

**PHYTOCHEMICAL PROFILING OF LOCALLY MARKETED *Moringa Oleifera* LEAVES  
IN BENIN CITY, EDO STATE.**

**BY**

**INEDEGBOR EJIODAMHEN SUCCESS**

**BMS2006673**

**DEPARTMENT OF MEDICAL BIOCHEMISTRY**

**SCHOOL OF BASIC MEDICAL SCIENCES**

**UNIVERSITY OF BENIN**

**NOVEMBER, 2025.**

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**A PROJECT SUBMITTED TO THE  
DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL  
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CITY.**

**NOVEMBER, 2025**

## CERTIFICATION

We the undersigned hereby certify that **INEDEGBOR EJIODAMHEN SUCCESS** with matriculation number **BMS2006673**, carried out this work, in the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin city and we approve same as adequate in scope and quality for the reward of Bachelor of Science (B.Sc.) degree in Medical Biochemistry.

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**DR. L.O. Agbontaen**

**(Project Supervisor)**

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**Date**

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**DR. N.B Aguebor-Ogie**

**(A.g Head of Department)**

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**Date**

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**External Examiner**

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**Date**

## **DEDICATION**

Firstly, I dedicate this work to God, my source of strength, inspiration, wisdom, knowledge and understanding, to my parents for their immense support during the course of my study here at the University of Benin and to the staff of the Department of Medical biochemistry who have taught me up to this point in my academic pursuit, equipping me with knowledge for both self and societal development.

## **ACKNOWLEDGMENT**

This project work is a product of much research, extensive discussion and analysis. I want to use this medium to acknowledge the input of various persons at the different stages of its development. I would first and foremost give glory to God for the successful completion of this work. I would like to appreciate my project Supervisor, Dr L.O. Agbontaen, for his help and guidance throughout the process of the development of this project. His guidance and encouragement has been instrumental in its completion. My sincere appreciation goes to my parents Mr and Mrs Oiyemhonlan for their love, financial support and prayers during the course of my academic journey. I will also like to appreciate my big bro Otis for his massive help and support both financially and education wise. A big shout out to my friend Ify, thank you for your support and always sticking by my side throughout our days in uniben, my appreciation also goes out to Dureka (my first friend in uniben), Miracle, Divine, Joy, Ofure, Benita, Timna, Marvellous etc and so many others I failed to mention you all have been a great help and support to the completion of this project. God bless you all.

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## ABSTRACT

This study examined the phytochemical content of *Moringa oleifera* leaves sold in local markets across Benin City, Edo State. Although *Moringa* is commonly used for food and traditional medicine, there is limited information on the quality of the leaves available to consumers in this area. Fresh samples were collected from different markets and analysed using standard qualitative and quantitative phytochemical screening methods.

The qualitative results showed the presence of major phytochemicals such as flavonoids, terpenoids, cardiac glycosides, tannins, steroids, alkaloids and phenols. Quantitative findings revealed that flavonoids were the most abundant (862.21  $\mu\text{g/ml}$ ), followed by cardiac glycosides (525.78  $\mu\text{g/ml}$ ), terpenoids (304.23  $\mu\text{g/ml}$ ), steroids (302.94  $\mu\text{g/ml}$ ) and tannins (126.56  $\mu\text{g/ml}$ ). These compounds are associated with antioxidant, anti-inflammatory and general health-promoting activities.

Overall, the study shows that *Moringa oleifera* leaves sold in Benin City still contain valuable bioactive compounds that support their traditional use. The findings provide useful baseline data, and further research is recommended to include chromatographic profiling and safety assessments for better quality control.

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background of the Study.

For thousands of years, human beings have depended on plants not only as food but also as natural sources of healing and wellness. Long before modern medicine became widespread, communities around the world used plants to treat illnesses and maintain good health (World Health Organization, 2013). Even today, in many parts of Africa and Asia, herbal medicine remains a major part of the healthcare system because it is affordable, effective, and closely tied to people's culture and traditions.

One of the plants that has gained strong attention in both traditional and scientific medicine is *Moringa oleifera*. It is commonly called the drumstick tree, miracle tree, or tree of life because almost every part of it—from the leaves to the seeds, flowers, and roots—can be used for food, medicine, or other purposes (Anwar *et al.*, 2007). The tree belongs to the family *Moringaceae* and grows easily in tropical and subtropical climates. Originally from northern India, *Moringa oleifera* has spread widely across the world, including many parts of Africa where it thrives naturally (Leone *et al.*, 2015).

In Nigeria, moringa is well known and commonly used as both food and medicine. It is often cultivated in home gardens or bought in markets, where the leaves are sold fresh, dried, or ground into powder. The plant grows easily even in poor soils and dry environments, which

makes it a reliable source of nutrition in many communities (Moyo *et al.*, 2011). The leaves are especially valued because they contain a wide range of essential nutrients—vitamins A, C, and E, minerals like calcium, potassium, and iron, and a good amount of protein (Mbikay, 2012). Because of this, moringa is sometimes referred to as a “superfood” that can help fight malnutrition and improve general health.

Apart from being nutritious, *Moringa oleifera* also contains powerful natural chemicals known as phytochemicals. These are special compounds that plants produce to protect themselves against diseases, pests, and harsh environmental conditions. When consumed by humans, many of these phytochemicals have protective and healing effects (Kasolo *et al.*, 2010). Common examples found in moringa include flavonoids, phenolic acids, tannins, saponins, alkaloids, and terpenoids (Sreelatha and Padma, 2009; Ben Salem *et al.*, 2017). These compounds are responsible for the plant’s antioxidant, antimicrobial, and anti-inflammatory properties, which make it useful in both traditional and scientific medicine.

In Benin City, located in Edo State, Nigeria, moringa leaves are widely available in local markets such as Ring Road Market, New Benin Market, and Oba Market. Many people in the city use the leaves as a health tonic, tea, or food ingredient to promote well-being. Despite its popularity and frequent use, there is still limited scientific information about the exact phytochemical composition of the moringa leaves sold in these markets. Since factors such as the soil type, harvesting time, storage, and drying method can affect the amount and type of phytochemicals in the leaves (Moyo *et al.*, 2011; Adjatin *et al.*, 2013), it becomes important to study and document the phytochemical profile of moringa obtained from these locations.

Phytochemical profiling simply means identifying and measuring the natural chemical compounds present in a plant. By analysing these compounds, scientists can understand why the plant is beneficial and how safe or effective it might be when used as food or medicine. Therefore, studying the phytochemical content of *Moringa oleifera* leaves from Benin City will provide valuable information to confirm its nutritional and medicinal value. It will also help to create awareness about the importance of proper handling and processing of moringa to preserve its quality and effectiveness.

### **1.1 Statement of the Problem.**

Although *Moringa Oleifera* is widely used and celebrated for its many health benefits, there is still a lack of scientific research that focuses on the specific phytochemical content of moringa leaves sold in Benin City. Most of the people who use moringa rely on traditional knowledge passed down through generations, but they often do not know whether the moringa they buy in the market contains the same amount of beneficial compounds as those reported in other scientific studies.

Environmental and handling factors such as the soil type, climate, storage conditions, and drying process can significantly influence the levels of phytochemicals in the plant (Kasolo *et al.*, 2010; Moyo *et al.*, 2011). Leaves that are poorly stored or exposed to sunlight for too long may lose some of their nutrients and active compounds. Because of these variations, it is difficult to ensure that all moringa leaves sold in the market have the same nutritional or medicinal quality.

This gap in knowledge means that many people may not be getting the full benefits of moringa, or in some cases, may be consuming products of lower quality without realizing it. Therefore, it

is necessary to carry out a phytochemical profiling study on moringa leaves collected from different parts of Benin City to determine the types and quantities of bioactive compounds they contain. This research will help confirm the quality and reliability of moringa used by the local population.

## **1.2 Aim of the Study.**

The aim of this study is to carry out a detailed phytochemical profiling of *Moringa oleifera* leaves obtained from selected areas in Benin City, Edo State, Nigeria, in order to determine their composition and evaluate their potential health value.

## **1.3 Objectives of the Study.**

To achieve the stated aim, the study seeks to:

1. Identify the types of phytochemicals present in *Moringa oleifera* leaves through qualitative screening.
2. Determine the quantity of selected phytochemicals such as phenols and flavonoids through quantitative analysis.
3. Assess how environmental and handling factors may influence the phytochemical content of moringa leaves sold in Benin markets.

## **1.4 Research Questions.**

This study will be guided by the following questions:

1. What phytochemicals are present in *Moringa oleifera* leaves found in Benin City?
2. In what quantities are these phytochemicals present?
3. What environmental or handling factors might affect the phytochemical composition of these leaves?

### **1.5 Scope of the Study.**

This research focuses exclusively on the leaves of *Moringa oleifera* collected from various markets within Benin City, Edo State. The study involves both qualitative and quantitative analyses of phytochemicals such as flavonoids, tannins, phenols, alkaloids, saponins, and terpenoids.

It does not cover other parts of the moringa plant such as seeds, roots, or flowers. Additionally, the study does not include any clinical or pharmacological testing on humans or animals. Instead, it is limited to laboratory-based evaluation of the chemical composition of moringa leaves.

The research also aims to link its findings to the traditional use of moringa in Benin City, helping to explain whether the chemical properties observed in the laboratory support the plant's long-standing reputation in traditional medicine.

### **1.6 Significance of the Study.**

This study is important for several reasons. Firstly, it provides scientific evidence about the chemical composition of moringa leaves commonly consumed in Benin City. This information

can help validate the traditional claims about the plant's nutritional and medicinal benefits. Secondly, it adds to the growing body of research on medicinal plants in Nigeria, which is essential for promoting local herbal knowledge and supporting national health policies that integrate traditional medicine into modern healthcare (Leone *et al.*, 2015).

Furthermore, understanding the phytochemical content of moringa leaves will help standardize its use in the preparation of herbal products, teas, and supplements. Many people use moringa daily, but few know the actual strength or concentration of its active compounds. With this study, consumers, herbal practitioners, and food scientists can have more reliable information about what the moringa leaves they use actually contain.

The findings of this research may also guide future studies on natural product formulation, drug development, and nutrition improvement, especially for addressing malnutrition or oxidative stress-related health issues (Anwar *et al.*, 2007; Mbikay, 2012). Lastly, the study will benefit the local community by increasing awareness of the importance of handling and storing moringa properly to preserve its quality, safety, and health benefits.

### **1.7 Limitations of the Study.**

- Although the study successfully determined the presence and quantity of several phytochemicals, it did not include biological or functional assays to test their actual effects.
- The sensitivity and accuracy of the analytical instruments used might limit the detection and quantification of certain phytochemicals.

- This study is limited only to the *Moringa oleifera* leaf sample and not other parts of the plant.
- The stability of the phytochemicals during extraction, storage and analysis might be a concern, potentially affecting the accuracy of the results.
- The study might focus on specific types of phytochemicals, potentially overlooking other bioactive compounds present in the plant.
- The phytochemical profile of Moringa Oleifera leaf might vary depending on the season, climate and environmental conditions, which could impact the study's findings.

## **CHAPTER TWO**

### **LITERATURE REVIEW**

#### **2.1 Overview of Moringa oleifera.**

*Moringa oleifera* is one of the most versatile and valuable trees known to humanity. It belongs to the family *Moringaceae* and is often called the “miracle tree” or “drumstick tree” because almost every part of it, leaves, pods, flowers, seeds, and roots, has nutritional, medicinal, or industrial uses (Anwar *et al.*, 2007). The tree is native to northern India but has spread widely across Africa, Asia, and South America. In Nigeria, it grows well in most ecological zones and is locally called “Zogale” (Hausa), “Okwe oyibo” (Igbo), and “Ewe igbale” (Yoruba) (Popoola and Obembe, 2013).

The tree can reach up to 12 meters in height and thrives even in poor soils. Its ability to survive drought conditions makes it an excellent choice for cultivation in both rural and urban areas such as Benin City. Over the years, *Moringa oleifera* has become increasingly popular not just among herbal medicine users but also among nutritionists and food technologists (Leone *et al.*, 2015). Because of its rapid growth, resilience, and rich nutrient composition, *Moringa oleifera* has been described as a sustainable food and health resource for developing countries. In recent years, its leaves have been turned into teas, powders, and capsules, used to improve health and combat malnutrition (Fahey, 2005).

In Benin City, the cultivation and sale of *Moringa oleifera* are common in local markets such as the Ring Road and Oba Market, where it is traded both as fresh leaves and in dried powdered form. Its increasing popularity in herbal medicine and everyday diets reflects how valuable it has become to human health and livelihood.



Figure

2.1: Showing the picture of a *Moringa Oleifera* tree (Miracle tree).

Source: Healthline articles.

### 2.1.1 Taxonomical Classification of *Moringa oleifera*

Kingdom: Plantae

Subkingdom: Tracheobionta (Vascular plants)

Superdivision: Spermatophyta (Seed-producing plants)

Division: Magnoliophyta (Flowering plants)

Class: Magnoliopsida (Dicotyledons)

Subclass: Dilleniidae

Order: Brassicales

Family: Moringaceae

Genus: *Moringa*

Species: *Moringa oleifera* Lam.

### **2.1.2 Nutritional Value of *Moringa oleifera* leaves.**

*Moringa oleifera* has earned global attention for its impressive nutritional composition. The leaves are exceptionally rich in proteins, vitamins, and minerals, making them one of the most nutrient-dense plants on Earth (Moyo *et al.*, 2011). The leaves contain high levels of vitamins A, C, and E, which are powerful antioxidants that protect the body from oxidative damage. In addition, they provide minerals such as calcium, potassium, magnesium, and iron that are vital for maintaining strong bones, proper muscle function, and blood formation (Fahey, 2005).

In many developing countries, *Moringa* leaves are used to combat malnutrition because of their high protein content up to 30% in dried leaf powder (Gopalakrishnan *et al.*, 2016). In Nigeria, especially around Benin City, mothers often add moringa powder to pap (ogi), soups, and stews to boost the nutritional value of meals for children and pregnant women. This simple practice helps to address nutrient deficiencies in communities where animal protein is expensive or unavailable (Adjatin *et al.*, 2013).

Beyond its nutrient content, *Moringa oleifera* also provides essential amino acids such as lysine, methionine, and tryptophan, which are necessary for body tissue repair and hormone balance. Its natural oils are rich in unsaturated fatty acids that support cardiovascular health (Leone *et al.*, 2015). Because of this, Moringa is not only considered a medicinal plant but also a functional food (a food that promotes health beyond basic nutrition).

When compared to common vegetables, Moringa leaves stand out for their nutritional superiority. For example, the leaves contain seven times more vitamin C than oranges and four times more calcium than milk (Gopalakrishnan *et al.*, 2016). This explains why nutritionists and public health organizations recommend its inclusion in daily diets.



Fig

ure 2.2: Showing the close up view of fresh organic Moringa leaves.

Source: Niteenrk (iStock).

### **2.1.3 Botanical Description of the Leaves of *Moringa oleifera***

#### **1. Leaf Type**

The leaves are compound, either bipinnate or tripinnate, depending on maturity and environmental conditions.

#### **2. Leaf Arrangement**

Leaves are arranged alternately along the stem.

#### **3. Leaf Size**

A fully developed leaf measures approximately 20–60 cm in length, with several pairs of pinnae.

#### **4. Rachis**

The central rachis is slender, green, and slightly flexible.

#### **5. Leaflets**

Leaflets are small, obovate to elliptical in shape. Each leaflet is typically 1–2 cm long and 0.5–1 cm wide. They are arranged in opposite pairs, with a single terminal leaflet.

#### **6. Leaflet Surface**

The upper surface is smooth and bright green, while the underside is paler.

The texture is thin and soft.

## 7. Margin, Apex, and Base

Margin: Entire (smooth)

Apex: Rounded to slightly obtuse

Base: Symmetrical and cuneate

## 8. Venation

Venation is pinnate, with a prominent midrib and fine lateral veins.

## 9. Petiole

Each compound leaf has a slender petiole, usually 3–6 cm long.

## 10. Deciduous Nature

*Moringa oleifera* is deciduous, so leaves are shed during dry or cold seasons.

## 11. Smell and Taste

When crushed, the leaves release a mild, characteristic smell and have a slightly bitter to pungent taste, especially when mature.

## **2.2 Phytochemical Constituents of *Moringa oleifera*.**

The term phytochemicals refers to naturally occurring chemical compounds in plants that contribute to their color, flavor, and disease-protective properties. *Moringa oleifera* leaves contain a rich variety of phytochemicals including flavonoids, tannins, saponins, alkaloids, terpenoids, and phenolic compounds (Kasolo *et al.*, 2010). These compounds are not nutrients

but play essential roles in protecting the body from harmful substances and improving overall health.

Flavonoids are one of the most important groups of phytochemicals in Moringa. They function as natural antioxidants that protect body cells from oxidative stress and damage caused by free radicals (Siddhuraju and Becker, 2003). These compounds are known to support heart health, strengthen the immune system, and even slow down the aging process. Tannins are another important component that gives Moringa its slightly bitter taste and are known for their antimicrobial and anti-inflammatory effects (Fahey, 2005).

Saponins have been reported to help lower cholesterol levels and improve immune responses, while alkaloids often show pharmacological properties, including pain relief and relaxation effects on the nervous system (Kasolo *et al.*, 2010). Terpenoids and phenolic compounds contribute to the plant's fragrance and antioxidant potential, which explains why Moringa extracts are now used in both herbal and cosmetic formulations.

The unique combination of these compounds gives *Moringa oleifera* its multiple health benefits and supports its role in both traditional and modern medicine. Many researchers have confirmed that the balance between these phytochemicals determines the plant's medicinal strength (Harborne, 1998).

### **2.2.1 Flavonoids**

*Moringa oleifera* leaves contain a high level of flavonoids, especially quercetin and kaempferol, which are some of the main contributors to the plant's strong antioxidant properties. These

compounds help neutralise harmful free radicals and have been linked with anti-inflammatory and heart-protective effects in several studies (Saini *et al.*, 2016; Kashyap *et al.*, 2022).

### **2.2.2 Phenolic acids**

The leaves also provide various phenolic acids, particularly chlorogenic acid, which plays a major role in the plant's antioxidant behaviour. These acids support the body's defence system by reducing oxidative stress and have been associated with anti-inflammatory and blood-pressure-lowering effects in research findings (Kashyap *et al.*, 2022; Pop *et al.*, 2022).

### **2.2.3. Glucosinolates and isothiocyanates**

Another important group of compounds in moringa leaves are glucosinolates and their breakdown products, known as isothiocyanates. Glucomoringin and niazimicin are among the most studied ones, and they have shown antimicrobial, anti-inflammatory and potential anticancer properties in laboratory experiments (Pop *et al.*, 2022; Saini *et al.*, 2016).

### **2.2.4. Alkaloids**

Alkaloids are also present in moringa leaves, although their quantity depends on the extraction method. These compounds have displayed antimicrobial activity and may have mild effects on the nervous system, according to experimental studies (Pareek *et al.*, 2023; Khalid *et al.*, 2023).

### **2.2.5. Saponins**

Saponins are naturally occurring compounds in moringa leaves and are known for their ability to lower cholesterol and support immune responses. They can also act as mild antimicrobial agents, and their levels vary with the preparation and extraction process (Pareek *et al.*, 2023).

### **2.2.6. Tannins**

Tannins are part of the plant's broader phenolic profile and contribute to moringa's antioxidant strength. They are commonly detected in phytochemical screenings and help the plant exhibit astringent and protective effects (Saini *et al.*, 2016; Pareek *et al.*, 2023).

### **2.2.7. Plant sterols and terpenoids (e.g., $\beta$ -sitosterol)**

Moringa leaves contain sterols such as  $\beta$ -sitosterol, which are associated with anti-inflammatory benefits and improved lipid balance. Terpenoids, also present in the leaves, contribute to both the plant's smell and its biological activities (Padayachee & Baijnath, 2020; Pop *et al.*, 2022).

## **2.3 Ethnomedicinal and Traditional Uses of *Moringa oleifera*.**

*Moringa oleifera* has been an integral part of traditional medicine for centuries, particularly in West Africa and Nigeria. In Benin City, herbal practitioners and local households commonly use the leaves, seeds, and roots for medicinal purposes. The leaves are often boiled into teas or added to soups to manage general weakness, digestive disorders, fevers, high blood pressure, and infections (Coppin, 2008; Adjatin *et al.*, 2013).

The seeds and roots also have traditional applications; seed oil, for example, is applied topically to wounds and skin irritations, while powdered roots are sometimes used as an anti-inflammatory agent. These traditional practices reflect accumulated indigenous knowledge and a deep understanding of the plant's effects, even before modern scientific validation (Kasolo *et al.*, 2010).

Globally, Moringa is used in Ayurvedic medicine in India to treat over 300 ailments, ranging from malnutrition to inflammatory diseases. Such uses highlight the plant's versatility and the scientific curiosity it has sparked over the past decades (Leone *et al.*, 2015). By profiling Moringa leaves from Benin City, researchers can bridge the gap between ethnomedicine and modern phytochemical science, validating local practices while offering insights into potential nutraceutical or therapeutic applications.

## **2.4 Environmental and Geographical Influence on Phytochemical Content: How Climate, Soil, and Location May Affect Bioactive Compounds.**

The production of phytochemicals in plants, including *Moringa oleifera*, can be influenced by environmental and geographical factors. Soil quality, rainfall, temperature, sunlight exposure, and altitude may affect the levels of bioactive compounds such as flavonoids, phenolic acids, and glucosinolates (Harborne, 1998; Leone *et al.*, 2015). For example, plants grown under moderate environmental stress often produce higher levels of phenolics as a protective response to UV exposure or nutrient limitation (Ghasemzadeh *et al.*, 2010).

Although Moringa is highly adaptable and can grow in a variety of soils and climates, quantitative studies specifically linking environmental conditions in Benin City to phytochemical variations are limited. This suggests a research gap that the current study seeks to address. By analyzing leaves collected locally, it is possible to explore how regional environmental factors may correlate with the diversity and concentration of phytochemicals in Moringa leaves.

## **2.5 Extraction and Analytical Methods for Phytochemical Profiling.**

The scientific profiling of phytochemicals in *Moringa oleifera* leaves requires systematic laboratory techniques. Extraction is usually the first step, which involves separating the bioactive compounds from the plant matrix. Different solvents, including water, ethanol, methanol, and acetone, are used depending on the polarity of the compounds being targeted (Anwar *et al.*, 2007). Polar solvents like methanol are excellent for extracting flavonoids and phenolic compounds, while non-polar solvents are used for terpenoids and certain alkaloids.

Qualitative analysis determines the presence of specific classes of compounds, such as alkaloids, tannins, saponins, and flavonoids. These tests often involve color reactions or precipitation assays, such as the ferric chloride test for tannins or the Dragendorff's test for alkaloids (Harborne, 1998).

Quantitative methods then measure the exact amounts of these compounds. For instance, total phenolic content (TPC) can be assessed using the Folin-Ciocalteu reagent and expressed in milligrams of gallic acid equivalents per gram of extract. Total flavonoid content (TFC) is often determined using aluminum chloride colorimetric methods and expressed as quercetin equivalents per gram of extract (Sreelatha and Padma, 2009).

Advanced techniques like High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS) allow for precise identification and quantification of individual bioactive molecules. These methods are especially important when comparing phytochemical profiles across regions or seasons, such as leaves sourced from different markets in Benin City (Vongsak *et al.*, 2013).



Figure 2.3: Showing the process of extraction of Moringa Oleifera leaf extracts

Source: Unveiling Moringa oleifera (potent source of antioxidant and antibacterial properties by (Deepali et al., 2025))

## 2.6 Nutraceutical and Food Applications of Moringa oleifera Leaves.

In addition to its traditional medicinal uses, *Moringa oleifera* has gained prominence as a nutraceutical—a food product that delivers both nutritional and therapeutic benefits. The

leaves, in particular, are rich in essential vitamins, minerals, proteins, and bioactive phytochemicals, making them highly suitable for incorporation into health-promoting foods and dietary supplements (Gopalakrishnan *et al.*, 2016).

In Benin City, small-scale producers and local markets have embraced the production of Moringa powders, capsules, and teas. These products are increasingly marketed not only for their nutrient content but also for their potential to boost immunity, provide antioxidant support, and maintain general wellness. Nutraceutical applications capitalize on the plant's high levels of flavonoids, phenolics, and vitamins, which collectively contribute to reducing oxidative stress and supporting metabolic health (Sreelatha and Padma, 2009).

Beyond direct consumption, Moringa leaf extracts are also explored for food preservation. Their natural antioxidant and antimicrobial properties can help prolong the shelf life of perishable foods, such as oils, meat products, and dairy, without relying on artificial chemicals (Falowo *et al.*, 2018). This is particularly important in regions like Benin City, where refrigeration is often limited and traditional preservation methods are commonly used. Incorporating Moringa into food formulations can therefore provide both nutritional enhancement and safety benefits.

Globally, researchers have experimented with incorporating Moringa into baked goods, beverages, and snack products. Studies indicate that up to 10% leaf powder can be added to foods without significantly altering taste or texture, while still delivering substantial health benefits (Leone *et al.*, 2015). These findings suggest that Moringa has significant potential as a functional food ingredient for both local consumption and commercial nutraceutical production.

## **2.7 Economic Importance and Community Impact of *Moringa oleifera*.**

*Moringa oleifera* is not only a health-promoting plant but also a valuable economic resource. In Benin City, the cultivation, processing, and sale of *Moringa* products—such as dried leaves, powders, teas, seeds, and oils—provide a source of income for small-scale farmers, market traders, and herbal medicine practitioners. The low maintenance requirements of *Moringa* trees, along with their rapid growth, make them particularly attractive to urban and peri-urban farmers (Leone *et al.*, 2015).

Market surveys indicate that dried leaf powder and teas are increasingly popular among health-conscious consumers in Benin City. Herbal vendors often combine *Moringa* with other local herbs to produce blended teas, marketed for boosting immunity and overall wellness. These activities generate microeconomic benefits, supporting livelihoods and contributing to household income, particularly for women who dominate the herbal medicine and food supplement trade (Adjatin *et al.*, 2013).

Moreover, *Moringa*'s multipurpose utility (from nutritional supplements to natural water purification agents) makes it a versatile tool for community development. The plant can help combat food insecurity, improve public health, and provide employment opportunities, making it a critical resource for sustainable development in the region (Coppin, 2008).

## **2.8 Summary of Related Studies on *Moringa oleifera* Leaves.**

A large body of research has investigated the nutritional and medicinal potential of *Moringa oleifera* leaves. Studies consistently report that the leaves are rich in proteins, vitamins,

minerals, and bioactive compounds such as flavonoids, phenolics, and saponins (Kasolo *et al.*, 2010; Gopalakrishnan *et al.*, 2016). These compounds contribute to antioxidant, antimicrobial, and anti-inflammatory activities, which justify the plant's use in traditional medicine and nutraceutical applications.

Globally, research has demonstrated that Moringa leaf extracts possess strong radical scavenging activity, comparable to synthetic antioxidants, and are effective in food preservation (Vongsak *et al.*, 2013; Falowo *et al.*, 2018). In Nigeria, studies in different regions (including Oyo State and Lagos) have confirmed the high nutritional value and bioactive properties of leaves, particularly in relation to combating malnutrition and supporting general wellness (Moyo *et al.*, 2011).

However, while research has been extensive in other regions, few studies have focused specifically on Benin City, where local cultivation practices, market conditions, and environmental factors may affect phytochemical content. Profiling Moringa leaves from this region can provide valuable data for comparing nutritional and medicinal quality across different ecological zones.

## **2.9 Research Gap in Phytochemical Profiling of *Moringa oleifera*.**

Despite the growing body of research on *Moringa oleifera*, a specific gap exists for leaves sourced from Benin City. Environmental factors such as soil type, rainfall, and urban pollution can significantly influence the quantity and quality of phytochemicals. Furthermore, differences in harvesting, drying, and storage methods used by local vendors can affect antioxidant capacity and nutritional value.

No comprehensive study has combined qualitative and quantitative phytochemical profiling with local ethnomedicinal insights and nutritional analysis for Benin City. Addressing this gap will provide an evidence-based understanding of the plant's potential for both community health and economic development, while also informing nutraceutical and food-based applications.

## **2.10 Theoretical Framework for Phytochemical Profiling of *Moringa oleifera*.**

The theoretical basis for this study is grounded in the Bioactive Compound Theory, which proposes that certain naturally occurring plant compounds, although not nutrients, can exert physiological effects on humans. According to this theory, the consumption of plant-based secondary metabolites; such as flavonoids, phenolic acids, and saponins, can influence metabolic pathways, oxidative stress, and overall health (Harborne, 1998).

Another relevant theoretical perspective is the Functional Food Theory, which emphasizes that foods can provide additional health benefits beyond basic nutrition. This theory supports the rationale for developing *Moringa*-based nutraceuticals, such as teas, capsules, or fortified food products, which deliver bioactive compounds in a convenient form (Gopalakrishnan *et al.*, 2016).

In combination, these theories guide the study's approach to profiling *Moringa oleifera* leaves. By identifying and quantifying phytochemicals, the study aligns with the idea that natural plant compounds can be harnessed for both nutrition and preventive health. Moreover, the inclusion of environmental and market-based considerations in Benin City emphasizes the practical

application of these theories, linking laboratory findings to real-world use in nutrition, herbal medicine, and food fortification.

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.1 REAGENTS**

- Distilled water
- Methanol
- Hydrochloric acid
- Sulphuric acid (H<sub>2</sub>SO<sub>4</sub>)
- Glacial acetic acid

- Ferric chloride
- Tripyridyl Triazine (TPTZ)
- Molybdate
- Sodium hydroxide (NaOH)
- Methylene red indicator
- Diethyl ether
- Petroleum ether

### **3.2 APPARATUS AND EQUIPMENTS**

- Test tubes
- Test tube racks
- Beaker (50ml, 100ml, and 200ml)
- Pipette
- Micro pipette
- Measuring cylinder
- Masking tape
- Funnels
- Sieve
- Whatman filter paper
- Electronic sensitive weighing balance

- Foil paper
- Glass stirrer
- Spatula
- Concentration jars
- Universal bottles
- Incubator
- Refrigerator
- Mortal and pestle
- Spectrophotometer
- Gloves
- Face mask
- EDTA bottle

### **3.3. METHOD**

#### **3.3.1 Collection of plant**

The leaves of *Moringa Oleifera* were collected from ring road market, Benin city, Edo State, Nigeria. The plant were identified and authenticated by Prof Akinnibosun Henry Adewale in the Department of Plant Biology and Biotechnology, University of Benin, Benin city, Edo State, Nigeria. The specimen was deposited at the University of Benin Herbarium with voucher number UBH-M340.

### **3.3.2 Sample preparation**

The collected leaves of *Moringa Oleifera* plant were washed with tap water, then with distilled water to remove any form of contaminants. The leaves were then cut into small pieces and weighed to 5kg with an analytical balance. It was homogenized in 100ml of methanol using a mortar and pestle till it was finely a smooth liquid.

The sample was then turned into a 100ml flat bottom flask and then kept at room temperature till it was required for analysis

### **3.4 QUALITATIVE PHYTOCHEMICAL SCREENING PROCEDURE**

Phytochemicals are bioactive constituents of medicinal plants which are not nutrients but very useful to the plants. Some bioactive constituents of methanolic extract were analysed qualitatively for Flavonoids, Tannins, Cardiac Glycosides, Saponins, Steroids, Terpenoids, Phenols, Phlobatanins, Coumarin, Anthraquinone and Alkaloids. Phytochemical screening was carried out on the samples after undergoing methanol extraction, using standard procedures to identify the secondary metabolites (Harborne 1973; Trease and Evans, 1989; Sofowora 1993).

#### **3.4.1 Test for flavonoids:**

5mL of 10% ammonia was added to 1ml portion of an aqueous filtrate of the extract. Then 1ml concentrated sulfuric acid was added. Observed yellow colour indicates the presence of flavonoids

#### **3.4.2 Test for tannins:**

1mL of (0.5g/5mL) ethanol extract was boiled in 2ml of water in a test tube and filtered. A 3drops of 0.1% ferric chloride was added and observed for brownish green to a blue-black colouration.

#### **3.4.3 Test for cardiac glycosides (Keller-Killiani test):**

1mL of 0.5g/5ml aqueous extract was treated with glacial acetic acid containing one drop of ferric chloride solution. 1mL of concentrated sulfuric was gently added. A browning at the interface indicated the presence of a deoxysugar characteristic of cardenolides. Hence, the presence of cardiac glycosides.

#### **3.4.4 Test for saponin (Frothing test):**

The ability of saponins to produce frothing in aqueous solution was used as a screening test for saponins. 1mL of extract (0.5g/5mL of distilled water) was mixed with 5mL of distilled water and shaken vigorously for a stable persistent froth, indicating the presence of saponin. This was further confirmed by adding 3 drops of olive oil and shaking vigorously after which it was observed for the formation of an emulsion.

#### **3.4.5 Test for steroids**

2mL of concentrated acetic anhydride was added to 0.5mL of (0.5g/5mL) ethanol extract of each sample with 2mL concentrated sulfuric acid. The colour changed from violet to blue or green colouration was positive for steroids.

#### **3.4.6 Test for terpenoids (Salkowski test):**

1mL of the extract in a test tube was mixed with 2mL of concentrated chloroform and 3ml of concentrated sulfuric acid. Reddish brown coloration at the interface confirmed the presence of terpenoids.

#### **3.4.7 Test for phenols:**

3 drops of 10% aqueous FeCl<sub>3</sub> solution were added in a test tube to 5mL of (0.5g/5mL) ethanol extract. Formation of blue or green coloration indicated the presence of phenols.

#### **3.4.8 Test for phlobatanins:**

3mL of (0.5g/5mL) ethanol extract was added to 2mL of 1% HCl and the extract was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatanins.

#### **3.4.9 Test for Coumarin:**

5mL of (0.5g/5mL) ethanol extract was dissolved in 2mL of hot distilled water and divided into two parts. Half of the volume was a control; the other part 0.5ml of 10% NH<sub>4</sub>OH was added.

#### **3.4.10 Test for alkaloids:**

Mayer's test: 1mL of (0.5g/5mL) ethanol extract was mixed with 3drops of Mayer's reagent. Cream coloured precipitate formation confirmed the presence of alkaloids.

#### **3.4.11 Test for anthraquinone:**

5mL of benzene was added to 1mL of (0.5g/5mL) ethanol extracts in a test tube and shaken vigorously in 2.5mL concentrated NH<sub>3</sub>. Formation of pink-red colouration at the lower phase was indicative of the presence of free Anthraquinone.

### **3.5 QUANTITATIVE DETERMINATION OF PHYTOCHEMICALS**

#### **3.5.1 Estimation of alkaloids (Madhu M. et al., 2016)**

To 1ml of test extract, 5ml of pH 4.7 phosphate buffer was added and 5ml BCG solution and shake a mixture with 4ml of chloroform. The extracts were collected in a 10ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 760nm against blank prepared as the above with extract. Atropine was used as a standard and compared the assay with atropine equivalent.

#### **3.5.2 Flavonoids (Madhu M. et al., 2016)**

Total flavonoid content was determined by Aluminum chloride method using Quercetin as a standard. 1ml of test sample and 4ml of water were added to a volumetric flask (10ml vol.). After 5mins, 0.3ml of 5% Sodium nitrite and 0.3ml of 10% Aluminum chloride were added. After 6mins incubation at room temperature, 2ml of 1M Sodium hydroxide was added to the reaction mixture. Immediately, the final volume was made up to 10ml with distilled H<sub>2</sub>O. The absorbance of the reaction mixture was read at 510nm against a blank spectrophotometrically.

#### **3.5.3 Steroids (Madhu M. et al., 2016)**

1ml of extract of steroid solution was transferred into 10ml volumetric flask. Sulphuric acid (4N, 2ml) and Iron (III) chloride (0.5% w/v 2ml) were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5ml). The mixture was heated with occasional shaking and diluted to the mark with diluted water. The absorbance was measured at 780 nm against the reagent blank. Stigmasterol was used as standard.

#### **3.5.4 Terpenoids (Alessandra M.P. et al., 2020)**

To 75ul plant extract, 250ul of vanillin solution (50mg/ml) and 500ul of Sulphuric acid (99.5%). The tube was heated in a water bath (60oC) for 20mins and then transferred into an ice bath followed by the addition of 2500ul of acetic acid (99.5%). The resulting solution was cooled for 20mins and absorbance was measured at 548nm. Beta-sitosterol was used as a standard.

#### **3.5.5 Coumarin (Ameen, O.A., et al. 2021)**

A 0.5ml of 5N NaOH was added to the solution of 1 ml of the extract (0.5g in 1 ml methanol). The mixture was heated at 80oC for 5mins. After cooled, 0.75ml of 5N H<sub>2</sub>SO<sub>4</sub> was added and mixed thoroughly, then, 0.25g of anhydrous NaHCO<sub>3</sub> was also added and transferred to the extractor and made up to 50 ml with pet-ether for 3hrs. About 20ml of H<sub>2</sub>O was added to the pet-ether extract and carefully evaporate the pet-ether in water bath at 50-55oC. The aqueous solution was transferred to a volumetric flask and made up to 50ml with mixing. 25ml of aqueous solution was pipetted into a flask and 1% Na<sub>2</sub>CO<sub>3</sub> solution was added and heated in a

water bath at 75°C for 15mins and cooled. 5 ml of the diazonium solution was added and stand 2 hours. The absorbance at 540nm against reagent blank. Esculin was used as standard.

### **3.5.6 Phenols (Tofighi, N. et al., 2016)**

The methanol solution of each sample (0.2 - 100ug/ml) was mixed with folin-ciocalteu reagent (2 ml, 1:10 diluted with distilled H<sub>2</sub>O). After 5mins, saturated NaHCO<sub>3</sub> solution (1.5ml, 60g/L distilled water) was added. The mixture were allowed to stand for 90mins at room temperature and absorbance of the solution was measured at 725nm. The same procedure was repeated for different concentrations of gallic acid solution (0.2-1.0ug/ml).

### **3.5.7 Cardiac glycosides (Tofighi, N. et al., 2016)**

10% extract was mixed with 10ml of freshly prepared Baljet's reagent (95ml of 1% picric of 5ml of 10%NaOH). After an hour, the mixture was diluted with 20ml distilled water and the absorbance was measured at 495nm. Securidaside was used as standard.

### **3.5.8 Tannins (Kritha Chandran C.I. and Indira G. 2016)**

Tannins was determined by folin ciocalteu method 0.1ml of sample extract was added to volumetric flask (10ml) containing 7.7ml of distilled water. The mixture was shaken well and kept at room temperature for 30mins, a set of reference standard solutions of tannic acid (20, 40, 60, 80, and 100ug/ml) in the same manner as described for sample extract. Absorbance for test and standard solutions were measured against reagent blank at 700nm.

## CHAPTER FOUR

### RESULTS

#### 4.1 QUALITATIVE PHYTOCHEMICAL SCREENING

**Table 4.1: Qualitative phytochemical analysis of methanol extract of Moringa Oleifera (leaves)**

METHANOL EXTRACT

| <b>PHYTOCHEMICALS</b> | <b>Qualitative results</b> |
|-----------------------|----------------------------|
| Flavonoids            | +++                        |
| Tannins               | ++                         |
| Cardiac glycosides    | ++                         |
| Phlobatanins          | -                          |
| Steroids              | +++                        |
| Terpenoids            | +++                        |
| Anthraquinones        | -                          |
| Saponins              | -                          |
| Coumarin              | -                          |

|           |   |
|-----------|---|
| Alkaloids | + |
|-----------|---|

Table 4.1: (+)= indicates presence of constituent, (-)= indicates absence of constituent

#### 4.2 QUANTITATIVE PHYTOCHEMICALS SCREENING

Table 4.2: Quantitative phytochemical constituents of methanol extract of *Moringa Oleifera* (leaves)

| Phytochemicals     | QUANTITATIVE RESULTS IN (ug/ml) |
|--------------------|---------------------------------|
| Flavonoids         | 862.21                          |
| Terpenoids         | 304.23                          |
| Cardiac glycosides | 525.78                          |
| Steroids           | 302.98                          |
| Tannins            | 126.56                          |

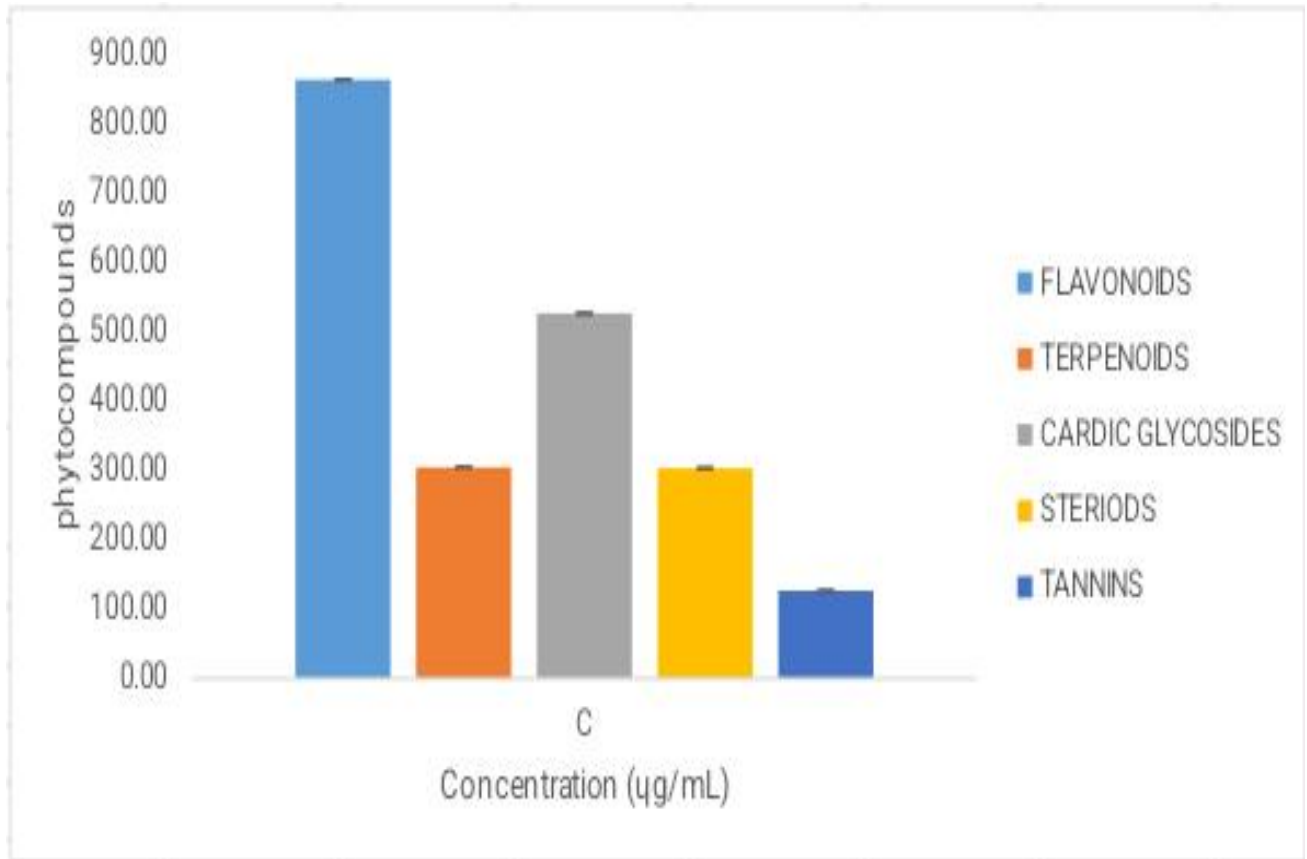


Figure 4.1 A Barchat Showing the quantity of each phytochemicals present in the leaves of Moringa Oleifera in ug/ml.

## CHAPTER FIVE

### DISCUSSION AND CONCLUSION

#### 5.1 Discussion

Table 1 shows the phytochemical screening of *Moringa Oleifera* sample where Flavonoids, Tannins, Cardic Glycosides, Steroids, Terpenoids, Alkaloids and phenols were found present, suggesting that the leaf still held bioactive compounds at the time of purchase. Meanwhile other groups such as phlobatanins, anthraquinones, saponins and Coumarin were not detected. The quantitative phytochemical screening results of Table 2 show that the leaves contain a wide range of bioactive compounds, with flavonoids appearing in the highest amount (862.21 µg/ml). This was followed by cardiac glycosides (525.78 µg/ml), terpenoids (304.23 µg/ml),

steroids (302.94 µg/ml) and tannins (126.56 µg/ml). The qualitative test also confirmed the presence of other useful phytochemicals such as alkaloids, phenols and saponins. These results align with the established understanding of moringa as a highly bioactive plant (Mdpi *et al.*, 2022; Sugabharathi *et al.*, 2018). Altogether, the findings affirm that *Moringa oleifera* leaves are chemically rich and support its long-standing traditional use in herbal medicine.

### **5.1.1 Flavonoids as the most abundant compound**

Flavonoids recorded the highest concentration among all the quantified metabolites, and this is not surprising because moringa leaves are widely recognised for their strong antioxidant properties. Compounds like quercetin and kaempferol, which fall under this group, help the body fight oxidative stress and inflammation. The high value obtained in this study suggests that the moringa leaves analysed were at a good physiological stage and were able to produce these protective compounds in large amounts. This further strengthens the idea that moringa can be a valuable natural source of antioxidants. This aligns with prior *in vitro* and *in vivo* studies linking moringa flavonoids to anti-inflammatory and antioxidant effects (Mdpi *et al.*, 2022).

### **5.1.2 High levels of cardiac glycosides**

Cardiac glycosides emerged as the second most abundant compound. Although moringa is not commonly known primarily for these compounds, their high presence in this study shows that the plant may possess more cardioactive properties than expected. Some earlier qualitative studies do report cardiac glycosides in moringa leaves (Adekanmi *et al.*, 2020), but in certain

comparative analyses, cardiac glycosides can be lower or even absent in particular plant parts (Etejere, Olayinka & Lawal, 2015). These compounds are generally associated with heart-related activities and may play a role in strengthening the heart muscles. Factors such as soil nutrients, plant age and the extraction method used may have contributed to the elevated levels recorded.

### **5.1.3 Moderate amounts of terpenoids and steroids**

Terpenoids and steroids were present in moderate but nearly equal amounts. These compounds are important in plants because they contribute to defence mechanisms, structural functions and therapeutic properties. Terpenoids help in protecting the plant from environmental stress, while steroids such as beta-sitosterol are associated with anti-inflammatory and cholesterol-lowering effects. Their moderate levels in this study agree with findings from other researchers who have also identified moringa as a good, but not excessive, source of these compounds.

### **5.1.4 Lower tannin concentration**

Tannins showed the lowest concentration among the quantified phytochemicals. This outcome is expected because moringa leaves naturally contain lower tannin levels compared with other plants. While tannins have antioxidant and astringent effects, very high levels can interfere with nutrient absorption. Therefore, the relatively low concentration found here suggests that moringa provides beneficial tannins without posing nutritional disadvantages.

### **5.1.5 Presence of other bioactive compounds**

Beyond the quantified compounds, the qualitative analysis identified several additional phytochemicals such as alkaloids, phenols and saponins. These compounds further enhance the medicinal value of the plant. Alkaloids are linked with antimicrobial activity, phenols contribute to antioxidant potential and saponins have been associated with immune support and cholesterol regulation. Their presence reinforces the idea that moringa leaves contain a broad spectrum of beneficial chemicals.

### **5.1.6 Interpreting the pattern of phytochemical distribution**

The distribution pattern observed, high flavonoids and cardiac glycosides, moderate terpenoids and steroids, and lower tannins reflects the balanced chemical makeup of *Moringa oleifera*.

This combination of compounds supports the plant's ability to provide multiple health benefits at once. Flavonoids explain its antioxidant strength, terpenoids and steroids contribute to anti-inflammatory and antimicrobial actions, while the presence of cardiac glycosides suggests added heart-related benefits. This synergy may be one of the reasons moringa is widely used in managing a variety of health conditions.

### **5.1.7 Comparisons with other research**

In a study by Adekanmi, (Adekanmi & Adekanmi, 2020), the authors reported the presence of flavonoids, steroids, terpenoids, cardiac glycosides, tannins, and alkaloids in *M. oleifera* leaves, though their quantitative measures (in percent) differ from my results. By contrast, (Etejere, Olayinka & Lawal, 2015) found that in *M. oleifera*, saponins were the most abundant in leaf

tissue, followed by flavonoids, with cardiac glycosides being present only in root in their samples.

Another work demonstrated that moringa leaf extracts (aqueous and ethanolic) produce a strong antimicrobial and antioxidant effect, qualitatively confirming terpenoids, glycosides, phenols, flavonoids, tannins, and more (PubMed, 2019).

These comparisons suggest that while my findings broadly align with published phytochemical profiles, the quantitative differences (especially in my high cardiac glycoside content) may reflect specific factors in my sample (geographic origin, growing conditions, solvent, etc.).

#### **5.1.8 Reasons for differences from other studies**

It is important to note that phytochemical concentrations often differ between studies. Factors such as geographical location, environmental conditions, harvest time, leaf maturity, drying procedures and extraction techniques all influence the amount of phytochemical produced or extracted. Therefore, the values obtained in this research are specific to the sample used and the methods applied, and slight variations from other reports are expected.

#### **5.2 CONCLUSION**

Overall, the findings of this study clearly show that *Moringa oleifera* leaves contain a rich and diverse array of phytochemicals. Flavonoids were the most abundant, highlighting the strong antioxidant potential of the plant. The presence of cardiac glycosides, terpenoids, steroids and tannins further demonstrates that moringa possesses multiple bioactive compounds that may

contribute to different therapeutic effects. The qualitative detection of alkaloids, phenols and saponins strengthens its reputation as a highly valuable medicinal plant.

In summary, the chemical profile revealed in this study supports the traditional and modern use of moringa in health management. The results also provide a useful scientific basis for future research and may guide further exploration of moringa's potential in pharmaceutical and nutraceutical applications.

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