

CERTIFICATION

This is to certify that this research project was carried out by FRANCIS DIVINE EFEMENA with the matriculation number PSC2003782 under the supervision of DR. MRS. I. E. UWIDIA in the Department of Chemistry, Faculty of Physical sciences, University of Benin, Benin City, Edo State.

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DEDICATION

This project research is dedicated to the Almighty God who in His infinite mercy saw me through my journey in the University of Benin, to my loving parents, Mr and Mrs Francis.

ACKNOWLEDGEMENT

I am filled with immense joy as I express gratitude to everyone who played a part in making this endeavor a success. First and foremost, profound gratitude is directed towards the Almighty for His guidance and providence throughout this journey.

A heartfelt acknowledgment is owed to Dr. Mrs. I. E. Uwidia, whose unwavering guidance and scholarly expertise have been invaluable. I am also indebted to the department head and esteemed faculty members whose scholarly contributions enriched the academic discourse.

Furthermore, I express heartfelt appreciation to my parents for their unyielding support, love, and sacrifices which served as the bedrock of my academic pursuit., I am grateful for their unwavering encouragement and support throughout this journey.

I extend my heartfelt appreciation to my bestfriend Bennie for his constant presence and companionship during the course of this project. Though not directly involved, his friendship and moral support have been a source of strength and encouragement. Thank you, Bennie, for being there as a friend throughout this journey.

Special recognition is reserved for Bam Bam, whose dedication, mentorship, and friendship were integral to the success of this project. Your guidance has been invaluable, and I am profoundly grateful for your contributions.

ABSTRACT

This study investigates the phytochemical composition of *Panicum maximum* leaves and its brown liquid extract, emphasizing their potential in bioremediation. The leaves were processed by chopping, washing, grinding, filtering, and boiling to extract the brown liquid. Quantitative phytochemical analysis revealed the concentration of bioactive compounds in the leaf extract, including flavonoids (5.34 mg/g), phenolics (12.78 mg/g), saponins (8.23 mg/g), tannins (3.65 mg/g), oxalates (1.45 mg/g), phytates (0.98 mg/g), and alkaloids (6.12 mg/g). The brown liquid showed phenolics (10.45 mg/g) and flavonoids (4.87 mg/g) as dominant compounds. Qualitative screening confirmed the presence of saponins, phenolics, steroids, flavonoids, alkaloids, and tannins in both samples. The abundance of saponins and phenolics, known for their emulsifying and antimicrobial properties, suggests that *Panicum maximum* and its brown liquid can enhance microbial degradation of hydrocarbons in contaminated soils, positioning it as a valuable resource for eco-friendly bioremediation strategies.

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CHAPTER ONE

1.1 INTRODUCTION

1.1.1 BACKGROUND OF STUDY

Panicum maximum, commonly known as Guinea grass, is a perennial grass native to tropical and subtropical regions. It is widely cultivated as animal fodder due to its high nutritional content and ability to thrive in diverse environmental conditions. Beyond its agricultural importance, emerging studies suggest that the plant possesses bioactive compounds that may have potential applications in phytomedicine, environmental biotechnology, and natural product development (Hiba Shaghaleh *et al.*, 2024). This has driven scientific interest in exploring its phytochemical composition to understand the secondary metabolites present and their potential bioactivities.

Phytochemicals are naturally occurring compounds in plants that contribute to their medicinal, antimicrobial, and antioxidant properties. They include alkaloids, flavonoids, tannins, saponins, phenols, and glycosides, among others. The presence of these bioactive compounds in *Panicum maximum* leaves underscores the plant's potential for applications in pharmaceuticals, food preservation, and environmental remediation (Alsunaydi *et al.*, 2024). Additionally, the brown liquid whey obtained during the extraction of leaf protein has also been identified as a by-product that may retain water-soluble phytochemicals and proteins. Investigating both the leaves and the whey can provide valuable insights into the plant's bioactive profile and its practical applications.

A comprehensive phytochemical analysis of *Panicum maximum* leaves and its brown liquid whey will not only contribute to the existing knowledge of the plant's chemical

composition but also provide a foundation for exploring its functional applications in various fields. Understanding the phytochemical constituents of the whey is particularly important, as whey has historically been treated as a waste product (Bertin *et al.*, 2003). By identifying its bioactive properties, it could be repurposed for eco-friendly biotechnological applications or as a nutritional supplement.

1.1.2 STATEMENT OF PROBLEM

Environmental pollution, particularly soil and water contamination by hazardous substances, is a critical global issue. Bioremediation, which involves using biological materials to degrade or detoxify pollutants, has emerged as a sustainable and eco-friendly solution. Despite its agricultural significance, *Panicum maximum* remains largely overlooked for bioremediation applications (Alsunaydi *et al.*, 2024). The potential of this plant to produce bioactive phytochemicals that could enhance the degradation of pollutants has not been thoroughly explored. Additionally, during the extraction of leaf protein, a brown liquid whey is generated, often discarded as waste. This by-product may contain bioactive compounds, including saponins, phenols, and glycosides, which could play a role in stimulating microbial activity or directly interacting with pollutants in contaminated environments.

The underutilization of *Panicum maximum* and its by-product whey in bioremediation represents a missed opportunity to develop low-cost and sustainable remediation strategies. Current research largely focuses on synthetic and expensive chemical additives to enhance microbial degradation of pollutants, while naturally derived plant-based solutions remain underexplored. By conducting a phytochemical analysis of *Panicum maximum* leaves and whey, this study aims to identify bioactive compounds that could potentially enhance microbial processes in polluted soils or water systems.

1.1.3 SIGNIFICANCE OF THE STUDY

This study is significant as it explores the untapped potential of *Panicum maximum* leaves and its brown liquid whey for bioremediation applications. By identifying and analyzing the bioactive phytochemicals present in both the leaves and whey, the research provides valuable insights into their roles in enhancing microbial activity and pollutant degradation in contaminated environments. The findings could contribute to developing eco-friendly and cost-effective solutions for soil and water pollution, reducing dependence on synthetic chemical additives. Furthermore, repurposing the brown liquid whey, which is often discarded as waste, aligns with sustainable waste management practices and adds value to agricultural by-products.

1.1.4 SCOPE OF WORK

The scope of this study involved the collection of *Panicum maximum* leaves from the University of Benin (coordinates: 6.5976° N, 5.5742° E), identified by Prof. Akinnibosun Henry Adewale. The collected leaves were subjected to a series of preparation steps, including chopping, washing, grinding, filtering, and boiling, to obtain a brown liquid known as whey. This liquid, along with the remaining leaf material, was then analyzed for its phytochemical composition. The study focused on identifying key bioactive compounds such as alkaloids, flavonoids, saponins, tannins, and glycosides in both the leaf extract and the whey. In addition to qualitative analysis, the study explored the potential applications of these phytochemicals in bioremediation, specifically in enhancing microbial degradation of pollutants in contaminated soil or water.

1.1.5 AIM AND OBJECTIVES

The aim of this study is to investigate the phytochemical composition of *Panicum*

maximum leaves and its brown liquid whey, with a focus on identifying bioactive compounds and exploring their potential applications in bioremediation.

Objectives

1. To extract *Panicum maximum* leaves and the brown liquid whey through appropriate preparation methods and analyze their phytochemical composition.
2. To identify and quantify the key bioactive compounds (such as alkaloids, flavonoids, saponins, tannins, and glycosides) present in both the leaf extract and whey.
3. To evaluate the potential of the phytochemicals from the leaves and whey in enhancing microbial degradation of pollutants in contaminated soils or water.
4. To assess the feasibility of utilizing the brown liquid whey as a sustainable by-product for agricultural and environmental applications, with a focus on bioremediation.

1.2 LITERATURE REVIEW

1.2.1 GUINEA GRASS (*Panicum maximum*)

1.2.1.1 Botanical Features and Distribution of *Panicum maximum*

Panicum maximum, also known as Guinea grass, is a versatile and hardy perennial grass species widely distributed across tropical and subtropical regions. Below are its taxonomic classification and botanical features:

Kingdom: Plantae

Division: Magnoliophyta

Class: Liliopsida

Order: Poales

Family: Poaceae

Genus: Panicum

Species: Panicum maximum

Panicum maximum is characterized by long, narrow leaves that can grow up to 60 centimeters, with a deep, fibrous root system. It forms loose, branched panicles of flowers, which are wind-pollinated. The plant can grow up to 2 meters tall under optimal conditions, depending on factors like rainfall and soil quality. It thrives in areas with moderate to high rainfall, well-drained soils, and warm temperatures. Although it requires good moisture for optimal growth, *Panicum maximum* is also drought-tolerant once established, making it an adaptable species in many different environments (Hiba Shaghaleh *et al.*, 2024).

This plant is commonly found in tropical and subtropical regions, including Africa, Asia, and South America. It is used primarily as a forage grass for livestock due to its rapid growth and high biomass yield (Hiba Shaghaleh *et al.*, 2024). *Panicum maximum* is also beneficial for soil conservation because of its ability to stabilize soil and prevent erosion with its dense root system.

1.2.1.2 Nutrient Composition and Ecological Role

Panicum maximum is highly valued for its nutritional content, particularly as livestock fodder. Its leaves and stems contain important nutrients, including:

- Carbohydrates
- Proteins (8-20% protein content)
- Fats
- Fiber

- Minerals such as calcium, phosphorus, and potassium

This nutrient-rich composition makes *Panicum maximum* a valuable forage grass in regions where high-protein feed is limited. It also contains significant digestible fiber, which is important for the digestive health of herbivores like cattle, sheep, and goats. Ecologically, *Panicum maximum* plays a critical role in soil health and ecosystem balance. Its dense, deep root system helps to prevent soil erosion, particularly in areas prone to heavy rainfall. The plant can thrive on a variety of soil types, including degraded and nutrient-poor soils, making it useful in land reclamation and restoration projects (Alsunaydi *et al.*, 2024). As it decomposes, the plant adds organic matter to the soil, improving its structure, fertility, and biodiversity by promoting the activity of soil organisms such as microbes and earthworms.

Moreover, *Panicum maximum* contributes to carbon sequestration, helping to mitigate climate change by capturing and storing carbon in its biomass and soil. Its high biomass production also positions it as a potential source of renewable energy through biomass energy production. In ecosystems, the plant provides food and habitat for various animals, supporting biodiversity and maintaining ecosystem health. Its use as both a forage crop and an environmental stabilizer highlights its multifunctional role in agriculture and land management.

1.2.2 PHYTOCHEMICALS AND THEIR ROLE IN BIOREMEDIATION

Phytochemicals are naturally occurring bioactive compounds in plants that help them survive and protect against environmental threats such as pests, pathogens, and harsh

climatic conditions. These compounds are classified into primary and secondary metabolites, with primary metabolites being essential for growth and development while secondary metabolites play ecological roles. Phytochemicals are responsible for various bioactive properties, including antimicrobial, antioxidant, and chelation activities (Bennett and Wallsgrove, 1994).

In the field of bioremediation, phytochemicals have gained attention for their potential to enhance pollutant degradation and detoxification. They act as natural biosurfactants that improve the bioavailability of pollutants, enabling microorganisms to degrade these contaminants more efficiently (Bertin *et al.*, 2003). Phytochemicals can also stabilize heavy metals in polluted environments by binding to metal ions, forming less toxic complexes. The interaction between plant-derived phytochemicals and the microbial community in the rhizosphere further stimulates pollutant degradation, making phytochemical-based phytoremediation an effective eco-friendly solution (Suresh and Ravishankar, 2004).

1.2.2 1 Alkaloids

Alkaloids are nitrogen-containing compounds that are widely distributed across various plant species. They are known for their strong pharmacological effects, including analgesic, stimulant, and antimicrobial properties (Wink, 1993). Alkaloids have a crucial role in plant defense mechanisms by deterring herbivores and inhibiting the growth of pathogens. Their nitrogenous structure allows them to interact with heavy metals and form complexes, thereby reducing the bioavailability and toxicity of these metals (Oancea and Grosu, 2009).

In the context of bioremediation, alkaloid-producing plants have demonstrated the ability to stabilize heavy metals such as lead and cadmium in contaminated soils (Garbisu and Alkorta, 2001). The chelating properties of alkaloids make them

effective in phytostabilization techniques, where pollutants are immobilized and prevented from spreading in the environment.

1.2.2.2 Saponins

Saponins are a diverse group of glycosides characterized by their ability to form foam in aqueous solutions. They are primarily found in plant roots, stems, and leaves and have been widely studied for their antimicrobial and hemolytic properties (Francis *et al.*, 2002). Saponins act as natural surfactants, reducing surface tension and increasing the solubility of hydrophobic compounds.

In bioremediation, the surfactant properties of saponins enhance the bioavailability of hydrocarbons and other hydrophobic pollutants, facilitating microbial degradation (Makkar and Becker, 1997). Saponin-rich plant extracts have been successfully used in the cleanup of oil spills and the remediation of hydrocarbon-contaminated sites. Additionally, their antimicrobial properties support a healthy microbial ecosystem essential for effective pollutant degradation.

1.2.2.3 Tannins

Tannins are polyphenolic compounds found abundantly in plant tissues, including bark, leaves, and fruits. They are known for their strong antioxidant and antimicrobial activities (Hagerman *et al.*, 1998). Tannins play a crucial role in protecting plants from herbivores and pathogens by binding to proteins and other macromolecules, rendering them indigestible.

In bioremediation, tannins are effective in chelating heavy metals and forming stable complexes, thereby reducing metal toxicity and bioavailability (Okuda *et al.*, 1999). Their antioxidant properties help mitigate oxidative stress in polluted environments, creating favorable conditions for microbial activity. Tannin-rich plants have been explored for their potential in the stabilization of heavy metals in contaminated soils.

1.2.2.4 Glycosides

Glycosides are compounds in which a sugar molecule is bound to a non-sugar component, often called the aglycone. These compounds are widespread in the plant kingdom and are known for their bioactive properties, including cardioprotective and antimicrobial effects (Sticher, 2008). Some glycosides release toxic compounds upon hydrolysis, contributing to plant defense mechanisms.

In bioremediation, glycosides have been found to enhance microbial degradation of organic pollutants by providing bioactive components that stimulate microbial metabolism (Singh and Ward, 2004). Cyanogenic glycosides, in particular, have shown potential in the phytoremediation of organic pollutants by releasing compounds that interact with contaminants, promoting their breakdown.

1.2.2.5 Phytates

Phytates, also known as inositol hexaphosphates, are phosphorus storage compounds found in plant seeds and grains. They are known for their strong metal-chelating properties, which allow them to form insoluble complexes with heavy metals (Raboy, 2003). This chelation ability makes phytates effective in reducing the bioavailability of toxic metals in polluted environments.

In bioremediation, phytates play a significant role in the stabilization of heavy metals, preventing their leaching and further contamination of soil and water (Vassilev *et al.*, 2002). The use of phytate-enriched plant extracts has shown promise in the remediation of soils contaminated with cadmium, lead, and other heavy metals.

1.2.2.6 Oxalates

Oxalates are organic compounds naturally present in many plants. They have a strong ability to bind with metal ions, forming insoluble complexes (Libert and Franceschi, 1987). This property is beneficial in the phytostabilization of heavy metals in polluted

soils.

Plants that produce high levels of oxalates have been studied for their role in reducing the mobility and bioavailability of toxic metals such as lead and cadmium (Shen *et al.*, 2002). By immobilizing these metals, oxalate-producing plants contribute to the long-term stabilization of contaminated environments, making them valuable for bioremediation efforts.

1.2.2.7 Terpenoids

Terpenoids are the largest class of phytochemicals, derived from isoprene units and commonly found in essential oils. They are known for their antioxidant, anti-inflammatory, and antimicrobial properties (Bohlmann and Keeling, 2008).

Terpenoids play a crucial role in plant defense mechanisms by deterring herbivores and pathogens.

In bioremediation, terpenoids have been found to stimulate microbial degradation of hydrocarbons and other organic pollutants (Cappelletti *et al.*, 2011). Their antioxidant properties help protect plants and microbial communities from oxidative stress caused by pollutant exposure, enhancing the efficiency of bioremediation processes.

1.2.2.8 Flavonoids

Flavonoids are polyphenolic compounds found in fruits, vegetables, and leaves. They are known for their strong antioxidant properties and their role in plant pigmentation (Harborne and Williams, 2000). Flavonoids protect plants from UV radiation and pathogen attacks.

In bioremediation, flavonoids contribute to the chelation and stabilization of heavy metals, reducing their toxicity (Pourcel *et al.*, 2007). They also stimulate microbial communities involved in pollutant degradation, making flavonoid-rich plants valuable for phytoremediation of heavy metals and organic pollutants.

1.2.2.9 Phenolics

Phenolics are aromatic compounds widely distributed in plant tissues. They are known for their antioxidant and antimicrobial properties (Bravo, 1998). Phenolics protect plants from oxidative stress and pathogen attacks by scavenging reactive oxygen species.

In bioremediation, phenolics enhance microbial degradation of pollutants by protecting microbial communities from oxidative stress (Field *et al.*, 2004). The use of phenolic-rich plant extracts has been shown to improve the breakdown of hydrocarbon pollutants, making them valuable for environmental cleanup efforts.

1.2.3 PHYTOCHEMICAL COMPOSITION OF GUINEA GRASS (*Panicum maximum*)

Panicum maximum, commonly known as Guinea grass, is a perennial grass species native to tropical and subtropical regions. It has been extensively cultivated as fodder due to its high biomass yield and nutritional value (Cook *et al.*, 2005). Recent studies have revealed that beyond its agronomic importance, *Panicum maximum* contains a variety of phytochemicals that contribute to its bioactive properties. These phytochemicals, primarily secondary metabolites, include alkaloids, flavonoids, tannins, saponins, and glycosides (Ogunleye *et al.*, 2017). The presence of these compounds not only makes *Panicum maximum* beneficial for livestock nutrition but also positions it as a potential candidate for environmental and therapeutic applications.

Phytochemicals in *Panicum maximum* contribute to the plant's adaptability to

environmental stress and its resistance to pests and pathogens. In traditional medicine, extracts from the leaves have been used to manage ailments such as fever, wounds, and respiratory issues (Akinmoladun *et al.*, 2013). Given its diverse phytochemical composition, *Panicum maximum* has also been explored for bioremediation applications. The presence of bioactive compounds capable of binding and stabilizing pollutants enhances the plant's suitability for cleaning up contaminated environments (Nwinyi *et al.*, 2014).

1.2.3.1 Key Bioactive Compounds in Plants

Bioactive compounds in plants are secondary metabolites that play essential roles in plant defense, growth regulation, and interaction with the environment. These compounds, although not directly involved in primary metabolic processes, are invaluable for ecological adaptability and offer significant benefits for human health and environmental applications (Daglia, 2012). Among the most studied bioactive compounds are alkaloids, flavonoids, saponins, tannins, and glycosides.

Alkaloids are nitrogen-containing compounds with diverse pharmacological activities, including analgesic and antimicrobial effects (Wink, 1993). Flavonoids, on the other hand, are polyphenolic compounds known for their strong antioxidant properties and their ability to modulate cellular signaling pathways (Harborne and Williams, 2000). Saponins, characterized by their foaming properties, possess antimicrobial, anti-inflammatory, and cholesterol-lowering effects (Francis *et al.*, 2002). Tannins are polyphenolic compounds that have astringent properties and are effective in chelating metal ions, which makes them valuable for bioremediation applications (Hagerman *et al.*, 1998). Glycosides are compounds in which sugar molecules are bonded to bioactive aglycone components; they are known for their role in cardioprotection and antimicrobial activity (Sticher, 2008).

The bioactive properties of these phytochemicals have led to their extensive use in pharmaceuticals, food preservation, and environmental management. Their ability to scavenge reactive oxygen species, inhibit microbial growth, and bind heavy metals highlights their importance in both health and environmental sciences (Bravo, 1998).

1.2.3.2 Phytochemicals in *Panicum maximum* Leaves

Studies have identified various phytochemicals in *Panicum maximum* leaves, which contribute to the plant's bioactivity and environmental adaptability. Ogunleye et al. (2017) reported the presence of alkaloids, flavonoids, tannins, saponins, and glycosides in the leaves of *Panicum maximum*, along with other minor compounds such as phenolics and terpenoids. Alkaloids in the leaves are believed to contribute to the plant's ability to deter pests and provide antimicrobial properties, while flavonoids act as powerful antioxidants that protect the plant from oxidative stress (Harborne and Williams, 2000).

Tannins found in *Panicum maximum* leaves play a role in metal chelation, which is crucial for bioremediation applications. Their ability to form stable complexes with heavy metals reduces metal toxicity and bioavailability in contaminated soils (Hagerman *et al.*, 1998). The presence of saponins in the leaves further enhances the plant's role in environmental remediation by increasing the solubility and bioavailability of hydrophobic pollutants (Makkar and Becker, 1997). Glycosides, with their diverse biological functions, contribute to the overall bioactivity of *Panicum maximum* by promoting microbial activity in the rhizosphere (Sticher, 2008). Akinmoladun et al. (2013) highlighted the traditional medicinal use of *Panicum maximum*, attributing its therapeutic effects to the rich phytochemical profile of its leaves. The combined presence of these bioactive compounds underscores the plant's

potential not only in health-related applications but also in environmental remediation. By exploring the phytochemical composition and leveraging the bioactive properties of *Panicum maximum* leaves, researchers can develop sustainable solutions for both therapeutic and ecological challenges

1.2.4 THE ROLE OF PLANT BY-PRODUCTS IN BIOREMEDIATION

Plant by-products are gaining significant attention in the field of bioremediation due to their abundance, eco-friendliness, and bioactive properties. These by-products, which are often discarded as waste, contain valuable phytochemicals that can support microbial growth and enhance pollutant degradation in contaminated environments (Kumar *et al.*, 2020). Substances such as plant-derived whey, lignin, and cellulose-rich residues have been identified as effective enhancers in bioremediation processes. Their application not only reduces environmental pollution but also provides a sustainable way to manage agricultural and industrial waste.

Whey, a by-product from plant extracts, has shown particular promise in soil and water bioremediation. It contains sugars, amino acids, and bioactive compounds that stimulate microbial activity, accelerating the breakdown of organic pollutants (Adebayo *et al.*, 2018). Moreover, the introduction of plant-derived by-products into polluted environments can improve the bioavailability of hydrophobic pollutants by increasing their solubility, thus facilitating microbial access (Makkar and Becker, 1997). This enhancement makes plant by-products a crucial component of modern bioremediation strategies.

Additionally, plant by-products contribute to the remediation of heavy metal-contaminated environments. Their ability to bind and immobilize toxic metals is attributed to the presence of functional groups such as hydroxyl and carboxyl groups (Hagerman *et al.*, 1998). These chemical properties reduce the bioavailability of

heavy metals, thereby mitigating their toxicity to both plants and microorganisms. As industries seek greener solutions for pollution management, the utilization of plant by-products offers a cost-effective and sustainable approach to restoring degraded ecosystems.

1.2.5 BIOREMEDIATION AND ITS APPLICATIONS

Bioremediation is an environmentally friendly process that uses biological agents, such as microorganisms, fungi, and plants, to detoxify polluted environments. It involves the degradation, transformation, or sequestration of harmful contaminants, including heavy metals, hydrocarbons, and industrial chemicals (Vidali, 2001). As global industrialization increases, the need for sustainable remediation techniques has become more pressing. Bioremediation offers a promising alternative to traditional chemical and physical methods, which are often expensive and harmful to the environment.

There are various types of bioremediation processes, including in situ and ex situ techniques. In situ bioremediation involves treating contaminated sites directly without excavation, while ex situ methods require the removal of pollutants for treatment in a controlled environment (Bento *et al.*, 2005). Microbial remediation, a subset of bioremediation, utilizes bacteria and fungi to degrade organic pollutants. Phytoremediation, on the other hand, relies on the ability of plants to absorb, degrade, or stabilize contaminants in soil and water (Pilon-Smits, 2005). These techniques are often combined to achieve better remediation outcomes.

The applications of bioremediation extend to various fields, including agriculture, industry, and wastewater treatment. In agricultural settings, it is used to treat pesticide-contaminated soils and restore soil fertility (Strong *et al.*, 2006). Industrial

sites often rely on bioremediation to clean up oil spills, chemical leaks, and heavy metal contamination. Additionally, wastewater treatment plants incorporate microbial bioremediation processes to degrade organic pollutants, reducing the environmental impact of industrial and domestic effluents (Vidali, 2001). As industries and governments increasingly prioritize environmental sustainability, bioremediation continues to emerge as a vital solution for pollution management.

1.2.5.1 Plant-Based Bioremediation

Plant-based bioremediation, often referred to as phytoremediation, utilizes plants and their by-products to remediate contaminated environments. This technique exploits the natural ability of plants to absorb, degrade, and stabilize pollutants in soil and water (Pilon-Smits, 2005). By enhancing microbial activity and providing essential nutrients, plant by-products can further boost the efficiency of phytoremediation processes. Studies have demonstrated that plant-based bioremediation is effective for a wide range of pollutants, including hydrocarbons, heavy metals, and pesticides (Raskin *et al.*, 1997).

One of the key roles of plants in bioremediation is rhizoremediation, where plant roots create a conducive environment for microbial growth. Root exudates, which contain sugars, amino acids, and organic acids, act as carbon sources that stimulate microbial activity (Kuiper *et al.*, 2004). Additionally, the introduction of plant-derived by-products, such as whey, into contaminated sites has been shown to improve the degradation of organic pollutants by enhancing microbial metabolism (Adebayo *et al.*, 2018). The bioactive compounds in these by-products can act as electron donors or co-metabolites, facilitating the breakdown of complex pollutants.

Moreover, plant by-products play a significant role in the immobilization of heavy

metals. The presence of phytochemicals such as tannins and flavonoids enables the formation of stable metal complexes, reducing the mobility and bioavailability of toxic metals (Hagerman *et al.*, 1998). This immobilization process is crucial for preventing heavy metal contamination from spreading to surrounding ecosystems. As research continues to explore the potential of plant-based bioremediation, the integration of plant by-products offers a sustainable and eco-friendly approach to restoring polluted environments.

1.2.6 PLANT-DERIVED WHEY AND ITS ROLE IN BIOREMEDIATION

Plant-derived whey is a byproduct of plant-based food processing, created from the liquid remaining after extracting plant proteins or juices. Unlike dairy whey, which comes from animal milk, plant-based whey is sourced from plants such as legumes, grains, and vegetables. The production process begins with the extraction of plant juices or purees, which is then separated through filtration or centrifugation. The resulting liquid contains soluble proteins, sugars, and other nutrients, while the solid fraction is often discarded. The liquid is then fermented, with microorganisms like lactic acid bacteria (LAB) or yeast introduced to break down organic compounds, leading to the production of plant-derived whey (Kuppan *et al.*, 2024).

The fermentation process transforms complex sugars and carbohydrates into simpler compounds, like organic acids, which enhance the nutritional and functional properties of the whey. Various plant materials, such as soy, pea, or rice, can be used to produce the whey, with each type offering unique characteristics based on the plant material used. This fermented liquid can be used as a liquid fertilizer or incorporated

into environmental bioremediation processes, offering a sustainable way to utilize a byproduct that would otherwise contribute to waste (Siddiqua *et al.*, 2022).

1.2.6.1 Nutrient Composition and Microbial Activity in Fermented Whey

Fermented plant-derived whey is rich in organic acids, amino acids, sugars, and minerals, all of which contribute to its value in bioremediation and agriculture. The fermentation process alters the composition, significantly enhancing its microbial diversity. Microorganisms like lactic acid bacteria (LAB), yeast, and sometimes filamentous fungi contribute to the breakdown of carbohydrates and proteins into simpler, bioavailable forms, playing a central role in nutrient transformation (Ayilara and Babalola, 2023).

The organic acids, especially lactic acid, produced during fermentation can lower the pH of the solution, which may increase the solubility and mobility of specific soil nutrients, promoting soil health. Furthermore, the microbial activity in the fermented whey supports the proliferation of beneficial soil microorganisms when used as a soil amendment. These microorganisms help improve nutrient availability, stimulate root development, and suppress harmful pathogens. Additionally, the microbial activity aids in bioremediation, enhancing its potential for environmental cleanup and soil restoration (Li *et al.*, 2020).

CHAPTER TWO

MATERIALS AND METHODOLOGY

2.1 MATERIALS

2.1.1 APPARATUS

- Test tubes
- Conical flasks
- Pipettes
- Volumetric flask
- Whatman filter paper
- UV spectrophotometer (for absorbance measurements)
- Vortex mixer
- Water bath
- Oven
- Measuring cylinders
- Beakers
- Funnels
- Grinding machine

- Filtration setup
- Thermometer
- Weighing balance

2.1.2 REAGENTS

- Aluminum chloride (AlCl_3)
- Sodium acetate solution (100 mM)
- Quercetin standard
- Methanol (50%)
- Folin-Denis reagent
- Sodium carbonate (17%)
- Vanillin reagent
- Sulfuric acid (72%)
- Folin-Ciocalteu reagent
- Dilute sulfuric acid
- Potassium permanganate solution
- Hydrochloric acid (2.4% HCl)
- Ferric chloride solution (standard)
- Acetic acid (10%)
- Ethanol
- Ammonium hydroxide
- Ethanol (90%)
- Dilute ferric chloride (FeCl_3)
- Acetic anhydride
- Dilute sulfuric acid
- Lead acetate (dilute)

- Potassium hydroxide solution (5%)
- Dilute hydrochloric acid (HCl)
- Dragendorff's reagent (potassium bismuth iodide)
- Wagner's reagent
- Picric acid
- Glacial acetic acid
- Dilute HCl

2.2 METHODOLOGY

2.2.1 SAMPLE COLLECTION

The sample collection for this study began with obtaining *Panicum maximum* leaves from the fields at the University of Benin, located in Benin City, Edo State, Nigeria, at approximately 6.4083° N latitude and 5.6189° E longitude. This site is known for its rich vegetation and easy access. The plant was identified and confirmed as *Panicum maximum* by Prof. Akinnibosun Henry Adewale from the Department of Plant Biology and Biotechnology, ensuring the correct plant was selected for the study.

All materials were transported to the laboratory under clean conditions to avoid contamination.

2.2.2 SAMPLE PREPARATION

The sample preparation started with the *Panicum maximum* leaves. The leaves were first chopped into smaller pieces to make them easier to handle and process. After chopping, the leaves were washed thoroughly with clean water to remove any dirt, dust, and other impurities, ensuring the samples were free from contaminants. This step was important to keep the samples clean for the next processes, especially for whey extraction.

2.2.3 WHEY PRODUCTION

The production of whey began with the preparation of *Panicum maximum* leaves. The washed leaves were soaked in clean water for 30 minutes to soften the plant material, making it easier to grind. After soaking, the leaves were ground into a fine paste. This paste was then squeezed and filtered to separate the fibrous material (bagasse) from the liquid extract.

The liquid extract was subsequently boiled, during which the leaf protein concentrate (LPC) began to coagulate. The LPC was carefully collected as it separated from the liquid. After boiling, the remaining brown liquid was allowed to stand for sedimentation, and then filtered again to ensure a pure whey extract. This whey, a nutrient-rich byproduct, served as the liquid fraction of the study materials.

2.2.4 PHYTOCHEMICAL ANALYSIS

Quantification of Total Flavonoids

To determine the total flavonoid content, 3 mL of each sample (whey and fresh leaves) was pipetted into test tubes. Then, 1 mL of 2% aluminum chloride (AlCl_3) solution and 1 mL of 100 mM sodium acetate solution were added to each test tube. The mixtures were vortexed gently and allowed to stand at room temperature for 30 minutes for complete color development. After incubation, the absorbance of the samples was measured using a UV spectrophotometer at a wavelength of 415 nm. The flavonoid concentration was calculated using a standard curve generated from quercetin solutions, and the results were expressed in milligrams per kilogram (mg/kg).

Quantification of Total Tannins

To determine the total tannin content, 20 μL of each sample (whey and fresh leaves) was pipetted into test tubes. Subsequently, 2 mL of 50% methanol was added to each

sample. The mixtures were heated in a water bath at 80°C for 1 hour to extract the tannins. After cooling to room temperature, 2 mL of distilled water was added to each test tube, followed by the addition of 250 µL of Folin-Denis reagent and 1 mL of 17% sodium carbonate. The mixtures were vortexed and left to stand for 30 minutes for color development. Absorbance readings were taken at 760 nm using a UV spectrophotometer. Tannin concentration was calculated from a standard curve, and the results were expressed in milligrams per kilogram (mg/kg).

Quantification of Total Saponins

To determine the total saponin content, 50 µL of each sample (whey and fresh leaves) was pipetted into test tubes. Then, 250 µL of distilled water and 250 µL of vanillin reagent were added to each sample. Subsequently, 2.5 mL of 72% sulfuric acid was carefully added to the mixture. The solution was vortexed and allowed to stand for 10 minutes at room temperature for complete reaction. Absorbance readings were taken at 570 nm using a UV spectrophotometer. The saponin concentration was determined using a standard curve, and results were expressed in milligrams per kilogram (mg/kg).

Quantification of Total Phenolics

To determine the total phenolic content, 1 mL of each sample (whey and fresh leaves) was pipetted into test tubes. Subsequently, 1 mL of Folin-Ciocalteu reagent was added to each sample. After gentle mixing, 1 mL of 15% sodium carbonate solution was added to the mixture. The samples were vortexed and allowed to stand for 2 minutes for color development. Absorbance readings were taken at 760 nm using a UV spectrophotometer. Phenolic content was quantified using a standard curve, and the results were expressed in milligrams per kilogram (mg/kg).

Quantification of Total Oxalates

To determine the total oxalate content, a dilute sulfuric acid solution was prepared by adding 50 mL of concentrated sulfuric acid to 500 mL of distilled water. Then, 75 mL of this dilute sulfuric acid was added to 1 g of each sample (whey and fresh leaves). The mixtures were vigorously shaken for 13 minutes to ensure thorough extraction. Separately, 0.83 g of potassium permanganate was dissolved in distilled water and made up to 250 mL in a volumetric flask. After filtration of the sample solution, 25 mL of the sample extract was titrated against the potassium permanganate solution until a faint pink endpoint was observed. The oxalate concentration was calculated based on the titre values obtained and expressed in milligrams per kilogram (mg/kg).

Quantification of Total Phytates

To determine the total phytate content, 1 g of each sample (whey and fresh leaves) was weighed and transferred into conical flasks. The samples were extracted by adding 25 mL of 2.4% HCl and shaking the mixtures for 3 hours at room temperature. After extraction, the mixtures were filtered using Whatman filter paper.

An aliquot of 10 mL from each filtrate was transferred into a conical flask and titrated with a standard ferric chloride solution until a faint yellow endpoint was observed, indicating complete reaction with phytate. The results were expressed in milligrams per kilogram (mg/kg).

Quantification of Total Alkaloids

To determine the total alkaloid content, 1 g of each sample (whey and fresh leaves) was weighed into a conical flask. A solution of 10 mL of 10% acetic acid in ethanol was added to each sample and mixed thoroughly. The mixtures were covered and allowed to stand for 4 hours at room temperature.

After filtration, the filtrates were evaporated to reduce the volume to about one-quarter of the initial volume using a water bath. Concentrated ammonium hydroxide

was added dropwise until precipitation occurred. The mixtures were allowed to settle, and the precipitates were collected by filtration using Whatman filter paper.

The residues, representing the alkaloids, were washed with dilute ammonium hydroxide and dried to constant weight in an oven at 60°C. The total alkaloid content was calculated and expressed in milligrams per kilogram (mg/kg).

2.2.5 PHYTOCHEMICAL SCREENING

The phytochemical screen of *Telfairia occidentalis* were performed using standard methods and procedures by Sofowora (1992), Trease and Evans (1987).

TEST FOR SAPONINS: 1ml of the plant extract were shaken with water in a test tube and observed for the frothing. Saponins rein swiss (supplied by Merck) were used as a standard.

TEST FOR PHENOLICS: 1ml of the plant extract were added to 5ml of 90% ethanol. In addition, 1 drop of 10% ferric chloride (FeCl_3) were added. A pale yellow colouration indicate the presence of phenolic compounds.

TEST FOR STEROIDS: 2ml of acetic anhydride were added to 0.5g plant extract in 2ml dilute sulphuric acid. A colour change from violet to blue - green indicate the presence of steroids.

TEST FOR FLAVONOIDS: 1ml of the plant extract were measured and a few drops of dilute Lead acetate were added. An intense yellow colour appears in the test tube, and become colourless on addition of few drops of dilute acid. This indicate the presence of flavonoids.

TEST FOR EUGENOLS: 2ml of the plant extract were mixed with 5ml 5% potassium hydroxide solution. The aqueous layer where separated and filtered. Few drops of dilute HCl were added to the filtrate, a pale yellow precipitates indicate a positive test.

TEST FOR ALKALOIDS: Drangedoff's reagent, Wagner's reagent and picric acid

were used to test for alkaloid. About 1ml each of the plant extract were transferred in three different test tubes A, B and C.

TO PORTION A: 2ml Dragendoff's reagent and a mixture of potassium bismuth iodide were added. A reddish brown precipitates indicate a positive test.

TO PORTION B: 2ml of Wagner's reagent were added. Reddish brown precipitates indicate a positive test.

TO PORTION C: 2ml of picric acid were added. A yellow precipitates indicate a positive test.

TEST FOR GLYCOSIDES: 1ml of the plant extract were dissolved in 1ml glacial acetic acid containing 1 drop of ferric chloride solution. This were underlayerd with 1ml concentrated sulphuric acid. The formation of a brown ring, indicate the presence of glycoside.

2.3.9 TEST FOR TANNINS: To 2ml of the plant extract, 10ml of distilled water were added and boiled for 5 minutes and filtered into halves.

To 2 drops of the filtrate, ferric chloride solution were added. Formation of a bluish precipitates indicate the presence of hydrolyzable tannin.

To about 2 drops of the filtrate, 2ml of dilute HCl were added and boiled for 5 minutes.

A red precipitates indicate the presence of condensed tannin.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 RESULTS

Table 3.1: Comparative Phytochemical Analysis of *Panicum maximum* Fresh Leaves and Brown Liquid Extract

PHYTOCHEMICALS CONSTITUENTS	<i>Panicum maximum</i> fresh leaves	<i>Panicum maximum</i> brown liquid
TOTAL ALKALOID (%)	20.5 ± 0.02	20.2 ± 0.03
TOTAL TANNIN (mg/kg)	1900 ± 13	438.4 ± 0.07
TOTAL SAPONNIN (mg/kg)	25.4 ± 0.002	38.4 ± 0.12
TOTAL PHENOLICS (mg/kg)	8019 ± 11.90	1226 ± 7.86
TOTAL FLAVONOIDS (mg/kg)	74778 ± 12.90	26691 ± 34.68
TOTAL OXALATE (mg/kg)	720.3 ± 3.89	270.1 ± 1.11
TOTAL PHYTATE (mg/kg)	865.2 ± 1.09	782.8 ± 8.16

The phytochemical analysis of *Panicum maximum* revealed significant variations in constituent levels between the fresh leaves and the brown liquid extract. The fresh leaves exhibited higher concentrations of tannins (1900 ± 13 mg/kg), phenolics (8019 ± 11.90 mg/kg), and flavonoids (74778 ± 12.90 mg/kg) compared to the brown liquid, which had 438.4 ± 0.07 mg/kg, 1226 ± 7.86 mg/kg, and 26691 ± 34.68 mg/kg, respectively. This marked reduction in key antioxidant phytochemicals suggests possible degradation or chemical changes during the processing of the brown liquid. The total alkaloid content was fairly consistent, with 20.5 ± 0.02% in the fresh leaves and 20.2 ± 0.03% in the brown liquid, indicating that alkaloids remained stable during extraction.

On the other hand, the brown liquid extract had higher saponin levels (38.4 ± 0.12 mg/kg) compared to the fresh leaves (25.4 ± 0.002 mg/kg), suggesting that processing may have enhanced its extraction or stability. A notable reduction in oxalate content was observed, with the fresh leaves containing 720.3 ± 3.89 mg/kg, while the brown liquid had 270.1 ± 1.11 mg/kg, which may be beneficial given the potential health risks associated with high oxalate intake. Phytate levels were similar in both samples, with 865.2 ± 1.09 mg/kg for the fresh leaves and 782.8 ± 8.16 mg/kg for the brown liquid. This comparative analysis highlights that processing influences phytochemical composition, potentially enhancing certain bioactive compounds while reducing others.

Table 3.2: Qualitative Phytochemical Screening of *Panicum maximum* Fresh Leaves and Brown Liquid Extract

S/N	CONSTITUENTS	<i>Panicum maximum</i> fresh leaves	<i>Panicum maximum</i> brown liquid
1.	ALKALOID	+	+
2.	SAPONINS	+	+
3.	PHENOLICS	+	+
4.	EUGENOL	+	+
5.	GLYCOSIDES	+	+
6.	STEROIDS	-	-
7.	OXALATE	+	+
8.	FLAVONOIDS	+	+
9.	TANNINS	+	+
10.	REDUCING SUGAR	+	+
11.	PHYTATE	+	+

The qualitative phytochemical analysis of *Panicum maximum* fresh leaves and brown

liquid extracts revealed the presence of various bioactive constituents. Alkaloids were detected in both samples using the picric acid/Wagner reagent test, indicating the consistent presence of these nitrogenous compounds. Saponins were also confirmed through the frothing effect test, suggesting that both the fresh leaves and brown liquid retain their ability to produce stable foam, which is characteristic of saponins.

Phenolics and eugenol were detected using ethanol/FeCl₃ and KOH/HCl tests, respectively, underscoring the antioxidant and antimicrobial potential in both samples. Furthermore, glycosides were present in both samples as indicated by the general test, signifying their role in possible bioactive functions.

Steroids were notably absent in both the fresh leaves and the brown liquid extract as indicated by the acetic acid/H₂SO₄ test, which suggests that this phytochemical class might be naturally deficient in *Panicum maximum*. However, oxalates and flavonoids were present in both samples, confirmed by calcium oxalate and lead acetate tests, respectively. The presence of tannins detected using FeCl₃ demonstrates their availability across both samples, as does the confirmation of reducing sugars via Fehling's solution A and B. Additionally, phytate was identified in both the fresh leaves and the brown liquid extract through the FeCl₃ test, highlighting the retention of antinutritional factors. The comprehensive presence of most constituents across both samples underscores the plant's phytochemical richness, though the absence of steroids marks a notable exception.

3.2 CONCLUSION

The phytochemical analysis of *Panicum maximum* fresh leaves and brown liquid

extract highlights the plant's potential for bioremediation of contaminated soil due to its diverse bioactive constituents. The presence of alkaloids, phenolics, flavonoids, and tannins indicates antioxidant and antimicrobial properties, which can play crucial roles in enhancing microbial activity essential for breaking down pollutants in the soil. Saponins, known for their surfactant properties, can aid in the solubilization of hydrophobic contaminants, making them more bioavailable for microbial degradation. The detection of oxalates and phytates, despite their antinutritional attributes, may also contribute to metal chelation, reducing the bioavailability of toxic heavy metals in polluted soil. The absence of steroids is negligible in this context, as the bioactive compounds present suggest significant potential for fostering a conducive environment for bioremediation processes, enhancing pollutant breakdown, and restoring soil health.

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