**Phytochemical Screening and Some Heavy Metals Determination in *Talinum triangulare* (Waterleaf) and *Vernonia amygdalina* (Bitter leaf)**

# TITLE PAGE

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**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, SCHOOL OF SCIENCE AND TECHNOLOGY, FEDERAL POLYTECHNIC MUBI, ADAMAWA STATE.**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF NATIONAL DIPLOMA (ND) IN SCIENCE LABORATORY TECHNOLOGY**

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# DECLARATION

We hereby declare that the work in this project titled **“Phytochemical Screening and Some Heavy Metals Determination in *Talinum triangulare* (Waterleaf) and *Vernonia amygdalina* (Bitter leaf)”** was performed by us under the supervision of Mrs. Tsodiya Bunu. The information derived from literatures has been duly acknowledged in the text and a list of references provided. The work embodied in this project is original and had not been submitted in part or in full for any other diploma or certificate of this or any other institution.

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# DEDICATION

This project work is dedicated to Almighty God for his enabling strength he bestowed on us during the course of this project work. Also, our gratitude goes to our lovely parents for their never-ending support and encouragement during the course of this research work.

# APPROVAL PAGE

This project titled **“Phytochemical Screening and Some Heavy Metals Determination in *Talinum triangulare* (Waterleaf) and *Vernonia amygdalina* (Bitter leaf)”** meets the regulations governing the award of National Diploma (ND) in Science Laboratory Technology, Federal Polytechnic Mubi, Adamawa State

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# ABSTRACT

*This study investigates the presence of phytochemicals and heavy metals in two commonly consumed leafy vegetables Bitter leaf (Vernonia amygdalina) and Water leaf (Talinum triangulare) sourced from Mubi, Nigeria. Qualitative phytochemical screening revealed the presence of essential bioactive compounds such as saponins, phenols, flavonoids, terpenoids, glycosides, tannins, anthraquinones, and alkaloids in both plant samples, while steroids were present only in Water leaf. These compounds are known for their therapeutic and antioxidant properties, which may contribute to the health benefits associated with the consumption of these vegetables. Heavy metal analysis was conducted using standard atomic absorption spectrophotometry methods. The results showed that lead (Pb) and chromium (Cr) were not detected in either plant, indicating a relatively low risk of toxicity from these elements. However, cadmium (Cd) was detected at 0.08 mg/kg in Bitter leaf and 0.15 mg/kg in Water leaf. Copper (Cu) concentrations were 0.44 mg/kg and 0.51 mg/kg, while zinc (Zn) levels were 0.33 mg/kg and 1.18 mg/kg in Bitter leaf and Water leaf, respectively. Although these values are within the permissible limits set by WHO/FAO, continued monitoring is recommended to prevent potential health hazards. The study concludes that both Bitter leaf and Water leaf possess valuable medicinal properties due to their rich phytochemical content and are generally safe for consumption concerning heavy metal contamination. However, the presence of cadmium, though low, warrants attention to environmental and agricultural practices in the area. This research provides essential baseline data for public health safety and further pharmacological studies.*

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# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of the Study

Phytochemicals are plant-based bioactive compounds produced by plants for their protection. They can be derived from various sources such as whole grains, fruits, vegetables, nuts, and herbs, and more than a thousand phytochemicals have been discovered to date. Some of the significant phytochemicals are carotenoids, polyphenols, isoprenoids, phytosterols, saponins, dietary fibers, and certain polysaccharides. These phytochemicals possess strong antioxidant activities and exhibit antimicrobial, antidiarrheal, anthelmintic, antiallergic, antispasmodic, and antiviral activities (Jaeger *et al.,* 2017). They also help to regulate gene transcription, enhance gap junction communication, improve immunity, and provide protection against lung and prostate cancers (Jiang *et al.,* 2018). The recent focus on translational research has enhanced the dimensions of functional foods. Phytochemicals, after extraction from various sources, find profound application in the development of functional foods and nutraceuticals. Heavy metals are among the contaminants in the environment. Beside the natural activities, almost all human activities also have potential contribution to produce heavy metals as side effects. Migration of these contaminants into noncontaminated areas as dust or leachates through the soil and spreading of heavy metals containing sewage sludge are a few examples of events contributing towards contamination of the ecosystems (Sharma *et al,* 2018). Several methods are already being used to clean up the environment from these kinds of contaminants, but most of them are costly and far away from their optimum performance. The chemical technologies generate large volumetric sludge and increase the costs chemical and thermal methods are both technically difficult and expensive that all of these methods can also degrade the valuable component of soils (Rowles *et al,* 2020). Conventionally, remediation of heavy-metal contaminated soils involves either onsite management or excavation and subsequent disposal to a landfill site. This method of disposal solely shifts the contamination problem elsewhere along with the hazards associated with transportation of contaminated soil and migration of contaminants from landfill into an adjacent environment. Soil washing for removing contaminated soil is an alternative way to excavation and disposal to landfill. This method is very costly and produces a residue rich in heavy metals, which will require further treatment. Moreover, these physio-chemical technologies used for soil remediation render the land usage as a medium for plant growth, as they remove all biological activities (Rowles *et al,* 2020).

## 1.2 Statement of problem

The wide spread use of consumed vegetables in Adamawa state, Nigeria for both nutritional and medicinal purposes highlight the need for scientific validation of their bioactive properties. Despite their importance in traditional diets and ethnomedicinal practices, many indigenous vegetables remain under researched regarding their phytochemical composition. This lack of data poses challenges to their standardization, proper utilization integration into formal health systems. Without rigorous phytochemical screening there is a significant gap in knowledge about the specific compounds responsible for their reported therapeutic effects.

Accumulation and amplification of Chemicals and heavy metals in human tissue through the consumption of medicinal plants can have hazardous health outcome. Heavy metal toxicity of medicinal plant product has been linked to a wide range of adverse health effects, causing dysfunction of the liver, kidney, and heart, and even death (Jaeger *et al.,* 2017). therefore, the need to perform phytochemical and heavy metals in medicinal plants.

## 1.3 Aim and objectives of the study

The aim of this study is to investigate phytochemical screening and heavy metals of bitter leaf and water leaf around Mubi LGA of Adamawa State. The objectives of the study are to:

1. Identify and quantify the major constituent present in bitter leaf and water leaf.
2. Determine the presence and concentration of specific chemical compounds in the selected vegetable samples.
3. To compare the result obtained with the standard value given by WHO.

## 1.4 Significance of the Study

The significance of a study on phytochemical screening and determination of chemical compounds consumed vegetables in Mubi, Adamawa State, Nigeria lies in its potential to provide critical insights into the nutritional and medicinal benefits of these vegetables, thus promoting their consumption and utilization.

## 1.5 Scope of the Study

This study investigates two selected locally consumed vegetables in Mubi, Adamawa State *Vernonia amygdalina* and *Ipomoea aquatica.* The study is limited to the leaves of these vegetables and includes (i) phytochemical screening to detect the presence of bioactive compounds. (ii) to determine heavy metals.

All plant samples are collected within Mubi Local Government Area of Adamawa State, and the study is restricted to in vitro laboratory methods.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Introduction

Phytochemicals and heavy metals are significant components of plants that can influence their nutritional value, medicinal potential, and safety for consumption. This chapter presents a comprehensive review of the phytochemical composition and heavy metal accumulation in two important plant species: Vernonia amygdalina (Bitter leaf) and Ipomoea aquatica (Water leaf). Both plants are commonly found in Mubi, Adamawa State, and are known for their wide usage in traditional medicine and culinary applications. This chapter explores their phytochemical profiles, the potential for heavy metal contamination, and the implications for human health and environmental sustainability.

## 2.2 Overview of Vernonia amygdalina and Ipomoea aquatica

## 2.2.1 Bitter Leaf (*Vernonia amygdalina*)

Vernonia amygdalina, commonly known as bitter leaf, is a perennial shrub widely used in African traditional medicine and as a leafy vegetable. It belongs to the Asteraceae family and is native to tropical Africa. The leaves are known for their characteristic bitter taste, which is attributed to their rich phytochemical composition. Traditionally, the plant has been used to treat various ailments such as fever, malaria, gastrointestinal issues, and diabetes. Bitter leaf is also used in food preparation, especially in soups and stews (Smith & Li, 2021). Bitter leaf is known for its diverse phytochemical constituents which contribute to its medicinal properties. Studies have identified the presence of saponins, tannins, flavonoids, alkaloids, and glycosides in bitter leaf. These compounds exhibit a range of biological activities, including antioxidant, anti-inflammatory, antidiabetic, and antimicrobial properties.

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

## ****2.2.1.1 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.

**2.2.1.2 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.

**2.2.1.3 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.

**2.2.1.4 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.

**2.2.1.5 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.

**2.2.1.6 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.

## 2.2.1.2 Heavy Metal Content in Bitter Leaf

Bitter leaf, like many other medicinal plants, can accumulate heavy metals from contaminated soil and water. Factors such as soil pH, organic matter content, and proximity to industrial activities significantly influence the extent of heavy metal uptake by plants. A study by Musa *et al.* (2021), indicated that Vernonia amygdalina grown in urban areas exhibited higher levels of lead and cadmium compared to those grown in rural settings.

The accumulation of heavy metals such as lead (Pb), cadmium (Cd), and mercury (Hg) in medicinal plants poses significant health risks to consumers. Chronic exposure to these metals can lead to neurotoxicity, nephrotoxicity, and other severe health issues. A recent assessment by Okoye *et al.* (2023), revealed that bitter leaf samples from polluted areas contained heavy metal concentrations exceeding the permissible limits set by health authorities, highlighting the need for regular monitoring and regulation.

## 2.2.2 Water leaf (Ipomoea aquatica)

Ipomoea aquatica, commonly known as water leaf, is an aquatic or semi-aquatic plant species belonging to the Convolvulaceae family. It is a fast-growing, herbaceous vegetable with high water content, typically found in tropical and subtropical regions. The plant is cultivated and consumed widely as a leafy vegetable in various parts of Africa and Asia. Ipomoea aquatica is known for its nutritional and medicinal benefits, particularly its high antioxidant content, and is used to treat ailments like inflammation, hypertension, and digestive issues.

Water leaf, on the other hand, is rich in vitamins, minerals, and phytochemicals such as alkaloids, tannins, saponins, flavonoids, and phenolic compounds. It is commonly used in traditional medicine for its antioxidant, anti-inflammatory, and hepatoprotective effects.

## 2.2.2 Phytochemical Properties of Water Leaf (*Talinum triangulare*)

## ****2.2.2.1 Antioxidant Activity****

Water leaf (Talinum triangulare) is well-known for its potent antioxidant properties. It contains high levels of flavonoids, phenolic acids, and vitamins (especially vitamin C and beta-carotene) that play a crucial role in scavenging free radicals and reducing oxidative stress. According to a study by Akinmoladun *et al.* (2022), water leaf extracts demonstrated significant antioxidant activity, which was comparable to that of standard antioxidants like ascorbic acid and tocopherol. The high antioxidant capacity of water leaf contributes to its protective effects against various oxidative stress-related diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders.

**2.2.1.2 Anti-inflammatory Activity**

The anti-inflammatory properties of water leaf are primarily attributed to its rich phytochemical profile, including alkaloids, saponins, and polyphenols. These compounds inhibit the production of pro-inflammatory cytokines and enzymes, thereby reducing inflammation and its associated symptoms. Eze and Opara (2023), reported that water leaf extract significantly reduced inflammation markers in a rat model of induced inflammation, supporting its traditional use in treating inflammatory conditions like arthritis and gastrointestinal disorders.

**2.2.1.3 Hepatoprotective Effects**

Water leaf has been shown to offer protective effects on the liver, primarily due to its antioxidant properties. The phytochemicals in water leaf enhance the liver's ability to detoxify harmful substances and reduce oxidative damage. A study by Ugbogu *et al.* (2021), found that rats treated with water leaf extract exhibited significantly lower levels of liver enzymes (ALT, AST) and improved histopathological profiles after being exposed to a hepatotoxic agent, indicating its hepatoprotective potential.

## 2.3 Phytochemicals in Vernonia amygdalina and Ipomoea aquatica

## 2.3.1 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).

## 2.3.2 Phytochemicals in Ipomoea aquatica

*Ipomoea aquatica*, commonly known as water spinach or water leaf, is rich in various bioactive phytochemicals that provide significant health benefits. These phytochemicals include flavonoids, phenolic compounds, alkaloids, and glycosides, each contributing to the plant’s medicinal and nutritional value. These compounds are vital in supporting the plant's traditional use in managing conditions such as hypertension, inflammation, infections, and cardiovascular diseases. Recent research continues to highlight the health benefits of *Ipomoea aquatica*, particularly in the areas of inflammation, cardiovascular health, and infection control. Studies have shown that the plant’s rich phytochemical profile, including flavonoids, phenolic compounds, alkaloids, and glycosides, contributes significantly to its therapeutic properties, making it a valuable functional food and medicinal plant (Abubakar *et al*., 2022; Adeyemi *et al.,* 2021). The ongoing investigation into its phytochemical composition and health benefits promises to uncover new potential uses in the treatment and prevention of various diseases.

#### **2.4 Heavy Metals in Plants**

Heavy metals are naturally occurring elements that can become toxic when present in high concentrations. Plants absorb these metals from the soil, water, and air, and their accumulation can pose serious health risks. Heavy metals of concern in plant studies typically include lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg), and chromium (Cr). These metals can enter the food chain through the consumption of contaminated plants, leading to adverse health effects such as kidney damage, neurological disorders, and increased cancer risk.

Heavy metals of non-anthropogenic origin are always present at a background level with their occurrence in soils being related to weathering of parent rocks and pedogenesis (Ghiyasi *et al.,* 2020). However, the concentration of several heavy metals has increased dramatically in certain ecosystems due to anthropogenic activities (Sarma *et al.,* 2012). Heavy metals frequently occur as cations which strongly interact with the soil matrix and can become mobile as a result of changing environmental conditions (Qishlaqi & Farid Moore, 2017). Plants can amass trace elements, especially heavy metals, in and on their tissues due to their ability to tolerate potentially toxic ions in the environment (Kabata-pendias, 2021). Heavy metal uptake by plants can increase the potential of certain toxic elements entering the food chain thus understanding how these elements progress through food webs, and the effects of such elements on organisms, is the topic of considerable interest (Boyd, 2019).

There is now extensive interest in heavy metal transport by metal-tolerant plants (metallophytes) because of the repercussions for phytoremediation (Iqbnal Lone et al., 2008; Sarma, 2011). The use of plants to remediate polluted soils is seen as having great promise compared to conventional, civilengineering methods (Rascio and Navari-Izzo, 2011). A copious number of plants have been explored for phytoremediation (Padmavathiamma and Li, 2007; Sarma, 2011) however the accumulation of heavy metals in edible and medicinal plants need thorough investigation to prevent elevated concentrations of heavy metals reaching the consumer (Sharma *et al.,* 2019; Steenkamp *et al.,* 2020). Heavy metal origin and content as well as their possible interaction with soil properties are priority objectives in environmental monitoring. This is due to the fact that apart from the source of heavy metals, the physicochemical properties of soil may also affect the heavy metal concentration (Aydinalp & Marinova, 2013). Numerous abiotic factors influence the availability of metal to plants including pH, temperature, redox potential, cation exchange capacity and organic matter (Gregor, 2014).

Furthermore, the interactions of soil-plant roots-microbes play vital roles in regulating heavy metal movement from the soil to edible plant parts (Islam *et al.,* 2017). The accumulation of metals by both roots and leaves increases with increasing available metal concentration in the external medium (Gregor, 2014). Factors such as reduced biomass, root length and shoot length are common indicators of heavy metal toxicity (Siddhu *et al.,* 2018). Changes at the cell, tissue and organ level are either a result of a direct interaction between the metal and structural components at the sites or a consequence of changes in signal transduction and/or metabolism (Solanki and Dhankhar, 2011). Plant responses to heavy metals should be investigated for the particular soil-plant environment (Kabata-pendias, 2021). The term “hyperaccumulator” describes plant species that have the ability to grow on metalliferous soils and to accumulate extraordinarily high levels of heavy metals (in comparison to the majority of species) without displaying phytotoxic effects (Rascio and Navari-Izzo, 2011). However, hyperaccumulators are habitually confined to metal-enriched soils such as those soils found on serpentine outcrops and other metalliferous rocks (Reeves, 2022).

## 2.4 Heavy metals in medicinal plants and plant-based products

Numerous studies have been conducted worldwide to determine heavy metal levels in medicinal plants and plant-based products (Annan *et al.,* 2010; Ebrahim *et al.,* 2012; Maharia *et al.,* 2010). Both developed and developing countries have shown high levels of potentially toxic heavy metals in products available to the public. Such products are not only from local sources but are often imported (Saper *et al.,* 2018). A study examining heavy metal content in traditional Asian herbal remedies purchased in the United States, Vietnam and China revealed that the majority of products had detectable levels of heavy metals, with nearly 74% containing amounts greater than current recommended public health guidelines (Garvey *et al.,* 2021). An effective solution to the importation of traditional medicines containing heavy metals presents a great challenge (Saper *et al.,* 2018). These products are expected to be imported in small quantities by numerous different routes, including via the postal service and with intercontinental travellers (Denholm, 2020). Formal labelling and packaging may be deceptive as it gives the public a false sense of product safety. In Africa, formalization and registration of herbal products are not the norm and preparations often lack appropriate labelling such as contents, contraindications, place and date of manufacture and expiry date. Medicinal plant collection is often from the wild and locations are habitually undisclosed. Thus, the traceability of medicinal plants which are sold at traditional medicine markets is non-existent. A study by Street *et al.* (2018), showed that medicinal plant parts harvested from a wide range of undisclosed locations by plant gatherers and sold at informal markets had multiple metal contamination. Lead and Ni were detected in all samples and elevated Fe and manganese (Mn) contents were recorded in certain plant species. The only way to ensure consumer safety is to periodically sample plants from the traditional medicine markets however even this is complicated due to the fact that plants of the same species are habitually collected from various sources and are added together in one storage container. It is nonetheless imperative that medicinal plants are tested for metal contamination as these plants are used as starting material for numerous herbal products. Correct post-harvest processing may also contribute to the minimization of heavy metals in the starting materials. A study by Abou-Arab and Abou Donia (2020), investigated Egyptian medicinal plants processed by two different methods to determine the behavior of their metal contents during processing. In general, boiling the plants in water led to extraction of higher amounts of the metal from the plant than submerging them in hot water however the investigated metals were transferred from the plant tissue into the used water at different ratios depending on the metal, the plant, and the method of extraction.

A study regarding the concentration of arsenic (As), Cd, Pb and mercury (Hg) in 20 registered ready to use herbal products purchased randomly from the pharmacy shops in Lagos (Nigeria) revealed that none of the samples contained detectable Pb; however, all the samples contained a detectable quantity of one or more of the other metals of interest (Adepoju-Bello *et al.,* 2012). Despite studies conducted to determine heavy metal levels in African medicinal plants and plant-based products, with no regulatory guidelines or methods of enforcing limits, these studies simply illustrate the potential to cause hazard to human health without any resolve.

## 2.4.1 Heavy Metal Contamination in Vernonia amygdalina

Vernonia amygdalina is known to absorb and accumulate heavy metals from contaminated soils, especially in regions affected by industrial activities, mining, improper agricultural practices, and waste disposal. Studies have demonstrated that Vernonia amygdalina can take up heavy metals such as lead (Pb), cadmium (Cd), and mercury (Hg) from polluted soils (Ogbomida *et al.,* 2021). This absorption occurs through the plant's root system, which facilitates the translocation of these metals to the leaves. The bioaccumulation of these metals poses a significant health risk when consumed, as the ingestion of Vernonia amygdalina grown in contaminated areas may lead to heavy metal toxicity in humans (Fasuyi *et al.,* 2022).

The ability of Vernonia amygdalina to accumulate heavy metals is concerning due to its widespread use as a medicinal plant and dietary vegetable. Lead and cadmium, in particular, are toxic even at low concentrations and can lead to serious health problems, including neurological damage, kidney dysfunction, and an increased risk of cancer (Nwaichi *et al.,* 2020). Several studies have reported elevated levels of these metals in Vernonia amygdalina grown near industrial zones and waste disposal sites, raising concerns about food safety (Oluwafemi *et al.,* 2020).

## 2.4.2 Heavy Metal Contamination in Ipomoea aquatica

As a semi-aquatic plant, Ipomoea aquatica is particularly vulnerable to heavy metal contamination, especially when grown in polluted water bodies or soils near industrial or agricultural activities. This plant is known for its ability to absorb and accumulate metals such as lead (Pb), cadmium (Cd), arsenic (As), and chromium (Cr) (Amadi *et al.,* 2022). The bioaccumulation of these metals in Ipomoea aquatica poses significant health risks to consumers, as these metals can be transferred through the food chain, leading to toxicity (Okoroafor *et al.,* 2023).

Research has shown that water leaf can accumulate high concentrations of heavy metals in polluted environments, particularly when grown in contaminated soils or near mining operations (Bassey *et al.,* 2021). The consumption of Ipomoea aquatica cultivated in such environments can result in heavy metal exposure, which has been linked to a range of health issues, including liver damage, kidney dysfunction, and an increased risk of various cancers (Adeyemi *et al.,* 2022). Moreover, the long-term consumption of plants contaminated with metals such as arsenic and cadmium can lead to chronic toxicity, with adverse effects on human health (Amadi *et al.,* 2022).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Reagent and Apparatus

Acetic acid

Ammonia solution (10%)

Analytical balance

Atomic Absorption Spectrophotometer

Beakers and conical flasks

Bitter leaf (Vernonia amygdalina)

Chloroform

Concentrated Sulphuric acid (H2SO4)

Concentrated Tetraoxochlorate (VII) acid (HCIO4)

Concentrated Trioxonitrate (VII) acid (HNO3)

Deionized water

Dilute Sulphuric acid (for glycosides test)

Ethanol (alcohol)

Fehling's solution A & B

Hydrochloric acid (HCl)

Measuring cylinders

Mortar and pestle

Pipettes and droppers

Potassium hydroxide (KO)

Sample bags/labels

Sodium hydroxide (NaOH)

Test tubes

UV-Spectrometer

Volumetric flask

Wagner's reagent

Water leaf (Talinum triangulare)

Whatman filter paper

## 3.2 Study Area

Mubi is the largest town in Adamawa state in terms of business establishment and infrastructural development. The study will be conducted at the Chemistry Laboratory department of Biochemistry Adamawa State University, Mubi, Adamawa State.

## 3.3 Sample Collection and Preparation

The vegetables plants samples for bitter leaf (*Vermonia* *amygdalina*) and water leaf (*Talinum* *triangulare*) were collected from the farm in Federal Polytechnic, Mubi, labeled and transported to the laboratory for subsequent digesting and analysis since the method chosen for a particular analysis is dependent on the reason for the analysis, wet ashing is used for the preparation of the medicinal plant sample to avoid loss of the metal by high temperature (dry) ashing. (Barth *et al.,* 2001)

## 3.4 Sample Digestion

The samples were air dried in the open and then grind in a blender. From the powder, 2g of each plant was weighed out and transferred into a flask. A mixture of concentrated trioxonitrate (vii) acid, HNO3 and tetraoxochlorate (vii) acid, HClO4 in the ratio of (4:1) was added and brought to boil for 4hrs, after the samples were completely digested, they were allowed to cool then filtered with Whatman filter paper and transferred into a 50ml volumetric flask and made up to mark with deionized water

The metal concentrations in these medical plants samples were determined using buck scientific atomic absorption Spectrophotometer 205. The heavy metals analyzed were Cr, Cu, Cd, ZN and Pb.

## 3.5 Phytochemical screening

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, Anthraquinone, Saponin and Alkaloids.

## 3.5.1 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.

## 3.5.2 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 was observed. To this, few drops of 70% dilute hydrochloric acid was added and yellow colour was disappeared. Formation and disappearance of yellow colour indicates the presence of flavonoids in the sample extract.

## 3.5.3 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.

## 3.5.4 Test for Anthraquinones

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

## 3.5.5 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.

## 3.5.6 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)

## 3.5.7 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.

0.5g of the extract was stirred with 10ml of distilled water and then filtered. Few drops of 5% FeCl3 reagent was added to the filtrate. Blue black or blue green coloration or precipitate was taken as an indication of the presence phenolics (AOAC, 2020).

## 3.5.8 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.

2ml of the methanolic extract was stirred with 2ml of distilled water and few drops of FeCl3 solution was added. The formation of a green precipitate was an indication for the presence of tannins (Singleton *et al.*, 2015).

## 3.5.9 Test for Terpenoids.

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

## 3.6 Elemental Analysis

5g of dried leaf powder with a mixture of nitric acid and hydrochloric acid to break down organic matter and release elements. The digested sample is filtered and diluted, then analyzed using a UV-Spectrometer. Absorbance values are measured and compared to standard calibration curves to determine the concentration of elements in the sample. Result was expressed in mg/kg, providing insights into the elemental composition of the leaves (AOAC 2020).

## 3.7 Statistical Analysis

Data obtained were subjected to statistical analysis to determine differences in heavy meta concentrations among the three fish species.

# CHAPTER FOUR

# RESULTS

## 4.1 Results

**4.1.1 Qualitative Analysis of phytochemicals**

**Table 4.1: Phytochemical Screening of Bitter leaf and Water leaf Extract**

|  |  |  |
| --- | --- | --- |
| **Test** | **Bitter leaf** | **Water leaf** |
| Saponin | + | + |
| Phenol | + | + |
| Flavonoid | + | + |
| Terpenoid | + | + |
| Glycoside | + | + |
| Steroids | - | + |
| Tannin | + | + |
| Anthraquinone | + | + |
| Alkaloid | + | + |

Key:

- Indicate Absence

+ Indicate Present

Table 4.1 reveals the qualitative phytochemical analysis reveals the presence of various bioactive compounds in both bitter leaf and water leaf extracts. Both plant extracts tested positive for saponins, phenols, flavonoids, terpenoids, glycosides, tannins, anthraquinones, and alkaloids, indicating their potential medicinal and antioxidant properties. However, steroids were absent in bitter leaf but present in water leaf, suggesting a slight variation in their phytochemical composition.

**4.1.2: Elemental Analysis of Heavy metals**

**Table 4.2: Heavy Metal Analysis in Bitter Leaf and Water Leaf Compared with WHO Standard Limits**

| **Heavy Metals** | **Bitter Leaf (mg/kg)** | **Water Leaf (mg/kg)** | **WHO Standard (mg/kg)** |
| --- | --- | --- | --- |
| Lead (Pb) | ND | ND | 0.3 |
| Cadmium (Cd) | 0.08±0.01 | 0.15±0.01 | 0.2 |
| Copper (Cu) | 0.44±0.01 | 0.51±0.01 | 10 |
| Zinc (Zn) | 0.33±0.01 | 1.18±0.01 | 60 |
| Chromium (Cr) | ND | ND | 2 |

Key: ND = Not Detected, ± = Standard error of mean (n = 3)

Table 4.2 presents the concentrations of selected heavy metals in bitter leaf and water leaf alongside WHO permissible limits. Lead (Pb) and chromium (Cr) were not detected (ND) in either of the samples, suggesting their absence or presence below detectable levels. Cadmium (Cd) was recorded at 0.003 mg/kg in bitter leaf and 0.006 mg/kg in water leaf, both below the WHO limit of 0.02 mg/kg. Copper (Cu) levels were 0.26 mg/kg in bitter leaf and 0.30 mg/kg in water leaf, compared to the WHO limit of 40 mg/kg. Zinc (Zn) was 0.47 mg/kg in bitter leaf and 0.69 mg/kg in water leaf, also well below the WHO limit of 60 mg/kg. These results indicate that the levels of heavy metals in both vegetables are within safe consumption limits.

## 4.2 Discussion

The phytochemical screening conducted in this study revealed the presence of numerous bioactive constituents in both *Vernonia amygdalina* (bitter leaf) and *Talinum triangulare* (water leaf). Specifically, both leaves contained saponins, phenols, flavonoids, terpenoids, glycosides, tannins, anthraquinones, and alkaloids. Notably, only water leaf exhibited the presence of steroids. This diverse phytochemical composition aligns with the findings of Adeyemo *et al.* (2024), who emphasized the therapeutic significance of flavonoids and saponins in local medicinal plants such as *Ficus exasperata*. These compounds are well documented for their antioxidant, antimicrobial, and anti-inflammatory properties, underscoring the medicinal potential of both leaves (Okonkwo & Ibrahim, 2023).

The elemental analysis for heavy metals revealed no detectable levels of lead (Pb) and chromium (Cr) in either leaf extract, which is significant from a public health perspective. However, cadmium (Cd), copper (Cu), and zinc (Zn) were present in both samples, with water leaf showing higher concentrations than bitter leaf. This trend is consistent with the findings of Kalagbor and Diri (2022), who reported higher accumulation of Zn and Cd in water leaf relative to bitter leaf in samples collected from Port Harcourt. They attributed this to environmental exposure and potential contamination from agricultural and industrial activities.

In a related study, Uwah *et al.* (2021) assessed trace metal content in *V. amygdalina* sourced from oil exploration areas in Ibeno, Akwa Ibom State. While trace amounts of Cd, Cu, and Zn were present, all were within permissible limits set by the World Health Organization (WHO), reaffirming the impact of environmental conditions on metal uptake. The present study’s findings—particularly the non-detection of Pb and Cr suggest that the leaves analyzed were harvested from relatively uncontaminated environments.

Broadening the context, a study by Eze *et al.* (2023) conducted a health risk assessment of bitter leaf grown in contaminated agricultural soils in Port Harcourt and identified potential toxicological concerns, especially for children (Hazard Index > 1). This highlights the necessity of regular monitoring of heavy metal concentrations in edible plants. Conversely, the relatively low levels observed in our current study suggest that the sampled locations are likely free from industrial or agricultural pollutants, making the leaves suitable for human consumption and therapeutic use.

Overall, the combination of rich phytochemical constituents and low levels of toxic heavy metals indicates that both *V. amygdalina* and *T. triangulare* hold substantial potential for use in traditional medicine and as dietary components. Nonetheless, the slightly elevated levels of Cd, Cu, and Zn in water leaf warrant periodic environmental assessments and controlled cultivation practices to ensure long-term safety and quality.

# CHAPTER FIVE

# Conclusion and recommendations

## 5.1 Conclusion

This study investigated the phytochemical composition and heavy metal content of Bitter leaf (*Vernonia amygdalina*) and Water leaf (*Talinum triangulare*) collected from Mubi. The qualitative phytochemical screening revealed the presence of various bioactive compounds such as saponins, phenols, flavonoids, terpenoids, glycosides, tannins, anthraquinones, and alkaloids in both plants, with steroids being absent in bitter leaf but present in water leaf. These compounds are known to possess diverse therapeutic effects, including antimicrobial, antioxidant, anti-inflammatory, and anti-cancer activities, underscoring the medicinal relevance of these plants.

In the heavy metal analysis, essential trace elements such as Copper (Cu) and Zinc (Zn) were detected in both plant samples, with higher concentrations in water leaf. However, toxic metals such as Lead (Pb) and Chromium (Cr) were not detected in either sample, which is a positive indication of minimal environmental contamination at the collection sites. The presence of Cadmium (Cd) at low levels (0.08 mg/kg in bitter leaf and 0.15 mg/kg in water leaf) raises mild concern, although the concentrations are within tolerable limits recommended by WHO/FAO. These results indicate that both plants are rich in beneficial phytochemicals and are relatively safe for consumption, though continuous environmental monitoring is necessary to prevent potential bioaccumulation of harmful metals.

## 5.2 Recommendations

1. Government and health agencies should ensure regular testing of soil and water in agricultural areas to detect and prevent heavy metal contamination that may affect edible plants.
2. Educating local farmers and the public on the importance of safe cultivation practices and the risks of using untreated wastewater and chemical fertilizers will help minimize contamination.
3. Future studies should carry out quantitative analysis of the phytochemicals present in the plant extracts to better understand their medicinal potential and safe dosage levels.
4. In vivo and in vitro toxicological studies are recommended to assess the long-term health effects of consuming these plants, particularly regarding cadmium content.
5. Similar studies should be conducted in other parts of Nigeria to compare levels of phytochemicals and heavy metals in the same plant species, thereby providing a broader ecological safety profile.
6. Since both plants contain valuable bioactive compounds, their use in traditional medicine should be promoted with adequate scientific backing. Conservation strategies should also be adopted to ensure their sustainable availability.

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