DETERMINATION OF PROXIMATE COMPOSITION, PHYTOCHEMICAL CONSTITUENTS, MINERAL NUTRIENT CONTENT, AND ANTIMICROBIAL ACTIVITY OF DEVIL BEAN (*Mucuna pruriens*) LEAVES

BY
EZENWALIE CHIEMELIE SOLOMON
PSC2105218

DEPARTMENT OF CHEMISTRY,

FACULTY OF PHYSICAL SCIENCE,

UNIVERSITY OF BENIN,

UGBOWO, BENIN CITY,

EDO STATE,

NIGERIA.

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PSC2105218

A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF CHEMISTRY, FACULTY OF PHYSICAL SCIENCES, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF

BACHELOR OF SCIENCE (B.Sc) IN INDUSTRIAL CHEMISTRY,

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CERTIFICATION

This is to certify that this research project was carried out by **EZENWALIE CHIEMELIE SOLOMON** with the matriculation number **PSC21052I8** under the supervision of Prof. D.E OGBEIFUN and Mrs. ANTHONIA OTOE in the Department of Chemistry, Faculty of Physical Sciences, University of Benin, Benin City, Edo State.

…….. ……

EZENWALIE CHIEMELIE SOLOMON DATE
(STUDENT)

…… ……...

Prof. D.E OGBEIFUN DATE
(PROJECT SUPERVISOR)

....

Mrs. ANTHONIA OTOE DATE
(CO-SUPERVISOR)

……... …….

Prof. E.E. IRABOR DATE
(H.O.D)

DEDICATION

I dedicate this work to the Almighty God, whose mercy, protection, grace, wisdom, and strength have sustained me throughout this journey.

This project is also dedicated to my beloved parents, Mr. and Mrs. UDOAMAKA EZENWALIE, for their prayers, sacrifices, and unwavering support which have been my greatest source of inspiration and encouragement.

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ABSTRACT

The study investigated the proximate composition, phytochemical constituents, mineral nutrient content, and antimicrobial activity of the aqueous leaf decoction of *Mucuna pruriens*, a plant of notable ethnomedicinal importance in Nigeria. Analyses were conducted using standard procedures. Proximate evaluation revealed 7.17% ash, 18.43% carbohydrates, 9.67% crude fat, 18.52% crude fibre, 37.88% crude protein, and 8.33% moisture, confirming the leaf as a protein- and fibre-rich nutritional resource. Phytochemical screening showed the presence of flavonoids, alkaloids, phenolic compounds, eugenols, and reducing sugars, while saponins, tannins, steroids, terpenoids, and glycosides were not detected. Mineral analysis indicated appreciable levels of sodium (1343.67 mg/kg), potassium (1018 mg/kg), calcium (238.33 mg/kg), magnesium (377 mg/kg), copper (23.33 mg/kg), iron (336.67 mg/kg), zinc (29.67 mg/kg), manganese (106.67 mg/kg), and nickel (11.33 mg/kg). Antimicrobial assays demonstrated concentration-dependent inhibition of *Escherichia coli* (15.0 mm), *Staphylococcus aureus* (14.1 mm), *Pseudomonas aeruginosa* (23.0 mm), and *Klebsiella pneumoniae* (22.0 mm) at 100 mg/mL. The minimum inhibitory concentration (MIC) was 25 mg/mL for all isolates, while the minimum bactericidal concentration (MBC) was 25 mg/mL for *S. aureus, P. aeruginosa*, and *K. pneumoniae*, but 75 mg/mL for *E. coli*. These findings validate the traditional medicinal use of *M. pruriens* leaves and demonstrate that decoction, a safe and culturally relevant extraction method, yields a nutritionally valuable, mineral-rich, and bactericidal extract with potential therapeutic applications.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Traditional medicine has been an essential component of healthcare systems across the world for centuries, particularly in developing nations. In Africa, an estimated 80% of the population depend on traditional medicine for their primary health needs (World Health Organization, 2019, Mbatha *et al.,* 2012). This form of medicine is deeply rooted in indigenous knowledge systems and relies heavily on medicinal plants for the treatment and prevention of various ailments. The World Health Organization (2020) defines traditional medicine as "the sum total of knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to various cultures, whether explicable or not, used in the prevention, and maintenance of health as well as in the diagnosis, improvement, or treatment of physical and mental illness."

The use of plants as medicine predates written human history, with archaeological findings indicating that humans were making use of medicinal plants during the Paleolithic era, approximately 60,000 years ago (Petrovska, 2012). This knowledge has been preserved, improved, and passed down from one generation to the next, forming the foundation of traditional medical systems across the world. In many African communities, traditional healers, also known as herbalists, serve as custodians of this knowledge and play an essential role in providing healthcare (Abdullahi, 2011).

The conditions commonly treated with traditional herbal remedies include gastrointestinal disorders, such as indigestion, dyspepsia, and ulcer-related symptoms (Wachtel-Galor and Benzie, 2011), respiratory tract infections, like coughs, colds, and acute bronchitis, especially in regions with limited access to pharmaceuticals (Agbabiaka *et al*. as cited in Hoang *et al*., 2023), paediatric ailments, particularly digestive problems and malaria in sub-Saharan African communities (Tugume and Nyakoojo, 2019), pain and inflammatory conditions, including arthritis, musculoskeletal pain, and headaches, with some formulations showing efficacy comparable to NSAIDs in clinical trials (Jahromi *et al*., 2021).

One of the rationale behind the exploration of plant-based medicinal agents is the growing threat of antimicrobial resistance (AMR) which has intensified interest in plant-based medicines as alternatives to conventional drugs. Plants contain a wide array of phytochemicals like alkaloids, terpenoids, flavonoids, and tannins, which have shown activity against resistant pathogens, sometimes even disrupting biofilms or efflux pumps (Ayaz *et al*., 2019; Rather *et al*., 2017). Nature's plant kingdom is a treasure trove of chemical diversity, yet countless species remain barely touched in the search for medicines. Within this rich biodiversity lie countless bioactive compounds, many with powerful health-boosting effects such as fighting inflammation and protecting the body against oxidative stress (Chaachouay and Zidane, 2024). Historically, natural products have played a major role in drug discovery. More than half of the medicines approved between 1981 and 2019 have more than half of their therapeutic agents derived directly from, or inspired by natural sources, showing just how important medicinal plants remain in driving the discovery of new drugs (Newman and Cragg, 2020). Many also work synergistically with modern drugs, boosting efficacy and reducing side effects (Ayaz *et al*., 2019). Economic and environmental benefits further support the use of plant-based medicines. They are often more affordable and accessible in resource-limited regions and promote sustainable practices while preserving indigenous knowledge. These features make them strong candidates for drug development, functional foods, and nutraceuticals. Thus, this study is aimed at phytochemical screening, proximate composition evaluation, mineral nutrients analysis and antimicrobial activity of *Mucuna pruriens* leaves.

1.1.1 BACKGROUND OF STUDY

In Nigeria, *Mucuna pruriens* is highly valued for its ethnomedicinal uses and is well-known among diverse ethnic groups, each referring to it by their own local names. Among the Yoruba people of southwestern Nigeria, it is popularly known as *Werepe* or *Ewe Ina*, names that reflect its distinctive itchy seed pods and its use in traditional herbal preparations. In southeastern region, the Igbo people call it *Agbala* or *Egbara*, recognizing the plant for its uses in enhancing fertility and calming the nerves (Avoseh *et al*., 2020; Ashidi *et al*.,2019; Onweluzo and Eilitta, 2003). In the northern Nigeria, particularly among the Hausa-speaking communities, it is called *Karara*, and it is utilized in folk remedies for combating various ailments such as male infertility and

general weakness. Certain experimental studies suggest it may have beneficial effects on hematological health (Barde *et al*., 2023).

Ethnomedicinally, decoctions and infusions of *Mucuna pruriens* leaves are used in the treatment of ailments like Parkinson's disease, infertility, snake bites, and microbial infections (Lampariello *et al*., 2012; Pathania *et al*., 2020). The renowned presence of L-Dopa (L-3,4-dihydroxyphenylalanine) in seeds is widely studied, but recently, the interest has shifted to the leaves, which offer a more accessible and sustainable plant part for bioactive extraction with low environmental impact (Wakeel *et al*., 2025).

The growing problem of antibiotic resistance has intensified the need for new sources of antimicrobial agents, especially from plants that are affordable and readily available. One such plant is *Mucuna pruriens*, which shows potential as a natural alternative or supplement to synthetic drugs. At the same time, it's important to evaluate both its beneficial nutrients and its anti-nutritional compounds such as L-DOPA (L-3,4-dihydroxyphenylalanine), tannins, phytates, saponins, and trypsin inhibitors to ensure that its use in human and animal diets is both safe and effective (Sowdhanya *et al*., 2024; Pathania *et al*., 2020).

Phytochemical screening of *Mucuna pruriens* leaves, using different solvent fractions (hexane, chloroform, ethyl acetate, and methanol), have revealed a rich variety of secondary metabolites including alkaloids, tannins, flavonoids, saponins, cardiac glycosides, anthraquinones, terpenoids, phenols, and steroids. Notably, the ethyl acetate fraction contained all the tested classes of compounds (Oyinloye *et al*., 2023).

The presence and concentrations of these compounds can differ depending on factors like extraction method, solvent used, growing conditions, and even the stage of the plant's maturity. For instance, a comparative phytochemical and biological activity analysis of *Mucuna pruriens* seed and leaf extracts showed that aqueous preparations (like decoctions) tended to be rich in flavonoids and tannins, which were closely linked to strong antioxidant, antimicrobial, and anti-inflammatory activities (Diniyah *et al*., 2023).

Mucuna pruriens leaves are a rich source of essential minerals such as potassium, magnesium, calcium, copper, sodium, iron, zinc, manganese, selenium, and phosphorus. They also provide

important vitamins such as A, B_1, C, and E, which contribute to both nutritional and antioxidant requirements (Enemchukwu *et al*., 2015). These minerals play crucial roles in the body, for example, iron supports oxygen transport, calcium and magnesium are important for bone strength and nerve function, zinc and copper aid enzyme activity, while sodium and potassium help maintain electrolyte balance. Together, they highlight the physiological roles of the nutrients found in the leaves.

Although *Mucuna pruriens* leaves are nutrient-rich, they also contain certain anti-nutritional compounds such as phytates, haemagglutinins, oxalates, trypsin inhibitors, and small amounts of hydrogen cyanide (Okunlola *et al*., 2023; Ezegbe *et al*., 2023). Appropriate processing techniques, particularly boiling, soaking, roasting, and fermentation, have been shown to greatly reduce the levels of these substances (Ezegbe *et al*., 2023). As a result, the leaves become safe and beneficial for consumption when traditional preparation methods such as decoction are followed, making them suitable for inclusion in both human diets and animal feed (Okunlola *et al*., 2023; Ezegbe *et al*., 2023).

Mucuna pruriens seeds contain substantial amounts of iron, calcium, potassium, and other essential minerals, often in equal or higher concentrations than more commonly consumed legumes such as soybean and lentils (Siddhuraju *et al*., 2000; Pathania *et al*., 2020). A comparative nutritional analysis revealed that *Mucuna pruriens* contain iron, potassium, and calcium, ranges that equal or exceed the lower ranges reported for soybean and lentil seeds (Siddhuraju *et al*., 2000). Consequently, this rich mineral content makes *M. pruriens* a promising functional food, capable of providing comparable or greater micronutrient value to commonly consumed legumes under appropriate processing and utilization (Pathania *et al*., 2020; Ezeagu *et al*., 2003).

Given its diverse properties, a systematic investigation of the phytochemical composition, proximate analysis, mineral nutrient content, and antimicrobial activity of *Mucuna pruriens* leaves is essential. Traditional preparation methods such as decoction remain widely used within communities and are simple techniques that ensure practical relevance. Conducting detailed studies not only fills existing knowledge gaps but also scientifically validates traditional uses and could reveal new applications of this underutilized plant in nutrition, public health, and medicine.

1.1.2 STATEMENT OF PROBLEM

Mucuna pruriens is well recognized for both its nutritional and therapeutic properties. However, while extensive research has spotlighted its seeds, especially for their rich L-DOPA content and associated neurological benefits, the leaves have received far less interest despite their widespread traditional use (Pathania *et al*., 2020). The leaves of *Mucuna pruriens* are widely used in traditional medicine, often prepared as decoctions to manage infections, inflammation, and metabolic disorders. Although these uses are well recognized in ethnomedicine, scientific studies confirming their effectiveness remain limited. Existing research has mostly focused on the chemical and nutritional composition of the leaves, with far less attention given to their actual biological activities (Enemchukwu *et al*., 2015).

The phytochemical profile of *Mucuna pruriens* leaves is known to be influenced significantly by geographic origin, environmental conditions, plant maturity, and extraction methods (Okunlola *et al*., 2020; Chikagwa-Malunga *et al*., 2009; Capo-Chichi *et al*., 2002). Nevertheless, systematic phytochemical screening of decoction extracts is still insufficient, limiting understanding of the specific compounds responsible for observed medicinal effects.

Preliminary evidence suggests that extracts of *Mucuna pruriens* leaves possess antimicrobial activity against various pathogens, but many studies rely on organic solvent extractions rather than decoction, the form traditionally prepared and consumed. The antimicrobial efficacy of decoction extracts remains inadequately characterized, hindering the potential integration of leaf decoctions into evidence-based phytotherapy.

The presence of anti-nutritional factors such as trypsin inhibitors and haemagglutinins in *Mucuna pruriens* leaves poses challenges to their safe use without proper processing (Enemchukwu *et al*., 2015). There is a critical need to assess how decoction influences these compounds and their impact on the nutritional and pharmacological qualities of the leaves.

1.1.3 JUSTIFICATION OF STUDY

This study is important because, although *Mucuna pruriens* leaves are widely used in traditional medicine, often prepared as decoctions, there is very little scientific research to support or explain their effects. Most studies so far have focused on the seeds for their L-DOPA (L-3,4-dihydroxyphenylalanine) content, while the leaves, which are more accessible and commonly used by communities, have been largely overlooked. This lack of attention leaves a major gap in knowledge and prevents their proper recognition and use in evidence-based healthcare.

This research also matters for nutritional security, especially in poor and vulnerable communities. The leaves are easy to access, renewable, and packed with proteins, minerals, and beneficial compounds. However, to use them safely and effectively, it is important to properly evaluate both their nutrients and anti-nutrients when prepared through traditional methods like decoction.

With antimicrobial resistance on the rise, there is increasing demand for affordable, plant-based alternatives. Investigating the antimicrobial effects of decoction extracts positions this study within urgent global health priorities while staying true to indigenous practices. At the same time, phytochemical screening will help establish the classes of compounds present, linking traditional claims with pharmacological potential and paving the way for nutraceutical or therapeutic applications.

1.1.4 SCOPE OF WORK

This research will determine the proximate composition, examine the phytochemical, nutritional, and antimicrobial properties of *Mucuna pruriens* leaves, using decoction method of extraction. Alongside the phytochemical screening, the research will assess the leaves' nutritional value by analyzing important macro-nutrients and micronutrients such as calcium, magnesium, potassium, iron, and zinc, since these elements are often overlooked in recent studies despite their dietary and health significance. The phytochemical analysis will follow established qualitative procedures to identify the presence of key groups of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, eugenols, steroids, terpenoids, phenolics, glycosides, and reducing sugars.

1.1.5 LIMITATIONS

The phytochemical and nutritional composition of *Mucuna pruriens* leaves greatly vary depending to genetic factors, environmental conditions, plant maturity, and harvesting techniques (Chinapolaiah *et al*., 2021; Shoge *et al*., 2025; Makhaye *et al*., 2021). This inherent diversity complicates the standardization and widespread applicability of findings from restricted sample sets, necessitating meticulous extrapolation to different populations or areas.

While decoction embodies traditional preparation techniques and holds ethnomedicinal significance, it predominantly removes water-soluble chemicals, potentially neglecting or destroying non-polar, lipophilic bioactive constituents crucial for the plant's comprehensive pharmacological effects. Moreover, the heat utilized in decoction may compromise heat-sensitive phytochemicals, perhaps leading to an underrepresentation of the plant's whole phytochemical variety and efficacy (González-Hernández *et al*., 2024).

The phytochemical screening methods employed are predominantly qualitative, demonstrating presence without quantifying quantities or bioavailability (Nortjie *et al*., 2022). This constrains the capacity to delineate accurate dose-response relationships, standardize extracts for therapeutic use, and compare results across investigations.

Antimicrobial assays, including agar well diffusion and MIC tests, often evaluate a limited spectrum of laboratory bacterial and fungus strains in vitro (González-Pastor *et al*., 2023). These assays may not precisely reflect the intricate in vivo environment, encompassing microbial biofilms, resistance mechanisms, and host-pathogen interactions, nor address the complete spectrum of clinically pertinent infections.

The study's results on phytochemical composition and antibacterial properties are predominantly in vitro, with limitations in forecasting in vivo effectiveness, bioavailability, pharmacokinetics, and safety, necessitating additional pharmacological or clinical validation (Chaves de Jesus *et al*., 2025). The absence of toxicity and dose-standardization studies limits direct clinical or therapeutic application.

Although decoction has been documented to diminish anti-nutritional factors like phytates and oxalates, the incomplete elimination or alteration of these compounds may still affect nutrient bioavailability and safety, aspects that this study may not comprehensively assess or quantify (Enemchukwu *et al.*, 2015). This restricts suggestions for immediate ingestion without further processing.

The experiment offers significant preliminary insights on the phytochemical, nutritional, and antibacterial characteristics of *Mucuna pruriens* leaves via decoction. Nonetheless, these findings should be approached with caution owing to biological heterogeneity, methodological limits, and analytical limitations, highlighting the necessity for additional in vivo research, comprehensive microbiological testing, and consistent extraction techniques.

1.1.6 AIM AND OBJECTIVES

1.1.6.1 Aim

This study aims to determine the proximate composition, phytochemical constituents, mineral nutrient content, and the antimicrobial activity of the aqueous extract of *Mucuna pruriens* leaves.

1.1.6.2 Specific Objectives

To achieve the aim above, the following specific objectives are set to:

i. Collect, dry and pulverize the leaves of *Mucuna pruriens*,
ii. Extract the leaves of *Mucuna pruriens* using distilled water,
iii. Analyze the pulverized material for proximate composition using standard methods,
iv. Determine the phytochemical constituents of the crude decoction extract using standard methods,
v. Analyze the mineral nutrient content of the crude decoction extract by digestion method,
vi. Evaluate the antimicrobial activity of the crude decoction extract.

1.2 LITERATURE REVIEW

1.2.1 MEDICINAL PLANTS

Medicinal plants have always occupied a central place in human history. Long before the era of synthetic pharmaceuticals, people relied on plants not just for food but also for healing. Across cultures, from African herbalists to Chinese traditional medicine and Ayurvedic practices in India, plants have been used to treat illness, restore vitality, and prevent disease. What makes these plants "medicinal" is their ability to produce a remarkable array of chemical compounds that can interact with the human body in ways that promote health. These compounds, often referred to as secondary metabolites, include alkaloids, flavonoids, tannins, terpenoids, and saponins, all of which contribute to the therapeutic potential of different species (WHO, 2019; Chandran *et al.*, 2020).

The link between medicinal plants and modern drug discovery is far stronger than many people realize. Some of the most important drugs in clinical use today originated directly from plants. Morphine, for instance, was isolated from the opium poppy (*Papaver somniferum*) and revolutionized pain management. Aspirin has its roots in willow bark extracts, while digoxin from foxglove (*Digitalis purpurea*) remains a cornerstone in treating heart conditions. Similarly, the anticancer drug paclitaxel (from *Taxus* species) and the powerful antimalarial artemisinin (from *Artemisia annua*) highlight how natural products can provide lifesaving solutions (Newman and Cragg, 2020). Even in the 21st century, researchers estimate that a significant proportion of new medicines are still inspired by natural compounds, underscoring the enduring value of plants as a biomedical resource (Atanasov *et al*., 2021).

Studying medicinal plants requires a blend of disciplines. Ethnobotany documents traditional uses and helps identify promising species. Phytochemistry focuses on extracting and identifying active compounds, often using techniques such as solvent extraction, chromatography, and spectroscopy. Pharmacology and toxicology then assess how these compounds work in the body, testing for antimicrobial, antioxidant, anti-inflammatory, anticancer, or cardioprotective effects. Increasingly, biotechnology also plays a role, with plant cell cultures and genetic engineering being used to enhance production of rare or valuable metabolites (Chandran *et al.*, 2020). These

interconnected fields share a common goal: to validate traditional knowledge, uncover mechanisms of action, and develop therapies that are both safe and effective.

Of course, interest in medicinal plants extends beyond pharmaceuticals. Today, plant-derived compounds are used in nutraceuticals, functional foods, and cosmetics. People are drawn to these products not only for their perceived naturalness but also for their cultural resonance and holistic appeal. This trend has fueled a growing global market for herbal supplements and plant-based health products (WHO, 2023). Yet with popularity comes challenges. One of the most pressing is quality and safety. Herbal products can vary widely in their chemical composition depending on where and how they are grown, harvested, and processed. In some cases, contamination with heavy metals, pesticides, or even adulteration with other substances poses real risks. The World Health Organization has emphasized the need for standardization and quality control through tools such as pharmacopoeial monographs and chromatographic fingerprints (WHO, 2011). Researchers like Ekor (2014) have also pointed out the importance of herb–drug interaction studies and stronger pharmacovigilance systems to protect consumers.

Another concern is sustainability. Many medicinal plants are collected from the wild, and with rising demand, overharvesting threatens biodiversity. Some species have already become endangered due to unsustainable collection practices. Conservationists and organizations like the International Union for Conservation of Nature (IUCN) advocate for cultivation, responsible harvesting, and fair benefit-sharing with communities that have long stewarded this knowledge (IUCN, 2010). Meanwhile, biotechnology offers alternatives, such as producing active compounds through tissue culture or engineered microbes, reducing pressure on wild populations while ensuring consistent yields (Chandran *et al.*, 2020).

Despite these challenges, the future of medicinal plant research is bright. New technologies in metabolomics, genomics, and artificial intelligence are speeding up the discovery process, allowing researchers to screen thousands of compounds quickly and identify those most likely to have therapeutic value. Equally important is the growing respect for traditional knowledge systems. By combining modern science with the wisdom of local healers and herbalists, researchers are not only discovering new medicines but also strengthening cultural heritage.

Medicinal plants are more than just relics of traditional medicine, they are active partners in modern health care and innovation. They embody the deep connection between biodiversity and human survival, reminding us that nature's pharmacy remains open and full of possibilities. Ensuring their sustainable use, rigorous study, and safe integration into modern health systems is not just a scientific task but also a moral one, balancing respect for tradition with the promise of innovation.

1.2.2 BOTANICAL CLASSIFICATION AND TAXONOMY OF *MUCUNA PRURIENS*

Fig 1.1 *Mucuna pruriens* leaves (Anosike *et al* 2019)

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Subfamily: Faboideae

Tribe: Phaseoleae

Genus: Mucuna

Species: Mucuna pruriens (L.) DC

One striking feature of *Mucuna pruriens* is the sheer number of names it carries in English. It is variously known as velvet bean, cowhage, cowitch, Lyon bean, itchy bean, lacuna bean, and buffalo bean. In South Asia, its traditional Sanskrit name *Kapikacchu* translates to "one that makes the monkey itch," a reference to the irritation caused by its hairy pods. It is also called *Kaunch* in Hindi and *Poonaikkali* in Tamil (Lampariello *et al*., 2012).

In West Africa, particularly Nigeria, the plant has significant cultural importance. Among the Yoruba, it is called *Werepe* or *Yerepe*, while the Igbo know it as *Agbala* or *Akurugba*. The Hausa call it *Karara*, and in Bini, it is called *Igbekpe*. These names are often linked with the itchy nature of its pods, also with its reputation as a strength-giving plant, commonly used in traditional remedies as an aphrodisiac, nerve tonic, and restorative food (Lampariello *et al*., 2012). The multiplicity of names across languages is a testament to its wide use and deep integration into both dietary and medicinal traditions.

Several infraspecific taxa, including subspecies and varieties such as *M. pruriens, var. utilis* and *var. pruriens*, have been identified, reflecting phenotypic and geographic variations.

The genus Mucuna is fairly diverse, comprising around 100–150 species distributed across the tropical and subtropical regions of Asia, Africa, and the Americas (Janardhanan *et al*., 2003). Among these, *M. pruriens* is the most well-studied, largely because of its rich nutritional profile and therapeutic compounds.

1.2.3 BOTANICAL DESCRIPTION

Mucuna pruriens is a vigorous, twining annual or perennial climbing legume that can reach up to 15 meters in length when supported by trees or trellises. Its stems are slender but tough, allowing it to climb and spread extensively. The plant bears trifoliate leaves (three leaflets per stalk), with

each leaflet being ovate to rhomboid in shape and covered in fine hairs that give them a slightly velvety texture (Janardhanan *et al*., 2003).

The flowers are large and striking, typically purple to deep violet, arranged in drooping clusters. They are typical of the pea family, with a prominent keel, wings, and standard petal structure, making the plant easily recognizable when in bloom.

The fruit is a pod, usually 4–13 cm long, densely covered with orange to brown hairs that cause intense itching upon contact with the skin. This irritation is due to mucunain, a proteolytic enzyme present in the trichomes. Inside the pods are hard, shiny seeds that range in color from black and brown to mottled white. These seeds are the most pharmacologically significant part of the plant, as they contain high levels of L-DOPA (levodopa), a direct precursor of dopamine widely used in the treatment of Parkinson's disease (Lampariello *et al*., 2012).

1.2.4 CULTIVATION AND DISTRIBUTION OF *MUCUNA PRURIENS*

The velvet bean (*Mucuna pruriens*) is a hardy tropical legume that farmers value for both its agricultural and medicinal uses. It grows best in warm, humid environments with well-drained soils and moderate acidity, and while it thrives under good rainfall, it cannot withstand frost or prolonged flooding (Heuzé *et al*., 2015). Cultivation is usually straightforward, often involving direct seeding, but the hard seed coat may require scarification to improve germination. Farmers either grow it alone or intercrop it with staples such as maize, where it is typically sown a few weeks later to avoid shading and competition. Its ability to produce abundant biomass and fix nitrogen makes it an important cover crop, restoring soil fertility while also suppressing weeds.

Although native to South and Southeast Asia, *M. pruriens* has spread widely across the tropics. It is now grown in Africa, Latin America, and the Caribbean, where it has been embraced for its multiple roles as food, forage, green manure, and a natural soil enhancer (Heuzé *et al*., 2015). In many regions, especially among smallholder farmers, the plant has become an integral part of sustainable farming systems. Its distribution today reflects not only its adaptability to different soils and climates but also centuries of cultural and agricultural exchange that have made it a global crop.

1.2.5 BIOACTIVE CONSTITUENTS OF MEDICINAL PLANTS

Recent studies consistently identify *Mucuna pruriens* leaves as a rich reservoir of diverse secondary metabolites known to confer health benefits, including alkaloids, phenolics, eugenols, flavonoids, tannins, saponins, glycosides, terpenoids, and steroids (Shoge *et al.*, 2025; Pathania *et al*., 2020). Preliminary phytochemical screening combined with advanced analytical methods such as Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared Spectroscopy (FT-IR) confirm the presence of numerous bioactive compounds that vary in abundance depending on solvent polarity and extraction method (Shoge *et al.*, 2025).

1.2.5.1 Alkaloids

Alkaloids are a broad and diverse group of naturally occurring organic molecules that contain nitrogen atoms, typically within heterocyclic rings. They are commonly distributed in the plant kingdom and are renowned for their strong biological and pharmacological activities. Many of these compounds influence both the central and peripheral nervous systems, exhibit antioxidant properties, and enhance the therapeutic potential of the plants in which they occur. Their chemical diversity allows them interaction with multiple molecular targets, making them relevant agents in drug discovery and traditional medicine.

In *Mucuna pruriens*, alkaloids are one of the key bioactive components responsible for the plant's medicinal properties. Phytochemical studies have confirmed that these nitrogen-containing compounds are present in both the leaves and seeds, alongside other metabolites such as flavonoids, tannins, and saponins (Enemchukwu *et al*., 2015; Misra and Wagner, 2004). The seeds are especially rich in L-DOPA, an alkaloid with well-documented neuroprotective and neurotransmitter-modulating effects. Meanwhile the leaves contain a mixture of water-soluble alkaloids that can be easily extracted through decoction or solvent-based methods (Muhartatik *et al*., 2023).

These alkaloids are associated with multiple health-promoting effects, such as antioxidant activity, protection of nerve cells, and potential modulation of enzyme systems. These properties help explain the long-standing use of *M. pruriens* in traditional medicine for managing neurological disorders, oxidative stress, and general vitality. The abundance and variety of alkaloids in this

plant not only supports its ethnomedicinal applications but also highlights its potential as a source of natural pharmacologically active compounds worthy of more scientific exploration.

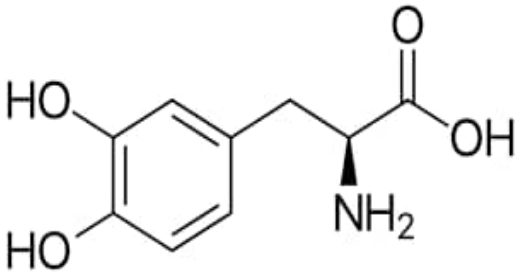


Fig 1.2 L-DOPA

1.2.5.2 Phenolic Compounds

Phenolic compounds are a diverse group of naturally occurring plant metabolites characterized by one or more hydroxyl groups attached to aromatic rings. These compounds are highly valued for their potent antioxidant properties, which allow them to neutralize harmful free radicals, chelate metal ions, and protect cellular structures from oxidative damage. Beyond their antioxidant activity, phenolics exhibit anti-inflammatory, antimicrobial, anti-diabetic, cardioprotective, and neuroprotective effects, making them essential contributors to the pharmacological and therapeutic potential of many plants. Their chemical diversity which includes simple phenolic acids, flavonoids, and complex polyphenols, enables a wide range of biological interactions and health benefits.

In *Mucuna pruriens*, phenolic compounds are abundant and central to the plant's medicinal properties. Leaf extracts have been found to contain significant amounts of gallic acid, caffeic acid, p-coumaric acid, quercetin, and (+)-catechin, among other phenolics (Njemuwa *et al*., 2019). These molecules are largely responsible for the strong antioxidant activity observed in aqueous extracts, which helps mitigate oxidative stress, protect against cellular damage, and support neurological and systemic health. Studies have shown that certain extracts of *Mucuna pruriens* leaves contain high levels of total phenolics and flavonoids, which are often associated with enhanced antioxidant capacity. A strong positive relationship between phenolic concentration and free radical–scavenging activity has been documented in plant studies more broadly.

The bioactivity of these phenolic compounds also explains why *Mucuna pruriens* has been used traditionally for managing conditions associated with oxidative stress, such as aging-related disorders, inflammation, and neurodegenerative diseases. Furthermore, phenolic compounds can act synergistically with other phytochemicals, including alkaloids and saponins, enhancing the overall therapeutic potential of the plant. The combination of high phenolic content, diverse bioactivity, and ease of extraction makes the leaves of *Mucuna pruriens* a particularly valuable source of natural antioxidants with wide-ranging health benefits.

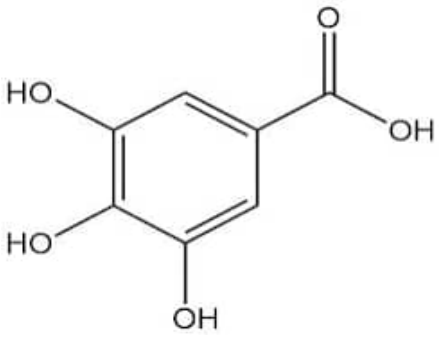
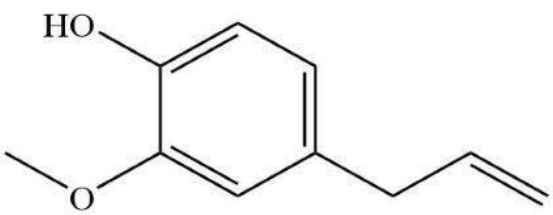


Fig 1.3 GALLIC ACID

1.2.5.3 Eugenols

Eugenol is a naturally occurring phenolic compound belonging to the class of essential oil components, known for its antimicrobial, anti-inflammatory, antioxidant, and analgesic properties. It is commonly found in various medicinal plants and is valued for its therapeutic potential. In medicinal plants, it contributes to protection against pathogens, mitigation of inflammation, and reduction of oxidative stress. It is used in food, cosmetics, and medicine as a flavoring, fragrance, antiseptic, and anesthetic agent. Its chemical structure, featuring a hydroxyl group attached to an aromatic ring, underlies many of these biological effects, making eugenol a key compound in both traditional and modern herbal medicine.



4-allyl-2-methoxyphenol

Fig 1.4

1.2.5.4 Flavonoids

Flavonoids are natural compounds widely found in plants, known for their health-promoting properties. Chemically, they have a structure of two aromatic rings connected by a three-carbon bridge, which allows them to form several types, including flavones, flavonols, flavanones, isoflavones, anthocyanins, and flavan-3-ols. The amount and type of flavonoids in plants can vary depending on species, growth conditions, and maturity.

In plants, flavonoids help attract pollinators by providing color, protect against UV damage, pathogens, and herbivores, and support growth and stress responses. For humans, they are important antioxidants, reducing free radicals and oxidative stress, which is linked to heart disease, diabetes, and neurodegenerative conditions. Flavonoids also have anti-inflammatory and antimicrobial effects, support cardiovascular health, and some can even mimic hormones or help fight cancer.

Flavonoids are present in fruits, vegetables, legumes, nuts, seeds, tea, and red wine. Regular consumption of these foods is associated with lower risks of chronic diseases and better overall health, making flavonoids an essential part of a balanced diet.

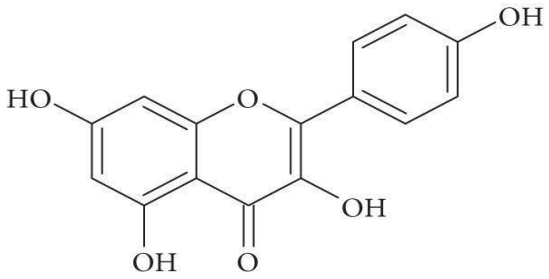


Fig 1.5 KAEMPFEROL

1.2.5.5 Reducing Sugars

Reducing sugars are a type of carbohydrate that possess free aldehyde or ketone groups, which allow them to act as reducing agents. These sugars play important roles in plant metabolism and also contribute to the nutritive value of plant extracts. In medicinal plants, reducing sugars can influence the pharmacological properties by interacting with other bioactive compounds.

They have been detected as significant phytochemical constituents of *Mucuna pruriens* leaves. Phytochemical screenings of *Mucuna pruriens* leaf extracts often reveal the presence of reducing sugars alongside alkaloids, flavonoids, tannins, and saponins (Eze *et al*., 2012). These sugars contribute not only to the nutritive value of the extract but may also play a role in its hypoglycemic activity.

Several studies indicate that the plant's extracts exhibit significant blood sugar-lowering effects, which are linked to the presence of reducing sugars that may enhance glucose metabolism and insulin secretion. The combination of reducing sugars with other bioactive compounds in the aqueous extract supports the traditional use of *Mucuna pruriens* in managing diabetes and related metabolic conditions (Eze *et al*., 2012; Njemuwa *et al*., 2019).

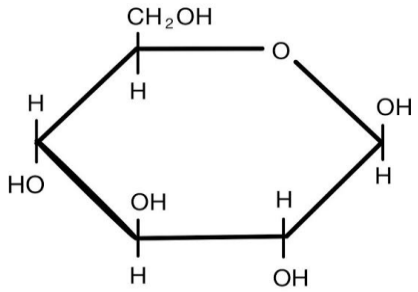


Fig 1.6 GLUCOSE

1.2.5.6 Tannins

Tannins are a group of polyphenolic compounds widely distributed in plants, known for their ability to bind and precipitate proteins. They possess astringent properties and exhibit various biological activities including antioxidant, antimicrobial, anti-inflammatory, and antidiabetic effects. Tannins contribute significantly to the pharmacological and therapeutic potential of medicinal plants.

In the aqueous and other extracts of *Mucuna pruriens* leaves, tannins have been consistently detected as important phytochemical constituents. Quantitative analyses reveal that the leaves contain a moderate amount of tannins, which play a role in the plant’s antioxidant and antimicrobial activities (Muhartatik *et al*. 2023; Njemuwa *et al*., 2019).

Studies report that tannins in *Mucuna pruriens* leaf extracts contribute to scavenging free radicals, inhibiting microbial growth, and reducing inflammation, which underlie some of the traditional uses of the plant in ethnomedicine for treating infections and inflammatory conditions (Indla *et al*., 2023; Njemuwa *et al*., 2019). The aqueous decoction method effectively extracts these tannins making the extract rich in these beneficial compounds.

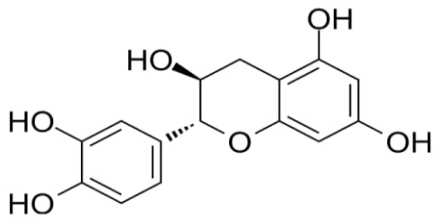


Fig 1.7 CATECHIN

1.2.5.7 Terpenoids

Terpenoids, also known as isoprenoids, are a large and diverse class of naturally occurring organic compounds derived from five-carbon isoprene units. These compounds are widely distributed in plants and are key contributors to their aroma, flavor, and therapeutic properties, including anti-inflammatory, antimicrobial, antioxidant, and anticancer activities.

In *Mucuna pruriens*, terpenoids have been identified as significant phytochemical constituents in various extracts, including aqueous and methanolic extracts of the leaves. Phytochemical analyses confirm the presence of terpenoids alongside other bioactive compounds (Indla *et al*., 2023; Borhade, 2017).

The presence of terpenoids in *Mucuna pruriens* leaf extracts contributes to the plant's antioxidant and antimicrobial activities. These terpenoids act by scavenging free radicals, reducing oxidative stress, and inhibiting microbial growth, thereby supporting the traditional use of the plant in managing infections and inflammatory conditions (Indla *et al*., 2023). Moreover, the decoction

method used for aqueous extraction effectively isolates these water-soluble terpenoids, making the extract rich in these beneficial compounds.

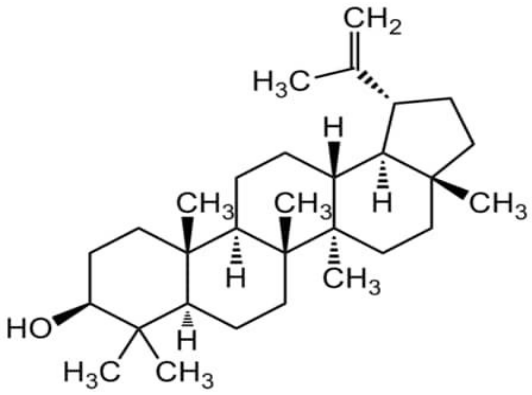


Fig 1.8 LUPEOL

1.2.5.8 Glycosides

Glycosides are compounds consisting of a sugar moiety bonded to a non-sugar component called an aglycone. In plants, they serve a variety of roles, including defense and pigmentation. In medicinal contexts, glycosides are known for their diverse pharmacological activities such as cardiotonic, anti-inflammatory, and analgesic effects.

Extracts of *Mucuna pruriens* leaves have been found to contain glycosides, which may contribute to the therapeutic potential of the plant by modulating biological pathways and enhancing the efficacy of other phytochemicals. These compounds may also play a role in regulating blood glucose and inflammation, supporting the traditional use of the plant in diabetes and inflammatory conditions (Njemuwa *et al*., 2019).

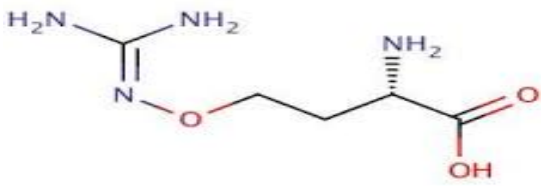


Fig 1.9 CANAVANIN

1.2.5.9 Saponins

Saponins are amphipathic glycosides characterized by their soap-like foaming properties when mixed with water. They are recognized for a range of biological activities, including immunomodulatory, antioxidant, antimicrobial, and cholesterol-lowering effects.

Saponins have been identified in *Mucuna pruriens* leaf extracts and are considered crucial to the plant's ability to combat oxidative stress and microbial infections. The presence of saponins enhances the extract's ability to stimulate the immune system and protect against pathogens, reinforcing the medicinal relevance of *Mucuna pruriens* leaves in traditional healthcare systems (Indla *et al*., 2023).

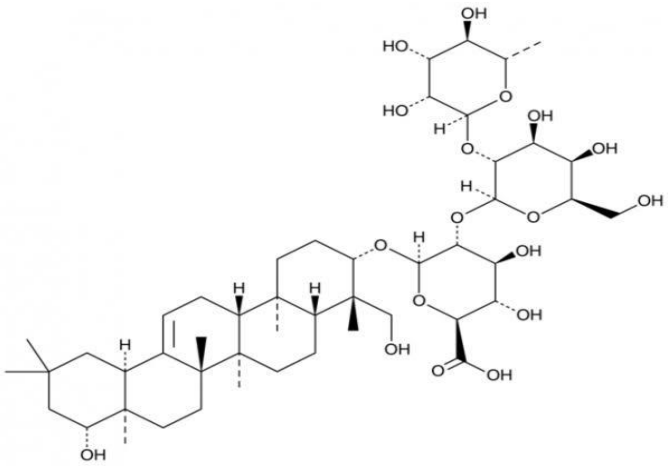


Fig 1.10 SOYASAPONIN

1.2.5.10 Steroids

Steroids are a group of naturally occurring compounds distinguished by a core structure of four interconnected carbon rings, three six-membered rings and one five-membered ring. This structure forms the foundation for a wide variety of molecules, including hormones, sterols, and plant secondary metabolites. Differences in functional groups and side chains attached to the rings create diversity in chemical properties and biological activity.

In plants, steroids are often referred to as phytosterols. They are essential for maintaining the stability and fluidity of cell membranes, regulating growth and development, and providing

defense against environmental stresses, such as pathogens, ultraviolet radiation, and herbivores. Beyond structural roles, plant steroids also participate in signaling processes, helping the plant adapt to changing conditions and stresses.

Phytosterols are also beneficial for human health. They can reduce the absorption of cholesterol in the intestine, lowering LDL cholesterol levels and supporting cardiovascular health. Additionally, plant steroids exhibit antioxidant and anti-inflammatory properties, which help protect against oxidative stress and chronic diseases like diabetes, cancer, and neurodegenerative disorders. Some steroids demonstrate antimicrobial and cytotoxic activities, further highlighting their therapeutic potential.

Mucuna pruriens leaf extracts contain steroids which contribute to their anti-inflammatory effects and may help in managing hormonal imbalances and metabolic disorders. These steroidal compounds complement the antioxidant properties of other phytochemicals in the extract, supporting the holistic medicinal use of the plant (Njemuwa *et al.*, 2019).

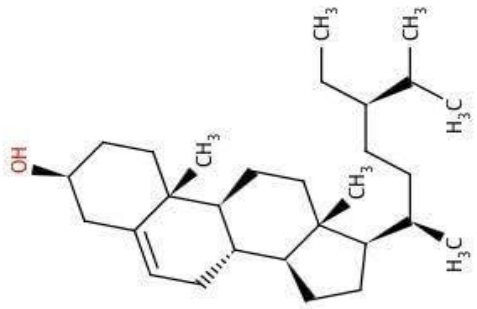


Fig 1.11 β-SITOSTEROL

1.2.6 PROXIMATE COMPOSITION AND NUTRITIONAL VALUE

The proximate composition of *Mucuna pruriens* provides a clear understanding of its nutritional quality, as it reflects the balance of key macronutrients such as proteins, fats, carbohydrates, fiber, and ash, which collectively determine its dietary value. Traditionally regarded as both a medicinal

and food plant, it has been the subject of nutritional assessments that reveal its potential as a source of nutrients and energy.

Proximate analyses have consistently demonstrated that the plant, particularly its leaves and seeds, is rich in protein. Protein levels reported in *M. pruriens* are relatively high compared to other tropical legumes, positioning it as a promising source of plant-based protein (Enemchukwu *et al*., 2015; Muhartatik *et al*., 2023). This protein richness supports its ethnomedicinal role in restoring vitality and strength, as well as its consideration as a potential feed ingredient in animal nutrition.

Carbohydrates make up another significant portion of its proximate composition. They provide energy but also influence the digestibility of the plant material. Studies show that the carbohydrate fraction of *M. pruriens* leaves is moderately high, offering both caloric value and metabolic support, which is particularly relevant in communities that traditionally incorporate the plant into food systems (Enemchukwu *et al*., 2015).

Crude fiber is also a notable component, contributing to digestive health by regulating bowel function and supporting beneficial gut microbiota. The fiber content of the leaves and seeds is consistent with what is typically observed in legumes, making it valuable for maintaining gastrointestinal health in both humans and animals (Muhartatik *et al*., 2023).

The fat or crude lipid content of *M. pruriens* is generally low, but this aligns with the nutritional profile of most legumes. While it does not serve as a major source of dietary fat, the lipids present are thought to contain bioactive compounds that may contribute to health benefits, including anti-inflammatory properties. The ash fraction, which reflects the mineral composition, indicates the presence of essential micronutrients necessary for metabolic and structural functions, though detailed mineral analyses often vary with soil and environmental conditions (Enemchukwu *et al*., 2015).

Taken together, these proximate values highlight the dual role of *M. pruriens* as both a medicinal resource and a nutritional supplement. Its high protein and moderate carbohydrate levels make it particularly useful for addressing dietary protein deficiencies, while its fiber and mineral contributions enhance overall dietary balance. The nutritional profile also supports the growing

interest in developing it into functional foods and feed formulations that not only provide nutrition but also deliver therapeutic benefits.

1.2.7 MINERAL NUTRIENT CONTENT

The mineral composition of *Mucuna pruriens* makes an important contribution to its nutritional and medicinal value. Although minerals are required only in small amounts, they are essential for metabolism, enzyme activity, structural integrity, and maintaining overall physiological balance. Studies on the leaves and seeds of *M. pruriens* consistently show that the plant contains a useful range of macro-minerals and micro-minerals, making it significant not only as a medicinal resource but also as a potential food ingredient.

Calcium and phosphorus are two minerals reported in high levels in *M. pruriens*. Calcium plays a central role in bone health, nerve transmission, and muscle function, while phosphorus is critical for energy production through ATP and contributes to the structural framework of nucleic acids and cell membranes. Their abundance in the leaves adds to the value of the plant in nutritional formulations (Enemchukwu *et al*., 2015).

Iron is another important mineral identified in the plant. It is indispensable for red blood cell formation and oxygen transport, which corresponds with the traditional use of the plant as a restorative tonic. The presence of iron also highlights its potential role in preventing iron-deficiency anemia and supporting recovery from fatigue or weakness (Enemchukwu *et al*., 2015).

Potassium and magnesium are also notable. Potassium helps regulate electrolyte balance, muscle contractions, and cardiovascular stability, while magnesium acts as a cofactor in numerous enzymatic reactions, particularly those involved in protein synthesis and energy metabolism. Their presence strengthens the case for *M. pruriens* as both a dietary component and a livestock feed resource (Muhartatik *et al*., 2023).

Trace elements such as zinc and copper, though present in smaller amounts, also enhance the plant's value. Zinc is involved in immune function, DNA repair, and wound healing, while copper supports antioxidant defense systems and connective tissue development. Together, these trace minerals contribute to the broader protective and therapeutic effects associated with *M. pruriens* (Indla *et al*., 2023).

The mineral content of *Mucuna pruriens* presents a balanced profile of essential nutrients. Combined with its rich protein and phytochemical composition, these minerals strengthen its role as a functional legume with benefits that extend beyond basic nutrition to therapeutic applications. This synergy between its nutrient and bioactive components highlights *M. pruriens* as a promising plant for both food and health-related use.

1.2.8 INTEREST IN *Mucuna Pruriens* AS FOOD AND HEALTH PRODUCT

In recent years, there has been a renewed global interest in *Mucuna pruriens* as both a food source and a foundation for health-related products. This attention is driven by the broader shift toward functional foods and nutraceuticals, where plants with dual nutritional and therapeutic properties are increasingly sought after. As a legume, *M. pruriens* holds a natural advantage due to its protein-rich seeds and leaves, but its uniqueness lies in the diversity of phytochemicals it contains, which distinguishes it from conventional pulses.

In the food sector, researchers and food technologists are exploring ways to incorporate it into formulations that extend beyond traditional diets. Its protein content makes it a promising candidate for plant-based protein supplements and fortified food products, particularly in regions facing food insecurity and malnutrition. Processing methods such as soaking, fermentation, roasting, and boiling are being refined to reduce anti-nutritional factors, thus improving its palatability and safety for wider human consumption. These efforts align with the growing consumer demand for sustainable, plant-derived proteins that can serve as alternatives to animal-based diets.

The health product industry has also turned its focus to *M. pruriens* due to its rich profile of bioactive compounds, particularly those with adaptogenic and neuroprotective properties. Extracts from the plant are increasingly used in dietary supplements aimed at supporting mood, stress resilience, and cognitive health. Its natural L-DOPA content has positioned it as a botanical candidate in formulations targeting neurological well-being, offering a plant-based alternative to synthetic compounds. Beyond neurological health, standardized extracts are being marketed for their role in boosting vitality, reproductive health, and general wellness.

Another area of interest lies in functional beverages and herbal teas developed from the plant's leaves and seeds. The infusion of its antioxidant-rich components into drinks caters to the expanding market of consumers seeking convenient, natural ways to combat oxidative stress and maintain long-term health. This reflects a shift toward preventive healthcare, where foods and beverages are chosen not just for nourishment but also for their capacity to reduce disease risk.

From a commercial standpoint, the plant's potential extends to both high-income and low-income settings. In developed countries, it is positioned within the nutraceutical and wellness markets, often in capsule or powdered extract form. In contrast, in developing regions, it is being investigated as a cost-effective food ingredient that can enhance diets lacking sufficient protein or micronutrients. This dual applicability underscores its versatility and the breadth of interest it commands globally.

The growing interest in *Mucuna pruriens* as food and health products reflects a convergence of nutritional science, ethnomedicine, and consumer trends. Its value is no longer seen solely through the lens of traditional use but is now framed within modern concepts of functional nutrition and holistic health. With ongoing research into processing methods, safety, and bioactive standardization, this plant is gradually moving from the margins of underutilized legumes into the mainstream of sustainable food and health innovation.

1.2.9 ANTI-NUTRITIONAL FACTORS AND PROCESSING EFFECTS

Mucuna pruriens (velvet bean) is well known for its medicinal and nutritional value, but alongside these benefits, it also contains some anti-nutritional factors that must be taken seriously. Anti-nutrients are natural plant compounds that interfere with how the body absorbs or uses nutrients, and if not managed properly, they can reduce the overall safety and quality of the plant when consumed.

The major anti-nutritional components found in it include L-DOPA, tannins, phytates, oxalates, and trypsin inhibitors. L-DOPA is a good example of a compound with two sides: it is highly valued as a therapeutic agent in Parkinson's disease, yet in excess, it can be harmful, causing nausea, vomiting, or even neurological issues when eaten without proper processing (Enemchukwu *et al*., 2015). Tannins reduce protein and mineral availability because they bind to

them, while phytates can chelate important minerals like iron, zinc, and calcium, preventing efficient absorption (Muhartatik *et al*., 2023). Oxalates, on the other hand, can form insoluble salts with calcium, which is a known risk factor for kidney stone formation. Trypsin inhibitors interfere with protein digestion by blocking the enzymes responsible for breaking down proteins, which can lower protein utilization from food.

The presence and concentration of these compounds are not fixed, they vary with the plant part used, where it was grown, and how it is prepared. For example, seeds typically contain higher levels of L-DOPA and trypsin inhibitors than the leaves. Fortunately, simple methods like boiling, soaking, or decoction significantly reduce these compounds, making the plant much safer to consume (Enemchukwu *et al*., 2015; Muhartatik *et al*., 2023). Traditional techniques such as roasting, fermentation, and germination also help reduce anti-nutritional factors, while at the same time improving protein digestibility and mineral availability.

Because of these concerns, proper handling and processing of *M. pruriens* is crucial, especially if it is to be used not just medicinally, but also as food, a nutraceutical, or a functional ingredient. By reducing the anti-nutrients, the plant can deliver its full benefits through its phytochemicals such as alkaloids, flavonoids, saponins, and phenolics without exposing consumers to unnecessary risks.

Mucuna pruriens is a highly valuable plant, but its anti-nutritional factors cannot be overlooked. With careful and appropriate preparation, these challenges can be minimized, allowing the plant to serve safely as both a functional food and a therapeutic resource.

1.2.10 HEALTH BENEFITS OF *MUCUNA PRURIENS*

1.2.10.1 Anti-ulcer Effect

Anti-ulcer activity refers to the ability of a substance to prevent or reduce the formation of gastric ulcers by protecting the stomach lining, lowering excess acid secretion, and reinforcing mucosal defense mechanisms. Ulcers are often triggered by factors such as ethanol exposure, stress, or hypersecretion of gastric acid, and plants with anti-ulcer potential are valued for their role in maintaining gastric integrity and preventing tissue damage.

Studies on *Mucuna pruriens* have consistently confirmed its gastroprotective effects. Sameet *et al*. (2016) demonstrated that seed extracts significantly lowered ulcer indices in animal models of ethanol- and pylorus ligation-induced ulcers. This effect was linked to enhanced mucosal defense and a reduction in gastric secretions. It is also reported that extracts from both leaves and seeds effectively reduced gastric lesion formation, attributing the protective action to antioxidant mechanisms and reinforcement of gastric mucosa against oxidative and acidic injury. Similarly, Golbabapour *et al*. (2013) showed that *M. pruriens* extracts not only decreased the severity of ethanol-induced gastric lesions in rats but also improved histological appearance of the mucosa, with additional confirmation of acute safety.

Together, these findings highlight that the anti-ulcer efficacy of *M. pruriens* is not the result of a single pathway but a convergence of actions including cytoprotection, antioxidant defense, acid suppression, and mucosal reinforcement. This multi-pronged mechanism makes the plant a compelling natural candidate for managing peptic ulcer disease and supports its place in traditional medicine as a safe and effective gastroprotective agent.

1.2.10.2 Anti-immumomodulatory Effect

The anti-immunomodulatory effect of *Mucuna pruriens* has been highlighted in both traditional accounts and modern scientific investigations. Immunomodulation refers to the ability of a substance to regulate or modify the immune system's activity, either by stimulating it in cases of immunosuppression or by downregulating it in situations of excessive immune response. Plants with immunomodulatory activity are of particular interest because they offer natural ways to restore immune balance without the adverse effects often seen in synthetic drugs.

Sathiyanarayanan and Arulmozhi (2007) reviewed the ethnopharmacological uses and pharmacological profile of *M. pruriens*, noting its role in enhancing immune function. They suggested that the plant's diverse bioactive compounds, including alkaloids and phenolics, contribute to its immunostimulatory properties, supporting traditional claims of its restorative and vitality-enhancing effects. More recently, Alabi *et al*. (2024) provided experimental evidence confirming the immunomodulatory action of the medicinal plant. Their findings showed that extracts of the plant were able to influence immune markers, improving both innate and adaptive responses, and helping to maintain immune homeostasis.

Together, these reports establish *M. pruriens* as a promising immunomodulatory agent, capable of supporting immune resilience through its phytochemical richness. Its dual potential, stimulating defense when weakened and regulating overactive responses, underscores its therapeutic relevance in maintaining overall health and managing immune-related conditions.

1.2.10.3 Anti-inflammatory Effect

Inflammation is a natural defense response of the body against injury, infection, or stress, but when it becomes excessive or chronic, it contributes to the progression of many diseases. Plants with anti-inflammatory potential are therefore valued in both traditional medicine and modern research for their ability to reduce tissue damage, modulate immune responses, and support healing.

Mucuna pruriens has been consistently reported to exhibit notable anti-inflammatory properties, largely attributed to its rich profile of bioactive compounds such as alkaloids, flavonoids, phenolic acids, and eugenol. These compounds act synergistically to suppress inflammatory mediators, scavenge free radicals, and protect tissues from oxidative damage. For instance, a research identified eugenol, quercetin, and catechins in its extracts, compounds well known for their capacity to modulate inflammatory pathways and reduce oxidative stress. Similarly, Enemchukwu *et al.* (2015) highlighted the presence of flavonoids and tannins in the leaves, which contribute directly to the anti-inflammatory response by stabilizing cell membranes and inhibiting enzymes linked to inflammation.

In addition, Sathiyanarayanan and Arulmozhi (2007) noted that the traditional applications of *M. pruriens* especially in treating conditions marked by pain, swelling, and oxidative stress, can be scientifically justified by its pharmacological activities. The antioxidant and free radical scavenging properties of its phytochemicals form the basis of its anti-inflammatory effect, preventing the escalation of oxidative stress into inflammatory damage.

These findings suggest that the anti-inflammatory activity of *M. pruriens* is multi-faceted, combining antioxidant action with suppression of inflammatory mediators. This makes the plant not only a valuable traditional remedy but also a promising natural source for developing therapeutic interventions against inflammatory disorders.

1.2.11 OTHER THERAPEUTIC POTENTIALS OF *MUCUNA PRURIENS*

Mucuna pruriens is more than a single-use herb: its chemical richness, particularly L-DOPA, diverse alkaloids, phenolics, flavonoids and terpenoids, gives it a wide therapeutic reach. Over the last two decades the plant has been examined for a range of potentials that complement and extend its traditional uses.

1.2.11.1 Neuroprotective and Antiparkinsonian Potential

The most widely recognized non-nutritional application of this plant is in neurology. The seeds are a natural source of L-DOPA, the biochemical precursor of dopamine; this single fact has driven much scientific and clinical interest in the plant (Lampariello *et al*., 2012). Preclinical work shows that whole-seed and seed-extract preparations can raise central dopamine levels and protect dopaminergic neurons in toxin-induced models of Parkinson's disease. In several comparative studies, seed extracts produced neuroprotective benefits that were as good as, or in some experimental settings broader than, equivalent doses of synthetic L-DOPA—possibly because other co-occurring phytochemicals (antioxidants, anti-inflammatory agents) reduce oxidative stress and inflammation in the brain (Kamkaen *et al*., 2022; Lampariello *et al*., 2012). Reviews and experimental papers therefore position *M. pruriens* as a promising adjunct or complementary source of dopaminergic support, while cautioning that controlled human trials and standardization are required before routine clinical substitution (Rai *et al*., 2017).

1.2.11.2 Antioxidant and Cytoprotective Effects (Systemic)

Across leaves, seeds and pods, *M. pruriens* consistently yields strong antioxidant activity in chemical and cellular assays. These activities are attributable to phenolic acids (e.g., gallic, caffeic acids), flavonoids (quercetin, catechins) and other radical-scavenging constituents identified in modern phytochemical screens (Enemchukwu *et al*., 2015; Indla *et al*., 2023; Muhartatik *et al*., 2023). The antioxidant profile explains a good deal of the plant's protective actions in organs and models, like reducing lipid peroxidation, preserving endogenous antioxidant enzymes (SOD, catalase, GSH), and thereby limiting oxidative injury in contexts ranging from neural tissue to gastric mucosa (Indla *et al*., 2023; Golbabapour *et al*., 2013). In short, antioxidant activity is a cross-cutting mechanism that helps rationalize many of *M. pruriens'* observed benefits.

1.2.11.3 Male Reproductive Health and Aphrodisiac Uses

Traditional systems (notably Ayurveda) have long used it as a "vitality" herb and male tonic; modern studies have explored this claim with supportive results. Experimental and some small clinical studies report improvements in sperm count, sperm motility, libido and serum testosterone following seed or seed-extract administration (Lampariello *et al*., 2012). Mechanistically, improved dopaminergic tone (via L-DOPA) and antioxidant protection of testicular tissue are the leading explanations for these effects. While the data are encouraging, larger, well-controlled clinical trials with standardized extracts are still needed to set dosing guidelines and confirm safety in long-term use.

1.2.11.4 Metabolic and Cardiometabolic Effects(Antidiabetic/Lipid-modulating)

Preclinical investigations indicate that *M. pruriens* extracts can favorably influence glucose and lipid metabolism. Animal studies report reductions in fasting glucose, improved glucose tolerance, and beneficial effects on serum lipids following extract treatment, effects that are plausibly secondary to antioxidant and anti-inflammatory actions as well as possible direct influences on insulin signaling. Such findings suggest potential for *M. pruriens* as a complementary nutraceutical in metabolic care, but they remain preliminary: human trials are sparse and heterogeneity of extracts complicates interpretation.

1.2.11.5 Analgesic and Antinociceptive Activity

Several experimental models of pain show that *M. pruriens* extracts reduce nociceptive responses. Ethanol leaf extracts reduced paw edema and nociception in rodent assays (egg-albumin, carrageenan and formalin models) and produced measurable analgesia without overt acute toxicity (Alabi *et al*., 2024). These analgesic effects are likely mediated by a combination of central and peripheral mechanisms—antioxidant reduction of inflammatory mediators, inhibition of pro-nociceptive signaling pathways, and possible effects on neurotransmitter systems (Alabi *et al*., 2024). The practical implication is that it could be a source of botanical analgesics or adjuvants in pain management after further toxicology and dose-finding work.

1.2.12 ANTIMICROBIAL ACTIVITY

Antimicrobial activity simply means the ability of a substance to stop microorganisms like bacteria, fungi, or even viruses from growing, or in some cases, to kill them completely. This property is very important in medicine and food preservation, especially now that antibiotic resistance is becoming a major problem worldwide. Medicinal plants are particularly interesting in this regard because they are naturally rich in bioactive compounds such as alkaloids, flavonoids, phenolics, saponins, and terpenoids. These compounds act in different ways: some damage microbial cell walls, some block enzymes or metabolic processes, while others generate reactive molecules that destroy microbial cells. Antimicrobial activity is one of the most valuable properties of medicinal plants and is central to the search for new drugs. By using standard screening methods, scientists can determine how strong plant extracts are and what microbes they can target. This not only helps to explain their traditional use but also provides a pathway for developing natural antimicrobial agents, something urgently needed in the fight against resistant pathogens.

Mucuna pruriens is one of those medicinal plants that has shown antimicrobial potential. Studies on its leaves, seeds, and pods have reported activity against both Gram-positive and Gram-negative bacteria, as well as some fungi. This scientific evidence supports its traditional use in treating infections and highlights its promise as a natural source of antimicrobial agents (Mohanasundari *et a*l., 2023).

1.2.12.1 Evaluation of Antimicrobial Activity

When testing plants for antimicrobial properties, researchers look at two main things. The first is the Minimum Inhibitory Concentration (MIC), which is the lowest concentration of the extract that can stop microbes from visibly growing. The second is the Minimum Bactericidal or Fungicidal Concentration (MBC/MFC), which shows the lowest amount needed to actually kill the microorganism. These values are important because they give an idea of how effective the extract is and what dosage might be needed for therapeutic use.

1.2.12.2 Methods for Antimicrobial Screening

There are several standard methods used to test plant extracts:

Agar Diffusion: This includes disk diffusion, where paper disks soaked in the extract are placed on microbial plates, and well diffusion, where the extract is poured into wells made in the agar. In both cases, clear zones around the disk or well show inhibition.

Broth Dilution: Extracts are diluted step by step in a liquid medium, and microbial growth is checked. This method is more precise for determining MIC values.

Agar Dilution: The extract is mixed directly into the agar at different concentrations, and then the microorganisms are added. Growth inhibition can be observed after incubation.

Time-Kill Assay: This method looks at how microbial numbers change over time when exposed to the extract, showing whether the effect is bacteriostatic (inhibiting) or bactericidal (killing).

Bioautography: Here, thin-layer chromatography (TLC) is combined with antimicrobial testing to identify which exact compounds in the extract are active against microbes.

Each method has its pros and cons. Diffusion methods are quick and cheap but less precise, while dilution methods are more reliable for measuring MIC and MBC/MFC. Usually, researchers start with diffusion tests for screening, then move on to dilution tests for accuracy.

1.2.13 EXTRACTION OF MEDICINAL PLANTS

Extraction of medicinal plants is a fundamental pharmaceutical and botanical process aimed at isolating the active phytochemicals or secondary metabolites responsible for therapeutic effects. It involves separating these biologically active compounds such as alkaloids, flavonoids, terpenoids, saponins, steroids, and glycosides from the inert or inactive matrix of the plant material using selective solvents and standard extraction procedures (Abubakar *et al*., 2020).

The process is essential because raw plant materials contain a complex mixture of bioactive and non-bioactive substances; effective extraction concentrates the desired constituents while eliminating unwanted materials. This separation is necessary whether the final product is intended

for direct use as a herbal preparation or for further processing into isolated compounds or standardized extracts (TNAU Agritech Portal).

Medicinal plant extraction is not merely about removing compounds from plants; it also ensures that the chemical integrity and biological efficacy of the active substances are maintained. Factors influencing the extraction process include:

Nature of Plant Material: This encompasses species, plant part used (leaf, root, stem, bark), moisture content, and particle size after grinding, all of which affect solvent penetration and extraction efficiency.

Solvent Selection: Choice of solvent is crucial and depends on the polarity of targeted bioactive constituents. Polar solvents like water, methanol, and ethanol extract hydrophilic compounds, while less polar solvents like hexane or dichloromethane are suited for lipophilic substances. Sometimes sequential extraction with solvents of increasing polarity is performed to fractionate compounds based on their solubility (Abubakar *et al*., 2020).

Extraction Conditions: Temperature, pH, solvent-to-material ratio, and extraction time must be optimized to maximize yield, avoid degradation, and preserve bioactivity. For example, excessively high temperatures may denature heat-sensitive compounds, while inappropriate pH levels may alter chemical structures (Abubakar *et al*., 2020).

Purpose of Extract: The intended use, whether as crude extracts, enriched fractions, or purified compounds, dictates how selective and exhaustive the extraction needs to be. Preparations for traditional herbal use may prioritize simplicity and safety, while extracts for pharmaceutical development require close control and standardization (TNAU Agritech Portal).

The products obtained from extraction, including tinctures, fluid extracts, decoctions, and powdered extracts are often impure mixtures containing a variety of compounds. Further refinement through chromatographic and spectroscopic techniques follows extraction to isolate individual chemical entities for analysis or drug development.

Medicinal plant extraction therefore is a multidisciplinary approach requiring knowledge of botany, chemistry, pharmacology, and process engineering. It is the cornerstone of ensuring that herbal drugs and phytopharmaceuticals meet quality, efficacy, and safety standards.

Key goals of plant extraction

The key goals of plant extraction are to separate and concentrate the medicinally active components or secondary metabolites, such as alkaloids, flavonoids, terpenes, saponins, steroids, and glycosides, from the inert or inactive parts of the plant. This is achieved through the use of selective solvents and standardized extraction methods. The extracted products are intended for therapeutic use as crude liquids, semisolids, or powders, or for further processing into purified compounds or standardized formulations. Extraction aims to obtain the therapeutically desired portion of the plant material while removing inert or unwanted substances, ensuring the quality and efficacy of the herbal preparation. The extracted material can then be used directly as herbal medicine, incorporated into pharmaceutical dosage forms, or subjected to fractionation and isolation to obtain individual bioactive molecules for drug development and research (TNAU Agritech Portal; Abubakar *et al*., 2020).

1.2.14 METHODS OF EXTRACTION

1.2.14.1 Decoction

Decoction is a traditional and widely used method of extracting bioactive compounds from medicinal plants by boiling the plant material in water. This process effectively dissolves water-soluble, heat-stable chemical constituents, particularly from tough and dense plant parts such as roots, barks, seeds, and rhizomes, which are otherwise difficult to extract through gentler methods like infusion or maceration (Abubakar *et al.*, 2020).

The decoction method is easy, cost-effective, and involves several key steps to maximize the yield of medicinal compounds:

Selection and Preparation of Plant Material: The plant parts intended for extraction, typically dried roots, barks, or seeds, are cleaned and then chopped, sliced, or powdered to increase their surface area, facilitating better contact with the solvent, usually water.

Boiling with Water: The prepared plant material is combined with a specific volume of water, commonly at ratios such as 1:4 or 1:16 (herb to water), in an appropriate vessel. The mixture is

then brought to a boil and simmered for a set duration, often between 15 to 45 minutes, depending on the nature of the plant material (Abubakar *et al.*, 2020; Vina Nha Trang, 2025).

Reduction and Concentration: During boiling, water volume typically reduces to about one-quarter of its original volume, concentrating the active substances into the liquid extract. This reduction concentrates the medicinal compounds to enhance potency (TNAU Agritech Portal).

Filtration: After boiling, the decoction is removed from heat and filtered or strained to remove solid residues, leaving a clear or slightly turbid aqueous extract termed decoction or "kashayam" in Ayurveda.

Storage and Usage: The resulting decoction is often consumed fresh or within 24 hours due to its perishable nature. It can also be stored in sterile, airtight glass containers to maintain stability. The extract may be used whole or further processed depending on medicinal requirements (Vina Nha Trang, 2025).

Decoction is a time-honored method of extracting medicinal compounds, especially useful for tough, woody, or dense plant materials. The combination of heat and prolonged exposure to water helps break down plant cell walls, releasing valuable bioactive compounds (Vina Nha Trang, 2025). This process efficiently draws out key phytochemicals such as tannins, alkaloids, glycosides, phenols, and other water-soluble substances, while also deactivating enzymes that could degrade these active ingredients. By boiling and reducing the liquid, decoctions yield concentrated extracts that are often stronger than infusions or cold extractions, making them particularly effective for treating persistent or chronic conditions. While heat-sensitive compounds like volatile oils may be partially lost, the method preserves heat-stable constituents that contribute both to therapeutic effects and nutritional benefits.

It has played a central role in systems like Ayurveda, Traditional Chinese Medicine, and indigenous herbal practices, where it is used to prepare tonics, detoxifying brews, and remedies for a variety of ailments, including digestive issues, respiratory problems, and fevers (Vina Nha Trang, 2025; TNAU Agritech Portal). Its use of plain water makes it affordable, environmentally friendly, and accessible, especially in resource-limited communities. The boiling process can also reduce or eliminate anti-nutritional factors such as phytates, oxalates, and protease inhibitors, enhancing both safety and nutrient availability (Enemchukwu *et al.*, 2015; Pathania *et al.*, 2020).

In contemporary herbal medicine, decoction continues to be valuable for producing safe, concentrated extracts rich in biologically active compounds with antimicrobial, anti-inflammatory, and antioxidant properties. By combining traditional knowledge with modern considerations of safety and potency, decoction offers a practical and culturally relevant approach for preparing herbal remedies, validating traditional uses, and supporting the development of effective medicinal and nutritional products.

The decoction method is suitable for extracting bioactive compounds from *Mucuna pruriens* leaves as it effectively extracts water-soluble, heat-stable phytochemicals such as phenols, polyphenols, tannins, flavonoids, saponins, steroids, and glycosides. These compounds are abundant in the leaves and contribute to its medicinal properties. This traditional extraction technique facilitates the release and concentration of these potent bioactive compounds, which are linked to various therapeutic effects including antioxidant, anti-microbial, anti-inflammatory, and anti-anaemic activities (Nweze *et al*., 2017; Abubakar *et al.*, 2020).

Among the leaf constituents, flavonoids and phenolic compounds are particularly important due to their strong antioxidant properties, helping to counteract oxidative stress and prevent redox-driven diseases. Decoction enhances the extraction of these compounds by prolonged boiling in water, which breaks down the plant cell walls, allowing the active substances to dissolve into the aqueous medium effectively. This is crucial for medicinal applications where efficacy depends on the concentration of these bioactives (Nweze *et al*., 2017).

Furthermore, decoction has been traditionally favored for *Mucuna pruriens* leaves because it supports the preparation of safe, consumable extracts that can be used to improve blood circulation and treat conditions like anemia. Studies indicate that the aqueous extracts (decoctions) of *Mucuna pruriens* leaves have shown hematopoietic and anti-anaemic effects, likely mediated by the phenolic and flavonoid compounds extracted through boiling. These compounds also exhibit antioxidant functions that protect red blood cells from oxidative damage (Nweze *et al.*, 2017; Madukwe *et al*., 2014).

1.2.14.2 Maceration

Maceration is a foundational and conventional extraction method used extensively for medicinal plants. It involves soaking coarsely powdered plant material in a selected solvent, commonly water, ethanol, or hydroalcoholic mixtures, at room temperature for a prolonged period, usually about three days. During this time, the solvent penetrates the plant tissues, dissolving the active phytochemicals which diffuse into the liquid phase. The mixture is periodically stirred or agitated to enhance compound release and ensure thorough extraction (Abubakar *et al*., 2020).

This method stands out for its simplicity, cost-effectiveness, and suitability for heat-sensitive (thermolabile) compounds. Because maceration is conducted at ambient temperature, it preserves delicate volatile oils, vitamins, and other bioactives that might degrade under heat. This makes it particularly useful for extracting antioxidants, flavonoids, alkaloids, and glycosides that are sensitive to thermal decomposition. It is simple but can be time-consuming and less efficient in extraction yield.

Maceration is flexible with respect to the solvent choice, enabling selective extraction depending on the polarity of targeted compounds. Water or hydroalcoholic mixtures are often used to obtain tinctures and fluid extracts for therapeutic formulations. After soaking, the plant residues (marc) are separated from the liquid extract (menstruum) via filtration or decantation, and the liquid extract can be concentrated or used directly.

1.2.14.3 Infusion

Infusion is a traditional extraction method used to obtain readily soluble bioactive compounds from medicinal plants. It involves pouring hot or cold solvent, typically water, but sometimes oil or alcohol, over finely powdered or coarsely chopped plant material and allowing it to steep for a short time, often minutes to hours. The extract, called an infusion, contains a dilute solution of water-soluble and heat-sensitive constituents like volatile oils, flavonoids, and alkaloids (Verep e*t al*., 2018; Abubakar *et al*., 2020).

The infusion process is especially suitable for extracting delicate plant parts such as leaves, flowers, or herbs, where prolonged heat exposure (as in decoction) might degrade active

compounds. It is a quick and convenient method often used to prepare fresh herbal teas or extracts immediately before use.

1.2.14.4 Percolation

Percolation is an extraction method involving the continuous passage of solvent through a column or bed of powdered medicinal plant material contained in a narrow, cone-shaped vessel called a percolator. The plant material is first moistened with solvent and allowed to swell for several hours, then placed in the percolator with the bottom closed. Extraction solvent is then poured from the top, saturating the material. The solvent slowly passes through the plant material by gravity, dissolving the bioactive compounds as it moves downward, and is collected from the bottom (Abubakar *et al*., 2020; TNAU Agritech Portal).

The solvent is continuously added until the desired volume is reached, after which the extract is filtered. The plant residues (marc) are sometimes pressed to recover remaining extract, and additional solvent may be added to obtain the required volume. Percolation ensures a steady flow of fresh solvent through the material, improving extraction efficiency compared to static soaking methods.

1.2.14.5 Digestion

Digestion is a form of extraction similar to maceration, but it involves the application of gentle heat during the process. In this method, powdered plant material is mixed with a solvent in a container, and the mixture is kept at a moderately elevated temperature, typically around 50°C using a water bath or an oven. The heat reduces the viscosity of the solvent and enhances the dissolution and removal of secondary metabolites from the plant matrix. This method is suitable for materials and compounds that can tolerate slight warming without degradation, increasing the efficiency of extraction compared to room temperature maceration (Abubakar *et al*., 2020).

Digestion is a variation of maceration involving gentle heating with solvent to enhance solubilization of certain compounds, yet it may cause degradation of thermo-labile substances.

1.2.14.6 Soxhlet Extraction

Soxhlet extraction is an advanced continuous solid-liquid extraction technique used to isolate soluble compounds from solid materials using a limited amount of solvent repeatedly. Developed by Franz von Soxhlet in 1879, it is widely applied in analytical chemistry, pharmacology, food science, and environmental analysis for effective extraction of target compounds such as lipids, fats, and bioactive constituents.

The extraction apparatus consists of a solvent reservoir flask connected to an extraction chamber containing the solid sample held in a porous cellulose thimble, and topped with a reflux condenser. The solvent in the flask is heated to boiling, producing vapor that rises through the apparatus and condenses in the condenser. The condensed solvent drips into the chamber, soaking the sample and dissolving soluble materials. When the solvent level reaches the top of a siphon tube, it automatically siphons back into the flask, carrying the extracted compounds with it. This cycle repeats continuously, allowing fresh solvent to repeatedly percolate through the sample without additional solvent use, thereby increasing extraction efficiency and yield.

Soxhlet extraction is particularly useful when the desired compounds have limited solubility in the solvent and are unstable at higher temperatures, as it provides gentle, continuous extraction at the solvent's boiling point. The process duration varies from several hours up to a day, depending on the sample matrix and target analyte.

Soxhlet extraction uses continuous reflux of organic solvents like ethanol or methanol, offering exhaustive extraction of both polar and non-polar phytochemicals but requires specialized apparatus and involves use of potentially toxic solvents limiting food-grade acceptance.

1.2.15 SPECTROSCOPY

Spectroscopy is a scientific technique used to study the interaction between electromagnetic radiation and matter. By analyzing how matter absorbs, emits, or scatters light, spectroscopy provides detailed information about the chemical composition, molecular structure, and physical properties of substances. The fundamental principle of spectroscopy is that atoms and molecules absorb or emit radiation at specific wavelengths that correspond to the energy differences between

their electronic, vibrational, or rotational states. This makes spectroscopy a powerful tool for qualitative and quantitative analysis in chemistry, and other sciences.

There are numerous types of spectroscopy, each based on the portion of the electromagnetic spectrum used and the type of interaction studied. UV-Visible spectroscopy measures absorption of ultraviolet or visible light, commonly used to identify chromophores and determine concentrations of compounds in solution. Infrared (IR) spectroscopy focuses on vibrational transitions of molecules, providing detailed information about functional groups and chemical bonds. Nuclear Magnetic Resonance (NMR) spectroscopy examines the interaction of atomic nuclei with magnetic fields, allowing the determination of molecular structures and dynamics. Mass spectrometry (MS), often coupled with spectroscopic techniques, measures the mass-to-charge ratio of ions, enabling identification and structural characterization of molecules. Other specialized methods include fluorescence spectroscopy, Raman spectroscopy, and atomic absorption spectroscopy, each providing unique insights into molecular and atomic behavior.

The applications of spectroscopy are vast. In pharmaceuticals, it is used for drug identification, purity assessment, and structural elucidation. In environmental science, spectroscopy detects pollutants and monitors water, air, and soil quality. In biochemistry, it helps analyze proteins, nucleic acids, and metabolites. Additionally, spectroscopy is integral to food science, forensic analysis, material characterization, and astrophysics, where it reveals the composition of distant stars and galaxies.

Modern spectroscopy continues to evolve with advancements in instrumentation, data analysis, and hybrid techniques. Coupling spectroscopy with chromatography, for instance, allows separation and identification of complex mixtures. High-resolution and multidimensional techniques improve sensitivity and structural detail, while portable spectrometers have expanded applications into field analysis and on-site testing.

1.2.15.1 Atomic Absorption Spectroscopy (AAS)

Atomic Absorption Spectroscopy (AAS) is an analytical technique extensively used for the qualitative and quantitative determination of mineral nutrients and trace metals in various samples, including medicinal plants. It operates on the principle that atoms of a specific element absorb light at characteristic wavelengths. When a sample is atomized, usually via a flame or graphite

furnace, the concentration of metal atoms is measured by the amount of light absorbed by these atoms from a light source, typically a hollow cathode lamp specific to each element. In mineral nutrient analysis, AAS provides precise measurements of essential and toxic metals such as copper, zinc, iron, lead, chromium, cadmium, nickel, and cobalt. The sample preparation often involves digestion through wet chemical methods (e.g., acid digestion) or dry ashing to convert the plant matrix into a liquid form suitable for atomization. The atomized sample interacts with light, and the decrease in light intensity is proportional to the concentration of the element in the sample.

AAS offers high sensitivity, selectivity, and accuracy, with detection limits in the microgram per liter (μg/L) to nanogram per liter (ng/L) range, depending on the technique used (flame or electrothermal atomization). Flame AAS is commonly used for higher concentration ranges (mg/L), whereas graphite furnace AAS extends detection limits to trace levels.

In medicinal plant analysis, AAS is crucial for determining both beneficial mineral content and potentially harmful heavy metal contaminants, ensuring quality control and safety in herbal products. Studies have shown successful application of AAS in profiling minerals and detecting toxic metals in various herbs, with wet digestion methods often preferred to prepare samples (Hina *et al*., 2023).

Flame AAS

Flame Atomic Absorption Spectroscopy (Flame AAS or FAAS) is an analytical technique used for quantitative determination of metal elements in liquid samples. The fundamental principle involves atomizing the sample in a flame to produce free ground-state atoms, which absorb light at characteristic wavelengths corresponding to specific metal elements.

In FAAS, a liquid sample containing metal ions is nebulized into a fine aerosol and introduced into a high-temperature flame, typically an air-acetylene or nitrous oxide-acetylene flame. The flame's thermal energy desolvates the aerosol, vaporizes the particles, and then atomizes the sample into free atoms. These ground-state atoms absorb light emitted by a hollow cathode lamp specific to the metal being analyzed. The decrease in intensity of transmitted light is measured by a detector and is proportional to the concentration of the particular metal in the sample.

The amount of light absorbed depends on the number of atoms in the ground state present in the flame, following Beer's Law. The electronic transitions occur when electrons absorb photons and move from lower to higher energy states, creating narrow atomic absorption lines unique to each element. The instrument uses a monochromator to isolate the specific wavelength and a detector to quantify absorption, providing precise metal quantification in parts per million (ppm) or parts per billion (ppb) ranges.

FAAS is widely used because it offers sensitivity, selectivity, and relatively fast analysis of metals such as lead, cadmium, chromium, copper, nickel, and zinc in environmental, biological, pharmaceutical, and food samples. The flame atomizer provides a steady-state signal, making it suitable for routine quantitative analysis. The flame's temperature and composition can be optimized depending on the metal's oxidation characteristics to maximize atomization efficiency and minimize interferences (Nwuga, 2022).

1.2.15.2 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a highly sensitive and versatile analytical technique used for detecting and quantifying trace elements and isotopes in various sample types. The core principle combines robust ionization from an inductively coupled plasma (ICP) with precise mass separation using mass spectrometry (MS).

In ICP-MS, samples, usually in liquid form, are introduced into a high-temperature argon plasma (over 6000 K), where the analyte atoms are atomized and ionized into positively charged ions. These ions are then extracted into the mass spectrometer, where they are separated based on their mass-to-charge ratio using a mass analyzer, commonly a quadrupole or sector field. The detector measures the ion intensity, which correlates directly to element concentration (Wilschefski and Baxter, 2019).

ICP-MS enables simultaneous multi-elemental analysis with very low detection limits, often reaching parts per trillion (ppt) for many metals and several non-metals. It provides isotopic information, facilitating applications such as isotope ratio studies and tracing elemental sources. Advanced configurations include laser ablation ICP-MS (LA-ICP-MS) for direct solid sampling, and hyphenated techniques like liquid chromatography-ICP-MS (LC-ICP-MS) for speciation analysis.

This technique is widely applied across environmental monitoring, food safety, clinical diagnostics, geology, forensics, and pharmaceuticals due to its accuracy, sensitivity, high throughput, and capability to analyze complex matrices (Wilschefski and Baxter, 2019).

1.2.15.3 Ultraviolet-Visible (UV-Vis) Spectroscopy

Ultraviolet-Visible (UV-Vis) Spectroscopy is an analytical technique that measures the absorption or transmission of ultraviolet (190–400 nm) and visible (400–800 nm) light by a sample. When a compound absorbs light in these regions, electronic transitions occur, such as electrons moving from ground to excited states. The amount of light absorbed at specific wavelengths can be related to the concentration and identity of the molecules present, following the Beer-Lambert law.

The core components of a UV-Vis spectrophotometer include a light source (usually deuterium for UV and tungsten for visible range), a monochromator to select specific wavelengths, a sample holder (cuvette), and a detector to measure transmitted light intensity. The resulting absorbance versus wavelength data is used for qualitative and quantitative analysis.

UV-Vis spectroscopy is widely applied in chemical, biological, pharmaceutical, and environmental fields. It helps in determining concentrations of nucleic acids, proteins, pharmaceuticals, and pigments, monitoring bacterial growth through optical density, assessing food and beverage quality, and studying chemical kinetics. It is non-destructive, rapid, cost-effective, and can analyze organic and inorganic compounds in liquids, solids, and thin films.

1.2.15.4 Infrared (IR) Spectroscopy

Infrared (IR) Spectroscopy is an analytical technique that measures the interaction between infrared radiation and matter, primarily to identify and characterize chemical substances by their molecular vibrations. It operates on the principle that molecules absorb infrared light at specific frequencies that correspond to the vibrations of their chemical bonds. When IR radiation matches the vibrational frequency of a bond in a molecule, absorption occurs, causing transitions from a ground vibrational state to an excited vibrational state.

A typical IR spectrum is a plot of absorbance (or transmittance) versus wavenumber (cm^{-1}), revealing peaks that correspond to different functional groups or bond types. The mid-infrared

region (approximately 4000 to 400 cm^{-1}) is most commonly used for studying fundamental vibrations including stretching and bending of bonds. The fingerprint region (around 1300 to 400 cm^{-1}) contains complex patterns unique to each molecule, useful for identification.

IR spectroscopy requires that the molecules have a dipole moment change during vibration, which allows interaction with the electromagnetic wave. Polar bonds such as O-H, C=O, and N-H show strong absorptions, whereas non-polar, symmetrical bonds like N≡N or O=O usually do not absorb IR radiation.

Instrumentation typically involves an IR spectrometer that directs a beam of IR radiation through a sample (solid, liquid, or gas) and measures the intensity of transmitted light at each wavelength. Fourier Transform Infrared (FTIR) spectroscopy is a common modern technique which enhances speed and resolution by collecting spectral data in the time domain and converting it to frequency domain.

IR spectroscopy is widely used in chemistry, materials science, and pharmaceuticals for qualitative analysis, functional group identification, purity testing, and monitoring chemical reactions.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALS

Medicinal Plant Sample

Mucuna pruriens (leaves)

2.1.1 SOLVENTS AND REAGENTS

The following solvents and reagents were used for the study. They were all of analytical grade.

For Extraction:

Distilled water

For Proximate Analysis:

Distilled water

Sodium hydroxide (NaOH)

Sulphuric acid (H_2SO_4)

Petroleum ether (BDH, England)

Kjehdals tab

For Phytochemical Screening:

Distilled water

Methanol (analytical grade)

Hydrochloric acid (HCl)

Mayer's reagent (potassium mercuric iodide)

Dragendorff's reagent (bismuth nitrate + potassium iodide)

Lead acetate

Wagner's reagent (iodine in potassium iodide)

Glacial acetic acid

Ferric chloride ($FeCl_3$)

Concentrated sulfuric acid (H_2SO_4)

Chloroform

Ammonia solution (10%)

Acetic anhydride

Picric acid

Benedict's reagent (Sodium citrate + Sodium carbonate + Copper (II) sulfate)

Ethanol (C_2H_5OH)

Potassium hydroxide (KOH) solution

For Mineral Nutrients Analysis:

Perchloric acid ($HClO_4$)

Nitric acid (HNO_3)

2.1.2 INSTRUMENT/APPARATUS

Traditional Instrument

Glass jar

Knives

Chopping board

Gas cooker

Turner

10L gallon

Airtight containers

Mechanical grinder

Laboratory Instrument

Freeze dryer

Analytical balance (OHAUS Model 2610)

Glass wares (beakers, standard flasks, test tubes, pipettes, rod)

Filter papers (what man no. 40)

Funnels

Spatula

Oven (Surgienfield Instrument, England)

Desiccator

Vacuum pump

Bunsen burner

Porcelain crucible

Litmus paper

Thimble

Soxhlet extractor

Heating mantle

Hot plate

Water bathe (Sanfa-Model No DK420)

Micro-Kjeldahl digestion flask

UV/visible spectrophotometer

Atomic Absorption Spectrometer

Petri dishes

Cork borer

Safety Instrument

Lab coat

Gloves

Fume cupboard

2.2 METHODS

2.2.1 Collection of Plant Sample

Fresh leaves of *Mucuna pruriens* were collected from farms located at Ekpoma in Esan West Local Government Area, Edo State. It was identified and authenticated by Prof. Akinnibosun Henry Adewale, at the herbarium unit of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria. It has voucher number; UHB-M358.

2.2.2 Treatment / Extraction (Decoction)

Fresh leaves of *Mucuna pruriens* were separated from their stem, and chopped into smaller size. The sample was then cleaned to remove any visible dirt, debris, or contaminants by washing them gently with distilled water. After that, the cleaned leaves were spread on a wooden board and allowed to air-dry for 21 days to remove excess moisture. After drying, the crispy-dried leaves were pulverized into fine powder using a mechanical grinder, and stored in an airtight container. This was used for proximate analysis.

A 1000g portion of the pulverized leaf sample was weighed out and put in a glass jar, then 10 liters of distilled water was added. The content of the jar was subjected to heat and the temperature was monitored. When the temperature got to 100°C, it was allowed to boil for 15 minutes, then heat was removed. The mixture was allowed to cool down, and was filtered through muslin cloth. The aqueous filtrate (extract) was collected in a gallon and stored in a freezer to avoid microbial growth or deterioration. The aqueous extract was concentrated using the freeze-drying method to get the crude extract. The crude extract was then used for phytochemical screening, antimicrobial activity, and mineral nutrient analysis.

The percentage yield was calculated by;

$$\%\ \text{yield} = \frac{\text{weight of extract}}{\text{weight of sample}}\ X\ 100 \qquad \text{..... (equation 1)}$$

2.3 PROXIMATE ANALYSIS

Proximate analysis was performed to determine the ash content, moisture content, crude fiber content, crude fat content, crude protein content, and total carbohydrates content using established standard methods (AOAC, 1984).

2.3.1 Ash Content

A 2g of the powdered sample was weighed out and placed into a porcelain crucible which was initially weighed. The crucible was incinerated in a preheated muffle furnace set at the temperature of 600°C for approximately three hours until whitish-grey ash was obtained. After that, the crucible and its content were transferred to a desiccator and allowed to cool, then they were weighed and the weight was noted. The percentage ash content was calculated from the relationship:

$$\text{Ash content}\ (\%) = \frac{\left((\text{wt of crucible} + \text{ash}) - (\text{wt of crucible})\right)}{\text{wt of sample}}\ X\ 100 \qquad \text{..... (equation 2)}$$

2.3.2 Moisture Content

A 2g of the powdered sample was weighed into an initially dried and weighed moisture can. The sample is then dried in an oven at 105°C for three hours. After three hours, it was cooled in a desiccator and weighed. The drying and weighing continues until a constant weight is obtained. The moisture content was determined as the percentage ratio of the change in weight to the original weight, given by the formula:

$$\text{Moisture content (\%)} = \frac{((\text{wt of sample + can before drying}) - (\text{wt of sample + can after drying}))}{Wt\ of\ sample}\ X\ 100$$

……. (equation 3)

2.3.3 Crude Fiber Determination

This determination was carried out according to the procedure of AOAC (1980). A 4g of the moisture-free (dried) sample was weighed into a 250mL beaker, and 50 mL of 4% H_2SO_4 was added followed by distilled water to a volume of 200mL. This was then heated to boiling and kept boiling for exactly 30 min on a Bunsen flame, with constant stirring using a rubber-tipped glass rod to remove all particles from sides of beaker. The volume was kept constant by addition of hot distilled water. After 30 min of boiling, the content was poured into a butchner funnel fitted with an ash less what man no. 40 filter paper and connected to a vacuum pump. The beaker was washed several times with hot distilled water and then transferred quantitatively with a jet of hot water. Washing continued on the funnel until the filtrate was acid-free as indicated by litmus paper. The acid-free residue was transferred quantitatively from the filter paper into the same beaker removing the last traces with 5% NaOH solution and hot water to a volume of 200mL. The mixture was boiled for 30 min with constant stirring as earlier described, keeping the volume constant with hot water. The mixture was then filtered and washed as earlier described until it was alkaline-free. Finally, the resultant residue was washed with two portions of 2mL 95% alcohol. Residues on filter paper were transferred to a pre-weighed porcelain crucible. The content of the crucible was then dried in an oven maintained at 110°C to a constant weight after cooling in a desiccator. The content of the crucible was then ignited in a muffle furnace at 550°C for 8 hours, cooled, and weighed. Triplicate determinations were carried out to ensure accuracy and precision, and the

mean value was calculated to provide a reliable estimate of the crude fiber content. The percentage crude fiber was therefore calculated using the formula:

$$\text{Crude Fiber content (\%)} = \frac{(X - Y)}{\text{Weight of sample}} \; X \; 100 \qquad \text{……. (equation 4)}$$

where;

X = Weight of insoluble matter (g)

Y = Weight of Ash (g)

2.3.4 Crude Fat Determination

The crude fat content of the leaves sample was determined using Pearson's method, a gravimetric technique that involves solvent extraction and evaporation (The method of Pearson (1973)). This method is based on the principle that non-polar components of samples are easily extracted into organic solvents.

A 3.0gram (Moist-free) sample was placed into fat-free thimbles. These were then weighed, plugged with wool, and introduced into soxhlet extractor containing 160mL petroleum ether (b.p 60-80°C). This process ensured the complete extraction of crude fats from the sample. A clean dry receiver flask was weighed and fitted to the extractor. The extraction unit was then assembled and cold water was allowed to circulate, while the temperature of the water bath was maintained at 60°C. Extraction was carried out for 8 hours and at the end of this time, the thimble containing the sample was removed and placed in an oven at 70°C for 3 hours and dried to reach a constant weight. The weight of the thimble and its content was obtained using a standard analytical balance to determine the weight of the extracted crude fat.

The weight difference between the initial and final weights of the thimble and its content represents the weight of the crude fat extracted from the sample. This value was used to calculate the percentage crude fat content, expressed as:

$$\text{Crude fat content (\%)} = \frac{Y - X}{\text{Weight of sample}} X \; 100 \qquad \text{……. (equation 5)}$$

where,

X = Weight of empty thimble

Y = Weight of thimble and oil (fat) extract

2.3.5 Crude Protein Determination

A modified method of micro-Kjeldahl as described by AOAC (1990) was used for crude protein determination.

Procedure for digestion:

3g of the defatted sample was weighed into micro-Kjeldahl digestion flask. 2g of catalyst mixture ($CuSO_4$: Na_2SO_4: SeO_2, 5:1:2 w/w) was added to the flask and then 10 mL nitrogen free conc. H_2SO_4 was also added to the flask. The flask was placed in inclined position on a heating mantle in a fume cupboard. Digestion was started at temperature of 30°C until frothing ceased and then heating was increased to 50°C for another 30 min and finally at full heating (100°C) until a clear solution was obtained. Simmering was continued below boiling point for another 30 min to ensure complete digestion and conversion of nitrogen to ammonium sulphate.

After digestion was completed, the sample was allowed to cool and then transferred to 100mL volumetric flask. It was diluted by adding distilled water to the mark to ensure uniform concentration. 5mL of the filtrate from the digest was transferred with the aid of a 10ml pipette into a 25mL standard flask. 2.5mL of the Alkaline Phenate was added and the solution shaken to mix properly. Then 1ml of Sodium Potassium Tartrate was added, shaken properly followed by the addition 2.5mL of sodium hypochlorite. There after the solution was made up to the 25mL mark with distilled water and the absorbance of the resultant solution measured with the aid of UV/visible spectrophotometer, at 630nm. The Nitrogen standards were treated the same way with the sample. Nessler's reagent was added to detect ammonium ions.

Calculation:

$$\%\text{Nitrogen} = \frac{\text{Instrument.Reading.X Slope Reciprocal X Color Vol.X Digest Vol.}}{\text{Weight of Sample X Aliquot Taken X 10000}} \quad \text{……. (equation 6)}$$

% Crude Protein = %Nitrogen x 6.25 (AOAC, 1975) ……. (equation 7)

2.3.6 Estimation of total carbohydrate

The total carbohydrate content of the sample was obtained by subtracting the sum of percentage crude protein, crude fat, moisture content, crude fiber, and ash content from 100.

2.4 PHYTOCHEMICAL SCREENING

Phytochemical screening of the aqueous extract was performed to identify and detect the presence of various secondary metabolites, including flavonoids, alkaloids, saponins, tannins, steroids, terpenoids, eugenols, glycosides, phenolic compounds, and reducing sugars using standard established procedures (Akindele *et al*., 2007; Harborne 1973; Trease and Evans, 2002, Sofowora 1993).

2.4.1 Test for Flavonoids

2mL of the extract was boiled in 10ml of distilled water and filtered. The filtrate was divided into two different portions, A and B of 5mL each.

To portions A: 10% Lead Acetate solution was added in few drops. A yellowish precipitate is required for a positive result.

To portions B: 5ml of 20% NaOH and few drops of dilute HCl were added to the solution. Formation of a colorless solution is required for a positive result.

2.4.2 Test for Alkaloids

Dragendoff's reagent, Wagner's reagent and Picric acid were used to test for alkaloids.

1ml of the extract was transferred into three different test tubes labelled A, B and C.

To test tube A: 2ml of Dragendoff's reagent (made of a mixture of potassium Bismuth Iodide Salt) was added. Reddish brown precipitate is required for a positive test.

To test tube B: 2ml of Wagner's reagent was added. Reddish brown precipitate is required for a positive test.

To test tube C: 2ml of Picric acid was added. A yellowish precipitate is indicative of a positive test.

2.4.3 Test for Saponins

0.5g of the extract was mixed with 5ml of water in a test-tube and shaken vigorously. It was observed for frothing. A stable, persistent froth is required for a positive test. Saponin rein Weiss (supplied by Merck) was used as standard.

2.4.4 Test for Tannins

To 2ml of the extract, 10ml of distilled water was added and heated for 5 minutes and then filtered into halves.

To about two drops of the filtrate, ferric chloride ($FeCl_3$) solution was added. The formation of a bluish precipitate is required for hydrolysable tannin.

To about five drops of the filtrate, 2ml of dilute HCl was added and boiled for 5 minutes. Red precipitate is required for a positive test.

2.4.5 Test for Steroids

2ml of acetic anhydride was added to 0.5g of extract in 2ml of dilute H_2SO_4. A color change from violet to blue or green is indicative of a positive test for steroids.

2.4.6 Test for Terpenoids

This is Salkowski test. 5ml of the extract was mixed with 2ml of chloroform and 3ml of conc. H_2SO_4 was carefully added down the side of the inner wall of the test tube to form a layer. A reddish-brown coloration on the interphase is indicative of a positive test for terpenoids.

2.4.7 Test for Eugenols

2ml of the extract was mixed with 5ml of 5% KOH solution. The aqueous layer was separated and filtered. Few drops of HCl were added to the filtrate. A pale-yellow precipitate is indicative of a positive result.

2.4.8 Test for Glycosides

1ml of the extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was under-layered with 1ml of conc. H_2SO_4. A brown ring obtained is indicative of the presence of glycoside.

2.4.9 Test for Phenolic Compounds

1ml of the aqueous extract was added to 5ml of 90% ethanol. In addition, 1 drop of 10% $FeCl_3$ was added. A pale-yellow coloration is indicative of a positive test.

2.4.10 Test for Reducing Sugars

2ml each of Fehling solution A and B was put in a test tube and boiled for one minute. To the test tube, 2ml of the extract was added and the mixture was heated in a water bath for five minutes. A brick red precipitate is indicative of a positive test.

2.5 MINERAL NUTRIENT ANALYSIS

The crude extract was digested to transform the target elements into their ionic forms, dissolved in the acid solution. This was achieved by weighing 1.0 g of the crude extract separately, into three flat bottom flasks. 10 mL of a mixture of conc. nitric acid (HNO_3) and perchloric acid ($HClO_4$) was added separately into the three flasks. These solutions were heated gently on a hot plate in the fume cupboard until clear solutions were obtained. The contents of the flasks were cooled, and filtered, into 100mL standard flasks using funnels and filter papers. After filtering, distilled water was added to the flasks to make the contents up to the mark. After digestion, the sample solutions were turned into clean sample bottles for AAS analysis.

Mineral level of the three sample bottles was assessed using Atomic Absorption Spectrometer as described by AOAC (2003).

2.6 MICROBIAL ASSAY

2.6.1 Collection of Microorganism

The antibacterial activities of the extracts were evaluated against a panel of local microbial isolates, comprising of four bacteria strains; two Gram-positive bacteria (Staphylococcus aureus, Escherichia coli) and two Gram-negative bacteria (pseudomonas aeruginosa and Klebsiella pneumoniae). These microbial strains were obtained from Department of Microbiology, Faculty of Life Science, University of Benin, Benin City, Edo State, Nigeria.

2.6.2 Preparation and Sterilization of Materials

Glassware plugged with cotton wool and packaged was sterilized in a hot air oven at 160°C for 1 hour after soaking and drying.

2.6.3 Preparation of Culture Media

Nutrient agar (pH 7.4) from Fluka England was used as the growth medium in this study. To prepare the agar, 40g of Mueller Hinton nutrient agar was accurately measured. The agar powder was completely dissolved in 1L of distilled water to create a homogeneous solution, sterilized in an autoclave at 121°C for 15 minutes to remove any microbial contaminants. The Seeded agar plates were prepared by pouring 20ml of molten agar into a sterile petri dish containing 0.1ml of microorganism culture allowing to set for 24 hours enabling the microorganisms to grow and form a uniform lawn.

2.6.4 Preparation of Test Solution of the Extract

Four different concentrations (25mg/ml, 50mg/ml, 75mg/ml, and 100mg/ml) of the extract were prepared. They were prepared by measuring out 25mg, 50mg, 75mg, and 100mg into separate test tubes and dissolving them with 1ml of distilled water each.

2.6.5 Determination of Antimicrobial Activity

The antimicrobial activity of the leaf extract was evaluated using the agar well diffusion method, in accordance with established guidelines of National Committee for Clinical Laboratory Standards (NCCLS, 2002).

The assay involved preparation of sterile agar plates. Sterilized Molten Mueller Hinton agar was poured into sterilized petri dishes and allowed to solidify. After solidification, the plates were seeded, i.e., 24 hours' nutrient broth grown pathogenic cultures were swabbed on the respective agar plates using sterilized cotton swabs. Then a sterile cork borer was used to bore 4 holes (wells) on each of the agar plate. Corresponding concentrations of the extract made were transferred to each well created in the agar plates aseptically using a sterile pipette. All the plates were labelled accordingly and later incubated at a temp of 37°C for 24 hours to allow for diffusion and interaction between the extracts and microorganisms. After incubation, zone of inhibition of each plate was measured using a graduated meter ruler, to assess antimicrobial activity. It was recorded in millimeter (mm). Standard antibiotics with known antimicrobial activity served as positive controls, while extraction solvent served as negative controls.

2.6.6 Determination of Minimum Inhibition Concentration (MIC)

The most sensitive extract as determined by the initial antimicrobial activity assay, was further evaluated for Minimum Inhibitory Concentration (MIC) using the agar well diffusion method. The inoculum of microorganisms was prepared from 18 hours' nutrient broth cultures. Serial concentrations of the leaf extract were prepared and inoculated with microorganisms. The test tubes containing the microbial cultures and leaf extract concentrations were incubated under controlled condition at 37°C for 18 hours. After incubation, the test tubes were examined for turbidity, which indicates microbial growth. The presence or absence of turbidity was recorded. All experiments were done in triplicates.

2.6.7 Determination of Minimum Bactericidal Concentration (MBC)

From the Minimum Inhibitory Concentration (MIC) tubes/wells that showed no visible growth, a loopful of the culture was streaked onto freshly prepared nutrient agar plates and incubated at 37 °C for 18–24 hours. After incubation, the plates were examined for bacterial colonies. The lowest concentration of the antimicrobial agent that showed no visible colony growth on the agar plates was recorded as the Minimum Bactericidal Concentration (MBC).

2.6.8 Antibiotic Susceptibility Test

Molten Mueller Hinton Agar was poured on sterile petri dishes and allowed to solidify. Upon solidifying, test bacteria isolates were streaked on it. After streaking, a sterile forcep was used to pick disc and placed on top or surface of the agar plate. All the plates were incubated at a temp of 37°C for 24 hours. After incubation, a graduated measuring rule was used to measure the diameter of the clear zone of inhibition and this was recorded in millimeter.

A clinical laboratory or National committee for Clinical laboratory standards (NCCLS) was used to evaluate the performance of each antibiotic (NCCLS 2002).

2.7 STATISTICAL ANALYSIS

Experimental values were expressed as mean ± Standard Error of the Mean (SEM). Comparison of mean values between various groups was performed by independent t-test and one-way analysis of variance (one-way ANOVA) followed by a post hoc test (multiple comparison).

CHAPTER THREE

RESULTS AND DISCUSSION

The results of the proximate analysis, Phytochemical screening, mineral nutrient analysis, and antimicrobial activity evaluation of *Mucuna pruriens* are presented below.

From the formula

Equation 1: $\% \boldsymbol{Yield} = \frac{\boldsymbol{weight\ of\ crude\ extract}}{\textbf{weight of sample}} \boldsymbol{x\ 100}$

The yield obtained was **5.8%**

3.1 PROXIMATE COMPOSITION ANALYSIS

The result of the proximate analysis is shown below in table 1:

Table 1: Proximate Composition of *Mucuna pruriens* leaf

Parameters (%)	***Mucuna Pruriens* Leaf**
Ash content	7.17±0.09
Carbohydrate content	18.43±0.17
Crude fat content	9.67±0.14
Crude fibre content	18.52±0.05
Crude protein	37.88±0.05
Moisture content	8.33±0.09

Mean±SEM of triplicate sample

The leaves of *Mucuna pruriens* contained 7.17% ash, 18.43% carbohydrates, 9.67% crude fat, 18.52% crude fibre, 37.88% crude protein, and 8.33% moisture as shown in table 1. When

compared with the findings of Enemchukwu *et al.* (2015), who reported 2.2% crude fat, 12.5% crude fibre, 35.0% crude protein, 7.8% ash, and 42.6% carbohydrate in aqueous leaf extract, some similarities and notable differences emerge. The protein value obtained in this study (37.88%) is slightly higher than their report (35.0%), confirming that the leaves are consistently rich in protein across different analyses. The crude fibre content (18.72%) was also higher than the 12.5% reported by Enemchukwu *et al*., reinforcing the leaves' value as a source of dietary fibre. However, the carbohydrate level in the present study (18.32%) was markedly lower than their finding of 42.6%, which may be attributed to differences in geographic location and sample preparation. Fat content in the current analysis (9.43%) was considerably higher than their reported 2.2%, suggesting variation in lipid retention depending on processing. In relation to Okunlola *et al.* (2023), who reported protein values between 16.80–18.59%, crude fibre ranging from 17.96–20.01%, fat between 4.12–4.26%, and ash values of 9.28–10.16%, the present study again shows a higher protein level (37.88%) compared to their range. This large difference may be due to differences in genetic accessions or environmental factors influencing protein accumulation. The fibre value (18.72%) closely aligns with their reported range (17.96–20.01%), suggesting consistency in the fibre-rich nature of *Mucuna pruriens* leaves. Fat content in this study (9.43%) was more than double the range given by Okunlola *et al*. (4.12–4.26%), indicating that lipid concentration may vary significantly among accessions. On the other hand, the ash value (7.02%) was lower than their reported 9.28–10.16%, suggesting slightly less mineral content in the sample analysed here.

Overall, the comparison shows that while the exact values vary across studies, *Mucuna pruriens* leaves consistently demonstrate high protein and fibre content, moderate fat, and appreciable mineral composition, supporting their nutritional and therapeutic relevance.

Moisture content plays a key role in defining the quality, stability, and overall usefulness of plant materials. The amount of water a sample holds affects not just its nutritional and therapeutic value but also how it can be handled, processed, and stored. In this study, the leaves were found to have low moisture content, a result that carries several important implications. Low moisture content extends shelf life by slowing the biochemical reactions that break down plant tissues, making it valuable for storage and transport. It also limits microbial growth, since bacteria, yeast, and fungi need water to survive, enhancing safety and reducing spoilage in herbal medicines. In addition, it helps stabilize sensitive compounds like phenolics, flavonoids, alkaloids, and vitamins, preserving both nutritional and therapeutic value for pharmaceutical and nutraceutical use. Low moisture further minimizes issues like browning or rancidity, ensuring better colour, texture, and aroma, which improves overall quality and consumer acceptability.

Ash refers to the inorganic residue left behind when all the organic matter in a plant sample has been burnt away. It essentially represents the total mineral content of the plant material. The high ash content observed in *Mucuna pruriens* leaves in this study points to a rich mineral profile that supports both nutritional and therapeutic applications. The leaves being mineral-rich, contain essential elements which are important for bone development, oxygen transport, nerve signalling, and enzymatic reactions. Their presence makes the plant especially valuable in plant-based diets, where it can help prevent conditions such as anaemia, osteoporosis, and general malnutrition. Beyond nutrition and therapy, ash content also serves as a marker of quality and authenticity. Consistent values confirm purity, while abnormal results may point to contamination or adulteration. This makes ash analysis important for standardizing *M. pruriens* in herbal and functional food products.

The crude fat factor of a plant material reflects its total lipids, such as triglycerides, fatty acids, waxes, sterols, and fat-soluble vitamins. The presence of a moderate level of crude fat in *Mucuna pruriens* leaves, as provided by this study, carries notable nutritional, physiological, and pharmacological significance. Nutritionally, it provides energy (about 9 kcal/g) without making the plant overly energy-dense, making it suitable even for those managing obesity or cardiovascular risks. It also supports the absorption of fat-soluble vitamins (A, D, E, K), which are vital for vision, immunity, antioxidant defence, and blood clotting. Biologically, moderate fat levels point to the presence of essential fatty acids like linoleic and linolenic acids, which maintain cell membranes, aid brain function, and regulate inflammation. Pharmacologically, lipids improve the absorption of bioactive compounds such as phenolics and terpenoids, enhancing therapeutic effects. Overall, this balanced fat content ensures nutritional adequacy while avoiding the risks linked to excessive fat intake.

Crude protein is often the most emphasized proximate parameter; it also has the highest percentage composition in this study. Protein is an essential macronutrient, needed for growth, repair of body tissues, enzyme function, and overall metabolic balance. In proximate analysis, crude protein is estimated from the nitrogen content of a sample, and a high value indicates that the plant contains abundant nitrogen-based compounds. The high protein level found by this study in the leaves of *Mucuna pruriens* therefore points to its important nutritional, therapeutic, and even agricultural benefits. The protein richness of *Mucuna pruriens* leaves gives it strong agricultural value. Its leaves serve as nutritious fodder that supports livestock growth, milk production, and resilience during periods of feed scarcity. As a legume, it also fixes atmospheric nitrogen, improving soil fertility and reducing dependence on synthetic fertilizers—an advantage for sustainable farming systems. From a nutritional perspective, the high protein content of the

leaves makes it an affordable source of essential amino acids, particularly where animal protein is limited. Adequate intake supports muscle development, enzyme and hormone production, immune defence, and helps prevent protein-energy malnutrition, thereby improving overall diet quality and health outcomes. Medicinally, *M. pruriens* leaves may exert antimicrobial, antioxidant, and immunomodulatory effects because of the proteins and peptides it contains. These proteins can also enhance the solubility and bioavailability of phytochemicals, increasing the leaves' therapeutic potential and expanding its applications in food and nutraceuticals. The high protein levels in the leaves highlight its relevance not only in human nutrition and medicine but also in agriculture and ecological sustainability.

Mucuna pruriens leaves, as found by this study, also contains high crude fibre which shows that it is a good source of dietary fibre, thus playing an important role in promoting gastrointestinal health. Fibre adds bulk to food, aids bowel movement, and prevents constipation. It also contributes to the regulation of blood sugar and cholesterol levels, thereby reducing the risk of metabolic disorders such as diabetes, obesity, and cardiovascular disease. In addition, high fibre content enhances satiety, supporting weight management. From an agricultural perspective, fibre-rich leaves can also improve animal digestion and serve as valuable roughage in livestock feed. Thus, the high crude fibre fraction in the leaves of *Mucuna pruriens* emphasizes both nutritional and functional benefits.

The low carbohydrate content in the leaves suggests that it is not a major source of dietary energy. While carbohydrates are the primary energy-yielding macronutrient, their low level in this sample indicates that the leaves may serve more as a source of supplementary nutrients such as protein, fibre, and minerals rather than as an energy staple. This makes the plant particularly suitable for individuals requiring low-calorie diets, such as those managing obesity, diabetes, or other

metabolic disorders. Additionally, low carbohydrate content reduces the risk of rapid postprandial blood sugar spikes, enhancing its potential dietary and therapeutic value.

3.2 QUALITATIVE PHYTOCHEMICAL SCREENING

The result of the phytochemical screening is shown in table 2 below:

Table 2: The Phytochemical Constituents of the decoction leaf extract

S/N	CONSTITUENTS	TEST	INFERENCE
1	FLAVONOIDS	LEAD ACETATE	+
2	ALKALOIDS	PICRIC ACID	+
3	SAPONINS	FROTHING	-
4	TANNINS	FERRIC CHLORIDE	-
5	STEROIDS	ACETIC ANHYDRIDE / SULPHURIC ACID	-
6	TERPENOIDS	SALKOWSKI'S TEST	-
7	EUGENOLS	POTASSIUM HYDROXIDE /HYDROCHLORIC ACID	+
8	GLYCOSIDES	GENERAL TEST	-
9	PHENOLIC COMPOUNDS	ETHANOL/FERRIC CHLORIDE	+
10	REDUCING SUGARS	FEHLING'S SOLUTION A and B	+

Key: (+) = Present

(-) = Absent

The results of the phytochemical analysis showed the presence of major classes of secondary metabolites such as flavonoids, alkaloids, eugenols, phenolic compounds, and reducing sugars in aqueous extract.

Phytochemicals are naturally occurring bioactive compounds produced by plants, usually as secondary metabolites that are not directly involved in primary processes like growth or reproduction. Unlike carbohydrates, proteins, and fats (which are primary metabolites needed for survival), phytochemicals often serve protective or adaptive roles in plants, acting as defense agents against pests, pathogens, and environmental stress. Common groups of phytochemicals include alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, glycosides, and phenolic compounds. These compounds are important in pharmacognosy and traditional medicine because many of them exert physiological effects in humans. Phytochemicals are considered responsible for much of the medicinal value attributed to herbs, including *Mucuna pruriens*.

The phytochemical screening of the decoction extract from *Mucuna pruriens* leaves, as shown in table 2, revealed a unique chemical composition characterized by the presence of flavonoids, alkaloids, phenolic compounds, eugenol, and reducing sugars, while glycosides, saponins, tannins, steroids, and terpenoids were not detected. Compared with the aqueous extract of Indla *et al*. (2023), which retained tannins, saponins, and glycosides, and the cold aqueous extract done by Agbafor and Nwachukwu (2011), which yielded tannins, saponins, glycosides, and terpenoids, the decoction stands out for its selectivity. Thermal processing may degrade heat-sensitive compounds such as certain tannins and saponins, while simultaneously enhancing the release of bound flavonoids and phenolic compounds (Liu *et al*., 2020, Duan *et a*l., 2021). It can also increase the level of detectable reducing sugars and liberate free eugenols from bound forms, although the volatility of eugenols may result in losses through evaporation at higher temperatures

(Song *et al*., 2021). Eugenol plays an important role in managing skin infections, reducing inflammatory, and promoting the healing of skin lesions. Nevertheless, using undiluted clove oil at high concentrations has been linked with adverse effects, and excessive intake of eugenol itself has been reported to cause toxicity (Nejad *et al*., 2017, Kamatou *et al*., 2012). It enhances the ability of various drugs to penetrate the skin. Beyond medicinal uses, it also finds application in agriculture, where it helps protect stored foods from microbial contamination by organisms such as *Lactobacillus* and *Listeria monocytogenes*, and it is further employed as both a pesticide and a fumigant (Kamatou *et al*., 2012). Sugars are essential in plants, serving not only as nutrients but also as key signaling and regulatory agents that influence gene activity tied to growth, defense against diseases, stress adaptation, and metabolic functions. Both reducing and non-reducing sugars are integral to core metabolic pathways and contribute to the formation of secondary metabolites, which enhance the medicinal value of plants (Rolland *et al*., 2002; Arsenault *et al*., 2010). The constituents exhibit significant correlations with antioxidant, neuroprotective, antimicrobial, and restorative effects, which correspond directly to the plant's ethnomedicinal uses.

Indla *et al*. 2023 demonstrated that various solvents can extract a wide range of phytochemicals; however, the exclusive use of water in decoction presents a distinct advantage. Water is widely available, non-toxic, and culturally acceptable, mitigating the health and environmental hazards associated with organic solvents like ethanol, chloroform, or petroleum ether. Therefore, decoction produces a therapeutically concentrated extract while providing a safer, practical, and community-oriented preparation method.

The decoction of *M. pruriens* leaves increases the plant's phytochemical potential, enriching compounds associated to oxidative stress and neurological health, while providing a safe and sustainable extraction method.

3.3 MINERAL NUTRIENTS ANALYSIS

The result of the mineral nutrients analysis is given in table 3:

Table 3: Mineral Nutrients Content of the decoction leaf extract

ELEMENTS	**INFERENCE (mg/Kg)**
SODIUM	1343.67±3.67
MAGNESIUM	377±3.22
POTASSIUM	1018±2.52
CALCIUM	238.33±1.45
COPPER	23.33±3.33
ZINC	29.67±0.33
IRON	336.67±14.53
MANGANESSE	106.67±3.33
NICKEL	11.33±0.33

Mean±SEM of the triplicate samples

Table 3 shows the mineral nutrients content of the decoction extract of *Mucuna pruriens* leaf. It contains 1,343.67 mg/kg Na, 377 mg/kg Mg, 1,018 mg/kg K, 238.33 mg/kg Ca, 23.33 mg/kg Cu, 29.67 mg/kg Zn, 336.67 mg/kg Fe, 106.67 mg/kg Mn, and 11.33 mg/kg Ni. The decoction of *Mucuna pruriens* leaves yielded high sodium and potassium, reinforcing the plant’s reputation as a restorative agent traditionally used to sustain strength and vitality. The magnesium content

supports its ethnomedicinal role in reducing fatigue and enhancing resilience (Chinapolaiah *et al*., 2021). Calcium concentration suggests a contribution to musculoskeletal health, aligning with its folkloric claim as a body fortifier. Notably, the elevated iron level validates traditional use in alleviating weakness and "blood loss," linking directly to its role in blood enrichment (Achikanu and Ani, 2024). Trace elements such as zinc, copper, and manganese, further confirm its association with immunity, fertility, and rejuvenation—attributes deeply rooted in its traditional applications. Even the moderate nickel level implies metabolic support, contributing to the plant's broad therapeutic profile.

Minerals are indispensable for human health because they sustain growth, repair, and the proper functioning of physiological systems. They act as cofactors in metabolic reactions, maintain cellular stability, and support immune resilience. Even in trace amounts, their absence can quickly lead to weakness, disease, or impaired development. Deficiencies are linked with malnutrition syndromes, compromised defense against infections, and diminished vitality. In traditional medicine, mineral-rich plants are often regarded as restorative agents and tonics, reflecting their role in strengthening the body, preventing illness, and enhancing recovery. This therapeutic perspective underscores the relevance of mineral analysis in validating the ethnomedicinal value of plants (Soetan *et al.*, 2010; Prashanth *et al*., 2015; Gharibzahedi and Jafari, 2017).

The measurable concentrations of minerals in *Mucuna pruriens* indicate its value as a source of essential mineral nutrients, and the indigenous preparation method of decoction effectively extracts physiologically relevant compounds. This confirms both the cultural authenticity of the method and its pharmacological significance, thereby emphasizing the importance of *M. pruriens* leaves in traditional medicine.

3.4 ANTIMICROBIAL ACTIVITY

The antimicrobial activity result is given in table 4a, 4b, and 4c below:

Table 4a: Antimicrobial activity of decoction extract of *Mucuna pruriens* leaf on selected isolated microbes represented by zone of inhibition in (mm) at different concentration in (mg/ml)

Isolated bacteria	Concentration in (mg/ml)				Control
					Distilled water(1ml)
	100	75	50	25	
Mucuna pruriens, Zone of inhibition in (mm)					
Escherichia Coli	15.0	11.4	9.5	9.0	-
Staphylococcus aureus	14.1	12.1	10	8.0	-
Pseudomonas aeruginosa	23.0	17.5	16.0	13.0	-
Klebsiella pneumoniae	22.0	18.0	15.0	11.1	-

Key: (-) = No activity, (≤15mm) = Resistant, (16-20mm) = Moderately sensitive, (≥21mm) = Highly sensitive (National Committee for Clinical Laboratory Standard (NCCLS), 2012).

Table 4b: Minimum Inhibitory Concentration (MIC) of decoction extract of *Mucuna pruriens* leaf on selected isolated microbes at different concentration in (mg/ml)

Isolated bacteria	Concentration in (mg/ml)				MIC
	100	75	50	25	
Mucuna pruriens, MIC					
Escherichia Coli	-	-	-	-	25
Staphylococcus aureus	-	-	-	-	25
Pseudomonas aeruginosa	-	-	-	-	25
Klebsiella pneumoniae	-	-	-	-	25

Key: (-) = No visible growth

Table 4c: Minimum Bactericidal Concentration (MBC) of decoction extract of *Mucuna pruriens* leaf on selected isolated microbes at different concentration in (mg/ml)

Isolated bacteria	Concentration in (mg/ml)				MBC
	100	75	50	25	
Mucuna pruriens, MBC					
Escherichia Coli	-	-	+	+	75
Staphylococcus aureus	-	-	-	-	25
Pseudomonas aeruginosa	-	-	-	-	25
Klebsiella pneumoniae	-	-	-	-	25

Key: (-) = No bacterial colony recovery
(+) = Colonies observed

The antibacterial activity of the decoction extract of *Mucuna pruriens* leaves was assessed against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae. The agar diffusion assay as shown by Table 4a demonstrated that the extract exerted inhibitory effects on all four organisms, and that activity was clearly concentration-dependent. At 100 mg/mL, the largest zone of inhibition was recorded against P. aeruginosa (23.0 mm), followed by K. pneumoniae (22.0 mm). E. coli and S. aureus showed comparatively smaller zones of 15.0 mm and 14.1 mm, respectively. The steady reduction in inhibition diameter with decreasing extract concentration confirms that the antimicrobial effect was dose related.

The broth dilution test as shown by Table 4b established the minimum inhibitory concentration (MIC) of the extract at 25 mg/mL for all isolates. This indicates that at this concentration, bacterial growth was suppressed uniformly across both Gram-positive and Gram-negative organisms, demonstrating broad-spectrum inhibitory capacity.

The minimum bactericidal concentration (MBC) values as shown by Table 4c revealed a slight divergence in susceptibility. For S. aureus, P. aeruginosa, and K. pneumoniae, the MBC was identical to the MIC (25 mg/mL), suggesting that the extract was bactericidal at this concentration. In contrast, E. coli required a higher concentration (75 mg/mL) to achieve bactericidal action. This finding implies that while the extract readily kills most of the organisms tested, its effect on E. coli at 25 mg/mL is primarily bacteriostatic, necessitating an increased dose to eliminate the cells completely. The higher tolerance observed in E. coli may be linked to its cell wall architecture and intrinsic resistance mechanisms, which often make Gram-negative bacteria less susceptible to plant-derived antimicrobials.

Comparison with the antibiotic sensitivity test highlights the practical relevance of these findings. Several standard antibiotics, including Ceftazidime, Cefuroxime, Erythromycin, and Streptomycin, failed to inhibit the test organisms. Even among the effective antibiotics, activity was inconsistent—Ciprofloxacin, Augmentin, and Gentamycin inhibited some isolates but not all. Particularly notable was the resistance of S. aureus to nearly all antibiotics tested, yet it was inhibited and killed by the *M. pruriens* decoction. This suggests that the extract contains bioactive compounds capable of overcoming resistance where conventional drugs are ineffective.

The results establish that the decoction of M. *pruriens* leaves possesses substantial antibacterial potential. The extract exhibited both bacteriostatic and bactericidal effects, with most organisms killed at 25 mg/mL and E. coli requiring 75 mg/mL. Compared with conventional antibiotics, the extract displayed a broader and more consistent spectrum of activity, including against resistant strains. These observations provide scientific support for the ethnomedicinal use of *M. pruriens*.

CONCLUSION

This study demonstrated that the leaves of *Mucuna pruriens* are a rich source of nutrients, phytochemicals, minerals, and bioactive compounds with therapeutic potential. The proximate composition confirmed the leaves as a valuable source of protein, fibre, fat, and ash, supporting their nutritional and functional significance in both human and animal diets. Phytochemical screening of the decoction extract revealed the presence of flavonoids, alkaloids, phenolic compounds, eugenols, and reducing sugars, which are known contributors to antimicrobial, antioxidant, and restorative activities. The mineral analysis highlighted appreciable levels of sodium, potassium, magnesium, calcium, iron, zinc, and other trace elements, underscoring the plant's role as a mineral-rich restorative agent traditionally linked to vitality and resilience.

The antimicrobial evaluation further established the potency of the leaf decoction against both Gram-positive and Gram-negative bacteria. The extract showed concentration-dependent inhibition of all tested isolates, with a uniform MIC of 25 mg/mL. While S. aureus, P. aeruginosa, and K. pneumoniae were killed at this concentration, E. coli required a higher MBC of 75 mg/mL, reflecting organism-specific tolerance. Compared with conventional antibiotics, which displayed inconsistent activity and frequent resistance, the decoction exhibited broader and more reliable antibacterial efficacy, particularly against drug-resistant S. aureus.

The findings validate the ethnomedicinal use of *M. pruriens* leaves and demonstrate that decoction, a safe, accessible, and culturally relevant method, yields extracts with strong nutritional, mineral, and antibacterial benefits. This work supports the integration of *M. pruriens* into both food and medicinal applications, and it provides a foundation for further research aimed at isolating active compounds, standardizing preparation methods, and exploring therapeutic development.

RECOMMENDATION

Based on the findings of this study, several recommendations are proposed to guide future work and practical applications. First, quantitative phytochemical screening should be carried out to isolate, characterize, and identify the specific bioactive compounds responsible for the antimicrobial activity of the decoction extract of *Mucuna pruriens* leaves. In addition, toxicological and safety studies are necessary to determine safe dosage ranges, possible side effects, and the long-term implications of consuming the decoction, particularly if it is to be developed into standardized herbal formulations.

Comparative studies with other extraction methods, such as methanol, ethanol, or acetone, should also be conducted using the leaves only, to establish whether decoction remains the most effective, safe, and practical approach for therapeutic purposes. Broader antimicrobial screening should be undertaken against a wider range of clinically relevant microorganisms, including fungi and multi-drug resistant bacterial strains, in order to validate and expand the spectrum of activity observed.

Furthermore, in vivo experiments and clinical evaluations are recommended to complement the in vitro results, thereby providing a clearer picture of the efficacy, pharmacokinetics, and pharmacodynamics of the decoction under biological conditions. Given the high nutritional and mineral content revealed in this study, nutraceutical and functional food applications of *M. pruriens* leaves should also be explored, especially in addressing protein-energy malnutrition, micronutrient deficiencies, and immune-related health challenges. Finally, the decoction method itself should be standardized, considering the leaf-to-water ratio, temperature, and extraction time, to ensure reproducibility, consistent quality, and the potential for commercialization of the extract for both dietary and therapeutic use.

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Antibiotic Sensitivity Test

		Bacteria			
Antibiotics	Potency	Escherichia Coli	Staphylococcus aureus	Pseudomonas aeruginosa	Klebsiella pneumoniae
Ofloxacin	10µg	$8_{(s)}$	$0_{(r)}$	$0_{(r)}$	$12_{(s)}$
Augmentin	30µg	$17_{(s)}$	$0_{(r)}$	$15_{(s)}$	$10_{(s)}$
Peflacine	10µg	$13_{(s)}$	$0_{(r)}$	$5_{(s)}$	$0_{(r)}$
Ceftazidime	30µg	$0_{(r)}$	$0_{(r)}$	$0_{(r)}$	$0_{(r)}$
Ciprofloxacin	10µg	$16_{(s)}$	$0_{(r)}$	$0_{(r)}$	$13_{(s)}$
Erythromycin	30µg	$0_{(r)}$	$0_{(r)}$	$0_{(r)}$	$0_{(r)}$
Streptomycin	30µg	$0_{(r)}$	$0_{(r)}$	$0_{(r)}$	$0_{(r)}$
Gentamycin	10µg	$10_{(s)}$	$0_{(r)}$	$11_{(s)}$	$11_{(s)}$
Cefuroxime	30µg	$0_{(r)}$	$0_{(r)}$	$0_{(r)}$	$0_{(r)}$

Key: s= sensitive

r= resistant

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London)
Faculty of Life Sciences,
Department of Plant Biology and Biotechnology,
P. M. B. 1154 Ugbowo, 300283 Benin City,
Edo State, Nigeria.

University of Benin

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name: - *Mucuna pruriens* (L.) DC.

Family: Fabaceae

Common Name: Velvet Bean, Cowitch, Lacuna bean, Devil Bean, Bengal velvet bean, Florida velvet bean

Voucher Number: UBH-M358

Students Names: Chemelie Solomon Ezenwalie and Efosa Samson

Plant Identification and Voucher Number Issued by:

30/06/2025

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London, LMBOSON, MECOSON, MAEIAN, MFBAN; Nigeria)