**PHYTOCHEMICAL CONSTITUENTS, NUTRITIONAL COMPOSITION, AND ANTI-INFLAMMATORY ACTIVITIES OF *Vernonia amygdalina* (BITTER LEAF)**

# TITLE PAGE

**BY**

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# DECLARATION

I Magira Kutarju with the registration number ST/CST/BC/HND/22/014 hereby declare that this work is the product of my own research effort, undertaken under the supervision of Mr. Abdulrazak Bello and has not been presented elsewhere for the award of any certificate. All sources of information have been duly distinguished and appropriately acknowledged.

Magira Kutarju …………………..

ST/CST/BC/HND/23/014 Sign/Date

# CERTIFICATION

This is to certify that this project entitled “**Phytochemical Constituents, Nutritional Composition, And Anti-Inflammatory Activities of *Vernonia amygdalina* (Bitter Leaf)**” was done by Magira Kutarju with Registration Number ST/CST/BC/HND/24/014. The work was examined and found to meet the requirements governing the award of Higher National Diploma (HND) in Science Laboratory Technology (Biochemistry Option) of the Federal Polytechnic, Mubi and is approved for its contribution to knowledge and literary presented.

Mr. Abdulrazak Bello …………………

(Project Supervisor) Sign/Date

Mr. Sudi P. D. …………………

(Head of Department) Sign/Date

**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 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 will be collected from parent trees on farms in Lokuwa ward, Mubi North Local Government and transported to the laboratory, where they will be washed and cut to eliminate dirt.

## 3.3 Nutritional Composition

Proximate nutrient composition analysis will be 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 will be used to accomplish this. Using copper sulphate as a catalyst, the nitrogen component of the protein in 5 g of the sample will be 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 will be 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 will be used to extract crude fat from the sample. The crude fat in the samples will be calculated using the weight of the fat obtained after evaporating the petroleum ether from the extract, and this will be stated as a percentage.

% crude fat = x 100

### 3.3.3 Ash Content

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

Ash content = x 100

### 3.3.4 Moisture

The ground sample will be 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 will be reported as a loss in weight.

Moisture content = x 100

### 3.3.5 Crude Fibre

The fibre content of samples will be determined using five grams of defatted samples extracted by acid digestion, filtration, and base digestion. At 550°C, the resultant leftovers will be eventually ignited. Fibre content will then be 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 will then be 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 will be carried out based on the method adopted by Evan *et al,* 1997. Simple chemical test will be 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 will be extracted in the chloroform and filtered. The filtrate will be 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 will be added to the extract, Portion of the extract will be added with few drops of 20% sodium hydroxide, formation of intense yellow colour observed. To this, few drops of 70% dilute hydrochloric acid will be 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) will be 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 will be added to 4ml of benzene and shaken, it will then be 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 will be 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 will be diluted with 10ml alcohol, boiled and filtered. 5ml of filtrate will be added to 2ml of ammonia. 5ml of chloroform will also be added and shaken gently; 10ml of acetic acid will be added. Then Wagner's reagent will also be added. Reddish brown precipitate will be 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 will be added with the 5 ml aqueous plant extract and evaporated on the water path and will then be 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 will be 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 will be applied to the extract drop by drop. The entire solution allowed to settle, and the precipitate will be 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 will be 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 will be 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 will be immediately measured at 510 nm in comparison to a prepared blank. Total flavonoids will be 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 will be 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 will be discarded, and the purification procedure will be repeated. After that, 60 milliliters of n-butanol extract were added. The extracted n-butanol will be rinsed twice with 10 mL of aqueous sodium chloride each time. In a water bath, the residual solution was heated. The sample will be dried in the oven to a constant weight after evaporation. The percentage of saponins will be 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 will be taken. All of the tubes received 8 mL of distilled water. Folin's Ciocalteau reagent (0.5 mL) will be also added to each tube (Malick and Singh, 1980). All of the tubes will be 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 will be put in the dark for one hour to incubate. At 660 nm, the color formed will be spectrophotometrically read. Tannic acid will be used to draw the standard curve. In a Shimadzu UV-1650 spectrophotometer, the O.D. will be 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 will be dissolved in 100 ml of distilled water. 5 ml of stock solution will be 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 will be weighed and transferred to a 250 ml conical flask and 75 ml water will be added. The flask would be heated gently and boiled for 30 min centrifuge at 2,000 rpm for 20 min and the supernatant will be collected in 100 ml volumetric flask and make up the volume. 1 ml of the sample extract will be transferred to 100 ml volumetric flask containing 75 ml water. 5 ml of folin denis reagent, 10 ml of sodium carbonate solution will be added and diluted to 100 ml with water. Shake well. The absorbance will be read at 700 nm after 30 min. If absorbance is greater than 0.7 make a 1 + 4 dilution of the sample. A blank will be prepared with water instead of the sample. A standard graph will be prepared by using 100 mg tannic acid. The tannins content of the sample will be 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 will be dissolved in 20 mL of chloroform and allowed to stand for 3 hours with occasional shaking. After filtration, the filtrate will be 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 will be done by measuring the absorbance at 530 nm against a reagent blank. A standard curve using cholesterol will be 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 will be neutralized with sodium hydroxide and extracted with chloroform. The chloroform layer was evaporated to dryness and the residue will be 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 will be soaked in 50 mL of distilled water and 50 mL of benzene in a 250 mL conical flask. The mixture will be 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 will be added, and the mixture will be 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 will be measured spectrophotometrically at 450 nm. The percentage of anthraquinones will be 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 will be subjected to statistical analysis using the special package for social sciences (SPSS). Data are expressed as mean ± standard deviation (SD) of triplicate.

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