INVESTIGATING THE INFLUENCE OF AQUEOUS PICRALIMA NITIDA ON SERUM CALCIUM AND URIC ACID LEVELS IN MALE WISTAR ALBINO RATS

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DEPARTMENT OF MEDICAL BIOCHEMISTRY

SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICINE

UNIVERSITY OF BENIN

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE, B.Sc. (HONS) MEDICAL BIOCHEMISTRY, OF THE UNIVERSITY OF BENIN, BENIN CITY.

MARCH, 2025.

CERTIFICATION

We, the undersigned, hereby certify that Miss ONYEBIGWA MIRABEL IFEANYICHUKWU carried out this work in the Department of Medical Biochemistry, University of Benin, Benin City, and we approve the same as adequate in scope and quality for the reward of Bachelors of Science Degree (B.Sc.) in Medical Biochemistry.

Signed:

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(External Examiner) **DATE**

DEDICATION

I would like to dedicate this work to the Almighty God, my source of inspiration, strength, wisdom, knowledge, and understanding. I also dedicate this project to myself for my resilience, hard work, and unwavering belief in my journey.

ACKNOWLEDGMENT

I would like to express my heartfelt gratitude to my family—my parents, my cousin Ama, and my aunty, Aunty Rukayat—whose unwavering support and assistance have been my greatest strength throughout my undergraduate journey. Their love, encouragement, and belief in me kept me going even during the most challenging times, and for that, I am deeply thankful.

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ABSTRACT

Traditional medicine has long played a crucial role in healthcare, particularly in Africa, where medicinal plants are widely used to treat various ailments. Picralima nitida, also known as the African bitter bean, is one such plant valued for its therapeutic properties, including its use in treating malaria, fever, and inflammatory conditions. Yet, regardless of its broad use in traditional medicine, scientific research on its biochemical effects is insufficient, particularly concerning its influence on mineral metabolism and metabolic health.

In this study, forty male Wistar albino rats were divided into control and experimental groups. The experimental groups received standard doses of aqueous Picralima nitida extract daily for a specific period, while the control group received no treatment. At the end of the study, serum calcium and uric acid concentrations were analyzed to determine potential alterations in mineral homeostasis.

As calcium is essential for bone health, nerve function, and enzymatic processes, and uric acid is a key metabolite linked to conditions such as gout and kidney disease, understanding how Picralima nitida affects these parameters is crucial.

The findings of this study will contribute to the scientific validation of Picralima nitida in traditional medicine, therefore determining whether its continued use is safe and beneficial for metabolic and mineral homeostasis. Additionally, it could open pathways for further research into its pharmacological applications, potentially leading to the development of standardized herbal treatments based on traditional knowledge.

CHAPTER ONE

INTRODUCTION

1.1. Background of Study

Medicinal plants have been widely studied for their potential pharmacological effects, and many traditional herbs are evaluated for their biochemical and physiological effects. One of these concerned plants is *Picralima nitida*, a member of the Apocynaceae family, often called Africa bitter bean. Originally from West Africa, Picralima nitida, called *Abeere* by Western African Yorubas, has long been used in traditional medicine to treat diseases such as fever, malaria, and inflammation (Etukodo, 2012). Biologically active compounds found in this tree, including alkaloids, flavonoids, and saponins, presented significant pharmacological properties, including pain relief effects and anti-inflammatory effects (Akinmoladun *et al*., 2019). Despite its traditional applications, the biochemical impact of Picralima nitida on important physiological signs, such as serum and uric acid calcium, has not been discovered.

Serum calcium is an important mineral that plays a basic role in bone metabolism, neurotransmitter, muscle contraction, and enzyme processes (Peacock, 2010). Any disturbance of calcium homeostasis or balance can cause metabolic disorders such as osteoporosis, kidney dysfunction, and cardiovascular disease. Similarly, uric acid is a metabolic product of purine metabolism and serves as an antioxidant and potential risk factor for gout, hypertension, and kidney deficiency (Richette and Bardin, 2010). With *Picralima nitida's* medicinal potential, it is essential to understand its effects on these biochemical parameters to assess safety and treatment.

Animal models, especially male albino rats, are often used in toxic and biochemical studies because of their genetic stability, physiological similarities to humans, and good metabolic records (Sengpta, 2013). In this study, forty Wistar mice have been chosen to study the potential changes of serum and uric acid after using Picralima nitida extract. Although previous studies have tested the general pharmacological effects of Picralima nitida, limited research is specifically mentioned about its impact on mineral balance and metabolites. This study aims to

fill this gap by evaluating biochemical changes caused by plant extracts in a controlled testing period.

1.2. Aim of study

To assess the effects of aqueous Picralima nitida extract on serum calcium and uric acid levels in male Wistar albino rats.

1.3. Objective of study

This study seeks to evaluate the biochemical effects of Picralima nitida extract on serum calcium and uric acid levels in male Wistar albino rats. By analyzing these parameters, the research aims to determine the plant's potential role in mineral homeostasis and metabolic regulation. The findings will contribute to the growing body of knowledge on the medicinal properties of Picralima nitida, providing a basis for further pharmacological and therapeutic investigations.

1.4. Scope of Study

This research focuses on evaluating the biochemical effects of aqueous Picralima nitida extract administered over the course of four weeks on the serum calcium and uric acid levels in male Wistar albino rats. This research will help demonstrate the biochemical impact of Picralima nitida on calcium and uric acid metabolism, potentially revealing its implications for bone health, renal function, and metabolic processes. This study is limited to an animal model and does not extend to human trials. It also focuses only on serum calcium and uric acid levels without assessing other potential biochemical or histopathological effects of Picralima nitida on organ function.

1.5. Significance of Study

By evaluating the effects of Picralima nitida on serum calcium and uric acid levels, this research provides essential biochemical insights into the medicinal properties of the plant. Picralima nitida has long been used in traditional medicine for its purported analgesic, antimalarial, and anti-inflammatory properties. However, scientific validation of its biochemical effects remains limited. This study will contribute to understanding how the plant extract interacts with mineral metabolism, offering valuable data on its potential physiological effects. By identifying specific biochemical changes induced by Picralima nitida, this research will help bridge the gap between traditional knowledge and modern pharmacological science.

Additionally, this study assesses the potential risks or benefits associated with the consumption of Picralima nitida, particularly concerning calcium and uric acid metabolism. Mineral homeostasis plays a critical role in various physiological processes, including bone health, nerve function, and metabolic regulation. Abnormal serum calcium levels may indicate potential

disruptions in bone remodeling or neuromuscular functions, while altered uric acid concentrations could be linked to metabolic disorders such as gout or kidney dysfunction. By determining whether Picralima nitida influences these parameters positively or negatively, this research provides crucial insights into its safety and potential therapeutic applications.

This study further contributes to the growing body of knowledge on herbal medicine and its implications for disease prevention and management. As the demand for natural and plant-based remedies continues to rise, scientific validation of herbal treatments becomes increasingly important. Understanding the biochemical effects of Picralima nitida will provide a more informed basis for its use in traditional and complementary medicine. If the plant demonstrates beneficial effects on calcium and uric acid regulation, it could be explored as a potential supplement for managing conditions related to mineral imbalances. Conversely, if adverse effects are observed, this research will highlight necessary precautions for its use.

Moreover, this research offers a foundation for future studies on Picralima nitida, including its possible applications in drug development and public health interventions. By establishing baseline data on its biochemical effects, this study paves the way for more extensive investigations into its pharmacological properties. Researchers can build upon these findings to explore its potential use in formulating novel therapeutic agents. If Picralima nitida proves beneficial in regulating mineral metabolism, it could be integrated into strategies for preventing or managing metabolic disorders. Such findings could influence both scientific research and public health policies regarding the use of medicinal plants.

Given the increasing global interest in herbal remedies and alternative medicine, this study serves as a stepping stone for further biochemical and clinical investigations into the therapeutic relevance of Picralima nitida. Many plant-based compounds have contributed significantly to modern medicine, and rigorous scientific evaluation is necessary to unlock their full potential. By providing empirical evidence on the effects of Picralima nitida, this research encourages future explorations into its broader pharmacological applications. Ultimately, the findings from this study may guide healthcare practitioners, researchers, and policymakers in making informed decisions regarding the use of Picralima nitida in both traditional and modern medical practices.

CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction

2.1.1 Overview of the importance of medicinal plant research

There are more than 300,000 higher plant species in the plant kingdom. Since then, these plants have been an important source of important chemical compounds with applications in agriculture and medicine. Medicinal plants have been shown to be crucial in the treatment of sickness, despite the fact that synthetic chemicals are the main focus of contemporary drug development (Rao and Satish, 2016). Various medicinal and aromatic plants have been used by cultures all over the world to treat ailments in their own unique methods. These herbs are utilized frequently due to their inherent healing properties and are prized for their capacity to assist with various health issues. They continue to play a key role in the development of novel medications and are essential suppliers of natural items used to treat a variety of ailments. Although a variety of bioactive compounds are naturally produced by plants, it frequently takes a lot of plant material to extract even a small amount of the desired molecule (Gómez and Luiz, 2018).

From medicinal plants that have long been utilized in agriculture and ethnomedicine, a number of natural products have been created. Random screening of plant extracts against pathogens has occasionally also been used to identify bioactive compounds (Harvey and Cree, 2010; Hupfeld and Efferth, 2009). Since many of these plants have biological activities that correspond with their ethnomedicinal uses, choosing medicinal plants based on their lengthy history of usage in traditional medicine has shown to be a successful strategy (Rout *et al*., 2009).

Biotechnology breakthroughs in recent years have improved research on medicinal plants. In order to increase the synthesis of bioactive substances and guarantee a more effective and environmentally friendly supply of medications produced from plants, researchers are increasingly using genetic engineering. Furthermore, by increasing the absorption and bioavailability of plant-based medicines, nanotechnology has transformed drug delivery systems. The use of medicinal plants in contemporary medication development has increased as a result of these developments.

Research on herbal remedies presents significant difficulties, despite their potential. Key plant species face threats from overharvesting and deforestation, which lowers biodiversity and restricts access to vital medicinal properties.

In conclusion, with ongoing research and technological innovations, medicinal plants will remain a cornerstone of pharmaceutical development and alternative medicine.

2.1.2 Relevance of Picralima nitida in traditional and modern medicine

Picralima nitida is a medicinal plant from the genus Picralima and plant family Apocynaceae. It can be found in tropical African nations like Ivory Coast, Nigeria, Uganda, and Gabon. The Yoruba people in the southwest of Nigeria call it Abeere. Because its seeds, bark, and leaves are thought to have potent antimalarial qualities, it is frequently used to treat fever and malaria. Also, due to its anti-inflammatory properties, the plant also relieves rheumatic pain, arthritis, and body pains. Its seeds are occasionally swallowed or steeped in water for the aim of controlling blood sugar levels in diabetics. It is recommended by traditional healers for intestinal worm infections, diarrhea, and stomach ulcers. Its extracts are also beneficial for treating bacterial and fungal diseases because of their antibacterial and antifungal qualities. It is thought to improve male fertility and sexual performance and is also prized as an aphrodisiac. It has even been used as a treatment for eczema and leprosy. The bark and leaves of the plant are applied topically to wounds, boils, and skin diseases to promote recovery. According to some herbalists, Picralima nitida boosts immunity in order to prevent infections and aids in the treatment of hypertension. It is sometimes used as a poison antidote and detoxifying agent. The plant does, however, contain alkaloids that can be poisonous if taken in excess; therefore, careful dosing and advice from a qualified herbalist are crucial despite its many therapeutic advantages.

Since alkaloids are the primary bioactive compounds known to have been extracted from the seeds of Picralima nitida, numerous studies have previously demonstrated that different extracts of this plant are good sources of phytochemicals such as glycosides, alkaloids, triterpenes, flavonoids, polyphenols, saponins, and tannins (Teugwa C.M., 2013). In a recent study of differentiated 3T3-L1 adipocytes, the indole alkaloid akuammicine, which was isolated from P. nitida, promoted the absorption of glucose. According to the findings, akuammicine may have contributed to the plant's accumulated antidiabetic potential in traditional medicine either alone or in combination with other bioactive chemicals (Shittu H *et al*., 2010).

The potential of Picralima nitida in contemporary drug development is shown by the scientific confirmation of its therapeutic qualities. To fully realize its potential while maintaining safety, more research is necessary, including clinical trials and pharmaceutical formulations. Picralima nitida is an achievable choice for the development of plant-based medicines because current research focuses on identifying its active components and assessing their modes of action.

2.1.3 Justification for studying the biochemical effects of Picralima nitida on serum calcium and uric acid

The potential impact of Picralima nitida on human health, specifically in relation to bone metabolism, renal function, and metabolic diseases, justifies the investigation of its biochemical effects on serum calcium and uric acid. It is important that we study the physiological and pathological effects of Picralima nitida because of its traditional use and new scientific findings that suggest it may affect calcium and uric acid equilibrium.

2.1.3.1 Traditional and Ethnopharmacological Relevance

In African traditional medicine, Picralima nitida has been used extensively to cure ailments like fever, inflammatory illnesses, pain, and malaria. The metabolism of calcium and uric acid is closely related to certain of these illnesses, especially inflammatory disorders and renal impairments. Its possible therapeutic or toxicological roles in calcium and purine metabolism can be better understood by researching its impacts on these biochemical parameters, which can help close the gap between traditional medicine and scientific validation.

2.1.3.2 Importance of Serum Calcium Regulation

Serum calcium is essential for several physiological functions, such as:

A. Mineralization and Bone Health: Calcium is necessary to preserve bone strength and density. Osteoporosis, rickets, and other metabolic bone problems can result from calcium imbalances. P. nitida's usage in traditional medicine to treat bone-related diseases may be affected if it affects calcium homeostasis.
B. Cardiovascular and Neuromuscular Functions: Calcium is essential for heart, muscle, and nerve signal transmission. While hypercalcemia (high calcium levels) leads to kidney stones, arterial calcification, and cognitive impairment, hypocalcemia (low calcium levels) can cause muscle spasms, cardiac arrhythmias, and neurological abnormalities.
C. Renal Function and Kidney Stone Development: Nephrocalcinosis, or calcium deposits in the kidneys, and kidney stone growth can result from abnormalities in calcium metabolism, which is mostly dependent on the kidneys.

By studying the effects of P. nitida on serum calcium, researchers can determine whether it poses a risk for calcium imbalance or offers potential therapeutic benefits in bone and renal health.

2.1.3.3 Uric Acid Metabolism and Its Clinical Implications

A waste product of purine metabolism, uric acid is mostly eliminated by the kidneys. Numerous illnesses are associated with abnormal uric acid levels:

A. Hyperuricemia with Gout: Gout, an excruciating inflammatory arthritis brought on by uric acid crystal deposition in joints, can result from elevated uric acid levels (hyperuricemia). As Picralima nitida has been used by practitioners of traditional medicine to treat inflammatory disorders, it is crucial to look into whether the plant makes uric acid accumulation worse or better.
B. Nephrolithiasis and Kidney Disease: Chronic hyperuricemia is associated with the development of kidney stones and chronic kidney disease (CKD).
C. Metabolic Disorders (Diabetes, Hypertension, and Cardiovascular Disease): Recent research has connected high uric acid levels to a higher risk for cardiovascular disease, insulin resistance, and hypertension.

Investigating P. nitida's effect on serum uric acid will help assess whether it has a hyperuricemic or hypouricemic effect, which is crucial for determining its safety and efficacy in managing metabolic diseases.

2.1.3.4 Toxicological Considerations and Safety Profile

The long-term effects of Picralima nitida on biochemical indicators like calcium and uric acid are still mostly unknown, despite its historical medical applications. Recognizing these effects is essential for

A. Finding Possible Toxicity: P. nitida may be a symptom of nephrotoxicity, hepatotoxicity, or metabolic abnormalities if it causes a marked disruption in calcium or uric acid levels. This study is required to determine whether the plant negatively impacts metabolic and renal health.
B. Determining Safe Dosage Levels: There is a risk of harm since traditional medicine frequently lacks defined dosages. By examining the effects of P. nitida on calcium and uric acid levels, acceptable consumption limits may be established, and possible adverse effects linked to prolonged or excessive use can be avoided.
C. Supporting Drug Development: Picralima nitida may be a source for the development of drugs that target metabolic diseases like gout, kidney stones, and osteoporosis if it is discovered to control calcium or uric acid levels in a positive way.

2.2 Botanical and Phytochemical Profile of Picralima nitida

2.2.1 Taxonomy and Morphology

2.2.1.1 Taxonomy of Picralima nitida

Picralima nitida belongs to the plant kingdom Plantae and is classified under the family Apocynaceae, which consists of many medicinally significant plants known for their alkaloid content. The plant is a monotypic species, meaning it is the only species within the Picralima genus. Below is its full taxonomic classification:

Kingdom: Plantae

Phylum: Tracheophyta (Vascular Plants)

Class: Magnoliopsida (Dicotyledons)

Order: Gentianales

Family: Apocynaceae

Genus: Picralima

Species: Picralima nitida

The Apocynaceae family, commonly referred to as the dogbane family, consists of flowering plants that include important medicinal and ornamental species. Many of these plants, including Picralima nitida, produce bioactive alkaloids that have significant pharmacological properties.

Figure 1.

Diagram showing Picralima nitida fruit (Ken., F, 2014)

2.2.1.2 Morphology of Picralima nitida

Picralima nitida is a small to medium-sized tree or shrub commonly found in the tropical rainforests of West and Central Africa. It is widely distributed in countries such as Nigeria, Ghana, Cameroon, Ivory Coast, Gabon, and Uganda, where it thrives in humid and shaded environments.

Growth Habit and General Appearance

The plant is a perennial evergreen tree that generally attains a height of 10 to 25 meters, featuring a well-branched canopy. The stem is straight and cylindrical, perhaps producing many branches at its apex. The bark is coarse, dark brown, and may exhibit a subtly fissured texture. The inner bark is fibrous and releases a milky latex upon incision, a characteristic trait of the Apocynaceae family.

The leaves of Picralima nitida are simple, opposite, and elliptical or oblong, featuring a smooth, leathery feel. The leaves are organized in pairs along the branches, featuring a strong core vein with minor veins radiating towards the edges. The leaf margins are complete, and the surface is lustrous, reflecting sunlight. Leaves generally range from 8 to 20 cm in length and 3 to 7 cm in width, featuring a short petiole. The leaves exhibit a dark green hue on the upper surface and a lighter green shade on the underside.

The flowers of Picralima nitida are little, aromatic, and range from white to yellowish hues. They develop in terminal or axillary clusters, indicating their presence at the branch tips or within the leaf axils. Each flower possesses five petals that are conjoined at the base, creating a tubular corolla. The petals are gently arched and radiate outward, imparting a star-like aspect to the bloom. The reproductive structures comprise one pistil and five stamens, contained within the flower's tube. Flowering predominantly transpires during the rainy season; however, the specific date may fluctuate based on environmental factors.

The fruit of Picralima nitida represents one of its most striking physical characteristics. It is a sizable, green, and somewhat fibrous capsule, resembling a little mango or an elongated apple. The fruit is ovoid or ellipsoidal, measuring 10 to 15 cm in length and 5 to 8 cm in diameter. As the fruit ripens, it transitions from green to a yellow or orange-brown coloration.

Within the fruit, several seeds are encased in a succulent, yellowish pulp. The seeds are round, compressed, and possess a rigid outer shell. They are frequently utilized in herbal therapy because of their elevated alkaloid concentration. The seeds possess a bitter taste, which is indicated by the name Picralima, originating from the Greek term pikros, signifying bitter.

The root structure of Picralima nitida is robust and fibrous, enabling it to secure itself effectively in tropical soils. The roots possess substantial quantities of bioactive chemicals that enhance their therapeutic application in traditional herbal treatments.

6. Latex Synthesis

Similar to other members of the Apocynaceae family, Picralima nitida exudes a viscous, white latex when its leaves, stems, or bark are incised. This latex comprises alkaloids and other bioactive compounds that enhance the plant's therapeutic properties. Nonetheless, latex may be harmful if ingested in substantial quantities.

2.2.2 Traditional and Medicinal Uses of *Picralima nitida*

2.2.2.1 Ethnobotanical Applications in African and Global Medicine

Picralima nitida, belonging to the Apocynaceae family, has been extensively employed in African traditional medicine for generations owing to its various pharmacological attributes. Indigenous communities throughout West and Central Africa, encompassing Nigeria, Ghana, Ivory Coast, Cameroon, and Gabon, have historically acknowledged its medicinal potential. The plant is referred to as "Abeere" by the Yoruba people of southwestern Nigeria and is widely utilized in traditional medicine.

In African ethnomedicine, Picralima nitida is commonly made as decoctions, infusions, or macerations using different plant parts, such as seeds, bark, leaves, and roots. These medications are conventionally used orally, topically, or via inhalation to address various diseases (Ogunwande *et al.*, 2020).

Scientific interest in Picralima nitida has transcended Africa, resulting in global research in phytopharmacology for prospective therapeutic applications. Contemporary herbal therapy assesses plant extracts for their antibacterial, anti-inflammatory, analgesic, and antimalarial activities (Okhale *et al.*, 2018). Certain researchers have investigated the feasibility of isolating and manufacturing its bioactive components for medicinal use.

2.2.2.1 Common Ailments Treated with Picralima nitida Extract

Traditional healers and herbal practitioners utilize Picralima nitida to address numerous diseases, including:

The plant's antiplasmodial properties render it a favored treatment for malaria. Alkaloids such akuammine and picraline are believed to account for its efficacy (Akinyemi *et al.,* 2019).

Decoctions derived from the seeds and bark are utilized for arthritis, rheumatism, headaches, and general body pain owing to their analgesic and anti-inflammatory qualities (Adotey *et al*., 2020).

Diabetes Management: The seeds and leaves are utilized to regulate blood sugar levels, with research indicating that their alkaloids affect glucose metabolism (Shittu *et al*., 2010).

Gastrointestinal Disorders: Traditional medicine practitioners utilize extracts for ailments like stomach ulcers, diarrhea, dysentery, and intestinal parasites (Egharevba *et al*., 2015).

Sexual Health and Fertility: The plant is considered to have aphrodisiac and fertility-enhancing characteristics, with its seeds being used to treat male sexual dysfunction and low sperm count (Oyelere *et al.*, 2016).

Hypertension and Cardiovascular Health: Some traditional uses include employing Picralima nitida to manage blood pressure and promote circulatory health (Okhale *et al.*, 2018).

Skin Conditions and Wound Healing: The bark and leaves are applied externally for wound healing, boils, eczema, and other skin infections due to their antimicrobial properties (Gbadamosi and Moody, 2021).

The plant is occasionally utilized as a tonic for overall immunological enhancement and cleansing, particularly in the prevention of infections (Akinmoladun *et al.*, 2019).

The various medical uses underscore the plant's significance in both ancient healing methods and contemporary herbal pharmacology.

2.2.3 Phytochemical Composition of Picralima nitida

2.2.3.1 Identification of Key Bioactive Compounds

The pharmacological attributes of Picralima nitida arise from its abundant phytochemical constituents, comprising alkaloids, flavonoids, tannins, saponins, glycosides, and triterpenes (Teugwa *et al.,* 2013). Phytochemical investigations have identified the subsequent beneficial compounds:

Alkaloids are the most potent bioactive components of Picralima nitida, accountable for numerous therapeutic actions. The principal alkaloids recognized comprise:

Akuammine is an indole alkaloid with analgesic, anti-inflammatory, and antimalarial properties.

Picraline demonstrates antibacterial and antipyretic effects.

Akuammicine has been demonstrated to improve glucose absorption, indicating possible antidiabetic properties (Shittu *et al.*, 2010).

Strictosidine is a precursor to several alkaloids exhibiting neuroprotective effects (Egharevba *et al.*, 2015).

Flavonoids, recognized for their antioxidant and anti-inflammatory characteristics, safeguard cells against oxidative stress and bolster immunological function.

Tannins possess astringent, antibacterial, and anti-diarrheal properties, rendering them advantageous for the treatment of gastrointestinal problems (Akinyemi *et al.,* 2019).

Saponins enhance the plant's antifungal properties, bolster the immune system, and reduce cholesterol levels.

Glycosides assist in regulating cardiovascular health and may enhance the antihypertensive properties of the plant.

Triterpenes are recognized for their anti-inflammatory and hepatoprotective properties, contributing to liver health and detoxification (Gbadamosi and Moody, 2021).

2.2.3.2 Documented Pharmacological Attributes of Picralima nitida Phytochemicals

Empirical research has yielded evidence substantiating the pharmacological properties of Picralima nitida. Several well-documented characteristics include:

Antimalarial Activity: Extracts of Picralima nitida have demonstrated significant antiplasmodial activity against Plasmodium falciparum, establishing it as a valuable plant for malaria treatment (Adotey *et al.*, 2020).

Alkaloids, including akuammine, exhibit analgesic and anti-inflammatory properties via interacting with opioid receptors, yielding pain relief akin to morphine but minimizing adverse effects (Okhale *et al.,* 2018).

The methanolic and ethanolic extracts of the plant exhibit antimicrobial and antifungal properties, effectively targeting bacterial strains including Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa, as well as the fungus Candida albicans (Ogunwande *et al.*, 2020).

Antidiabetic Potential: Research indicates that the alkaloids of Picralima nitida can improve insulin sensitivity and glucose absorption, corroborating its traditional application in diabetes management (Shittu *et al*., 2010).

Cardioprotective and Antihypertensive Effects: Extracts have exhibited the capacity to reduce blood pressure and heart rate, potentially assisting in the management of hypertension (Akinmoladun *et al.*, 2019).

Neuroprotective and Sedative Properties: Certain alkaloids in Picralima nitida engage with central nervous system receptors, suggesting possible applications in the treatment of anxiety, depression, and neurological illnesses (Egharevba *et al*., 2015).

The presence of flavonoids and polyphenols mitigates oxidative stress, a significant contributor to aging, cardiovascular illnesses, and neurological disorders (Gbadamosi and Moody, 2021).

The considerable phytochemical diversity of Picralima nitida enhances its numerous therapeutic uses. The abundant alkaloid composition supports its powerful antimalarial, analgesic, antidiabetic, antibacterial, and anti-inflammatory effects. The plant remains a valuable ethnobotanical resource in African medicine and a promising candidate for further pharmaceutical research and drug development.

2.3 Calcium and Its Biochemical Importance

2.3.1 The Function of Calcium in Biochemical Systems

Calcium (Ca^{2+}) is a vital mineral that is integral to various metabolic processes. Calcium functions as a structural element, intracellular messenger, enzyme cofactor, and signaling molecule in numerous physiological processes. Calcium homeostasis is strictly maintained to guarantee appropriate cellular function, as its imbalance can lead to severe health issues.

i) Structural Element in Osteogenesis and Dentinogenesis

Calcium is the principal mineral in bones and teeth, constituting hydroxyapatite crystals ($Ca_{10}(PO_4)_6(OH)_2$) that confer stiffness and strength.

Approximately 99% of the body's calcium is sequestered in bones, serving as a reservoir for the regulation of calcium homeostasis.

ii) Function in Muscle Contraction

Calcium ions are crucial for muscular contraction. The procedure adheres to the following steps:

Calcium ions (Ca^{2+}) attach to troponin, inducing a conformational alteration that facilitates the interaction between actin and myosin.

Muscle fibers contract, and relaxation ensues as Ca^{2+} is reabsorbed into storage.

iii) Intracellular Signaling and Functions of Second Messengers

Calcium functions as a secondary messenger in various cellular signaling pathways, including:

Release of neurotransmitters in neurons

Secretion of hormones (e.g., insulin release from pancreatic beta cells)

Activation of T-cells in the immunological response

Apoptosis (planned cellular demise)

iv) Enzyme Activation and Cofactor Functions

Calcium serves as a cofactor for numerous enzymes, including:

Calmodulin-dependent enzymes (e.g., CaM Kinase, Myosin Light Chain Kinase)

Proteolytic enzymes (e.g., calpain)

Coagulation enzymes (e.g., Factor IV in the hemostatic cascade)

v) Hemostasis (Coagulation Cascade)

Calcium is essential in the blood coagulation process, particularly in the activation of clotting proteins like prothrombin to thrombin. It facilitates the creation of a stable fibrin clot, hence reducing excessive hemorrhage.

vi) Neuronal Function and Synaptic Transmission

Calcium is essential for synaptic transmission within the neurological system.

Voltage-gated calcium channels (VGCCs) facilitate the inflow of Ca^{2+} into neurons.

This initiates the secretion of neurotransmitters (e.g., dopamine, serotonin) into synapses.

Calcium also has a role in long-term potentiation (LTP), a mechanism associated with learning and memory.

vii) Regulation of the Cell Cycle and Apoptosis

Calcium plays a pivotal role in regulating the cell cycle and is essential for cell division, including mitosis and meiosis.

Increased intracellular Ca^{2+} can trigger apoptosis, an essential mechanism for eliminating damaged or senescent cells.

2.3.2 Serum Calcium Metrics in Biochemical Evaluation

2.3.2.1 Total Calcium Concentration Parameters

2.3.2.1.1 Definition and Normal Range of Total Serum Calcium Concentration

Total serum calcium denotes the aggregate of all calcium types found in the blood plasma. It comprises:

A. Ionized (Unbound) Calcium (Ca^{2+}): The biologically active form (about 50%)
B. Protein-bound calcium: Primarily associated with albumin (about 40%)
C. Calcium Complexed with Anions: Including phosphate, bicarbonate, sulfate, and citrate (about 10%)

The standard reference range for total serum calcium in healthy people is generally **8.5–10.5 mg/dL (2.12–2.62 mmol/L),** while modest variations may occur based on laboratory techniques and demographic factors (Lian *et al.*, 2017).

2.3.2.1.2 Regulation of Serum Calcium Homeostasis

Calcium homeostasis is meticulously managed by a complex interplay of hormonal and physiological processes to sustain stable serum calcium levels. The principal regulators comprise of:

i) Parathyroid Hormone (PTH)

Secreted by the parathyroid glands in reaction to diminished calcium levels.

Elevates serum calcium through:

Inducing bone resorption (calcium liberation from bones)

Augmenting calcium reabsorption in the renal system

Enhancing intestinal calcium absorption through elevated calcitriol synthesis

ii) Vitamin D (Calcitriol, 1,25-dihydroxyvitamin D_3)

Produced in the skin (via UV radiation) or acquired from food sources.

Facilitates intestinal calcium absorption and augments bone resorption during periods of low calcium levels.

iii)Calcitonin

Secreted by the thyroid gland (C cells) in reaction to elevated serum calcium levels.

Decreases calcium levels by inhibiting osteoclast activity, hence lowering bone resorption.

The kidneys filter approximately 10 grams of calcium daily, with around 98% reabsorbed in the tubules under hormonal regulation.

2.3.2.1.3 Determinants of Total Serum Calcium Levels

a) Albumin concentrations and protein binding affinity

Approximately 40% of serum calcium is bound to albumin; thus, hypoalbuminemia (low albumin levels) may lead to inaccurately low total calcium readings, even when ionized calcium levels are normal (Dickerson *et al*., 2007).

b) pH and Acid-Base Equilibrium:

Alkalosis (elevated blood pH) promotes calcium binding to albumin, resulting in decreased ionized calcium levels. Acidosis (reduced blood pH) diminishes albumin binding, resulting in elevated free calcium levels.

c) Dietary Calcium Intake and Absorption:

Calcium absorption is contingent upon dietary intake, vitamin D concentrations, and gastrointestinal health. Malabsorption disorders, such as celiac disease and Crohn's disease, can hinder calcium absorption.

d) Hormonal Dysregulations and Endocrine Pathologies

Hyperparathyroidism → Elevated PTH → Hypercalcemia

Hypoparathyroidism → Decreased PTH → Hypocalcemia

e) Chronic Kidney Disease (CKD) leads to impaired vitamin D metabolism, resulting in calcium dysregulation.

2.3.2.1.4 Clinical Importance of Abnormal Total Serum Calcium Concentrations

a) Hypercalcemia (Serum Calcium Level Exceeding 10.5 mg/dL)

Etiology:

Primary hyperparathyroidism (excessive secretion of parathyroid hormone)

Cancers (bone metastases, multiple myeloma)

Vitamin D toxicity

Extended immobilization (bone resorption)

Symptoms include fatigue, weakness, constipation, nephrolithiasis, disorientation, and heart arrhythmias.

Management includes intravenous fluids, bisphosphonates, calcitonin, and addressing the underlying cause.

b) Hypocalcemia (Serum Calcium <8.5 mg/dL)

Etiology:

Hypoparathyroidism (diminished PTH secretion)

Deficiency of vitamin D

Chronic renal illness (impaired calcium metabolism)

Severe hypomagnesemia (Mg^{2+} is essential for the release of parathyroid hormone)

Symptoms include muscle cramps, tetany (Trousseau's and Chvostek's symptoms), paresthesia, seizures, and heart arrhythmias.

Management: Administration of calcium supplements (oral or intravenous), vitamin D therapy, and addressing underlying problems.

2.3.2.1.5 Techniques for Assessing Total Serum Calcium

Colorimetric Assay (Arsenazo III, O-Cresolphthalein Complexone Method) → Standard laboratory procedure.

Atomic Absorption Spectroscopy (AAS) → More accurate, utilized in research.

Ionized Calcium Measurement → Yields the most precise physiological value, particularly in critically ill individuals.

The total serum calcium concentration is an essential biochemical indicator of calcium homeostasis and general health. It exists in several forms, with ionized calcium being the most physiologically active. The levels are meticulously managed by parathyroid hormone (PTH), vitamin D, calcitonin, and renal function. Serum calcium abnormalities may signify endocrine diseases, renal impairment, neoplasms, or dietary inadequacies. Comprehending these changes and their clinical implications is crucial for the diagnosis and management of metabolic and systemic disorders.

2.3.2.4 Sources of Calcium in the Diet

Calcium is a vital mineral necessary for numerous physiological functions, including osseous development, muscular contraction, neural transmission, and hemostasis. The recommended dietary allowance (RDA) for calcium differs based on age, sex, and physiological states, including pregnancy and lactation. Principal dietary sources comprise:

Dairy Products: Milk, cheese, and yogurt are among the most abundant sources of bioavailable calcium.

Leafy green vegetables such as kale, bok choy, and broccoli offer modest calcium content; however, certain greens like spinach possess oxalates that diminish calcium absorption.

Fortified Foods: Cereals, plant-based milk (such as almond, soy, and oat milk), and orange juice are frequently enriched with calcium. Nuts and seeds, such as almonds, sesame seeds, and chia seeds, possess moderate quantities of calcium.

Fish and Seafood: Sardines and salmon, particularly with bones, are outstanding providers of calcium. Legumes and Soy Products: Beans, lentils, tofu, and tempeh supply plant-derived calcium.

2.3.2.5 Calcium Absorption and Metabolism

Calcium absorption predominantly transpires in the small intestine, namely inside the duodenum and jejunum, via two mechanisms:

i) Active Transport (Vitamin D-Dependent Pathway): This process entails calcium-binding proteins (calbindins) and is modulated by 1,25-dihydroxyvitamin D (calcitriol), the bioactive form of vitamin D (Christakos *et al.*, 2011).

ii) Passive Diffusion: Transpires along a concentration gradient, significantly influencing processes when calcium intake is elevated.

Calcium is carried in the bloodstream in three forms following absorption:

i) Ionized Calcium

ii)Protein-Bound Calcium

iii) Complexed Calcium

Calcium metabolism deposition bones, renal excretion, and hormonal control to sustain homeostasis (Bronner and Pansu, 2013).

2.3.2.6 Hormonal Regulation of Calcium Homeostasis

Calcium homeostasis is meticulously managed by three principal hormones: parathyroid hormone (PTH), vitamin D (calcitriol), and calcitonin. These hormones influence the bones, kidneys, and intestines to regulate serum calcium concentrations within a restricted range (8.5–10.5 mg/dL).

i) In the parathyroid hormone (PTH), calcium is:

Synthesized by: Parathyroid glands.

Secretion Stimulus: Decreased blood calcium levels (hypocalcemia).

Activities:

Bone: Enhances osteoclast activity, resulting in the release of calcium into the bloodstream.

ii) The Kidneys:

Increases calcium reabsorption and promotes the transformation of 25-hydroxyvitamin D into 1,25-dihydroxyvitamin D (calcitriol), the bioactive form of vitamin D.

iii) The Intestines: Indirectly enhances calcium absorption by boosting vitamin D synthesis (Goltzman, 2018).

Vitamin D (Calcitriol)is: Synthesized from cholesterol-derived precursors in the skin upon UV exposure or acquired through dietary sources.

Activated in: The liver (conversion to 25-hydroxyvitamin D) and kidneys (final activation to 1,25-dihydroxyvitamin D).

Actions:

a) Augments intestinal calcium absorption through the upregulation of calbindin expression.
b) Enhances calcium reabsorption in the renal system.
c) Supports bone mineralization by ensuring sufficient calcium and phosphate concentrations (Bouillon and Carmeliet, 2018).

Calcitonin is:

Produced by: Parafollicular (C-cells) of the thyroid gland.

Stimulus for Secretion: Elevated blood calcium concentrations (hypercalcemia).

Actions:

a) Bone: Suppresses osteoclast activity, hence decreasing bone resorption.
b) Kidneys: Increases calcium excretion.
c) Overall Impact: Calcitonin reduces blood calcium levels; nonetheless, its significance in calcium homeostasis is subordinate to that of parathyroid hormone (PTH) and vitamin D (Masi and Brandi, 2022).

2.3.2.6. Pathological Conditions Influencing Calcium Homeostasis

Disruptions in calcium homeostasis may result in numerous disorders, categorized as hypocalcemia (reduced calcium levels) and hypercalcemia (elevated calcium levels).

i) Hypocalcemia (Decreased Serum Calcium Concentrations)

Hypocalcemia manifests when blood calcium concentrations drop below 8.5 mg/dL, resulting in neuromuscular excitability, tetany, and cardiac impairment. Prevalent factors encompass:

a) Hypoparathyroidism

Causation: Autoimmune destruction, surgical excision of the parathyroid glands, or congenital anomalies.

Effect: Diminished PTH secretion results in compromised calcium mobilization from bones, reduced intestinal absorption, and heightened renal calcium excretion.

Manifestations: Muscle cramps, paresthesia, seizures, and a prolonged QT interval on electrocardiogram (Clarke *et al*., 2016).

b) Vitamin D Deficiency (Rickets and Osteomalacia)

Cause: Insufficient food consumption, limited solar exposure, chronic kidney disease (CKD), or hepatic dysfunction impairing vitamin D metabolism.

Effect: Reduced intestinal calcium absorption, resulting in bone demineralization.

Symptoms: Ostealgia, skeletal malformations (rickets in pediatric patients), and heightened susceptibility to fractures (Holick, 2017).

c) Chronic Kidney Disease (CKD) with Secondary Hyperparathyroidism

Cause: Diminished renal function results in decreased calcitriol production and phosphate retention, instigating secondary hyperparathyroidism. Excessive release of parathyroid hormone (PTH) results in bone resorption and renal osteodystrophy.

Symptoms: Ostealgia, fractures, and vascular calcifications (Evenepoel *et al.*, 2021).

ii)Hypercalcemia (Elevated Serum Calcium Concentrations)

Hypercalcemia arises when serum calcium concentrations surpass 10.5 mg/dL, potentially resulting in neurological dysfunction, nephrolithiasis, and cardiovascular complications. Prevalent etiologies encompass:

a) Primary Hyperparathyroidism

Cause: Hyperactivity of parathyroid glands resulting from adenomas, hyperplasia, or cancer.

Effect: Elevated PTH secretion enhances bone resorption, renal calcium reabsorption, and intestine absorption.

Symptoms: "Bones, stones, groans, and psychiatric overtones" (osteoporosis, nephrolithiasis, abdominal discomfort, and neuropsychiatric disturbances) (Marcocci *et al*., 2018).

b) Hypercalcemia Associated with Malignancy

Cause: Tumors (lung, breast, multiple myeloma) release parathyroid hormone-related protein (PTHrP), which mimics the effect of parathyroid hormone (PTH).

Outcome: Enhanced bone resorption and renal calcium retention.

Symptoms: Exhaustion, nausea, disorientation, excessive urination, and arrhythmias (Stewart, 2005)

c) Vitamin D Toxicity

Cause: Excessive supplementing or granulomatous illnesses (e.g., sarcoidosis, tuberculosis) resulting in the overproduction of calcitriol.

Effect: Enhanced intestinal calcium absorption.

Symptoms: Hypercalciuria, nephrolithiasis, muscular weakness, and cardiac problems (Sharma *et al*., 2019).

Calcium is essential for numerous physiological activities, and its homeostasis is meticulously managed by parathyroid hormone (PTH), vitamin D, and calcitonin. Disruptions in calcium metabolism can result in serious illnesses, including osteoporosis, renal failure, and problems associated with hypercalcemia. Comprehending the dietary origins, metabolism, and hormonal control of calcium is crucial for the prevention and management of illnesses associated with calcium dysregulation.

2.4 Uric Acid and Its Biochemical Function

2.4.1 Uric Acid Metabolism

Uric acid is the ultimate byproduct of purine metabolism in humans and certain apes. In contrast to the majority of mammals, which convert uric acid to allantoin through the enzyme uricase, humans are devoid of this enzyme due to evolutionary gene silence. Consequently, uric acid is predominantly eliminated by the kidneys and intestines (Maiuolo *et al.*, 2016).

Disruptions in uric acid metabolism may result in hyperuricemia, gout, nephrolithiasis, and further metabolic diseases. This essay offers a detailed summary of uric acid metabolism, encompassing its metabolic routes, control, excretion, and related disorders.

2.4.1.2 Purine Metabolism and Uric Acid Synthesis

Purines are vital nitrogenous bases integral to the production of DNA, RNA, ATP, NAD+, and coenzymes. Purine metabolism encompasses its synthesis, interconversion, and breakdown, culminating in uric acid as the final excretory product in humans and certain primates (Maiuolo *et al*., 2016).

Abnormalities in purine metabolism may result in hyperuricemia, gout, Lesch-Nyhan syndrome, and immunodeficiencies. This essay examines the de novo and salvage pathways of purine metabolism and the biochemical process that culminates in uric acid production.

Purine metabolism transpires through three primary pathways:

2.4.1.2.1 De Novo Synthesis

Purine nucleotides (adenine and guanine) are produced from ribose-5-phosphate, amino acids (glycine, glutamine, aspartate), carbon dioxide, and tetrahydrofolate derivatives (Wang *et al.,* 2021).

Stages of De Novo Purine Biosynthesis:

i) Activation of Ribose-5-Phosphate

Enzyme: Phosphoribosyl pyrophosphate (PRPP) synthetase catalyzes the conversion of ribose-5-phosphate to PRPP.

PRPP is a crucial precursor for the production of purines and pyrimidines.

ii) Dedication to Purine Biosynthesis

Enzyme: Glutamine phosphoribosyl pyrophosphate amidotransferase (GPAT) catalyzes the conversion of PRPP to phosphoribosylamine.

(PRA).

This constitutes the rate-limiting step in purine biosynthesis.

iii) Transformation of IMP to AMP and GMP

IMP to AMP (needs aspartate and GMP)

iv) Conversion of IMP to GMP (necessitates glutamine and ATP)

Regulation:

AMP and GMP hinder their own synthesis at branching points.

PRPP synthetase and GPAT are subject to feedback inhibition by IMP, AMP, and GMP.

2.4.1.2.2 Salvage Pathway

The salvage route reconstitutes free purines (adenine, guanine, and hypoxanthine) into nucleotides, thereby preserving energy.

i) Adenine Reclamation

Enzyme: Adenine Phosphoribosyltransferase (APRT)

Reaction: Adenine + PRPP → AMP

ii) Salvage of Guanine and Hypoxanthine

Enzyme: Hypoxanthine-guanine phosphoribosyltransferase (HGPRT)

Lesch-Nyhan syndrome, characterized by full HGPRT loss, results in hyperuricemia, severe gout, and self-mutilating behavior (Torres *et al*., 2019).

2.4.1.2.3 Degradation of Purines and Synthesis of Uric Acid

Purine nucleotides are metabolized into uric acid, which is eliminated by urine.

Stages of Purine Catabolism

i) Decomposition of AMP and GMP

AMP is converted to Inosine through the action of AMP deaminase, which subsequently transforms into Hypoxanthine.

GMP is converted to Guanosine through the action of 5'-nucleotidase, which subsequently yields Guanine.

ii) Transformation to Xanthine

Hypoxanthine is converted to xanthine through the action of xanthine oxidase.

Guanine is converted to xanthine through the action of guanine deaminase.

iii) Conclusive Oxidation to Uric Acid

Xanthine is converted to uric acid through the action of xanthine oxidase.

This primarily transpires in the liver.

Regulation:

Xanthine oxidase activity regulates uric acid concentrations.

Allopurinol inhibits xanthine oxidase, hence decreasing uric acid production, and is utilized in the treatment of gout.

2.4.1.2 Function of the Liver and Kidneys in Uric Acid Regulation

The liver and kidneys are essential for the metabolism and regulation of uric acid levels in the body.

i) Function of the Liver:

The liver serves as the principal location for purine metabolism, wherein purines derived from nucleic acids are decomposed into uric acid.

Enzymes like xanthine oxidase facilitate the oxidation of hypoxanthine to xanthine, and subsequently to uric acid.

Uric acid is thereafter released into the bloodstream for conveyance to the kidneys.

ii) Function of the Kidneys:

The kidneys modulate uric acid concentrations by filtration, reabsorption, secretion, and excretion.

Approximately 100% of uric acid is filtered by the glomeruli and subsequently reabsorbed in the proximal tubules.

A fraction is actively reabsorbed into the renal tubules, while the residual uric acid is eliminated in urine.

Impaired renal function can diminish uric acid excretion, resulting in hyperuricemia and diseases such as gout and nephrolithiasis.

Consequently, the liver produces uric acid, whereas the kidneys govern its excretion, thereby preserving homeostasis within the body.

2.4.2 Serum Acid Metrics in Biochemical Investigations

2.4.2.1 Aggregate Uric Acid Concentration

Total uric acid concentration denotes the quantity of uric acid in the blood serum or plasma, indicating the equilibrium among purine metabolism, hepatic synthesis, renal excretion, and intestinal clearance.

Standard Reference Range:

Males: 3.4–7.0 mg/dL (202–416 µmol/L)

Women: 2.4–6.0 mg/dL (143–357 µmol/L)

Children: 2.0–5.5 mg/dL (119–327 µmol/L)

Determinants of Total Uric Acid Levels:

i) Consumption of high-purine meals (e.g., red meat, shellfish, alcohol) might elevate uric acid levels.

ii) Metabolic Rate: Elevated cellular turnover (e.g., cancer, hemolysis) results in increased uric acid synthesis.

iii) Renal Function: Diminished kidney function decreases uric acid excretion, resulting in hyperuricemia.

iv) Genetic Factors: Variations in uric acid transporters (e.g., URAT1, GLUT9) affect serum concentrations.

v) Pharmaceuticals: Diuretics, aspirin, and chemotherapeutic agents can modify uric acid metabolism.

Clinical Importance:

Hyperuricemia (>7.0 mg/dL) is linked to gout, nephrolithiasis, metabolic syndrome, and cardiovascular disorders. Symptoms include: Painful urine and blood in urine as signs of kidney stones. Fatigue, and swelling in legs, may occur with high blood pressure and diabeties. This can be managed by change of diet, increase in hydration, and limitation of sugar and fructose intake. In severe conditions, Xanthine oxidase inhibiting medications can be used.

Hypouricemia (<2.0 mg/dL) may suggest liver illness, increased uric acid excretion, or xanthine oxidase deficiency. Symptoms include: Frequent urination, fatigue and weakness, excercise-induced kidney injury which can occur in athletes with genetic disorders affecting uric acid metabolism. It can be managed by treatment of underlying causes, maintaining proper nutrition, and monitoring athletes for complications.

Regular monitoring of total uric acid concentration is critical for detecting and controlling metabolic diseases and kidney function problems.

2.4.2.1.2 Uric acid clearance and renal function

Uric acid clearance refers to the process by which the kidneys filter, reabsorb, produce, and ultimately eliminate uric acid from the blood into the urine. It is a vital function in maintaining purine metabolism balance and preventing hyperuricemia or hypouricemia.

2.4.2.1.2.2 Renal Handling of Uric Acid

Uric acid is predominantly removed by the kidneys through a four-step process:

i) Glomerular Filtration: About 100% of uric acid in circulation is readily filtered through the glomerulus.

ii) Tubular Reabsorption: Nearly 90% of filtered uric acid is reabsorbed in the proximal tubules via urate transporters (URAT1, GLUT9) to prevent excessive loss.

iii) Tubular Secretion: Some uric acid is actively secreted back into the tubules, allowing for additional control.

iv) Excretion: Under normal conditions, only 10% of filtered uric acid is excreted in the urine.

2.4.2.3 The Ratio of Urea to Uric Acid and Its Significance

The urea-to-uric acid ratio is a biochemical metric that assesses the concentration of urea and uric acid in blood or urine.

i) Physiological Importance

Urea and uric acid are both nitrogenous waste products.

Urea is the principal end product of protein metabolism, synthesized in the liver through the urea cycle.

Uric acid is a byproduct of purine metabolism and is predominantly regulated by the kidneys.

ii) Significance in Clinical Practice

The standard urea-to-uric acid ratio typically ranges from 10:1 to 20:1 in healthy individuals.

Augmented Ratio:

Observed in high-protein diets, dehydration, renal dysfunction, and urea cycle abnormalities (resulting in excessive urea production).

Chronic kidney disease (CKD) diminishes uric acid clearance, resulting in an elevated ratio.

Reduced Ratio:

Noted in hepatic dysfunction (e.g., cirrhosis, liver failure) where urea production is compromised.

Hyperuricemia, as observed in conditions such as gout, leukemia, or tumor lysis syndrome, leads to elevated uric acid production and a diminished ratio.

Diagnostic Application

The urea-to-uric acid ratio aids in distinguishing the causes of renal failure, evaluating metabolic diseases, and monitoring irregularities in protein and purine metabolism.

It is especially beneficial for assessing renal function, nutritional health, and conditions such as gout and hyperuricemia.

Comprehending these aspects is essential for the management of illnesses such as gout, hyperuricemia, and renal problems, in addition to informing dietary and lifestyle modifications.

2.5 Prior Research on Picralima nitida

Prior Investigations of Picralima nitida: Biochemical and Pharmacological Perspectives, Impact on Mineral Metabolism, and Prospective Research Avenues

In recent decades, Picralima nitida has attracted interest for its various pharmacological characteristics. Initial biochemical investigations concentrated on the separation and characterization of bioactive alkaloids, including akuammine, akuammicine, and picraline, which are associated with significant antimalarial, analgesic, and anti-inflammatory properties (Shittu *et*

al., 2010; Rao and Satish, 2016). These experiments established a solid basis by demonstrating that extracts from different areas of the plant showed considerable biological activities both in vitro and in vivo.

Regarding mineral metabolism, comparatively few research have investigated the impact of P. nitida on serum calcium and uric acid levels. Initial research indicates that the phytochemicals of the plant may influence pathways associated with bone.

Mineralization and renal function. Some studies have observed changes in calcium homeostasis in animal models after the administration of P. nitida extracts, suggesting potential effects on osteoblast and osteoclast activity, as well as renal calcium regulation. There is emerging evidence that its bioactive components may impact purine metabolism, potentially affecting serum uric acid levels. Nevertheless, these findings frequently represent secondary observations in studies primarily aimed at evaluating antidiabetic or anti-inflammatory effects, resulting in a significant gap in our comprehension of the plant's contribution to mineral equilibrium.

Notwithstanding encouraging signs, considerable research deficiencies persist. Firstly, the absence of standardized extraction methods, dosing regimens, and controlled experimental designs complicates inter-study comparisons. Secondly, although many reports outline the general pharmacological effects of P. nitida, few have specifically examined its influence on mineral metabolism. The exact mechanisms—whether through direct interaction with osteocytes or regulation of renal transporters—remain predominantly conjectural. The long-term safety profiles and potential toxicity associated with mineral dysregulation have not been thoroughly assessed.

In light of these deficiencies, additional research is necessary. Subsequent research should focus on:

i) Clarifying Mechanisms: Perform comprehensive in vitro and in vivo studies to elucidate the molecular processes by which P. nitida affects calcium and uric acid balance.

ii) Assessing Long-Term Effects: Examine the enduring influence of P. nitida ingestion on skeletal health and renal function in animal models, followed by a progression to clinical studies.

iii) Isolating Active Constituents: Identify and describe particular bioactive chemicals responsible for any reported impacts on mineral metabolism, which may open the way for new treatment medicines targeting osteoporosis, hyperuricemia, or similar illnesses.

In summary, while earlier biochemical and pharmacological research has established the framework for understanding the wide medicinal effect of Picralima nitida, its specific effects on mineral metabolism continues to remain an open and intriguing subject for further experiments and studies.

2.6 Justifications for this study

Previous studies on Picralima nitida have emphasized its extensive pharmacological activity, including antimalarial, analgesic, and anti-inflammatory actions. However, many of these investigations have been restricted by non-standardized extraction procedures, irregular dosage regimens, and a significant focus on general bioactivity rather than on specific biochemical markers. In particular, the effects of Picralima nitida on mineral metabolism, specifically serum calcium and uric acid levels, which are the main points of this toxicology study, have not been comprehensively explored despite their clinical relevance in bone health, renal function, and metabolic control.

Investigating serum calcium and uric acid levels in an animal model, such as male Wistar albino rats, is significant for various reasons. First, these characteristics serve as sensitive indicators of abnormalities in mineral homeostasis, which can underlie illnesses including osteoporosis, nephrolithiasis, and gout. Second, animal models provide a controlled experimental setting that enables the accurate evaluation of biochemical changes generated by Picralima nitida extract.

Such a method helps to distinguish the plant's potential medicinal benefits and its toxicological dangers.

This study has been done to bridge the gap in the present medicinal literature by focusing primarily on the biochemical effects of Picralima nitida on serum calcium and uric acid levels. By standardizing extraction processes, dosing, and by adopting a well-characterized animal model, the study seeks to yield reliable and clinically relevant findings. These discoveries will not only support traditional medicinal claims but also lead to the development of plant-based therapeutic medicines targeting metabolic and renal illnesses, while finding safe dosages for this plant extract. Ultimately, the findings are intended to expand our understanding of the plant's pharmacological profile and promote future research on its possible incorporation into modern medicinal procedures.

2.7 Summary of Literature Review

The literature study offers a comprehensive examination of Picralima nitida as a medicinal plant with notable pharmacological potential, especially regarding mineral metabolism. The review commences with an examination of medicinal plant research, emphasizing the worldwide significance of plants as sources of innovative bioactive chemicals utilized in the treatment of diverse disorders. Comprehensive taxonomic and morphological descriptions of P. nitida highlight its unique identity within the Apocynaceae family, while its traditional applications in

African communities, spanning antimalarial and analgesic uses to remedies for gastrointestinal issues and reproductive health, are thoroughly examined.

The paper additionally analyzes the phytochemical composition of P. nitida, emphasizing its principal elements, including alkaloids, flavonoids, tannins, saponins, and glycosides. These chemicals support the plant's documented pharmacological characteristics, including anti-inflammatory, antidiabetic, and antibacterial actions. Despite extensive study on its overall bioactivity, a significant gap persists concerning its specific effects on mineral metabolism, especially serum calcium and uric acid concentrations.

It also examines essential principles of calcium and uric acid metabolism. It delineates the functions of total, ionized, and protein-bound calcium in physiological processes, along with the mechanisms governing uric acid generation from purine breakdown and its renal excretion. The interaction of dietary components, hormonal regulation (particularly parathyroid hormone, vitamin D, and calcitonin), and renal function in sustaining calcium and uric acid homeostasis is emphasized.

This literature emphasizes the need for targeted investigation on the biochemical impacts of Picralima nitida on mineral metabolism. Utilizing a standardized animal model, such as male Wistar albino rats, future research could address current gaps, substantiate traditional medicinal assertions, and potentially facilitate the creation of innovative, plant-derived therapeutic agents for issues pertaining to bone health, renal function, and metabolic disorders.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 EQUIPMENT AND APPARATUS:

a) Hand Gloves
b) Cotton wool
c) Chloroform
d) Dissecting set
e) Methylated spirit
f) Laboratory coat
g) Sample container (Lithium heparin)
h) Refrigerator (Hisense Refrigerator, Model REF302DR)
i) Weighing scale (NEXT-SHINE, china, POC-P225-CA1)
j) Scissors
k) Gavage
l) Syringe (5ml and 10ml)
m) Lancets
n) Plain containers
o) EDTA containers
p) Knife

3.2 MACHINES

The following machines were used for the study

a) Glucometer (ACCU-Check, United Kingdom)
b) Weighing scale, (NEXT-SHINE, China, Model: POC-P225-CA1)
c) Storage system (Haier Thermocool chest freezer (Model: HTF-319H))
d) Grinder (SY-18B Industrial Dry Herbs Grinder)
e) Freeze Dryer (Biobase BK-FD10s Freeze Dryer (Xi'an, China))

3.3 ANIMAL EXPERIMENTAL STUDY

Forty (40) physiologically normal male albino Wistar rats without any morbid disorders were used as the subjects of this study. These rats were obtained from the Department of Anatomy, University of Benin, Nigeria. Upon arrival, the rats were acclimatized for one week in a standard animal house under controlled environmental conditions, including a temperature of 22 ± 2°C, relative humidity of 50-60%, and a 12-hour light/dark cycle. The rats were housed in clean, well-ventilated plastic cages with soft bedding saw dusts, which was replaced regularly to maintain proper sanitation. They were fed with commercial chicken grower mash and had unrestricted access to clean water throughout the study period.

The rats were randomly divided into five groups based on their initial weight ranges: Group A (56.9g - 64.9g), Group B (66.4g - 72.7g), Group C (76.7g - 77.2g), Group D (77.2g - 80.76g), and Group E (82.9g - 90.93g). Each group consisted of eight rats, ensuring even distribution across the weight ranges. To facilitate easy identification, the rats were individually labeled using colored markers (GV) on specific body parts, including the hand, leg, back, head, and tail. Each rat was assigned a unique identification based on the part of the body where the color was applied.

During the experiment, the rats were closely monitored for any signs of distress, behavioral changes, or alterations in their physical condition. Their weights were recorded weekly to observe any significant changes that might indicate the effects of the experimental procedures. The rats were kept under optimal care, and all handling procedures were designed to minimize stress and maintain their well-being.

At the end of the experimental period, the rats were sacrificed humanely using chloroform anesthesia, following ethical guidelines for animal research. Blood samples were collected via cardiac puncture where necessary, Samples were carefully stored in labeled lithium heparin containers and kept in a refrigerator to preserve their integrity until analysis.

This study was conducted following the ethical standards outlined by the relevant ethics board of the University of Benin. Throughout the study, efforts were made to ensure the humane treatment of the animals and to adhere strictly to ethical practices in biomedical research.

3.4 AQUEOUS EXTRACT PREPARATION

The *Picralima nitida* fruits used in this study were commercially obtained from the Ediaken (Uselu) market area of Benin City, Nigeria. A total of forty (40) fruits of varying sizes, each weighing between 0.2 - 0.5 kg, were purchased. The fruits were then transported to the Department of Plant Biology and Biotechnology, University of Benin, where they were identified and authenticated at the department's herbarium.

Following proper identification, the fruits were taken to the Department of Medical Biochemistry for further processing. The fruits were thoroughly washed with clean water to remove dirt and debris, ensuring that they were free of contaminants. Using sharp stainless-steel knives, the fruits were carefully peeled to remove the rinds. The white pulp was cut into smaller pieces while ensuring that the seeds were completely removed to avoid any interference with the extraction process.

The cut pulp pieces were spread on clean trays and exposed to direct sunlight for **three (3) weeks** to achieve adequate drying. During this period, the pulp was regularly turned to ensure even drying and to prevent microbial growth. The drying process continued until the moisture content was visibly reduced, and the pulp had become significantly dry and brittle.

Once the drying process was complete, the dried pulp was mechanically ground into a fine powder using a SY-18B Industrial Dry Herbs Grinder. The grinding process was thorough to ensure that a smooth, fine powder was obtained, which would facilitate efficient extraction of bioactive compounds. The powdered pulp was then subjected to freeze-drying for an additional **one (1) week** to further reduce the moisture content and preserve the extract's integrity.

For the aqueous extraction, the powdered sample was soaked in distilled water at a ratio of 1:10 kg/L, following the method described by *De Campos et al.* (2020). The mixture was continuously stirred for **72 hours** to ensure maximum solubility of the bioactive components. After the soaking period, the slurry was filtered through several layers, including filter paper, cotton wool, and muslin cloth, to obtain a clear filtrate.

The clear filtrate was then freeze-dried using a Biobase BK-FD10s Freeze Dryer (Xi'an, China). This process involved sublimating the water content under low temperatures and pressure, resulting in a stable, dry extract. At the end of the freeze-drying process, a total yield of 637.6 g (12.6%) of the extract was obtained. The freeze-dried extract was stored in an airtight container at 40°C until further use to maintain its stability and prevent contamination.

Phytochemical analysis of the freeze-dried aqueous extract revealed the presence of significant bioactive compounds. The extract was found to contain 39.24 ± 0.45 mg GAE/g extract of total phenols and 24.39 ± 0.74 mg QE/g extract of flavonoids, as reported by *Ilenowa et al.* (2024). These bioactive components are known for their antioxidant properties and contribute to the therapeutic potential of the extract.

The preparation method ensured that the extract retained its bioactive properties while providing a safe and effective preparation for experimental use.

3.5 EXPERIMENTAL DESIGN

Forty (40) physiologically normal male albino Wistar rats, weighing between 56.9 g and 90.3 g, were used for this experimental study. The rats were obtained from the Department of Anatomy, University of Benin, Nigeria, and were acclimatized for three weeks. During the acclimatization period, the rats were housed in well-ventilated plastic cages, maintained under standard laboratory conditions with a 12-hour light/dark cycle, an ambient temperature of 25 ± 2°C, and relative humidity of 50-60%. The cages were regularly cleaned, and sawdust bedding was changed periodically to maintain hygiene.

The rats were fed commercial rat feed (Chicken Grower Feed, Top Feeds Ltd, Nigeria) with an approximate nutrient composition of 16% crude protein, 7% crude fiber, 3% fat, 1% calcium, and 0.45% phosphorus. Clean drinking water was provided ad libitum. The initial fasting blood glucose levels of the rats were measured using a glucometer, with values ranging from 41 mg/dL to 136 mg/dL.

After acclimatization, the rats were grouped into five experimental groups based on their weights:

Group A: 56.9 g - 64.9 g
Group B: 66.4 g - 72.7 g
Group C: 76.7 g - 77.2 g
Group D: 77.2 g - 80.76 g
Group E: 82.9 g - 90.93 g

On the fourth week, the rats were marked using Gentian Violet (GV) for proper identification. Markings were made on specific body parts (head, hand, leg, tail, back, hand and back, head and back, and plain) to facilitate monitoring of individual weights, glucose levels, and extract dosage calculations.

3.6 ADMINISTRATION OF AQUEOUS EXTRACT

The aqueous extract of *Picralima nitida* fruit pulp, prepared as previously described, was administered to the experimental groups. Group A served as the control and received only water and feed, providing a baseline for comparison to determine the effects of the extract.

Weight was used to allocate the animals into five groups of eight (8) each. The Oral gavage technique (Diehl *et al*., 2001; Turner *et al.,* 2011b) was used in this study to introduce the aqueous fruit pulp extract of P. nitida.

Groups B, C, D, and E received the extract via oral gavage in addition to feed and water, with extract dosages of 200 mg/kg, 250 mg/kg, 400 mg/kg, and 500 mg/kg, respectively.

Throughout the trial, no signs of poisoning and animal death were observed

3.7 DOSAGE CALCULATION

The dosage of the extract for each rat was calculated based on its body weight. An example of the calculation method is provided below:

For a rat in Group B weighing 154 g with a dosage of 200 mg/kg:

> 200 mg of extract is required for 1 kg of body weight.
> Since 1 kg = 1000 g, the calculation for the 154 g rat is as follows:

$$\text{Extract required}(X) = \frac{200\ \text{mg} \times 154\ \text{g}}{1000\ \text{g}}$$

$$X = 30.8\ \text{mg}$$

To convert the required extract amount into a solution volume for administration:

vert the required extract amount into a solution volume for administration:

1 g of extract is dissolved in 10 ml of water (since 1000 mg = 1 g and 1 g = 10 ml).

$$\text{Volume}(V) = \frac{30.8\ \text{mg} \times 10\ \text{ml}}{1000\ \text{mg}}$$

$$V = 0.30\ \text{ml}$$

This method was used for all rats in Groups B, C, D, and E, ensuring that each rat received the appropriate extract dosage according to its body weight.

3.8 WEIGHT AND BLOOD GLUCOSE EVALUATION

The rats were administered their respective doses of the extract daily for four weeks. Throughout this period, their body weights and glucose levels were monitored at the beginning and end of the study.
The glucometer reading were obtained after an overnight fast (ACCU-Check, United Kingdom). A lancet was used to obtain blood samples from the ends of the rats' tails, and the glucose levels were recorded.

The rats were weighed using High precision strain gauge sensor, Multi function scale (NEXT-SHINE, China, Model: POC-P225-CA1).

Note: Below are the various results of the each individual rats ranging from the initial weights, final weights, differences in weights. And also the values for initial glucose, final glucose and difference in glucose.

3.9 BLOOD COLLECTION

Good hygiene was maintained in the animal house, with regular cleaning of cages and replacement of sawdust bedding. The health and well-being of the rats were closely observed, and food and water were replenished daily.

On the 31st day, the rats fasted overnight before being re-weighed and sacrificed. Anesthesia was induced using chloroform to minimize pain and distress. The animals were euthanized in a chloroform chamber and were laterally opened at the abdominal cavity. Blood samples were collected through cardiac puncture and stored in different containers for specific analyses:

Plain bottles: For biochemical assays

EDTA bottles: For hematological studies

Lithium heparin bottles: To maintain plasma integrity

3.10 BIOCHEMICAL ASSAY

3.10.1 ESTIMATION OF URIC ACID

BY ENZYMATIC COLORIMETRIC METHOD

INTRODUCTION:

Uric acid and its salts are end products of purine metabolism. In gout, the most common complication of hyperuricemia (increased serum levels of uric acid) is the formation of monosodium urate crystals around the joints. Further elevated blood concentrations of uric acid are renal diseases with decreased excretion of waste products, starvation, drug abuse, increased alcohol consumption, as well as the use of certain medication. High uric acid levels also constitute an indirect risk factor for coronary heart disease. Hypouricemia is seldom observed and is associated with rare hereditary metabolic disorders.

PRINCIPLE:

Uricase acts on uric acid to produce allantoin, carbon dioxide, and hydrogen peroxide. The hydrogen peroxide, in the presence of peroxidase (POD), reacts with 4-aminoantipyrine (4-AA) and N-ethyl-N-(hydroxy-3-sulfopropyl)-m-toluidine (TOOS) to form a blue-violet dye (indamine), which is read at 520 nm.

Uric Acid + H_2O + O_2 >> Uricase> > Allatonin + CO_2 + H_2O

H_2O + TOOS + 4-AA >>POD>> Indamine + 3 H_2O

PROCEDURE

	BLANK	STANDARD	SAMPLE
REAGENT	1000 µL	1000 µL	1000 µL
STANDARD	-	10 µL	-
SAMPLE	-	-	10 µL

1. Mix and incubate for 5 minutes at **37°C**.
2. Measure the absorbance of the sample and standard against the reagent blank at **520 nm**.

CALCULATION: abs of test/abs of standard x conc of acid.

REFERENCE RANGE:

2.0 – 7.0 mg/dL

3.10.2 ESTIMATION OF CALCIUM

BY CALCIUM-O-CRESOLPHTHALEIN COMPLEXONE METHOD

INTRODUCTION:

Calcium is the mineral present in the largest amount in the body (1150g). Approximately 99% of total body calcium is deposited in the skeleton. A higher proportion of non-skeletal calcium is

present within cells than in extracellular fluids, and most of this intracellular calcium is bound to proteins in the cell membrane. Intracellular ionized calcium is physiologically active and functions as an intracellular messenger by binding to or being released from specific intracellular proteins, a process that changes protein conformations and hence its activity or function.

PRINCIPLE:

Calcium forms a purple-colored complex with ortho-cresolphthalein complexone in an alkaline medium. The inclusion of HCl helps to release calcium bound to proteins, and 8-hydroxyquinoline eliminates the interference by magnesium. 2-amino, 2-methyl, 1-propanol (AMP) provides the proper alkaline medium for the color reaction. The intensity of the color is measured at 540 nm.

	BLANK	STANDARD	SAMPLE
REAGENT	1000µL	1000µL	1000µL
STANDARD		25µL	
SAMPLE			25µL

1. Mix and incubate for **5 minutes at 37°C**.
2. Measure absorbance of the sample and standard against the reagent blank at **540 nm**.

CALCULATION: abs of test/abs of standard x conc of acid.

REFERENCE RANGE: 8.0 – 10.5 mg/dL

CHAPTER FOUR

RESULTS

4.1 URIC ACID LEVELS

The mean uric acid levels of the different groups are shown in Table 4.1. There was no statistically significant difference among the groups ($p > 0.05$), as determined by one-way ANOVA ($F = 1.46$, $p = 0.24$). Tukey's multiple comparison test also confirmed that no pairwise comparisons were significant.

Table 4.1: Uric Acid Levels (mg/dL) in Experimental Groups

Groups	**Uric Acid (mg/dL)**
Group 1 (Normal Control)	4.07±0.29[a]
Group 2 (200 mg/kg P. nitida extract)	4.55±0.33[a]
Group 3 (250 mg/kg P. nitida extract)	4.96±0.32[a]
Group 4(400 mg/kg P. nitida extract)	4.31±0.31[a]
Group 5(500 mg/kg P. nitida extract)	4.91±0.31[a]
F- value	1.46
P-value	0.24

Values are expressed as mean ± SEM.

Groups sharing the same superscript letter ([a]) indicate no significant difference ($p > 0.05$).

4.2 SERUM CALCIUM LEVELS

The mean serum calcium levels of the experimental groups are displayed in Table 4.2. Statistical analysis showed no significant difference between the groups ($F = 0.59$, $p = 0.67$), indicating that treatment did not significantly alter calcium levels.

Table 4.2: Serum Calcium Levels (mg/dL) in Experimental Groups

Groups	Serum Calcium (mg/dL)
Group 1 (Normal Control)	6.77±0.30[a]
Group 2 (200 mg/kg P. nitida extract)	6.36±0.33[a]
Group 3 (250 mg/kg P. nitida extract)	6.51±0.31[a]
Group 4(400 mg/kg P. nitida extract)	6.94±0.31[a]
Group 5(500 mg/kg P. nitida extract)	6.80±0.29[a]
F-value	0.59
P-value	0.67

Values are expressed as mean ± SEM.

Groups sharing the same superscript letter ([a]) indicate no significant difference ($p > 0.05$).

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

The results of this study indicate that oral administration of Picralima nitida extract at different doses did not significantly alter uric acid or serum calcium levels in the experimental groups. Statistical analysis using one-way ANOVA confirmed no significant difference in uric acid levels ($F = 1.46$, $p = 0.24$) or serum calcium levels ($F = 0.59$, $p = 0.67$), suggesting that P. nitida extract does not have a notable effect on these biochemical markers.

Serum Uric Levels

Uric acid is a key metabolic product that plays a role in both oxidative stress and purine metabolism (Maiuolo *et al.,* 2016). Elevated uric acid levels, known as hyperuricemia, are associated with gout, kidney dysfunction, and cardiovascular diseases (So and Thorens, 2010). In this study, all treatment groups showed uric acid levels within a similar range as the control group, with no statistically significant differences. This suggests that P. nitida extract does not promote hyperuricemia or hypouricemia. Previous studies have demonstrated that plant extracts with antioxidant properties can either reduce or stabilize uric acid levels depending on their bioactive components (Gowda *et al.,* 2010). The findings of this study align with research showing that some plant-based therapies do not necessarily modulate uric acid metabolism unless they contain specific uricostatic or uricosuric compounds (George *et al.*, 2019).

Despite the lack of significant changes, the slight numerical increase in uric acid levels observed

in some groups, particularly at 250 mg/kg and 500 mg/kg, may indicate a dose-dependent trend. However, since the differences were not statistically significant, this could be attributed to normal biological variations rather than a direct pharmacological effect of P. nitida extract. Further research with a larger sample size or longer duration may help clarify any potential long-term effects of the extract on uric acid metabolism.

Serum Calcium Levels

Calcium homeostasis is tightly regulated by the endocrine system, primarily through the actions of parathyroid hormone, calcitonin, and vitamin D (Peacock, 2010). Disruptions in calcium levels can lead to conditions such as osteoporosis, muscle dysfunction, and neurological impairments (Bronner and Pansu, 1999). In this study, no significant differences were observed in serum calcium levels between control and treatment groups, indicating that P. nitida extract does not interfere with calcium metabolism.

Previous research on medicinal plant extracts suggests that bioactive compounds such as alkaloids, flavonoids, and tannins may influence calcium homeostasis, either by enhancing absorption or affecting renal excretion (Gupta *et al.,* 2018). However, the lack of effect in this study suggests that P. nitida does not contain bioactive compounds that significantly impact calcium regulation, or its effects may be too subtle to detect within the experimental timeframe. Similar findings have been reported in studies on other plant extracts that did not influence serum calcium levels despite their purported medicinal properties (Adeyemi *et al,.*2020). Moreover, the slight numerical differences observed across groups could be attributed to normal physiological variations rather than a direct consequence of P. nitida administration. This is

further supported by the fact that all values remained within the expected physiological range for normal calcium levels. While the extract appears to have no detrimental effects on calcium homeostasis, further investigations could explore its potential interactions with other minerals or its effects over a prolonged duration.

5.2 Conclusion

This study demonstrates that administration of P. nitida extract at doses ranging from 200 mg/kg to 500 mg/kg does not significantly impact uric acid or serum calcium levels in the experimental groups. The results suggest that the extract is unlikely to contribute to hyperuricemia or hypocalcemia, supporting its potential safety with respect to these biochemical parameters. While this finding is promising, further studies are needed to examine other metabolic and physiological effects of P. nitida, particularly its impact on kidney function, liver enzymes, and oxidative stress markers. Future research should also consider longer treatment durations, larger sample sizes, and different experimental models to provide a more comprehensive understanding of the extract's pharmacological properties.

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