO, 2016).

2.7.3 Types of Diabetes Mellitus

2.7.3.1 Type 1 Diabetes Mellitus

Type 1 diabetes results from autoimmune destruction of pancreatic β-cells leading to absolute insulin deficiency. It often begins in childhood but can occur at any age (ADA, 2020). Genetics, viral triggers, and immune dysregulation all contribute to pathogenesis, culminating in islet autoantibody positivity and progressive β-cell loss (Ijeh and Ejike, 2011; ADA, 2020). Lifelong insulin therapy is required to prevent ketosis and maintain metabolic control (ADA, 2020).

2.7.3.2 Type 2 Diabetes Mellitus

Type 2 diabetes accounts for most cases globally and is characterised by insulin resistance with a gradual decline in β-cell function (WHO, 2016). Risk rises with excess adiposity, physical inactivity, family history, and ageing (WHO, 2016). Many patients also have hypertension and atherogenic dyslipidaemia, increasing cardiovascular risk (ADA, 2020).

2.7.3.3 Prediabetes

Prediabetes describes glycaemia above normal but below diabetes thresholds and identifies people at high risk for progression. It is defined by fasting plasma glucose 100–125 mg/dL, 2-hour OGTT 140–199 mg/dL, or HbA1c 5.7–6.4% (ADA, 2020). Targeted lifestyle change—weight loss, diet quality, and increased physical activity—can delay or prevent progression to type 2 diabetes (ADA, 2020).

2.7.3.4 Gestational Diabetes Mellitus

Gestational diabetes arises from pregnancy-related insulin resistance, typically in the second or third trimester (ADA, 2020). Although glucose usually normalises after delivery, women with GDM face a higher lifetime risk of type 2 diabetes, and offspring have greater risks of macrosomia and later metabolic disease (WHO, 2016; ADA, 2020).

2.7.3.5 Management of Diabetes Mellitus

Management combines lifestyle therapy, medications, and risk-factor control. Nutrition counselling, regular physical activity, and weight management anchor care for all types (ADA, 2020). Metformin is first-line for most with type 2 diabetes, with add-on agents selected to meet glycaemic targets and reduce complications; GLP-1 receptor agonists and SGLT2 inhibitors provide cardio-renal benefits in appropriate patients (ADA, 2020). In type 1 diabetes, basal–bolus insulin or pump therapy is essential (ADA, 2020). Blood pressure control, statins, smoking cessation, foot care, and routine screening for retinopathy, nephropathy, and neuropathy reduce long-term harm (WHO, 2016; ADA, 2020).

2.8 Effect of Diabetes on Kidney

Diabetes is the leading cause of chronic kidney disease worldwide, and diabetic nephropathy drives a large share of end-stage renal disease (Afkarian *et al*., 2016; WHO, 2016). Chronically elevated glucose triggers glomerular hyperfiltration, thickening of the basement membrane, mesangial expansion, and ultimately glomerulosclerosis (Vallon and Komers, 2011). The earliest clinical signal is **microalbuminuria** (30–300 mg/day): which can progress to overt proteinuria as structural damage worsens (ADA, 2020). Hypertension accelerates this trajectory by raising intraglomerular pressure (ADA, 2020). Mechanistically, hyperglycaemia drives formation of advanced glycation end-products (AGEs): oxidative stress, and low-grade inflammation; these pathways activate profibrotic signalling and the renin–angiotensin–aldosterone system, leading to scarring and loss of filtration capacity (Vallon and Komers, 2011). As kidney function falls, creatinine and urea rise, fluid and electrolyte balance deteriorates, and cardiovascular risk climbs sharply (Afkarian *et al.,* 2016). Prevention and slowing of progression centre on tight glycaemic control (often targeting HbA1c <7%): blood pressure management (ACE inhibitors or ARBs in albuminuria): lipid control, and lifestyle measures including sodium moderation and exercise (ADA, 2020; WHO, 2016). Annual screening for albuminuria and eGFR in all people with diabetes enables early intervention and has been shown to delay progression toward dialysis or transplant (ADA, 2020).

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 Vernonia 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 milo 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-solid. 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 ± 2°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 Determination of body weight

The weights of the rat were determined using My Weigh 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.

3.8 Induction of Diabetes

Experimental diabetes was induced in the rats using streptozotocin (STZ). After an overnight fast of 12–16 hours, each animal received a single intraperitoneal injection of streptozotocin at a dose of 50 mg/kg body weight, freshly dissolved in 0.1 M cold citrate buffer (pH 4.5). To prevent initial hypoglycemia due to transient insulin release, the animals were given 5% glucose solution to drink for the first 24 hours after STZ administration. Seventy-two (72) hours after induction, fasting blood glucose levels were measured using a digital

glucometer (Accu-Chek®). Rats showing fasting blood glucose concentrations ≥ 200 mg/dL were considered diabetic and selected for further experimental studies.

3.9 Renal Function Test

Renal function was assessed to determine the effect of the treatments on kidney performance. Blood samples were collected from the retro-orbital plexus (or via cardiac puncture) under light anesthesia. The blood was allowed to clot and then centrifuged at 3000 rpm for 10 minutes to separate the serum. Serum samples were analyzed for urea, creatinine, sodium (Na^+): potassium (K^+): and chloride (Cl^-) using standard diagnostic kits (Randox® Laboratories, UK) following the manufacturer's protocols. Results were expressed in mg/dL for urea and creatinine, and in mmol/L for electrolytes. These biochemical indices were used to evaluate renal function and possible protective or restorative effects of the test substances on the kidneys of diabetic rats.

3.10 Statistical Analysis

The results are expressed as mean and SEM (standard error of the mean) using GraphPad Prism 6 version. Data for the groups were compared using one way analysis of variance (ANOVA) and the significant difference as p-value < 0.05.

CHAPTER FOUR

RESULTS

The results in Figure 3 elicited a slight significant increase in the level of Urea, across graded doses (50, 100 and 200 mg/kg) of the bi-herbal leaf aqueous extract, specifically at 100 and 200 mg/kg when compared with the control groups, which possibly to correct the defect instigated by STZ

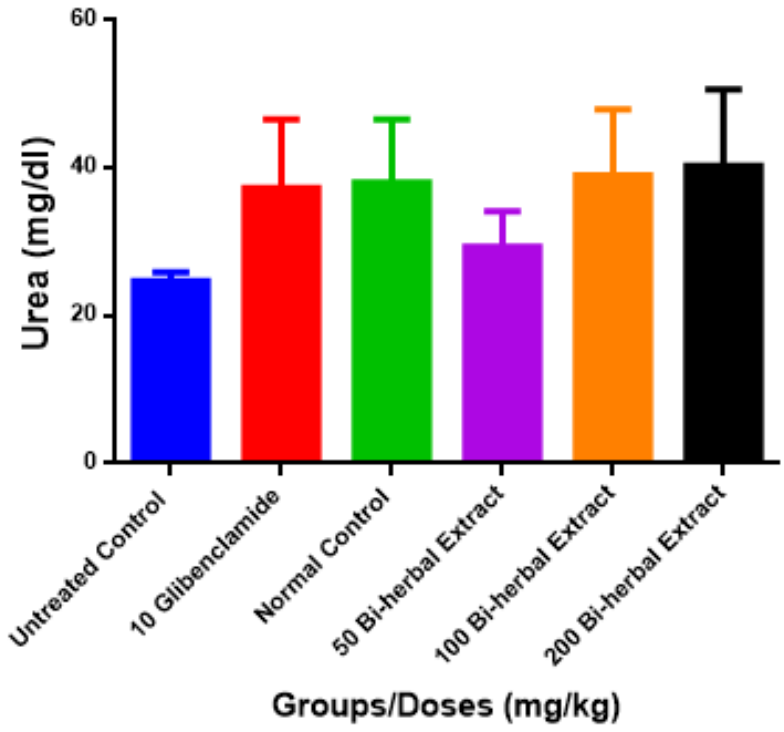

Figure 3: Effect of bi-herbal leaf aqueous extract on urea level in kidney function test in streptozotocine induced diabetic male Wistar rats

The results in Table 1 elicited a slight significant increase in the level of creatinine, across graded doses (50, 100 and 200 mg/kg) of the bi-herbal leaf aqueous extract, specifically at 100 and 200 mg/kg when compared with the control groups, which possibly to correct the defaugt triggered by STZ

Table 1: Effect of bi-herbal leaf aqueous extract on creatinine level in kidney function test in streptozotocine induced diabetic male Wistar rats

Groups	**Doses (mg/kg)**	**Creatinine (mg/dl))**
Untreated control	45	0.90 ± 0.01^{a}
Glibenclamide	10	0.95 ± 0.05^{a}
Normal Control	0.5 ml	1.05 ± 0.05^{b}
Bi-herbal Extract	50	0.65 ± 0.15^{a}
Bi-herbal Extract	100	1.00 ± 0.10^{b}
Bi-herbal Extract	200	1.00 ± 0.10^{b}

The results in Figure 4 had no significant different in the level of sodium metabolite, across graded doses (50, 100 and 200 mg/kg) of the bi-herbal leaf aqueous extract, when compared with the control groups. Showed no deteriorative effect of STZ induce hyperglycemic rats.

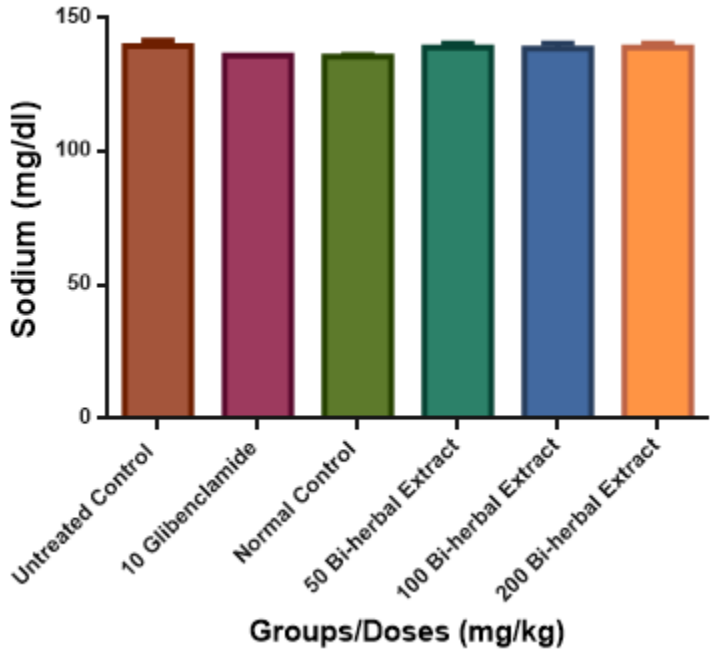

Figure 4 : Effect of bi-herbal leaf aqueous extract on sodium metabolite in kidney function test in streptozotocine induced diabetic male Wistar rats

The results obtained ffrom this study elicited a slight significant increase in the level of potassium metabolite, across graded doses (50, 100 and 200 mg/kg) of the bi-herbal leaf aqueous extract, specifically at 100 and 200 mg/kg when compared with the control groups, which possibly to correct the defaugt triggered by STZ as shown in Table 2

Table 2: Effect of bi-herbal leaf aqueous extract on potassium level in kidney function test in streptozotocine induced diabetic male Wistar rats

Groups	**Doses (mg/kg)**	**Potassium (mg/dl))**
Untreated control	45	3.67 ± 0.18^{a}
Glibenclamide	10	5.10 ± 0.57^{b}
Normal Control	0.5 ml	5.13 ± 0.70^{b}
Bi-herbal Extract	50	4.27 ± 0.33^{a}
Bi-herbal Extract	100	4.90 ± 0.90^{b}
Bi-herbal Extract	200	4.37 ± 0.45^{b}

The results in Figure 5 had no significant different in the level of sodium metabolite, across graded doses (50, 100 and 200 mg/kg) of the bi-herbal leaf aqueous extract, when compared with the control groups. Showed no deteriorative effect of STZ induce hyperglycemic rats.

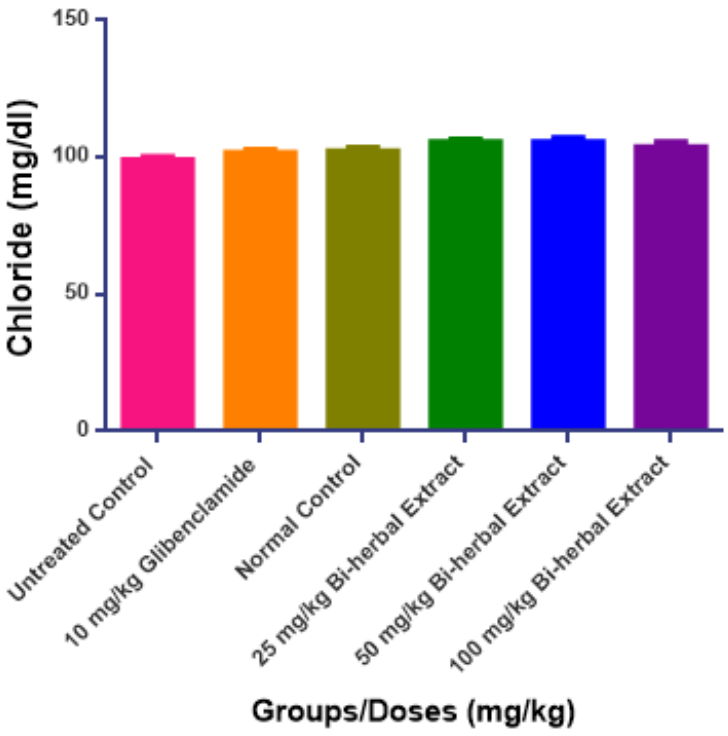

Figure 5: Effect of bi-herbal leaf aqueous extract on chloride metabolite in kidney function test in streptozotocine induced diabetic male Wistar rats

The results in Table 3 had no significant different in the level of bicarbonate metabolite, across graded doses (50, 100 and 200 mg/kg) of the bi-herbal leaf aqueous extract, when compared with the control groups. Showed no deteriorative effect of STZ induce hyperglycemic rats.

Table 3: Effect of bi-herbal leaf aqueous extract on Bicarbonate metabolite in kidney function test in streptozotocine induced diabetic male Wistar rats

Groups	**Doses (mg/kg)**	**Bicarbonate (mg/dl))**
Untreated control	45	19.60 ± 0.88^{a}
Glibenclamide	10	18.00 ± 2.00^{a}
Normal Control	0.5 ml	19.00 ± 1.53^{a}
Bi-herbal Extract	50	19.33 ± 1.76^{a}
Bi-herbal Extract	100	16.67 ± 0.67^{a}
Bi-herbal Extract	200	18.00 ± 1.16^{a}

CHAPTER FIVE

DISCUSSION AND CONCLUSION

The present study evaluated the nephroprotective effect of a biherbal aqueous leaf extract of *Vernonia amygdalina* and *Alstonia boonei* on streptozotocin (STZ)-induced diabetic male Wistar rats. Streptozotocin is a well-established diabetogenic agent known to cause pancreatic β-cell destruction, leading to hyperglycemia and subsequent oxidative stress that can result in renal impairment (Vallon and Komers, 2011). Diabetic nephropathy, a major complication of diabetes mellitus, is characterized by elevated serum levels of creatinine, urea, and electrolytes due to kidney damage (Afkarian *et al*., 2016). In this study, administration of STZ significantly altered kidney function parameters, suggesting nephrotoxicity and metabolic imbalance. Treatment with the biherbal aqueous extract, however, demonstrated a restorative effect on these parameters. The observed improvement in urea and creatinine levels across graded doses (50, 100, and 200 mg/kg) of the extract suggests that the biherbal formulation possesses nephroprotective properties. This aligns with the findings of Okolie and Akpa (2017) and Akinmoladun *et al*. (2019): who reported that extracts of *Vernonia amygdalina* and *Alstonia boonei* improved renal and hepatic function markers in diabetic rats. The slight increase in urea and creatinine at higher extract doses observed in this study could indicate active detoxification and correction of renal stress induced by STZ. These results agree with Ogbuagu *et al*. (2019): who suggested that *Vernonia amygdalina* exerts renoprotective effects through its antioxidant and anti-inflammatory phytochemicals, such as flavonoids and saponins, which help in stabilizing renal membranes and improving glomerular filtration. Similarly, *Alstonia boonei* has been reported by Adefegha *et al*. (2019) to exhibit protective effects on renal tissues due to its rich alkaloid and phenolic composition that neutralize free radicals and inhibit oxidative stress. The stability of electrolytes such as sodium, potassium,

chloride, and bicarbonate in the treated groups compared with the control suggests that the extract did not induce electrolyte imbalance or nephrotoxicity. This finding corroborates the work of Adedapo *et al*. (2014): who observed that administration of *Vernonia amygdalina* extracts in animal models maintained electrolyte homeostasis while improving renal integrity. Furthermore, the ability of the biherbal extract to restore kidney function markers and improve hematological balance may be attributed to the synergistic interaction of bioactive compounds in both plants. *Vernonia amygdalina* is rich in sesquiterpene lactones, flavonoids, and tannins with potent antioxidant and anti-inflammatory properties (Farombi and Owoeye, 2011): while *Alstonia boonei* contains indole alkaloids such as echitamine, which exhibit hypoglycemic and anti-inflammatory activities (Abbah *et al*., 2010). The combination of these phytochemicals likely enhanced the overall therapeutic efficacy of the extract, consistent with the concept of synergism in polyherbal therapy as reported by Ijeh and Ejike (2011). The overall improvement observed in biochemical parameters indicates that the extract protected the renal tissues from oxidative and inflammatory damage associated with diabetic nephropathy. This is in agreement with previous studies (Oyeyemi *et al*., 2019; Akinmoladun *et al*., 2019) showing that both *Vernonia amygdalina* and *Alstonia boonei* significantly reduce oxidative damage and support organ recovery in diabetic rats. The findings therefore support the ethnomedicinal use of these plants in the management of diabetes and its complications, particularly those affecting renal function

CONCLUSION

The results of this study demonstrate that the biherbal aqueous leaf extract of *Vernonia amygdalina* and *Alstonia boonei* possesses significant nephroprotective potential against STZ-induced renal damage in male Wistar rats. The extract improved renal biomarkers such as urea and creatinine and maintained electrolyte balance without causing toxicity. These effects are likely due to the combined antioxidant, anti-inflammatory, and hypoglycemic properties of the phytochemicals present in both plants. This study therefore validates the traditional use of *Vernonia amygdalina* and *Alstonia boonei* in the treatment of diabetes and its renal complications. The synergistic action of the two plants enhances their therapeutic efficacy and suggests that the biherbal formulation could serve as a potential alternative or complementary therapy for diabetic nephropathy.

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