**CHAPTER ONE**

**INTRODUCTION**

**1.1 Background of the Study**

The use of plants as sources of food and medicine is as old as human civilization. In recent years, there has been a growing global interest in the use of plant-derived compounds for promoting health and treating diseases due to the adverse effects associated with synthetic drugs (Okagu et al., 2021). One such plant that has received considerable scientific attention is the bitter leaf (*Vernonia amygdalina*), a perennial shrub widely distributed across tropical Africa.

Bitter leaf is a vital part of many African diets and traditional healing practices. It is primarily valued for its distinct bitter taste and rich medicinal properties. In traditional medicine, the leaves are commonly used to treat malaria, typhoid, gastrointestinal disorders, diabetes, and inflammation-related conditions (Ogunyemi *et al.,* 2023). Modern studies have confirmed that these health benefits are largely due to the presence of diverse phytochemicals such as flavonoids, saponins, alkaloids, tannins, and terpenoids (Egbuna *et al.,* 2022).

Phytochemicals are non-nutritive plant chemicals that have protective or disease-preventing properties. Several bioactive compounds isolated from bitter leaf have demonstrated significant antioxidant, antimicrobial, anticancer, and particularly, anti-inflammatory activities (Obasi et al., 2020). These properties make bitter leaf a promising candidate for the development of plant-based therapies against chronic diseases, many of which are driven by oxidative stress and inflammation.

Besides its medicinal properties, bitter leaf is highly nutritious. It contains essential vitamins such as Vitamin A, Vitamin C, and Vitamin E, minerals like calcium, potassium, magnesium, and iron, as well as dietary fiber and proteins (Akinmoladun et al., 2021). This nutritional richness makes it valuable in combating malnutrition and boosting immune function, especially in low-resource settings.

Inflammation is a natural biological response to harmful stimuli, such as pathogens, damaged cells, or irritants. However, chronic inflammation is now recognized as a key contributor to the development of several non-communicable diseases, including cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders (Libby, 2021). With the limitations and side effects of many conventional anti-inflammatory drugs, there is a pressing need for safer, natural alternatives. Plants like bitter leaf offer a promising source of such alternatives due to their multi-targeted mechanisms and lower toxicity profiles (Nwafor *et al.,* 2022).

Recent scientific investigations have focused on validating the anti-inflammatory potential of bitter leaf extracts through in vitro and in vivo studies. For example, a study by Ibrahim *et al.* (2023) demonstrated that aqueous extracts of *Vernonia amygdalina* significantly reduced inflammatory markers in rat models, suggesting potential therapeutic applications for inflammatory diseases.

Given the increasing global emphasis on natural health products and evidence-based herbal medicine, it becomes essential to scientifically evaluate and document the phytochemical composition, nutritional value, and anti-inflammatory properties of bitter leaf. Such studies not only confirm traditional uses but also pave the way for the development of novel nutraceuticals and pharmaceuticals derived from indigenous plants.

**1.2 Statement of the Problem**

Despite the recognized traditional use of bitter leaf in managing various ailments, there is still limited detailed scientific documentation regarding its phytochemical composition, nutritional benefits, and mechanisms underlying its anti-inflammatory effects. Many individuals consume bitter leaf based on traditional beliefs without standardized information on its active components or verified health benefits.

Furthermore, chronic inflammatory diseases continue to pose major public health challenges worldwide. There is a critical need to explore natural, affordable, and accessible anti-inflammatory agents. Therefore, it is essential to scientifically validate the traditional claims surrounding bitter leaf to ensure its safe and effective use in disease prevention and management.

**1.3 Aim and Objectives of the Study**

**1.3.1 Aim**

The aim of this study is to evaluate the phytochemical constituents, nutritional composition, and anti-inflammatory activities of bitter leaf (*Vernonia amygdalina*).

**1.3.2 Objectives**

Specifically, the study seeks to:

1. Identify and quantify the phytochemical compounds present in bitter leaf.
2. Analyze the nutritional content of bitter leaf, including proteins, vitamins, minerals, and fiber.
3. Determine the anti-inflammatory activities of bitter leaf through laboratory testing methods.

**1.4 Significance of the Study**

This study will contribute significantly to the body of knowledge on the medicinal and nutritional importance of bitter leaf. It will provide scientific evidence supporting its traditional uses, promote the use of natural therapies in managing inflammation, and potentially guide the development of phytochemical-based anti-inflammatory drugs. Additionally, it will encourage dietary diversification and the inclusion of nutrient-rich plants in everyday nutrition, especially in developing countries where access to conventional medicine may be limited.

**1.5 Scope of the Study**

This study is focused on investigating the phytochemical composition, nutritional value, and anti-inflammatory activities of bitter leaf (*Vernonia amygdalina*). It involves the collection, preparation, and extraction of bitter leaf samples using standard laboratory procedures. The phytochemical screening will qualitatively and quantitatively assess the presence of important bioactive compounds such as alkaloids, flavonoids, tannins, saponins, terpenoids, and phenols. In addition, the nutritional analysis will cover the determination of macronutrients (proteins, fats, carbohydrates, fiber) and micronutrients (calcium, magnesium, iron, zinc) essential for human health. The study will also evaluate the anti-inflammatory potential of bitter leaf extracts through selected in vitro methods like protein denaturation inhibition assays, providing a scientific basis for its traditional use against inflammatory diseases.

However, the study is limited to bitter leaf and does not extend to other related plant species or varieties. It will focus solely on laboratory-based analyses without conducting clinical trials on humans or extensive in vivo animal testing. Only selected phytochemical and nutritional parameters will be assessed based on available resources and equipment. Environmental factors such as soil type, seasonal variations, and geographical differences influencing the chemical profile of bitter leaf are beyond the scope of this research. This defined boundary ensures that the study remains manageable, focused, and achievable within the available timeframe and logistical constraints.

**CHAPTER TWO**

**LITERATURE REVIEW**

**2.1 Introduction**

*Vernonia amygdalina* Delile, commonly referred to as bitter leaf, is a perennial shrub that belongs to the family Asteraceae. Native to tropical Africa, it is widely distributed across sub-Saharan regions where it plays a dual role as both a food and a medicinal plant. It has earned considerable attention from researchers due to its rich phytochemical content, nutritional value, and broad pharmacological activities, notably its anti-inflammatory, antioxidant, antidiabetic, antimalarial, and antimicrobial properties (Ijeh & Ejike, 2022; Ukoha *et al.,* 2023).

Bitter leaf is used in various forms decoctions, infusions, extracts, or directly as leafy vegetables in soups. In traditional medicine, it has been used to manage conditions such as malaria, diabetes, high blood pressure, gastrointestinal disorders, and inflammation (Nwachukwu et al., 2023). The pharmacological efficacy of *V. amygdalina* is largely attributed to its bioactive secondary metabolites such as flavonoids, alkaloids, saponins, tannins, terpenoids, and phenolics, which have been extensively studied (Adegbite & Ajayi, 2023; Oboh *et al.,* 2024).

Recent studies have shown that extracts from *V. amygdalina* modulate inflammatory pathways through mechanisms involving the inhibition of pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6, as well as suppression of oxidative stress-induced damage (Olamide & Enyinnaya, 2023). These findings support the ethnomedicinal use of the plant in treating inflammatory diseases such as arthritis and inflammatory bowel syndrome.

In terms of nutritional content, *V. amygdalina* leaves are rich in proteins, dietary fiber, essential minerals (such as calcium, iron, and magnesium), and vitamins (notably A, C, and E), making it an important functional food for improving health outcomes in low-resource communities (Onyechi & Oguejiofor, 2022; Ezeonu *et al.,* 2023). The nutritional and medicinal relevance of *V. amygdalina* has also encouraged its incorporation into dietary interventions aimed at combating malnutrition and oxidative stress-related diseases.

Despite its wide application, there are still gaps in knowledge concerning standardized dosages, compound isolation, and molecular mechanisms of action. Therefore, this literature review explores the phytochemical constituents, nutritional composition, and anti-inflammatory activity of *Vernonia amygdalina*, with the aim of consolidating existing research and identifying areas requiring further investigation.

## 2.2 Botanical Description and Ethnomedicinal Uses of *Vernonia amygdalina*

Vernonia amygdalina Delile, commonly referred to as bitter leaf, is a hardy perennial shrub that grows between 2 to 5 meters in height. It is characterized by rough, dark green leaves that are elliptical to lanceolate in shape and extremely bitter in taste due to the presence of sesquiterpene lactones such as vernodalin and vernomygdin (Oboh *et al.,* 2023). The plant belongs to the family **Asteraceae** and is commonly found growing wild or cultivated in home gardens throughout **tropical sub-Saharan Africa**, particularly in Nigeria, Cameroon, Ghana, and Uganda (Ezeonu *et al.,* 2023). It thrives in various ecological zones and soil types, which contributes to its wide availability and traditional importance.

Botanically, V. amygdalina has a soft, woody stem that becomes more robust with maturity. The leaves are simple, opposite, and about 6–20 cm long. The flowers are small and purple, usually arranged in terminal inflorescences. The plant reproduces through seeds or stem cuttings and requires minimal agronomic input, making it a sustainable source of leafy greens and medicinal agents in both urban and rural settings (Adegbite & Ajayi, 2023).

In African traditional medicine, Vernonia amygdalina has been used extensively for centuries to treat a wide array of health conditions. Traditional healers employ various parts of the plant—primarily the leaves but also the roots and bark for the preparation of decoctions, infusions, and extracts. The most common applications include the treatment of **malaria, fever, gastrointestinal disturbances (such as diarrhea and dysentery), hepatitis, diabetes, and inflammatory conditions** (Okeke *et al.,* 2022; Nwachukwu *et al.,* 2023).

Bitter leaf juice is often consumed to cleanse the gastrointestinal tract and boost the immune system, especially in convalescent patients. In Nigeria, the leaves are squeezed and washed in water to reduce their bitterness before being used in soups or as oral decoctions to treat parasitic infections and blood sugar imbalances (Olorunfemi et al., 2022).

Additionally, V. amygdalina is employed in post-partum care, where its decoctions are used to promote uterine contraction and reduce the risk of postnatal infection (Ukoha et al., 2023). In some regions, the roots and bark are chewed or boiled to relieve toothache, respiratory infections, and cough (Onyechi & Oguejiofor, 2022).

Scientific studies have corroborated many of these traditional uses, linking them to the plant’s **bioactive compounds** including **flavonoids, alkaloids, saponins, tannins, terpenoids, and phenolic acids,** which have demonstrated antimicrobial, anti-inflammatory, and antipyretic effects in vitro and in vivo (Olamide & Enyinnaya, 2023). Such findings validate the therapeutic potential of V. amygdalina and underscore the need for further pharmacological standardization and clinical trials.

## 2.3 Phytochemical Properties of Bitter Leaf (*Vernonia amygdalina*)

## ****2.3.1 Antioxidant Activity****

Bitter leaf (Vernonia amygdalina) is renowned for its high antioxidant content, primarily attributed to its rich composition of flavonoids, phenolics, and other phytochemicals. These antioxidants play a crucial role in neutralizing free radicals, thereby mitigating oxidative stress and protecting the body from various diseases, including cancer and cardiovascular disorders. A study by Ibrahim *et al*. (2023), demonstrated that the methanolic extract of Vernonia amygdalina exhibited significant radical-scavenging activity, comparable to standard antioxidants like ascorbic acid and quercetin.

**2.3.2 Antimicrobial Activity**

Bitter leaf exhibits potent antimicrobial properties against a broad spectrum of pathogens, including bacteria, fungi, and protozoa. The antimicrobial activity is largely due to the presence of bioactive compounds such as alkaloids, tannins, and saponins. Oboh *et al.* (2022), found that extracts of Vernonia amygdalina were effective against Escherichia coli, Staphylococcus aureus, and Candida albicans, suggesting its potential use in treating infections caused by these pathogens.

**2.3.3 Antidiabetic Activity**

Research has shown that Vernonia amygdalina can play a significant role in managing diabetes mellitus. The saponins, flavonoids, and glycosides in bitter leaf are believed to enhance insulin sensitivity and modulate carbohydrate metabolism. A study by Ekpo *et al.* (2021), reported that diabetic rats treated with bitter leaf extract showed a significant reduction in blood glucose levels and improved lipid profiles, highlighting its antidiabetic potential.

**2.3.4 Anti-inflammatory Activity**

The anti-inflammatory properties of bitter leaf are attributed to its ability to inhibit the synthesis of pro-inflammatory cytokines and enzymes. These properties make it useful in managing inflammatory conditions such as arthritis. According to a study by Eze *et al.* (2023), the administration of bitter leaf extract resulted in a marked decrease in inflammation markers in an animal model of induced inflammation.

**2.3.5 Anti-cancer Activity**

Bitter leaf has also been investigated for its potential anti-cancer properties. The phytochemicals in bitter leaf, such as flavonoids and sesquiterpene lactones, have been shown to induce apoptosis and inhibit the proliferation of cancer cells. Recent research by Adeoye *et al.* (2023), demonstrated that extracts of Vernonia amygdalina significantly inhibited the growth of breast cancer cells in vitro, suggesting its potential as a complementary therapy in cancer treatment.

**2.3.6 Hepatoprotective Effects**

The hepatoprotective effects of bitter leaf are well-documented. The leaf's antioxidant properties help in detoxifying the liver and protecting it from damage caused by toxins. A study by Amadi *et al.* (2022), showed that rats treated with a hepatotoxic agent and subsequently administered Vernonia amygdalina extract had significantly lower liver enzyme levels and improved histopathological profiles compared to untreated controls.

## 2.4 Phytochemicals in Vernonia amygdalina

Phytochemicals are bioactive compounds found in plants that have been shown to provide numerous health benefits, often acting as antioxidants, antimicrobial agents, and anti-inflammatory compounds. In Vernonia amygdalina, these phytochemicals are responsible for the plant’s extensive medicinal use in traditional and modern health applications. The key phytochemicals in Vernonia amygdalina include flavonoids, alkaloids, saponins, tannins, phenolic compounds, and terpenoids, each contributing uniquely to the plant's health-promoting properties. Recent research has confirmed the health benefits of these phytochemicals in Vernonia amygdalina, particularly in the management of chronic diseases. Studies have highlighted its potential in managing conditions like diabetes, cardiovascular diseases, and cancer due to its rich composition of antioxidants and anti-inflammatory agents (Oluwafemi *et al.,* 2020; Adebayo *et al.,* 2022). The ongoing exploration of Vernonia amygdalina's phytochemical profile continues to uncover new therapeutic potentials, especially in areas where conventional medical treatments may be inaccessible (Okoye *et al.,* 2022).

## 2.4.1 Flavonoids

Flavonoids are a class of polyphenolic compounds found in plants, known for their strong antioxidant properties. In Vernonia amygdalina, flavonoids are among the most abundant phytochemicals and play a pivotal role in neutralizing free radicals, thereby protecting cells from oxidative damage. Free radicals can cause cellular dysfunction, leading to chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders (Oluwafemi *et al.,* 2020). The antioxidant action of flavonoids in Vernonia amygdalina also contributes to its anti-inflammatory properties, making it effective in managing inflammatory conditions like arthritis and other chronic inflammatory diseases (Ezeonu *et al.,* 2021).

In addition to their antioxidant effects, flavonoids in Vernonia amygdalina have shown promise in antimalarial and anticancer activities. Several studies have highlighted the role of flavonoids in inhibiting the growth of cancer cells by inducing apoptosis (programmed cell death) and preventing the proliferation of cancerous tissues (Adebayo et al., 2022). Furthermore, these compounds contribute to the plant's effectiveness in treating malaria by inhibiting the development of the malaria parasite Plasmodium falciparum (Nwaichi & Nwachukwu, 2021).

## 2.4.2 Alkaloids

Alkaloids are nitrogen-containing compounds that are known for their potent pharmacological activities, particularly their antimicrobial and analgesic properties. The presence of alkaloids in Vernonia amygdalina makes the plant effective against a variety of bacterial, fungal, and parasitic infections (Okoye et al., 2022). Alkaloids have been shown to disrupt the cell membranes of microorganisms, leading to their inhibition or destruction. This antimicrobial action is particularly valuable in the treatment of gastrointestinal infections and skin diseases, which are commonly treated with bitter leaf extracts (Fasuyi *et al.,* 2022).

Additionally, alkaloids in Vernonia amygdalina contribute to its analgesic properties. They act on the central nervous system to alleviate pain, making the plant useful in traditional medicine for treating conditions associated with pain and discomfort, such as headaches, toothaches, and menstrual cramps (Oluwafemi *et al.,* 2020).

## 2.4.3 Saponins

Saponins are glycosides that contribute to Vernonia amygdalina's bitterness and are known for their role in boosting the immune system and lowering cholesterol levels. Saponins work by stimulating the production of antibodies and other immune responses, enhancing the body's defense against infections (Ezeonu *et al.,* 2021). This immune-boosting effect is one reason Vernonia amygdalina is often used to treat common illnesses like colds, flu, and malaria in traditional African medicine (Adebayo *et al.,* 2022).

Moreover, saponins have been shown to bind with cholesterol in the intestines, preventing its absorption into the bloodstream. This cholesterol-lowering effect reduces the risk of cardiovascular diseases, such as atherosclerosis and hypertension (Oluwafemi et al., 2020). Saponins also possess anti-cancer properties, as they have been shown to inhibit the growth of cancer cells and reduce tumor size in animal studies (Nwaichi & Nwachukwu, 2021).

## 2.4.4 Tannins

Tannins are polyphenolic compounds with astringent properties, which make them effective in inhibiting the growth of microorganisms. In Vernonia amygdalina, tannins contribute to the plant’s antimicrobial potential by creating an inhospitable environment for bacteria and fungi (Okoye *et al*., 2022). Tannins bind to proteins in the cell membranes of pathogens, leading to their death or preventing their reproduction.

The astringent nature of tannins also aids in wound healing and helps to reduce inflammation, making Vernonia amygdalina extracts useful in the treatment of skin infections, ulcers, and other inflammatory conditions (Oluwafemi *et al.,* 2020). Tannins also play a role in the plant's ability to manage gastrointestinal issues, such as diarrhea and dysentery, by inhibiting the growth of pathogenic bacteria in the digestive tract (Ezeonu *et al.,* 2021).

## 2.4.5 Phenolic Compounds

Phenolic compounds are one of the most important groups of phytochemicals due to their antioxidant and anti-inflammatory effects. In Vernonia amygdalina, phenolic compounds help neutralize free radicals, reducing oxidative stress and the risk of developing chronic conditions such as cancer, cardiovascular diseases, and diabetes (Adebayo *et al.,* 2022). These compounds also exhibit anti-inflammatory properties, which contribute to the plant’s effectiveness in treating inflammatory disorders like arthritis, asthma, and inflammatory bowel diseases (Nwaichi & Nwachukwu, 2021).

Phenolics have also been shown to inhibit the growth of cancer cells by disrupting the cell cycle and inducing apoptosis (Oluwafemi et al., 2020). This makes Vernonia amygdalina a valuable plant in cancer prevention and treatment, especially in regions where access to conventional cancer therapies may be limited (Okoye *et al.,* 2022).

## 2.4.6 Terpenoids

Terpenoids, also known as isoprenoids, are another class of bioactive compounds found in Vernonia amygdalina. Terpenoids have been associated with a wide range of medicinal properties, including anti-inflammatory, antimalarial, and antibacterial activities. These compounds play a critical role in the plant's traditional use for treating malaria and other infectious diseases (Fasuyi *et al.,* 2022).

Terpenoids in Vernonia amygdalina help reduce inflammation by inhibiting the production of pro-inflammatory cytokines and enzymes, making the plant effective in managing inflammatory conditions like rheumatoid arthritis (Nwaichi & Nwachukwu, 2021). Additionally, the antimalarial properties of terpenoids have been well-documented, with studies showing that they help inhibit the growth of the Plasmodium parasite, which causes malaria (Adebayo *et al.,* 2022).

The antibacterial effects of terpenoids have also been demonstrated in studies showing their ability to disrupt bacterial cell membranes and inhibit bacterial enzyme activity. This makes Vernonia amygdalina an effective treatment for bacterial infections, including those affecting the skin, respiratory system, and digestive tract (Okoye *et al.,* 2022).

**2.5 Nutritional Composition of *Vernonia amygdalina***

In addition to its extensive pharmacological properties, Vernonia amygdalina plays a crucial role in human nutrition, particularly in sub-Saharan Africa where it is widely consumed as a leafy vegetable. It is frequently incorporated into traditional diets in Nigeria, Cameroon, and Ghana as part of soups, stews, or teas. The leaves, though bitter, are nutritionally dense and serve as a significant source of macro- and micronutrients essential for maintaining health, especially in communities facing food insecurity or malnutrition (Ezeonu *et al.,* 2023; Nwafor *et al.,* 2023).

## ****2.5.1 Proximate Composition****

Numerous studies have analyzed the proximate (basic nutritional) components of V. amygdalina, with findings indicating that it is a high-moisture, moderate-protein, and fiber-rich vegetable. On average, the composition per 100 grams of fresh leaves includes:

1. **Moisture content**: 70–85%
2. **Crude protein**: 5–12%
3. **Carbohydrates**: 6–14%
4. **Crude fiber**: 8–15%
5. **Ash content**: 3–6%
6. **Fat (lipid) content**: 1–3%

These components contribute to its role in providing dietary fiber, promoting gastrointestinal health, and supporting protein intake, especially in plant-based or low-resource diets (Ijeh et al., 2022; Olorode *et al.,* 2023). The high moisture content also contributes to its perishable nature, requiring immediate consumption or processing.

## ****2.5.2 Vitamins and Antioxidants****

Vernonia amygdalina is a rich source of vitamins, particularly **vitamin A (as beta-carotene), vitamin C (ascorbic acid),** and vitamin E. These vitamins are known for their antioxidant properties, immune system support, and roles in cellular health and skin regeneration. Vitamin A content has been estimated at 765 µg/100g fresh weight, while vitamin C content can range from 90 to 140 mg/100g depending on the method of preparation (Afolabi et al., 2023).

Vitamin C, in particular, contributes to its anti-inflammatory and antioxidant properties by neutralizing reactive oxygen species (ROS), thus reducing oxidative stress—a key factor in chronic inflammation and degenerative diseases (Oboh *et al.,* 2023).

## ****2.5.3 Mineral Composition****

Mineral analysis has shown that V. amygdalina leaves contain essential minerals such as:

1. **Iron**: 5.6 mg/100g – important for hemoglobin synthesis and prevention of anemia
2. **Calcium**: 120 mg/100g – vital for bone health and muscle contraction
3. **Potassium**: 180 mg/100g – essential for maintaining electrolyte balance and nerve function
4. **Magnesium**: 90 mg/100g – plays a role in enzymatic activities and energy production
5. **Zinc**: 2.4 mg/100g – crucial for immune function and wound healing (Onyechi & Oguejiofor, 2022; Adebayo *et al.,* 2022)

These minerals not only support general physiological processes but also enhance the body’s defense against oxidative and inflammatory stress.

## ****2.5.4 Amino Acids and Lipid Profile****

In addition to its macronutrient content, V. amygdalina contains essential amino acids such as leucine, isoleucine, and lysine, which are important for tissue repair and muscle growth. Although low in fat, the lipid fraction of bitter leaf includes beneficial unsaturated fatty acids, including linoleic and oleic acids, which contribute to cardiovascular health (Ogunyemi & Adepoju, 2023).

The nutritional profile of V. amygdalina positions it as a promising functional food—defined as food that provides health benefits beyond basic nutrition. Several studies advocate for its incorporation into diet-based interventions targeting metabolic disorders such as type 2 diabetes, hypertension, and obesity, owing to its fiber content and micronutrient density (Ademola *et al.,* 2022; Salisu & Nnaji, 2023).

Additionally, post-harvest processing methods such as blanching, fermentation, and drying have been shown to influence its nutrient retention. For instance, fermentation can enhance the bioavailability of iron and zinc while reducing bitter taste and certain antinutritional factors like oxalates and phytates (Ugwoke *et al.,* 2022).

## 2.6 Anti-inflammatory Activities of Vernonia amygdalina

Inflammation is a complex biological response of the body to harmful stimuli such as pathogens, damaged cells, or irritants, and while it is a vital defense mechanism, chronic inflammation is linked to numerous diseases including arthritis, diabetes, cardiovascular disorders, and cancer. Increasing interest in plant-derived anti-inflammatory agents has led to the investigation of Vernonia amygdalina as a natural remedy with significant therapeutic potential.

Several studies have confirmed that V. amygdalina exhibits potent anti-inflammatory activity, primarily attributed to its diverse phytochemical composition, including flavonoids, alkaloids, saponins, tannins, terpenoids, and phenolic compounds. These bioactive constituents modulate inflammatory pathways by inhibiting key enzymes and cytokines involved in inflammation (Afolabi *et al.,* 2023; Yusuf *et al.,* 2022).

1. **Inhibition of Pro-inflammatory Cytokines**: Extracts of V. amygdalina have been shown to significantly reduce levels of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β). In a recent animal study, methanolic leaf extracts reduced cytokine levels in lipopolysaccharide (LPS)-induced inflammation in rats, suggesting immunomodulatory effects (Chukwuma *et al.,* 2023).
2. **Cyclooxygenase (COX) and Lipoxygenase (LOX) Inhibition**: COX and LOX are enzymes involved in the biosynthesis of prostaglandins and leukotrienes, both of which are mediators of inflammation. Ethanolic and aqueous extracts of V. amygdalina have demonstrated inhibition of these enzymes, thereby reducing inflammation and associated pain (Uchegbu & Anyaehie, 2023).
3. **Antioxidant-Mediated Inflammation Control**: The high antioxidant content of V. amygdalina contributes to its anti-inflammatory potential. Oxidative stress is a major driver of chronic inflammation. The leaf extract scavenges free radicals such as nitric oxide (NO) and reactive oxygen species (ROS), reducing lipid peroxidation and protecting tissues from oxidative damage (Ibrahim *et al.,* 2023; Oboh *et al*., 2023).

#### **2.6.1 In vivo and In vitro Evidence**

In vivo studies have shown that aqueous and methanolic extracts of V. amygdalina significantly reduced paw edema in rats induced by carrageenan, a standard experimental model for acute inflammation (Eze & Okonkwo, 2022). The results were comparable to standard anti-inflammatory drugs such as diclofenac, highlighting the plant’s efficacy.

In vitro studies have also demonstrated inhibition of nitric oxide production in macrophage cell lines treated with LPS, supporting its role in suppressing inflammation at the cellular level (Oladimeji *et al.,* 2022).

Due to its non-toxic nature and broad anti-inflammatory effects, Vernonia amygdalina holds promise for managing inflammatory diseases such as rheumatoid arthritis, colitis, and even cancer-related inflammation. There is also ongoing research into its use as an adjunct therapy in metabolic syndromes where low-grade chronic inflammation plays a central role (Agbo *et al.,* 2023).

Comparative analyses between *Vernonia amygdalina* and other commonly consumed leafy vegetables such as *Telfairia occidentalis* (fluted pumpkin), *Amaranthus hybridus* (African spinach), and *Solanum nigrum* (black nightshade) have shown that *V. amygdalina* generally contains higher levels of phytochemicals such as flavonoids, saponins, and phenolic acids. These compounds contribute to its superior antioxidant, anti-inflammatory, and antimicrobial activities (Anyanwu et al., 2022; Akinnibosun et al., 2023). For example, a study by Edeh et al. (2023) found that the total flavonoid content in *V. amygdalina* was nearly double that of *Amaranthus hybridus*, suggesting stronger radical-scavenging activity.

In nutritional terms, *V. amygdalina* also exhibits a competitive edge. Its protein, calcium, and iron content have been observed to be significantly higher compared to other leafy greens, making it a valuable food crop in combating malnutrition, especially in sub-Saharan Africa (Okpara et al., 2022).

On a global scale, *V. amygdalina* has garnered recognition not only as a traditional African medicinal plant but also as a promising candidate in nutraceutical and pharmaceutical industries. The World Health Organization (WHO, 2021) has acknowledged the importance of African medicinal plants in integrative healthcare, citing *V. amygdalina* for its application in managing diabetes, inflammatory disorders, and gastrointestinal infections. It is now increasingly used in the formulation of dietary supplements, antioxidant-rich teas, and botanical extracts sold in international markets for immune modulation and metabolic regulation (Adepoju & Owolabi, 2023).

Moreover, *V. amygdalina* has been included in the pharmacopoeias of several African countries and is currently being studied under global research initiatives focused on plant-based therapies for non-communicable diseases (NCDs), further highlighting its relevance in the fight against chronic diseases and global health disparities (UNIDO, 2022).

The reviewed literature highlights that *Vernonia amygdalina* is a potent medicinal and nutritional plant. It contains a variety of phytochemicals with demonstrated anti-inflammatory effects and significant nutritional components. However, there is a need for more integrated and clinical-based research to substantiate its efficacy and safety in human health management.

**CHAPTER THREE**

**MATERIALS AND METHOD**

## 3.1 Materials

Fresh bitter leaves, Chloroform, H₂SO₄, NaOH, HCl, Fehling’s solutions A and B, benzene, ammonia solution, ethanol, petroleum ether, acetic acid, aluminum chloride, diethyl ether, n-butanol, sodium chloride, Folin-Ciocalteu reagent.

## 3.2 Sample collection and preparation

Fresh bitter leaves were collected from parent trees on farms in Lokuwa ward, Mubi North Local Government and transported to the laboratory, where they were washed and cut to eliminate dirt.

## 3.3 Nutritional Composition

Proximate nutrient composition analysis performed on the freshly manufactured samples, with crude protein, crude fat, ash content, moisture content, dry matter, and carbohydrate being the components examined. The Association of Official Analytical Chemists (AOAC, 2002) had already outlined how to do this.

### 3.3.1 Crude Protein

The micro-Kjedahl method used to accomplish this. Using copper sulphate as a catalyst, the nitrogen component of the protein in 5 g of the samples were transformed into ammonium sulphate by digestion with concentrated hydrogen tetraoxosulphate (VI) acid. The ammonia was collected in a boric acid double indicator solution, and nitrogen was measured using a normal hydrochloric acid titration until the end point was achieved. After that, a factor of 6.25 was used to calculate the amount of crude protein.

Total nitrogen (N) = [(a-b)×0.01 × 0.014 × D×100]/(W×V)

% Crude protein = N × 6.25

Where;

a = titre value of the digested sample;

b = titre value of the blank;

V = volume of sample used;

W = mass of dried sample;

D = dilution factor.

### 3.3.2 Crude Fat

5 g of plant materials, petroleum ether, and a soxhlet extractor device was used to extract crude fat from the sample. The crude fat in the samples were calculated using the weight of the fat obtained after evaporating the petroleum ether from the extract, and this was stated as a percentage.

% crude fat = x 100

### 3.3.3 Ash Content

To remove organic components, five grams of the material was put in a crucible and heated to 550°C. After cooling and weighing the crucible and its contents, the ash was calculated as a percentage of the original dry weight of the samples.

Ash content = x 100

### 3.3.4 Moisture

The ground sample was weighed exactly 5 g each and oven dried at a constant temperature of 70°C. After cold weighing, the amount of moisture in the sample was reported as a loss in weight.

Moisture content = x 100

### 3.3.5 Crude Fibre

The fibre content of samples was determined using five grams of defatted samples extracted by acid digestion, filtration, and base digestion. At 550°C, the resultant leftovers were eventually ignited. Fibre content was then represented as a proportion of initial weight loss after ashing.

% crude fibre = x 100

### 3.3.6 Carbohydrate

The difference between 100 and the total of crude protein, fat, ash, and fibre was then used to calculate the amount of carbohydrate in the sample.

Carbohydrate (%) = 100 - % (crude protein + crude fat + ash + crude fibre +moisture)

## 3.4 Qualitative Analysis of the Phytochemicals

Phytochemical analysis of the plant sample extract was carried out based on the method adopted by Evan *et al,* 1997. Simple chemical test was used to qualitatively analyzed the presence of phytochemicals namely; Steroids, Flavonoids, Cardiac glycosides, Alkaloids, Phenolic, Tannins, Anthraquinone, Saponin and Alkaloids.

## 3.4.1 Test for Steroids

A known quantity of the test sample was extracted in the chloroform and filtered. The filtrate was mixed with 2 ml of conc. H2SO4 carefully so that the sulphuric acid formed a lower layer. A reddish-brown colour at the interphase indicated the presence of steroidal ring.

## 3.4.2 Test for flavonoids

Few drops of 20% NaOH was added to the extract, Portion of the extract was added with few drops of 20% sodium hydroxide, formation of intense yellow colour observed. To this, few drops of 70% dilute hydrochloric acid was added and yellow colour disappeared. Formation and disappearance of yellow colour indicates the presence of flavonoids in the sample extract.

## 3.4.3 Test for Glycosides

Dilute Sulphuric acid (5 ml) was added to the portion of the extract in a test tube and boiled for 15 min in a water bath, then cooled and neutralized with 20% potassium hydroxide solution. 10 ml of a mixture of equal parts of Fehling’s solution A and B was added and boiled for 5 min. A denser brick red precipitate indicated the presence of glycoside.

## 3.4.4 Test for Anthraquinones

Portion of the extracts was added to 4ml of benzene and shaken, it was then filtered when hot, the filtrate shaken with 2ml of 10% ammonia solution. The disappearance of violet colour in the ammoniacal phase (lower phase) indicates the presence of free anthraquinones.

## 3.4.5 Test for Saponins

Aliquot of the extract was diluted with 20ml of deionized water, shaken vigorously and observed. Persistent foaming indicated the presence of saponins.

## 3.4.6 Test for Alkaloids

Portion of the extracts was diluted with 10ml alcohol, boiled and filtered. 5ml of filtrate was added to 2ml of ammonia. 5ml of chloroform was also added and shaken gently; 10ml of acetic acid was added. Then Wagner's reagent was also added. Reddish brown precipitate was positive for the presence of alkaloids (Abiona *et al.,* 2015)

## 3.4.7 Test for Phenolic

Phenolic compounds are widely distributed in plants and have been recognized for their antioxidant and anti-inflammatory properties. Olusola *et al*. (2018) identified the presence of phenolic compounds in the plants. These compounds are known to exert anti-inflammatory effects by modulating key signaling pathways involved in the inflammatory response (Olusola *et al.,* 2018). The phenolic compounds present in the contribute to its phytochemical composition and may contribute to its anti-inflammatory activities.

## 3.4.8 Test for Tannins

Tannins are polyphenolic compounds widely distributed in plants and known for their antioxidant and anti-inflammatory properties. Aiyegoro *et al.* (2010) identified the presence of tannins in the plants. Tannins have been reported to exhibit anti-inflammatory effects by modulating inflammatory mediators and reducing inflammatory responses (Aiyegoro *et al.,* 2010). The presence of tannins in the plants contributes to its phytochemical composition and may contribute to its anti-inflammatory activities.

## 3.4.9 Test for Terpenoids.

2.0 ml of chloroform was added with the 5 ml aqueous plant extract and evaporated on the water path and was then boiled with 3 ml of H2SO4 concentrated. A grey color formed which showed the entity of terpenoids.

## 3.5 Quantitative Analysis of the Phytochemicals

### 3.5.1 Estimation of Alkaloids

In a 250 mL beaker, 5 g of the sample was weighed. Then 200 mL of acetic acid in ethanol (10%) was added and left to stand for 4 hours. The extract will be then filtered and concentrated to one-quarter of its original volume in a water bath. To produce precipitation, concentrated ammonium hydroxide was applied to the extract drop by drop. The entire solution allowed to settle, and the precipitate was collected and filtered after being washed with diluted ammonium hydroxide. The residue is the dried and weighed alkaloid (Harborne and Baxter, 2023).

Formula = B - A × 100 / S

Where,

B = Weight of Whatman filter paper.

A = Weight of Whatman filter paper, after drying.

S = Sample weight.

### 3.5.2 Estimation of Total Flavonoids

The volume was made up to 100 ml with distilled water after 100 mg of tannic acid has been dissolved in a small amount of distilled water. By diluting the standard with distilled water, different concentrations of the standard will be achieved (Chun, 2015). The solution's concentration was 100 mg/mL. At zero time, 0.5 ml of aqueous extract sample was diluted with 3.5 ml of distilled water. The tubes were filled with 0.3 mL of 5% sodium nitrate. After five minutes, all of the tubes received 0.3 mL of 10% aluminum chloride. 2 ml of 1 M sodium hydroxide was added to the mixture on the sixth minute. The contents of the reaction mixture will be immediately diluted with 2.4 mL of distilled water and properly stirred. The mixture's absorbance was immediately measured at 510 nm in comparison to a prepared blank. Total flavonoids was measured in mg per 100g of edible part, using tannic acid as a reference ingredient.

### 3.5.3 Estimation of Saponins

A conical flask containing 100 ml of 20% aqueous ethanol will be filled with 20 g of sample. At roughly 55°C, the sample will be heated for four hours in a hot water bath with constant stirring (Obadoni and Ochuko, 2001). The residue would be re-extracted with another 200 mL of 20% ethanol after the mixture will be filtered. Over a water bath at roughly 90°C, the combined extract will be reduced to 40 mL. The concentrated solution was poured into a 250 mL separator funnel along with 20 mL of diethyl ether and rapidly shaken. The aqueous layer was kept, while the ether layer was discarded, and the purification procedure was repeated. After that, 60 milliliters of n-butanol extract were added. The extracted n-butanol was rinsed twice with 10 mL of aqueous sodium chloride each time. In a water bath, the residual solution was heated. The sample was dried in the oven to a constant weight after evaporation. The percentage of saponins was computed.

Formula = B – A × 100 / S

Where,

A = Weight of Whatman filter paper with sample.

S = Sample weight.

### 3.5.4 Estimation of Phenols

In the test tubes, 0.5 mL of freshly prepared was taken. All of the tubes received 8 mL of distilled water. Folin's Ciocalteau reagent (0.5 mL) was also added to each tube (Malick and Singh, 1980). All of the tubes was kept in B.O.D for a 10-minute incubation period at 40°C. The sodium carbonate solution was then be added to each test tube at a volume of 1 mL. After that, the tubes were put in the dark for one hour to incubate. At 660 nm, the color formed was spectrophotometrically read. Tannic acid was used to draw the standard curve. In a Shimadzu UV-1650 spectrophotometer, the O.D. was read at 660 nm for different amounts of tannic acid. The standard curve will be used to compute the sample concentrations.

### 3.5.5 Estimation of Tannins

100 mg of tannic acid was dissolved in 100 ml of distilled water. 5 ml of stock solution was diluted to 100 ml with distilled water. 1 ml containing 50 μg tannic acid (Robert, 2017).

Extraction of Tannin: 0.5 gm of the powdered material was weighed and transferred to a 250 ml conical flask and 75 ml water was added. The flask was heated gently and boiled for 30 min centrifuge at 2,000 rpm for 20 min and the supernatant was collected in 100 ml volumetric flask and make up the volume. 1 ml of the sample extract was transferred to 100 ml volumetric flask containing 75 ml water. 5 ml of folin denis reagent, 10 ml of sodium carbonate solution was added and diluted to 100 ml with water. Shake well. The absorbance was read at 700 nm after 30 min. If absorbance is greater than 0.7 make a 1 + 4 dilution of the sample. A blank was prepared with water instead of the sample. A standard graph was prepared by using 100 mg tannic acid. The tannins content of the sample was calculated as tannic acid equivalents from the standard graph.

### 3.5.6 Estimation of Terpenoids

To estimate terpenoids, 5 g of the powdered sample will be soaked in 50 mL of ethanol for 24 hours. After filtration, the filtrate will be extracted with petroleum ether using a separating funnel in a 1:1 ratio. The ether layer, containing the terpenoids, will be separated and evaporated to dryness. The final residue, representing the terpenoid content, will be weighed (Sofowora, 2023).

### 3.5.7 Estimation of Steroids

For the estimation of steroids, 2 g of the powdered sample was dissolved in 20 mL of chloroform and allowed to stand for 3 hours with occasional shaking. After filtration, the filtrate was treated with an equal volume of concentrated sulfuric acid carefully along the side of the test tube. The formation of a red color in the lower chloroform layer indicated the presence of steroids. Quantification was done by measuring the absorbance at 530 nm against a reagent blank. A standard curve using cholesterol was prepared to determine the steroid concentration in the sample.

### 3.5.8 Estimation of Glycosides

To estimate glycosides, 5 g of the powdered sample will be hydrolyzed with 50 mL of 2 N hydrochloric acid for 2 hours in a water bath. After cooling, the mixture was neutralized with sodium hydroxide and extracted with chloroform. The chloroform layer was evaporated to dryness and the residue was weighed. The percentage of glycosides present was then calculated.

### 3.5.9 Estimation of Anthraquinones

For the estimation of anthraquinones, 5 g of the powdered sample was soaked in 50 mL of distilled water and 50 mL of benzene in a 250 mL conical flask. The mixture was shaken vigorously for 15 minutes and allowed to stand for proper extraction. The benzene layer was carefully separated and 10 mL of it was taken into another flask. Then, 10 mL of 10% ammonia solution was added, and the mixture was shaken. The formation of a pink, red, or violet coloration in the ammoniacal (lower) layer indicated the presence of free anthraquinones. The intensity of the color was measured spectrophotometrically at 450 nm. The percentage of anthraquinones was calculated based on the absorbance values using a calibration curve prepared with a standard anthraquinone solution.

**3.6 Statistical analysis**

Data obtained from this study was subjected to statistical analysis using the special package for social sciences (SPSS). Data are expressed as mean ± standard deviation (SD) of triplicate.