

**ANTIBACTERIAL ACTIVITY OF MUSA SPP (BANANA STEM)
EXTRACT AGAINST SELECTED CLINICAL ISOLATE**

BY

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DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY.

NOVEMBER, 2025

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**A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF
MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF
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REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.
Hons) IN MICROBIOLOGY**

NOVEMBER, 2025

CERTIFICATION

This is to certify that this project work was carried out by **APEJI JOSEPHINE ONIVENU** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

.....
PROF. (Mrs) I.S OBUEKWE
(Project Supervisor)

.....
DATE

.....
PROF. E.O. IGBINOSA
(Head of Department)

.....
DATE

DEDICATION

This project work is dedicated to God for His faithfulness, my lovely mother, Aunty Glad and Pastor Aliu Joshua for their love.

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ABSTRACT

Increase in antibiotic resistance of infectious bacteria to conventional antibiotics has emerged as a threat to public health and this has led to the growing interest rate in plants as herbal sources of antimicrobial agents. Among such plants, *Musa spp* (banana stem) has been widely used in traditional medicine for its therapeutic properties. This study explored the antibacterial activity of *Musa spp* stem extracts against selected bacteria.

Fresh stem of *Musa spp* were cut, cleaned, air-dried and pulverized. The powdered sample was soaked using ethanol and distilled water and extracted to obtain the ethanolic and aqueous extracts. The antibacterial activity of the extracts were assessed against selected bacteria (*Escherechia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*), obtained from the University of Benin Teaching Hospital using Agar well diffusion method.

The phytochemical screening of the aqueous and ethanol extracts, revealed the presence of tannins, flavonoids, terpenoids, steroids and saponins at varying degrees with the ethanolic extract showing stronger phytochemical activity, compared to the aqueous extract. The bacterial evaluation of the extract revealed that both extracts, showed dose dependent antibacterial activity with inhibition zones, decreasing at lower concentrations. For *Staphylococcus aureus*, ethanolic extract showed moderate inhibition at 11.00mm and no inhibition for aqueous extract. *Pseudomonas aeruginosa* showed moderate inhibition for both the aqueous- 11.00mm and ethanol- 10.00mm extract at 50% concentration and 10.00mm at 25% aqueous concentration. *Escherechia coli* showed both ethanolic and aqueous inhibition at only 50% concentration at 11.00mm and 9.00mm respectively. The Minimum Inhibitory Concentration of both aqueous and ethanolic extracts showed that *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherechia coli*, showed bacteriostatic activity at 50mg/ml for the ethanolic extract while only *P. aeruginosa* inhibited at 50mg/ml for the aqueous extract. The Minimum Bactericidal Concentration showed that only ethanolic extract at 50mg/ml showed bacteriacidal effect on *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherechia coli* showed bacteriostatic activity. This study further emphasizes the antibacterial potential of *Musa spp* stem extracts against selected bacteria. This supports its basis as an alternative to synthetic antibiotics as a medicinal plant.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study.

Bacteria are the most prevalent form of microorganisms (Soni *et al.*, 2024). Some of these bacteria are pathogenic for human health, causing infectious diseases. c Increase in antibiotic resistance among various infectious bacteria to conventional antibiotic drugs has emerged as a threat to public health all over the world at a terrifying rate (Tanwar *et al.*, 2014). As a result of this, there is need for more effective antibiotics agents and this has led to the growing rate of interest in herbal sources of antibiotics (Renuka ., 2025). India is the largest producer of medicinal plants and has a rich heritage of knowledge on herbal products derived from these plants and their application to treat various ailments (Patra *et al.*, 2020). Many reports are available on the antimicrobial activity of plant extracts on human pathogenic bacteria (Pothiraj *et al.*, 2021). Plants contain a wide variety of antimicrobial agents called Phytochemicals which have demonstrated significant potential in treating infectious diseases (Barbieri *et al.*, 2017). Recent researches highlights the significant role of these phytochemicals found in plants in preventing infections diseases (Yang and Ling., 2024).

The banana plant is a giant herbaceous flowering plant, belonging to the Musaceae family *Musa acuminata* being the specie of interest. (Maseko *et al.*, 2024). The plant is grown in over 135 countries of the world with major producing countries being India, China, Southeast Asia, Africa and Latin America (Sidhu and Zafar, 2018). Though it's ancestral home is the Indo- Malesian region, particularly Southeast Asia (Kennedy, 2009). Banana is rich in potassium, calcium, iron and vitamins A, B, C and D. The root, peels, leaves and seed mucilage also serves medicinal purposes (Sumpathy *et al.*, 2021).The extracts of core of the stem are found to be useful in

dissolving the stones in the kidney and urinary bladder and reducing the weight. To flush the urinary blocks, the inflorescence mixed with coconut oil and spices is used. Banana flower is traditionally used to treat many illnesses such as heart pain, diarrhea, asthma and stomach cramps (Sumathy *et al.*, 2021). The banana plant (*Musa spp.*), widely cultivated in tropical and subtropical regions of Africa, Asia, South America, and the Pacific Islands (Sau *et al.*, 2023), belongs to the Musaceae family is one of the most important fruit crops globally due to its nutritional, economic, and industrial significance. While the fruit is the most consumed and commercially valuable part, other components of the plant, such as the pseudostem commonly referred to as the banana stem, leaves and peel have gained increasing scientific interest for their underexplored potential in food, pharmaceutical, and environmental applications (Rani *et al.*, 2022).

The banana stem is a fibrous, water-rich, cylindrical structure that forms the trunk-like pseudostem of the plant. Traditionally considered agricultural waste in many banana-producing countries, the stem is now recognized as a valuable biomass resource due to its richness in dietary fiber, bioactive compounds, and phytochemicals including polyphenols, flavonoids, glucosides, tannins and alkaloids, saponins, flavonoids and phenolglucosides and vitamins (Mohapatra *et al.*, 2023). Several studies have highlighted its potential in producing functional food products, biodegradable packaging, textiles, and biofuels (Nandhini *et al.*, 2021; Sridevi and Sivakumar, 2020). Nutritionally, banana stem juice is widely consumed in parts of Asia for its purported health benefits, particularly in managing kidney stones, urinary tract infections, and blood sugar levels (Chandran *et al.*, 2021). The high fiber content, especially insoluble fiber like lignin and cellulose, has been shown to support gastrointestinal health and reduce the risk of metabolic disorders (Kaur *et al.*, 2023).

1.2 Aim and Objective of study.

Aim:

The aim of this study was to evaluate the antibacterial activity of banana stem extract on some clinical isolates.

Objectives:

1. evaluate the phytochemical activity of aqueous and ethanol extract of *Musa acuminata*.
2. carryout antibacterial potential of *Musa acuminata* extract on selected bacterial isolates.
3. evaluate the Minimum Inhibitory Concentration(MIC) of the extract and Minimum Bacteriocidal Concentration(MBC) of *Musa acuminata* extract on selected bacterial isolates.

CHAPTER TWO.

LITERATURE REVIEW.

2.1 Overview of the global challenge of bacterial infections.

The problem of bacterial infections which is a leading cause of morbidity and mortality in our world today. The number of resistant bacteria and other bacteria, rapidly becoming resistant to conventional antibiotics is of global health concern. The problem of Antimicrobial Resistance has become a global health issue, driven by the misuse and overuse of antibiotics in different sectors, leading to the emergence of resistant microorganisms. The history of AMR, dates back with the rise of Multi Drug Resistant pathogens. The rise of MDR bacteria which pose a serious health threat to clinical treatment has been linked to the misuse of conventional antibiotics on multiple occasions. There is high rate of resistance to antibiotics commonly used to treat common bacteria infections such as Hospital Acquired Infections, Urinary Tract Infections and diarrhea indicating that effective antibiotics are running out. The bacterium *Staphylococcus aureus* can be found as normal flora on the skin and is also responsible for infection in healthcare facilities and also in the community. Methicillin Resistant Staphylococcus Aureus (MRSA) has shown to have a higher mortality rate in patients than patients infected with drug sensitive strain. Drug resistant strains of *Pseudomonas aeruginosa* is responsible for approximately 11% of Hospital Acquired Infections with a steady increase in the mortality rate As antimicrobial resistance is on the rise in the different parts of the world, it is becoming more crucial to not only

find new antimicrobial agents but also slow down or stop the resistance of these microorganisms (Sirwan *et al.*, 2024, Chinemerem *et al.*, 2022).

As the problem of antibiotic resistance and multi drug resistance increases, research is made for alternative sources of antibiotics such as traditional antibacterial therapies. The presence of bioactive molecules called phytochemicals found in medicinal plants substances such as tannins, lectin, terpenoids, flavonoids, alkaloids and phenolic compounds. These bioactive substances possess the capacity to demonstrate bacteriocidal and bacteriostatic impact on bacteria, resistant to a variety of conventional antibiotics. A major advantage of these phytochemicals for antimicrobial activity is their capacity to engage a variety of bacteria. This multi target affinity of these phytochemicals, make it challenging for the bacteria to produce resistance mechanism (Beatriz. N *et al.*, 2023). These phytochemicals from natural sources have a variety of chemical composition and mode of action that can suppress bacterial growth. It has been shown that these phytochemicals target a variety of bacterial cell pathways, reducing the likelihood of resistant development by bacteria (Ankumoni *et al.*, 2025). Plant derived substances are compared to their synthetic counterparts and are typically more attainable, safer to use in terms of side effects and easier to find, thereby creating natural antibacterial agent possessing compounds having antibacterial properties (Bahaman *et al.*, 2021).

2.2 The Banana Plant (*Musa* spp.)

The banana plant (*Musa* spp.) is among the most vital and widely cultivated fruit crops in tropical and subtropical regions around the world (Joshi *et al.*, 2023). It is highly valued not only for its substantial contribution to global agricultural economies but also for its diverse nutritional, medicinal, and ecological benefits. Belonging to the family Musaceae, the genus *Musa* includes a

wide array of cultivars that fall into two major categories: dessert bananas, which are typically sweet and consumed raw, and plantains, which are starchy and usually cooked before consumption (Elayabalan *et al.*, 2017). These categories are primarily distinguished by differences in their sugar and starch compositions, making them suitable for varied culinary and industrial uses.

From a botanical standpoint, bananas are often mistakenly referred to as trees; however, they are actually large herbaceous perennials. Unlike woody plants, bananas lack a true woody trunk. Instead, what appears to be a trunk is a pseudostem, a structure formed by the concentric layering of tightly packed leaf sheaths. This pseudostem provides the plant with both support and flexibility, enabling it to grow to considerable heights typically ranging from 2 to 9 meters, depending on the specific cultivar and the environmental conditions under which it is cultivated (Nelson *et al.*, 2006).

Bananas are cultivated not only for their fruit, which is a staple food in many parts of the world, but also for their utility in traditional medicine, fiber production, and soil conservation (Ranjha *et al.*, 2022). Their leaves are commonly used for wrapping food, serving as biodegradable plates, and even for roofing in rural communities. The plant also plays a critical ecological role, especially in intercropping systems, where it helps prevent soil erosion and maintains biodiversity. As a result of these diverse applications and its adaptability to various climates and soil types, the banana plant remains a cornerstone of sustainable agriculture in many developing countries (Ronald, 2011).

The banana plant is taxonomically classified as follows:

- **Kingdom:** Plantae
- **Clade:** Angiosperms
- **Clade:** Monocots
- **Order:** Zingiberales
- **Family:** Musaceae
- **Genus:** *Musa*
- **Species:** Various species and hybrids, commonly *Musa acuminata* and *Musa balbisiana*, including numerous cultivars and hybrids such as *Musa* × *paradisiaca* (Imam and Akter, 2011).

Banana is known by various local names across different cultures and languages in the tropical regions where it is widely cultivated. For instance, in parts of West Africa, it is commonly called “Ogede” in Yoruba, “Igwa” or “Unere” in Igbo, and “ayaba” in Hausa. In Southeast Asia, the banana plant holds numerous local names reflecting its cultural importance, such as “Pisang” in Malay and Indonesian, and “Kela” in several Indian languages (Opatola *et al.*, 2025; Uma *et al.*, 2019).

The genus *Musa* was first formally described by the botanist Carl Linnaeus in the 18th century, and subsequent taxonomic revisions have refined its classification as new species and cultivars were documented. Over time, the banana plant has been extensively studied not only for its agricultural significance but also for its ethnobotanical uses, nutritional benefits, and medicinal properties (Price, 1995; Nelson *et al.*, 2006).

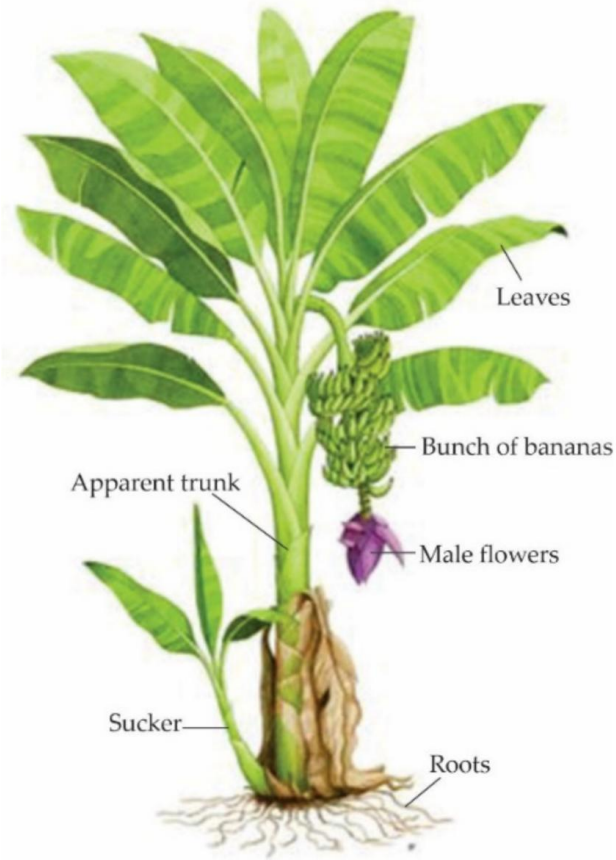


Figure 1: Photo of a banana plant.



Figure 2. Photo of banana (*Musa* spp) showing the stem

2.2.1. Origin and Distribution

The banana is believed to have originated in Southeast Asia, particularly the Indo-Malaysian region, where wild species of *Musa acuminata* and *Musa balbisiana* still grow in abundance (Halder *et al.*, 2024). These wild progenitors are the primary ancestors of most cultivated bananas, which are sterile triploids (AAB, AAA, ABB genome groups) resulting from natural hybridization and human selection (Perrier *et al.*, 2011; Campos *et al.*, 2017). From its center of origin, banana cultivation spread across India, Africa, Latin America, and the Caribbean. Today, bananas are grown in over 130 countries, with India, China, Indonesia, the Philippines, and Brazil being some of the largest producers globally (Evans *et al.*, 2020; Scott, 2021).

2.2.2. Botanical and Morphological Features

Although commonly referred to as a “tree,” the banana plant is technically a monocotyledonous herb (Martin *et al.*, 2013). It has no woody stem but grows from a corm, with its pseudostem composed of overlapping leaf bases. The plant produces a single inflorescence per life cycle, from which a large bunch of bananas develops. The fruit is technically a berry, formed parthenocarpically (without fertilization), which is why commercial bananas are seedless (Lobo and Rojas, 2020). The lifecycle of the banana plant typically includes vegetative growth, flowering, fruiting, and senescence, after which it is replaced by a sucker or shoot from the base, allowing for continuous cultivation through vegetative propagation (Sau *et al.*, 2023).

2.2.3. Nutritional and Economic Importance

Bananas play a critical role in global nutrition and food security, especially in tropical and subtropical regions where they are cultivated and consumed extensively (Scott, 2021). They are a

nutrient-dense food, providing a quick source of energy primarily due to their high carbohydrate content, particularly in the form of natural sugars such as glucose, fructose, and sucrose (Vicente *et al.*, 2022). In addition to being energy-rich, bananas are an excellent source of dietary fiber, which aids digestion and promotes gut health. They are also rich in potassium, a mineral vital for maintaining proper heart function, fluid balance, and muscle contractions (Vicente *et al.*, 2022). Furthermore, bananas supply significant amounts of vitamin C, an antioxidant important for immune defense and tissue repair, and vitamin B6, which supports brain development and the metabolism of proteins and amino acids (Wall, 2006; Oyeyinka and Afolayan, 2019).

In many developing countries, bananas are not merely a snack or complementary food but a staple that contributes substantially to daily caloric intake (Ranjha *et al.*, 2022). In particular, plantains a starchier and less sweet variety of banana are a dietary cornerstone in parts of Africa, Latin America, and the Caribbean. They are typically cooked through boiling, frying, or roasting, and are used in a wide range of traditional dishes. Their versatility and affordability make them essential for both rural and urban households.

From an economic perspective, bananas hold the distinction of being the most widely traded fresh fruit in the world. According to recent reports by the Food and Agriculture Organization (FAO), global banana exports surpass 18 million tonnes annually, representing a multibillion-dollar industry that spans continents (FAO, 2022). The banana trade is especially crucial for the livelihoods of millions of smallholder farmers, particularly in countries across Latin America, Africa, and Asia, where bananas serve as both a subsistence crop and a cash crop. In addition to farm-level employment, the banana industry supports numerous sectors along the value chain, including transportation, processing, packaging, and export services.

Despite its immense importance, the banana industry faces a range of serious challenges. Chief among these are plant diseases, particularly Panama disease, caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (TR4), and Black Sigatoka, a fungal leaf spot disease that significantly reduces yields and fruit quality (Munhoz *et al.*, 2024). These diseases are difficult to control due to the clonal propagation of most commercial banana varieties, especially the widely grown Cavendish cultivar, which lacks genetic diversity and thus exhibits uniform susceptibility. The spread of such pathogens poses a major threat to global banana production, jeopardizing both food security and the livelihoods of those dependent on this critical crop (Ploetz, 2006).

2.2.4. Traditional and Medicinal Uses

Beyond their well-established role as a staple food crop, various parts of the banana plant (*Musa* spp.) have long been utilized in traditional and indigenous medical systems around the world. From Asia to Africa and Latin America, banana plants have been regarded as versatile botanical resources with therapeutic potential, used either directly or in processed forms for the treatment and management of various ailments. These ethnomedicinal applications are increasingly gaining scientific attention due to their bioactive properties, which are supported by a growing body of phytochemical and pharmacological research (Ranjha *et al.*, 2022).

2.2.4.1. Banana Leaves

Banana leaves are widely used in folk medicine and cultural practices, particularly in South and Southeast Asia, as well as parts of Africa. Traditionally, fresh banana leaves are applied externally as wound dressings and burn coverings due to their cooling, soothing, and mildly

antiseptic properties (Guenova *et al.*, 2013). The leaves are believed to reduce inflammation, promote moisture retention, and minimize the risk of infection (Kumar *et al.*, 2012). Additionally, banana leaves are sometimes used in the preparation of poultices and wraps for skin irritations, insect bites, and rashes, offering both a barrier and a healing agent derived from the natural compounds present in the leaf (Kumar *et al.*, 2012).

2.2.4.2. Banana Flowers (Inflorescence)

The banana flower, also known as the banana blossom or heart, is an edible part of the plant commonly consumed as a vegetable in many Asian cuisines. Medicinally, it holds a prominent place in traditional medicine due to its high content of antioxidants, flavonoids, tannins, and polyphenols (Ranjha *et al.*, 2022). Research has highlighted the antidiabetic potential of banana flower extracts, which may help regulate blood sugar levels by enhancing insulin sensitivity and reducing oxidative stress (Anbuselvi and Balamurugan, 2014). It also exhibits antibacterial and anti-inflammatory properties, making it useful in the management of infections, digestive issues, and menstrual disorders. In traditional South Indian and Thai medicine, banana flowers are used to alleviate excessive bleeding, ulcer pain, and gastrointestinal disturbances.

2.2.4.3. Banana Peel

Often discarded as waste, the banana peel is now recognized as a rich source of bioactive compounds, including polyphenols, carotenoids, flavonoids, and triterpenoids, which contribute to its pharmacological activities. Several studies have shown that banana peel extracts possess antimicrobial properties, particularly against common pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* (Sulaiman *et al.*, 2011; Sirajudin *et al.*, 2014; Hanafy *et*

al., 2021;). In traditional medicine, banana peels have been applied topically for treating minor burns, acne, and skin infections, as well as for relieving itching and pain from insect bites or allergies. Their anti-inflammatory and wound-healing potential is attributed to the combined effect of antioxidant compounds and natural emollients present in the peel (Kumar *et al.*, 2012).

2.2.4.4. Roots, Rhizomes, and Stem Sap

Less commonly known but equally important in traditional medicine are the roots, rhizomes, and stem sap of the banana plant. In Ayurvedic medicine, decoctions made from banana roots are used to treat digestive disorders, diarrhea, dysentery, and intestinal ulcers (Kumar *et al.*, 2012). The stem sap, extracted from the inner core of the banana pseudostem, is traditionally consumed to detoxify the body, alleviate kidney stones, and manage high blood pressure (Kendole *et al.*, 2022). In various African and Indian healing traditions, the sap is also used to treat fevers, urinary tract infections, and gastric inflammation (Maridass, 2008). These applications are supported by the presence of alkaloids, sterols, and saponins that exhibit antipyretic and antimicrobial effects.

The traditional uses of banana plant parts are now being validated by modern pharmacological research, which seeks to isolate and characterize the active constituents responsible for these therapeutic effects. As a result, banana-derived extracts are being investigated for their potential in nutraceuticals, cosmeceuticals, and pharmaceutical formulations. Furthermore, these ethnomedicinal practices emphasize the importance of integrating traditional knowledge with scientific inquiry, not only to preserve cultural heritage but also to uncover sustainable and affordable healthcare solutions based on natural products.

Banana cultivation also contributes to soil stabilization and agroforestry systems. Due to their rapid growth and large leaf surface area, banana plants help reduce erosion and can be integrated with other crops in sustainable farming practices. Additionally, post-harvest residues such as pseudostems and peels are increasingly being studied for biogas production, composting, and as animal feed, adding value to what was once considered agricultural waste (Mohapatra *et al.*, 2010).

2.3 Phytochemical Constituents of banana plant (*Musa spp*)

2.3.1 Overview of Phytochemicals in Medicinal Plants

Phytochemicals are naturally occurring bioactive compounds found in plants that contribute to their color, flavor, and resistance to diseases. More importantly, they have profound implications for human health, as many possess antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (Dutta *et al.*, 2019). These compounds are broadly categorized into alkaloids, flavonoids, tannins, saponins, phenolics, terpenoids, and glycosides (Pandey and Rizvi, 2009). In medicinal plants, phytochemicals serve as the cornerstone of traditional therapies, providing therapeutic benefits that justify their use in managing diseases ranging from diabetes and hypertension to infections and cancer (Okwu, 2004).

The continuous scientific exploration of phytochemicals has led to the isolation of potent bioactive compounds now used in modern pharmaceuticals. For example, quinine (from *Cinchona spp.*) and artemisinin (from *Artemisia annua*) are plant-derived compounds that revolutionized malaria treatment (Newman and Cragg, 2020). Banana leaves, beyond their agricultural and cultural uses, are a rich source of bioactive phytochemicals that contribute to

their medicinal properties. These compounds fall broadly into phenolics (including flavonoids and tannins), alkaloids, saponins, terpenoids, and steroids. Each group has distinct chemical characteristics and biological activities that have been validated through numerous phytochemical and pharmacological studies.

2.3.1 Phenolic Compounds and Flavonoids

Phenolics constitute one of the most abundant and biologically significant groups of phytochemicals present in banana leaves (*Musa* spp.), playing a pivotal role in the plant's defense mechanisms and potential health benefits. Chemically, phenolic compounds are defined by the presence of one or more aromatic rings bonded to hydroxyl groups (Elayabalan *et al.*, 2017). This unique molecular configuration imparts the ability to donate hydrogen atoms and readily scavenge reactive free radicals, which are unstable molecules that cause oxidative damage to cells and biomolecules. This free radical neutralization underpins the antioxidant properties of phenolics, a function extensively documented in the literature (Rice-Evans *et al.*, 1997). The antioxidative activity of these compounds is fundamental in protecting biological systems from oxidative stress, which is implicated in the etiology of several chronic and degenerative diseases, including cancer, diabetes, and cardiovascular disorders.

Extensive phytochemical analyses of banana leaves have revealed the presence of various phenolic acids such as gallic acid, caffeic acid, and ferulic acid (Anand *et al.*, 2020). These phenolic acids serve as powerful antioxidants by directly neutralizing reactive oxygen species (ROS), thus preventing lipid peroxidation and damage to DNA and proteins. By mitigating

oxidative stress, these acids contribute to the maintenance of cellular integrity and function, potentially reducing the risk or progression of oxidative-stress related pathologies (Kumar *et al.*, 2020). The presence of such phenolic acids in banana leaves adds to their therapeutic value and supports their use in traditional medicine for wound healing and inflammation reduction.

Among the phenolics, flavonoids represent a major subclass and are characterized by a distinctive C6-C3-C6 carbon framework that forms two aromatic rings linked by a three-carbon bridge, often arranged in a heterocyclic ring. In banana leaves, several flavonoids have been identified, including quercetin, kaempferol, luteolin, and catechins (Singh *et al.*, 2019). These polyphenolic compounds are recognized for a broad spectrum of biological activities. Their antioxidant capacity stems from their ability to stabilize free radicals through electron delocalization, thereby protecting vital cellular components such as lipids, proteins, and nucleic acids from oxidative damage. Additionally, flavonoids exert significant anti-inflammatory effects by modulating intracellular signaling pathways—most notably the nuclear factor kappa B (NF- κ B) pathway. By inhibiting NF- κ B activation, flavonoids reduce the production and release of pro-inflammatory cytokines, which are key mediators in inflammatory processes and chronic inflammatory diseases (Middleton *et al.*, 2000).

Flavonoids also demonstrate potent antimicrobial activities. They interact with bacterial cell membranes, causing increased permeability and membrane disruption, which leads to leakage of cellular contents and bacterial death. Furthermore, flavonoids inhibit nucleic acid synthesis within microbial cells, impairing their replication and survival. These mechanisms are effective against a wide range of bacterial pathogens, including both Gram-positive and Gram-negative strains, making flavonoids valuable natural antimicrobial agents (Cushnie and Lamb, 2005).

Empirical studies underscore the significance of these phenolic compounds in banana leaves. Gupta and Sharma (2017) reported that banana leaf extracts exhibited high total phenolic content (TPC), which showed a strong positive correlation with antioxidant activities measured by assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging tests. These findings highlight the capacity of banana leaves to counteract oxidative stress effectively and suggest their promising application in developing natural antioxidant therapies or supplements.

2.3.2 Tannins

Tannins are high molecular weight polyphenols capable of forming complexes with proteins and metal ions. They are categorized into hydrolyzable tannins (esters of gallic or ellagic acid with sugars) and condensed tannins (proanthocyanidins) (Haslam, 1998). Banana leaves predominantly contain condensed tannins, which are polymeric flavonoids.

The astringent property of tannins is due to their ability to precipitate proteins, which is therapeutically useful in wound healing by forming a protective layer over damaged tissue and reducing inflammation (Fraga-Corral *et al.*, 2021). Moreover, tannins exhibit strong antimicrobial activity through multiple mechanisms including enzyme inhibition, membrane disruption, and metal ion deprivation essential for microbial growth (Lemire *et al.*, 2013). Sivasamugham *et al.*, (2021) demonstrated that tannins extracted from banana leaves inhibited the growth of several pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli*. This corroborates traditional uses of banana leaves for topical application in infections and injuries.

Furthermore, tannins act as antioxidants by donating electrons to neutralize free radicals and chelate pro-oxidant metal ions (iron and copper), which catalyze ROS formation (Pietta, 2000). This dual action enhances their therapeutic potential in inflammatory and degenerative diseases.

2.3.3 Alkaloids

Alkaloids are nitrogen-containing heterocyclic compounds derived biosynthetically from amino acids. They exhibit diverse pharmacological activities including analgesic, anti-malarial, antitumor, and antibacterial effects (Amoa Onguéné *et al.*, 2013). In banana leaves, alkaloids are less abundant but still significant. Their presence has been confirmed by qualitative phytochemical screening (Harith, 2018). Alkaloids may contribute to the leaves' reported analgesic and antimicrobial effects. The antimicrobial mechanism of alkaloids often involves DNA intercalation or inhibition of enzyme systems critical for microbial survival (Cushnie *et al.*, 2014). In addition, some alkaloids modulate the central nervous system, which supports the ethnomedicinal use of banana leaves in pain management. Research on *Musa* spp. alkaloids remains limited, suggesting a need for further isolation, characterization, and bioactivity testing of these compounds to elucidate their exact roles.

2.3.4 Saponins

Saponins are a distinctive class of phytochemicals characterized as amphipathic glycosides, consisting of a hydrophobic aglycone core—known as a sapogenin—bonded to one or more

hydrophilic sugar chains. This unique molecular architecture confers saponins with surfactant properties, which manifest as their well-known soap-like foaming ability when agitated in aqueous solutions (Francis *et al.*, 2002). In banana leaves (*Musa* spp.), phytochemical investigations have consistently identified the presence of saponins, suggesting that these compounds contribute significantly to the biological activities attributed to the plant.

One of the most notable bioactivities of saponins is their antimicrobial effect. The mechanism primarily involves interaction with sterols embedded within the lipid bilayer of microbial cell membranes. By binding to these membrane sterols, saponins disrupt membrane integrity, increasing permeability and ultimately causing leakage of cellular contents, which results in microbial cell lysis and death (Sparg *et al.*, 2004). This mode of action is effective against a broad spectrum of microorganisms, including fungi and bacteria. Supporting this, research by Harith , (2018) demonstrated that banana leaf extracts rich in saponins significantly inhibited the growth of fungal pathogens, underscoring the antifungal potential of these compounds and validating traditional uses of banana leaves in managing microbial infections.

Beyond their antimicrobial properties, saponins also exert immunomodulatory effects that enhance the body's defense mechanisms. These compounds stimulate immune responses by promoting cytokine production and activating macrophages, which are key players in the innate immune system (Sun *et al.*, 2009; Shen *et al.*, 2023). Through this immune activation, saponins contribute to improved pathogen clearance and can potentially modulate inflammatory processes, making them valuable agents in both preventive and therapeutic contexts.

Additionally, saponins have been shown to influence lipid metabolism, particularly through cholesterol-lowering activities. When consumed, saponins bind to cholesterol molecules in the

gastrointestinal tract, forming insoluble complexes that reduce the absorption of dietary cholesterol (Matsuura, 2001). This reduction in cholesterol uptake can lead to decreased serum cholesterol levels, thereby conferring cardiovascular benefits. The ability of banana leaf saponins to act as natural hypocholesterolemic agents further expands their therapeutic relevance, especially in populations at risk of hypercholesterolemia and related cardiovascular diseases.

2.3.5 Terpenoids and Steroids

Terpenoids, also referred to as isoprenoids, represent a vast and structurally diverse class of naturally occurring organic compounds synthesized through the assembly of five-carbon isoprene units. These compounds are categorized based on the number of isoprene units they contain, ranging from monoterpenes (two isoprene units) to sesquiterpenes (three units), diterpenes (four units), and triterpenes (six units). Among terpenoids, steroids form a distinct subgroup characterized by a tetracyclic cyclopentanoperhydrophenanthrene ring system, which is fundamental to their biological functions and structural rigidity (Gershenzon and Dudareva, 2007).

Banana leaves (*Musa* spp.) are known to contain various volatile terpenoids that contribute not only to their characteristic aroma but also to their medicinal properties (Elayabalan *et al.*, 2017). These terpenoids have attracted scientific interest due to their broad spectrum of pharmacological activities (Singh *et al.*, 2019). One of the most well-documented effects of terpenoids is their potent anti-inflammatory action. Mechanistically, terpenoids inhibit key inflammatory mediators such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), enzymes responsible for producing pro-inflammatory prostaglandins and nitric oxide, respectively (Wagner and Ulrich-Merzenich, 2009). By downregulating these enzymes, terpenoids mitigate

the inflammatory response, offering therapeutic potential in the management of inflammatory disorders.

In addition to anti-inflammatory properties, terpenoids possess antiviral activities (Hortelano, 2009). Certain terpenoids interfere with the viral replication cycle by targeting various stages such as viral entry, genome replication, or protein synthesis. This antiviral potential has been explored against a range of viruses, making terpenoids promising candidates for developing novel antiviral agents (Chattopadhyay *et al.*, 2009). Although specific studies on banana leaf terpenoids against viruses are limited, their structural similarity to other bioactive terpenoids suggests comparable efficacy.

Moreover, some triterpenoids isolated from banana leaves exhibit anticancer properties (Salama *et al.*, 2020). These compounds have been shown to induce apoptosis programmed cell death and cause cell cycle arrest in cancerous cells, thereby inhibiting tumor growth and proliferation (Shanmugam *et al.*, 2012). The molecular mechanisms involve modulation of signaling pathways that regulate cell survival and death, highlighting the potential of banana leaf-derived triterpenoids as natural chemopreventive agents.

Steroids, as a subset of terpenoids present in banana leaves, are hypothesized to contribute additional health benefits. Their steroidal structure enables hormone-like activity, which can influence physiological processes such as inflammation and cellular homeostasis. Furthermore, steroids play a critical role in stabilizing cellular membranes, potentially enhancing the anti-inflammatory effects observed in banana leaf extracts by maintaining membrane integrity and modulating membrane-bound receptor functions.

2.4. Health Benefits Associated with the Phytochemicals

Banana (*Musa spp.*) leaves are a valuable source of various bioactive phytochemicals including flavonoids, polyphenols, saponins, terpenoids, tannins, alkaloids, and steroids. These compounds confer multiple health benefits through a wide range of pharmacological activities. The interplay of these compounds contributes to the therapeutic efficacy of banana leaf extracts in traditional and modern medicine. Below is an in-depth examination of the key health benefits attributed to the phytochemicals found in banana leaves.

2.4.1. Antibacterial Properties

Banana (*Musa spp.*) leaves have been widely acknowledged not just for their traditional culinary use, but also for their potential medicinal properties. Among these is their notable antibacterial activity, which has attracted scientific attention in recent years. The antibacterial properties of banana leaf extract are primarily attributed to the presence of a diverse range of phytochemicals such as flavonoids, phenolic compounds, tannins, saponins, alkaloids, and terpenoids. These bioactive constituents have been shown to possess the ability to inhibit the growth of both Gram-positive and Gram-negative bacteria.

Research conducted by Salama *et al.* (2020) revealed that banana leaves are rich in phenolic and flavonoid content, which are compounds known to exhibit strong antimicrobial effects. These compounds can interfere with bacterial cell wall synthesis, disrupt membrane integrity and inhibit essential enzymatic functions within the microbial cell. Similarly, Sivasamugham *et al.* (2021); Asuquo and Udobi, (2016) and Eedee *et al.*, (2022) demonstrated that ethanolic extracts of banana leaves produced significant zones of inhibition when tested against common bacterial

pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* using the agar well diffusion method. The most pronounced activity was observed against *S. aureus*, suggesting that banana leaf extract could be especially effective against Gram-positive organisms.

In support of this, Maharani *et al.* (2019) investigated the antibacterial potential of methanolic banana leaf extract and found it to be effective against *Bacillus subtilis*, *Klebsiella pneumoniae*, and *E. coli*. The study emphasized the role of methanol as an efficient solvent for extracting active compounds from the leaves, which contributed to the strong antibacterial activity observed. The underlying mechanism of action, as suggested by Prakash *et al.* (2018), involves the disruption of bacterial membranes, leading to leakage of intracellular contents and eventual cell death. Other mechanisms include inhibition of DNA synthesis and chelation of essential metal ions necessary for bacterial metabolism.

These findings indicate that banana leaf extract possesses broad-spectrum antibacterial activity and could serve as a natural alternative or complementary agent to synthetic antibiotics, especially in the face of rising antibiotic resistance. It also presents opportunities for its application in food preservation, wound healing products, and organic antimicrobial formulations.

2.4.2. Antioxidant Activity

Phytochemicals such as flavonoids (e.g., quercetin, catechins), phenolic acids, and tannins present in banana leaves are known for their strong antioxidant properties. These compounds help in neutralizing reactive oxygen species (ROS) that cause oxidative stress in biological systems. Oxidative stress plays a key role in the progression of several chronic diseases,

including cardiovascular diseases, diabetes, cancer, and neurodegenerative disorders (Pietta, 2000).

Banana leaf extracts have been found to exhibit significant free radical scavenging activity in various in vitro assays, indicating their antioxidant potential (Sulaiman *et al.*, 2011). This activity is primarily due to the polyphenolic content which donates hydrogen atoms to stabilize free radicals. Regular intake of antioxidant-rich extracts from banana leaves could potentially mitigate cellular damage and delay the onset of age-related diseases.

2.4.3. Anti-inflammatory Properties

Inflammation is a physiological response to injury or infection, but chronic inflammation contributes to many degenerative conditions. Phytochemicals such as flavonoids, terpenoids, and plant-derived steroids present in banana leaves have demonstrated notable anti-inflammatory effects (Mathew and Negi, 2017). These compounds act by modulating inflammatory mediators such as prostaglandins, cytokines, nitric oxide (NO), and enzymes like cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) (Wagner and Ulrich-Merzenich, 2009).

By inhibiting the expression of pro-inflammatory genes and enzymes, these phytochemicals help in controlling inflammatory pathways. This property makes banana leaf extracts potentially useful for managing conditions such as arthritis, asthma, and inflammatory bowel diseases. Moreover, the membrane-stabilizing effects of steroids in the leaves also contribute to the anti-

inflammatory response by reducing capillary permeability and leukocyte migration (Kumar *et al.*, 2020).

2.4.4. Antifungal Properties

Banana leaves, particularly from *Musa paradisiaca* (plantain), have demonstrated significant antifungal properties, making them a potential natural remedy against various fungal infections. These properties are attributed to the bioactive compounds present in the leaves, such as alkaloids, flavonoids, polyphenols, tannins, and saponins.

Several studies have demonstrated the inhibitory effects of banana leaf extracts on *Candida albicans*. In a study utilizing hydroethanolic extracts of *Musa paradisiaca* peel, varying concentrations were tested against *C. albicans* strain ATCC 10231. The results indicated that the 50% extract concentration produced the largest inhibition halo (12.5 mm), surpassing the standard antifungal agent nystatin, which had an inhibition halo of 8.9 mm. Statistical analysis confirmed significant differences in antifungal activity across the different concentrations (Loyaga-Castillo *et al.*, 2020). A study by Prabawati *et al.*, (2023) focused on the efficacy of banana leaf oil extract against *C. albicans* associated with diaper rash. The findings revealed that a 25% concentration of the extract effectively inhibited fungal growth, highlighting its potential as a natural remedy for candidiasis dermatitis.

Beyond *Candida albicans*, banana leaf extracts have shown inhibitory effects against other fungal pathogens. Methanolic extracts of banana leaves demonstrated significant antifungal activity against *Fusarium culmorum* and *Rhizoctonia solani*, with inhibition rates increasing alongside extract concentration. Additionally, studies have reported that banana leaf extracts can

suppress the growth of *Aspergillus niger*, *Aspergillus oryzae*, *Rhizopus stolonifer*, and *Penicillium notatum*.

The antifungal activity of banana leaf extracts is attributed to their rich phytochemical composition. Compounds such as ellagic acid, gallic acid, ferulic acid, rutin, myricetin, and naringenin have been identified in banana leaf extracts (Mostafa, 2021). These compounds are known for their antimicrobial activities, which include disrupting fungal cell membranes, inhibiting enzyme functions, and interfering with nucleic acid synthesis.

Studies conducted by Sasidharan *et al.* (2010) showed that both methanolic and aqueous extracts of banana leaves were effective against fungal species such as *Aspergillus niger*, *Candida albicans*, and *Fusarium oxysporum*. Methanolic extracts, in particular, demonstrated higher antifungal activity, which can be attributed to the better solubility of active compounds in organic solvents. This finding is consistent with other studies that suggest organic solvents enhance the extraction of phenolic and flavonoid compounds from plant materials.

2.4.5. Antiviral Activity

Though research on the antiviral properties of banana leaf phytochemicals is still emerging, terpenoids present in the leaves have shown promising activity against various viruses. Terpenoids can interfere with different stages of the viral replication cycle including attachment, penetration, and replication (Chattopadhyay *et al.*, 2009). Their lipophilic nature enables them to interact with viral envelopes and host cell membranes, disrupting viral entry.

The volatile oils and aromatic compounds found in banana leaves may contain monoterpenes and sesquiterpenes which have been documented in other plants for their antiviral activity. These

findings suggest that banana leaves could serve as a supplementary treatment option for viral infections, particularly when used in synergy with conventional drugs.

2.4.6. Antidiabetic Potential

Banana leaves have long been employed in traditional medicine for the management of diabetes, and modern scientific investigations have begun to substantiate these ethnomedical claims. The antidiabetic effects of banana leaf extract are primarily attributed to the presence of bioactive phytochemicals such as flavonoids and alkaloids, which play crucial roles in modulating glucose metabolism. These compounds act by inhibiting carbohydrate-hydrolyzing enzymes, particularly α -amylase and α -glucosidase. This enzymatic inhibition slows the breakdown of complex carbohydrates and subsequently reduces glucose absorption in the intestinal tract, thereby moderating postprandial blood glucose spikes (Tiwari *et al.*, 2011).

In addition to their inhibitory action on digestive enzymes, the phytochemicals in banana leaves also enhance insulin sensitivity and facilitate improved glucose uptake in peripheral tissues such as skeletal muscles and adipose tissues (Abdel-Gabbar *et al.*, 2023). This dual mechanism of action both delaying glucose entry into the bloodstream and enhancing cellular glucose utilization—provides a comprehensive approach to glycemic control. Moreover, the antioxidant properties of the leaf extract are particularly beneficial in protecting pancreatic β -cells from oxidative stress-induced damage, a common pathological feature in both Type 1 and Type 2 diabetes (Oyeyinka and Afolayan, 2020).

By addressing hyperglycemia through multiple biological targets, banana leaf extract presents a promising natural adjunct for diabetes management. Its use may also contribute to the reduction of long-term complications associated with poorly controlled blood glucose levels, including neuropathy, retinopathy, and nephropathy.

2.4.7. Cardiovascular Benefits

Banana leaves contain a variety of bioactive compounds including flavonoids, phenolic acids, tannins, alkaloids, and phytosterols (Someya, Yoshiki, and Okubo, 2002; Mohapatra *et al.*, 2010). These compounds exhibit strong antioxidant properties, which are critical in combating oxidative stress—a major factor in the pathogenesis of atherosclerosis and other cardiovascular conditions (Reuter *et al.*, 2010). The antioxidant activity of banana leaf extract helps neutralize reactive oxygen species (ROS), thereby protecting vascular endothelial cells from oxidative damage and maintaining vascular integrity (Bucalen *et al.*, 2023; Oyeyinka and Afolayan, 2020).

In addition to antioxidant effects, banana leaf extract has demonstrated significant anti-inflammatory activity. Chronic inflammation is well-recognized as a key driver in the initiation and progression of CVDs, particularly through the upregulation of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) and interleukins (IL-6, IL-1 β) (Libby, 2021). Phytochemicals in banana leaf extract inhibit these cytokines and suppress enzymes like cyclooxygenase-2 (COX-2), which are involved in inflammatory pathways contributing to endothelial dysfunction and plaque formation (Pan *et al.*, 2010; Kandaswami *et al.*, 2005). Banana leaf extract also exhibits vasorelaxant effects, which improve endothelial function and promote healthy blood pressure regulation. The flavonoids and polyphenols in the extract enhance nitric oxide (NO) bioavailability, a critical vasodilator responsible for maintaining

vascular tone and preventing hypertension (Moncada and Higgs, 1993; Verma *et al.*, 2018). Improved NO production reduces vascular resistance and protects against vascular stiffness, a hallmark of many cardiovascular disorders (López *et al.*, 2017).

2.4.8. Anticancer Properties

Banana leaf extract is rich in phenolic compounds, flavonoids, tannins, and terpenoids, which are renowned for their antioxidant and anti-inflammatory properties (Someya, Yoshiki, and Okubo, 2002). These compounds play a significant role in neutralizing reactive oxygen species (ROS), which are known to damage cellular components and trigger the initiation and progression of cancer (Reuter *et al.*, 2010). The antioxidant capacity of banana leaf extract helps prevent oxidative DNA damage, which is a critical event in the transformation of normal cells into malignant ones.

Experimental studies have provided compelling evidence supporting the cytotoxic effects of banana leaf extract on various cancer cell lines. For instance, research by Nirmala *et al.* (2011) demonstrated that methanolic extracts of banana leaf induced significant growth inhibition in MCF-7 breast cancer cells, attributed to the activation of apoptotic pathways. Similarly, Chabuck *et al.* (2013) observed dose-dependent antiproliferative effects of banana leaf extract on colon (HT-29) and liver (HepG2) cancer cell lines, suggesting that the bioactive compounds in the extract may cause cell cycle arrest and promote apoptosis.

The anti-inflammatory properties of banana leaf extract also contribute to its anticancer effects. Chronic inflammation is recognized as a key driver of tumor development and progression. Bioactive compounds in banana leaf extract—such as flavonoids—are known to suppress pro-

inflammatory cytokines and inhibit enzymes like cyclooxygenase-2 (COX-2), which are often overexpressed in cancerous tissues (Pan *et al.*, 2010). This anti-inflammatory mechanism not only reduces the tumor-promoting microenvironment but may also inhibit angiogenesis and metastasis. Moreover, banana leaf extract has shown potential immunomodulatory effects, which may enhance the body's natural ability to combat cancerous cells. Through modulation of immune responses and oxidative balance, the extract may help reinforce innate surveillance mechanisms against tumor formation (Kandaswami *et al.*, 2005).

While these *in vitro* and *in vivo* findings are promising, it is important to note that clinical trials in humans are still limited. Further research is needed to isolate and characterize the specific active compounds responsible for the anticancer effects, determine effective dosage regimens, and ensure safety for therapeutic use. Nonetheless, the natural abundance, safety profile, and bioactive potential of banana leaf extract make it an attractive candidate for future development as a complementary agent in cancer prevention and therapy.

2.4.9. Wound healing properties

Several studies have demonstrated the wound healing potential of banana leaf extracts. Agarwal *et al.* (2009) investigated the wound healing activity of both methanolic and aqueous extracts of plantain banana (*Musa sapientum* var. *paradisiaca*) in rat models. Their findings revealed that treatment with these extracts significantly increased the levels of hydroxyproline, hexuronic acid, hexosamine, and superoxide dismutase—biomarkers associated with collagen synthesis and antioxidant defense. Additionally, the extracts improved wound breaking strength and elevated

reduced glutathione levels, while effectively reducing wound area, scar area, and lipid peroxidation. These effects were largely attributed to the antioxidant properties of the plantain extracts, which help mitigate oxidative stress at the wound site, thereby promoting faster and more effective tissue repair (Agarwal et al., 2009).

Supporting these findings, Pushpangadan and George (1995) also reported accelerated wound contraction and epithelialization in wounds treated with banana leaf extracts, attributing this to the presence of bioactive compounds such as flavonoids, tannins, and phenolics that stimulate fibroblast proliferation and collagen synthesis. In a similar vein, Wound healing studies Amutha and Selvakumari (2016) demonstrated that the topical application of banana leaf extract improved granulation tissue formation and enhanced the tensile strength of healed wounds in animal models. Sharma et al. (2014), who documented the inhibition of bacterial growth by banana leaf phytochemicals, further contributing to the wound healing process by reducing infection-related delays. Collectively, these studies reinforce the traditional use of banana leaves in wound management and underscore their potential as a natural source of compounds that accelerate wound healing through antioxidant, antimicrobial, and tissue regenerative mechanisms.

CHAPTER THREE.

MATERIALS AND METHODS.

3.1 Collection of Plant Sample.

Fresh banana (*Musa* spp) stem were locally sourced from home garden in Osasogie, Benin City, Edo state, Southern Nigeria. The stems were chosen based on the freshness and absence of visible spoilage or infection. The stems were peeled carefully with a clean sterile knife and chopped into smaller pieces and these smaller pieces were washed using clean water. The

chopped pieces of banana stem was dried under the sun for 5-7 days until completely dried. The dried chopped stems was blended to powder form at a local market with a blending machine. The blended powder is sieved to obtain banana stem powder and the powder is stored in an airtight container until needed for further analysis (Xu *et al.*, 2015).

3.2 Sample Extraction.

Aqueous and ethanolic extraction of banana stem powder was carried out using 500g of the sample. The measured sample was soaked in water and alcohol (Aqueous and Ethanol extraction) and then filtered with Whatman No 1 filter paper and evaporated to obtain extract (Abdul-Hammed *et al.*, 2021).

3.3 Microorganisms used.

Different strains of bacteria were obtained from clinical samples at the University of Benin Teaching Hospital, Medical Microbiology Department. Organisms involved are: *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Subcultures were maintained on Nutrient Agar (NA) slants and incubated at 25–28°C for 18- 72 hours to ensure microbial cell activity. The cultures were then preserved at 4°C and to continue viability for antibacterial assay.

3.4 Apparatus and Equipment

Experiment was conducted using the following equipments:

Test tubes and conical flasks (500 ml and 1000 ml), Petri dishes and measuring cylinders, Bunsen burner, inoculating loops, and pipettes, Sensitivity discs, Incubator and autoclave, Rotary evaporator (for concentration of extracts) and Water bath.

3.5 Preparation and Sterilization of Materials.

All glassware, culture media, and tools used for microbial assays were sterilized by autoclaving at 121°C and 15 psi pressure for 15 minutes. Media were prepared according to manufacturers' instructions. Work benches and instruments were disinfected with 70% ethanol before and after procedures to maintain aseptic conditions (Pelczar et al., 2008).

3.6 Preparation and sterilisation of culture media.

All culture media were aseptically prepared following manufacturer's instruction. After preparation, media was sterilized at 121°C, 15ps: for 15 minutes unless otherwise advised by manufacturer.

3.6.1.Nutrient agar

Twenty-eight grams (28g) of nutrient agar was dissolved in 1000ml of distilled water in a conical flask corked with cotton wool and foil paper. Agar powder was allowed to dissolve and then sterilized in an autoclave at 121°C for 15 minutes after which it was allowed to cool before pouring into sterile petri dishes.

3.6.2.Mueller Hinton agar

Thirty eight grams (38g) of Muller Hinton agar was dissolved in 1000ml of distilled water and boiled to completely dissolve agar. Autoclaving to sterilize was done for 15 minutes at 121°C (15 psi). Upon cooling, media was poured in to Petri dishes.

3.7.Confirmatory Test for Isolates

3.7.1.Morphological Analysis.

Gram Staining

Gram staining technique is a differential staining technique to distinguish between Gram-positive and Gram-negative bacteria. Gram positive organisms retain primary stain while Gram-negative lose primary stain when decolourized. Gram staining is based on the principle of difference in cell wall composition; the procedure of Gram staining is as follows:

A smear of the bacteria isolate was made on grease free slide and heat fix by passing over flame. The smear was flooded with crystal violet which is the primary stain for 1min then rinsed with distilled water. Subsequently the slides was flooded with Lugol's iodine solution for 30sec and then will rinsed off with distilled water. 95% alcohol was used for decolorization for 10sec and immediately was rinsed off with distilled water. Finally, the smear was counter stained with saffranin for 1min and rinsed off. The slides were allowed to air dry before observing under the microscope using an oil immersion objective lens of $\times 100$ magnifications to view the slides.

3.7.2. Biochemical identification

Biochemical test was carried out so as to help in the identification of the bacteria isolates as phenotypic (cultural) characteristics is not sufficient. The various biochemical test carried out are shown below;

3.7.2.1. Oxidase test

This is mainly used to differentiate between *Pseudomonas* from other Gram-negative rods. Oxidase test was carried out to identify bacteria species that produced cytochrome oxidase

enzyme. *Staphylococcus aureus* and *Escherichia coli* which are Gram-positive and Gram-negative respectively were employed as control. A piece of filter paper using sterilized wire loop 2-3 drops of freshly prepared oxidase reagent (1% aqueous tetramethyl-3-phenyl nediamine dichloride) was added. A positive oxidase test was indicated by purple colouration within 10 seconds.

3.7.2.2.Urease test.

This is used to test organisms that have the ability to produce the enzyme urease which catalyzes the breakdown of urea to produce ammonia. The test is usually used to differentiate organisms like *Proteus mirabilis* from other non-urease positive organism. A sterilized medium was dispensed into test tubes aseptically and the test bacteria isolated were inoculated into the medium and incubated at 37 °C for 24 hours. A change in colour from yellow to red-pink confirmed the presence of urease.

3.7.2.3.Indole production test

This test was used to determine which of the isolates has the ability to split indole from tryptophan present in peptone water. The test is usually used in differentiating Gram-negative Bacilli especially those of enterobacteriaceae. Five grams of commercially available peptone broth was dissolved in 1litre of distilled water. The medium was then sterilized by autoclaving at 121 degree centigrade for 15 minutes. The 4 ml of the medium was dispensed into sterile test tube and each of the bacterial isolates was inoculated into the peptone broth. The inoculated media was incubated at 37 °C for 24 hours after which few drops of KOVAC reagent was added. KOVAC reagents consist of 150 ml of amylalcohol, 10 g dimethylaminobenzaldehyde and 150

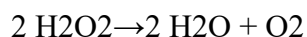
ml of concentrated hydrochloric acid. Positive test was indicated by the red colouration that occurred immediately at the upper part of the test tube.

3.7.2.4.Citrate utilization test

This test was used to identify which of the isolate can utilize citrate as the sole source of carbon for metabolism. The medium used for this test is Simon's citrate agar. In the preparation, 22 g of commercially available Simon's citrate agar was dissolved in a litre of distilled water and sterilized by autoclaving at 121 °C for 15 minutes. The medium was dispensed into test tubes and the test organism was inoculated by stab-inoculating the medium on the tubes using sterile straight inoculation wire containing culture. The tubes were incubated at 37 °C for about 24 hours. Positive result was indicated by a change in colour from green to bright blue colouration.

3.7.2.5.Catalase test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdowns of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide was added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive.



3.7.2.6.Sugar fermentation and production of gases using Triple sugar iron agar (TSI)

TSI was prepared following manufacturer's instruction and the prepared media was placed in a test tube and kept in a slant position for it to solidify. The slant and butt of the medium was inoculated with the test bacterium using a sterile loop and it was incubated for 18- 24 hours. The results was read on the basis of acid or alkaline production in the slant or butt region of the tube

and gas production was confirmed by the presence of crack or air bubbles in the slant or but region. More so, production of hydrogen sulphide was confirmed by the blackening of the medium. A prepared laboratory chat was used for result interpretation in line with microbiological standard protocol as well as other biochemical tests carried out on the isolates to confirm or ascertain their identity.

3.8 Determination of phytochemicals.

3.8.1.Detection of Flavinoids.

0.3g of extract was dissolved in 3ml of 95% ethanol and heated.A small magnesium metal (0.1g) was added to the mixture followed by the addition of 3 drops of concentrated HCl. The occurrence of an orange colouration indicates the presence of flavonoids compounds

3.8.2.Detection of Saponins.

0.3 g of extract was dissolved in 3ml of 95% ethanol and 20ml of the extract was added into a test tube and shaken vigorously. The test tube was allowed to stand on the bench for a minute. The presence of formation of stable froth, indicated positive results.

3.8.3.Detection of tannins.

1g of the extract was dissolved in 20ml of distilled water and filtered. Three drops of 10% FeCl₃ were added to 2ml of the filtrate. The appearance of blackish -blue or blackish - green colouration shows the presence of tannins (Anameze *et al.*, 2023)

3.8.4.Detection of terpenoids.

2ml of the extract was added to 2ml of acetic acid and sulphuric acid was added. The formation of a violet ring precipitate indicates the presence of terpenoids.

3.8.5.Detection of Steroids.

0.5g of extract was mixed with 2ml of acetic anhydride followed by 2ml of sulphuric acid. There is no colour change observed, which indicates the presence of steroids.

3.9 Antimicrobial sensitivity of extract.

3.9.1.Inoculation of plates

This was done by the modified method of Acar and Goldstein using flood-inoculation technique. Bacterial suspension having turbidity equivalent to 0.5 McFarland was freshly prepared and 2 ml of this was transferred onto the Mueller Hinton Agar plate and distributed gently over surface of medium with gentle rocking. The excess fluid was removed from the plate and the plate was kept in incubator at 37°C for 30 minutes for drying before application of discs.

3.9.2.Agar Well Dilution

This was carried out using the modified method of (Bauer *et al.*, 2012). Mueller Hinton Agar was prepared and after sterilization, cooled and poured into the petri dishes and allow to solidify. Upon solidification of the agar, sterile corn-bore of 6mm was used to make well into the agar plates, about 4mm deep. The bacteriocin in different concentrations was transferred aseptically into the hole and labeled accordingly, and the plate was flooded with bacteria isolates. The petri dishes was incubated at 37°C up to 24 hours after which the radius of the zone of growth of the isolates was measured using graduated millimeter (mm).

3.9.3.Determination of Minimum Inhibitory Concentration (MIC) and MBC using Broth Dilution Method.

The Broth dilution method (Nagalakshmi *et al.*, 2021) was used for the determination of Minimum Inhibitory Concentration (MIC) of the extract against bacteria from wounds. The extracts was diluted into various concentrations 6.25µg/ml, 12.5µg/ml, 25µg/ml and 50 µg/ml in a sterile Nutrient broth in test tubes. Using standard wire loop (Hi-media), a loopful of the bacterial culture was inoculated into test tubes containing various concentrations of oil extract in Nutrient broth. The tubes were incubated at 37 °C for 24 hours and thereafter observed for growth or turbidity

CHAPTER FOUR

RESULTS

The results of this study, presents the antibacterial activity of *Musa spp* both aqueous and ethanolic stem extracts on selected bacterial isolates

Table 4.1 shows the phytochemical composition of *Musa spp* extracts. It revealed the presence of flavonoids, tannins, saponins, terpenoids and steroids. Tannins and saponins showed to be moderately present in the aqueous extract, flavonoids and terpenoids showed moderate presence in the ethanol extract while steroid showed to be slightly present in both extracts.

Table 4.2 shows the confirmatory cultural, morphological and biochemical tests for the bacterial isolates. Isolate 1 exhibited flat cream coloured colonies, gram negative rods, indole and positive, confirming *Escherechia coli*. Isolate 2, exhibited small green pigmented irregular colonies, positive for urease and citrate, confirming *Pseudomonas aeruginosa*. Isolate 3 exhibited golden-yellow, raised colonies with gram positive cocci in clusters, fermenting mannitol and catalase positive, confirming *Staphylococcus aureus*.

Table 4.3 shows the Antibacterial potential of aqueous and ethanolic extracts of *Musa spp* against bacterial isolates. For *Staphylococcus aureus*, ethanolic extract showed moderate inhibition at 11.00mm and no inhibition for aqueous extract. *Pseudomonas aeruginosa* showed moderate inhibition for both the aqueous- 11.00mm and ethanol- 10.00mm extract at 50% concentration and 10.00mm at 25% aqueous concentration. *Escherechia coli* showed both ethanolic and aqueous inhibition at only 50% concentration at 11.00mm and 9.00mm respectively.

Table 4.4 shows the Minimum Inhibitory Concentration of both aqueous and ethanolic extracts of *Musa spp* against bacterial isolates. *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherechia coli*, showed bacteriostatic activity at 50mg/ml for the ethanolic extract while only *P. aeruginosa* inhibited at 50mg/ml for the aqueous extract.

Table 4.5 shows the Minimum Bactericidal Concentration of *Musa spp* against bacterial isolates. This table showed that only the ethanolic extract at 50mg/ml showed bacteriacidal effect on *Pseudomonas aeruginosa*.

Table 4.1. Cultural, Morphological and Biochemical Characteristics of Bacterial Isolates

Cultural	1	2	3
Elevation	Flat	Flat	Raised

Margin	Undulate	Undulate	Entire
Color	Cream	Green	Cream
Shape	Circular	Irregular	Circular
Size	Medium	Small	Medium
Morphological			
Gram stain	-	-	+
Cell type	Rod	Rod	Cocci
Arrangement	disperse	Disperse	Cluster
Color	pink	Pink	Purple
Spore staining	-	-	-
Biochemical			
KOH test	+	+	-
Catalase	+	+	+
Indole	+	-	-
Citrate	-	-	+
Oxidase	-	+	-
Motility	+	+	+
Urease	-	+	-
Glucose	+	-	+
Sucrose	+	-	+
Lactose	+	-	+
Mannitol	-	-	+
Gas formation	+	-	+
H₂S formation	-	-	+
Identity	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>

Table 4.2 Phytochemical screening of *Musa spp* (banana stem) extracts.

PHYTOCHEMICALS	Aqueous	Ethanol
Tannins	++	+
Steriods	+	+
Saponins	++	+
Flavonoids	+	++
Terpernoids	+	++

Key: + = slightly present

++= moderately present

- = absent

Table 4.3: Antimicrobial potential of aqueous and ethanolic extracts of *Musa spp* (banana stem) on selected bacterial isolates

ZONES OF INHIBITION (mm)					
Test Organism	Extract	50%	25%	12.5%	6.25%
<i>Staphylococcus aureus</i>	Ethanolic	11.00mm	-	-	-
	Aqueous	-	-	-	-
<i>Escherichia coli</i>	Ethanolic	11.00mm	-	-	-
	Aqueous	9.00mm	-	-	-
<i>Pseudomonas aeruginosa</i>	Ethanolic	10.00mm	-	-	-
	Aqueous	11.00mm	10.00mm	-	-

Table 4.4: Minimum inhibitory concentration of *Musa spp* (banana stem) extracts

	Ethanol extract	Aqueous extract
<i>Staphylococcus aureus</i>	50 mg/ml	-
<i>Escherichia coli</i>	50 mg/ml	-
<i>Pseudomonas aeruginosa</i>	50 mg/ml	50 mg/ml

Table 4.5: Minimum bactericidal concentration of banana stem (*Musa spp*) extracts on bacterial isolates

	Ethanol extract	Aqueous extract
<i>Staphylococcus aureus</i>	static	Static
<i>Escherichia coli</i>	static	Static
<i>Pseudomonas aeruginosa</i>	50mg/ml	Static

CHAPTER FIVE.

DISCUSSION.

The banana plant (*Musa spp*) does not only produce edible fruit, but its other components are also very useful in traditional medicine. The banana stem has been employed in wound healing, managing Urinary tract infections and blood sugar levels (Chandran *et al.*, 2021). Banana stem extracts have shown antibacterial effects against gram positive- *Staphylococcus aureus* and *Enterococcus faecalis* and gram negative - *Escherichia coli* and *Pseudomonas aeruginosa*. This study investigated the Antibacterial potential of aqueous and ethanolic extracts from banana stem on selected clinically relevant bacterial isolates - *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*.

Previous studies carried out on this subject have shown the effectiveness of banana stem against infections are due to the presence of phytochemicals such as saponins, flavinoids, tannins e.t.c. Phytochemical analysis of stem extracts revealed the presence of these phytochemicals which further proved previous research. Flavonoids are known for their antioxidant, anti-inflammatory and antiviral activity (Ullah *et al.*, 2020). Terpenoids and tannins are widely known for their antibacterial activity, they act by inhibiting bacterial cell wall.

According to Dutta (1993), the kind of solvent used determines the active ingredients extracted and thus its activity against microorganisms. Both aqueous and ethanolic extracts of both plants under consideration showed antimicrobial activity against selected isolates to varying degrees. Ethanolic extracts proved to be more potent, demonstrating broader zones of inhibition and

bactericidal activity. Ethanol has a higher polarity index than water allowing it to extract more phenolic and flavonoid compounds thus making it a superior extraction solvent.

Banana (*Musa spp*) stem ethanol extract demonstrated higher antibacterial activity against gram positive - *Staphylococcus aureus*, which zone of inhibition was measured 11mm compared to aqueous extract which did not inhibit and gram negative *Escherichia coli*, having a greater zone of inhibition 10mm than aqueous extract 9mm. However, aqueous extract inhibited the gram negative - *Pseudomonas aeruginosa* at a lower concentration of 25% as compared to the ethanolic which inhibited at only 50% concentration and *Escherichia coli* which inhibited at 50% concentration. Ethanolic abstract for the three isolates, inhibited at only 50% concentration *Pseudomonas aeruginosa* showed weak inhibition at 10mm, *Staphylococcus aureus* showed moderate inhibition at 11mm and *Escherichia coli* showed moderate inhibition at 11mm respectively. However, upon further testing of Minimum Bactericidal Concentration ethanolic extract, showed bacteriacidal activity at 500mg/ml while aqueous extract showed no bacteriacidal activity.

Broader zones of inhibition were observed in ethanolic extracts, which suggests a wider inhibition zone, particularly in the gram positive *Staphylococcus aureus* indicating that the bioactive components against the gram positive bacterial was adequately extracted by ethanol unlike the aqueous extract that did not inhibit *Staphylococcus aureus* at all. Flavonoids having antibacterial properties as well as antioxidant exert antibacterial properties by particularly against gram positive bacteria like *Staphylococcus aureus* by inhibiting nucleic acids synthesis and blocking energy metabolism enzymes. *Escherichia coli* showed a broader zones of inhibition in the ethanolic extract than the aqueous extract, this is due to the presence of terpenoids, which have

a broad range of antibacterial activities and their lipophilic nature, which makes them more extractable in ethanol (Wagner and Ulrich-Merzenich, 2009) and the presence of steriods which aid the Antibacterial properties of ethanolic extract (Matthew and Negi, 2017). *Pseudomonas aeruginosa* on the other hand, inhibited better in aqeous extract than in the ethanolic extract and even at a lower concentration 25% concentration than the ethanolic extract. This further confirms the presence of tannins and saponins, which are more extractable in water due to their high solubility in water. They act by forming strong complexes with the bacterial cell wall protein enzymes, damaging the cell wall and disrupting cell integrity, leading to cell lysis, due to their ability to interact with the lipid-rich outer membrane of the call wall of the bacteria (Sparg *et al* ., 2004).

Across board, both ethanolic and aqeous extract showed bacteriostatic activity at 500mg/ml. However, for aqeous extract, *Staphylococcus aureus* and *Escherechia coli*, showed not inhibition. Minimum Bacteriacidal Concentration was observed for only *Pseudomonas aeruginosa* ethanolic extract. This demonstrates that ethanol is a more sufficient solvent for extracting Antibacterial agents from *Musa* spp. The higher efficacy of ethanol, aligns with previous finding that solvent polarity and compound solubility significantly influence extraction efficacy and antimicrobial potency (Dutta, 1993; Ullah *et al.*, 2020).

The findings align with reports from Maharani *et al.* (2019) and Sivasamugham *et al.* (2021), which demonstrated that ethanolic banana extracts exhibit stronger antibacterial activity than aqueous extract. The observed inhibition zones (9–11 mm) suggest moderate antibacterial activity, which, although weaker than standard antibiotics, indicates promising natural antibacterial potential, especially considering its non-toxic and renewable nature.

5.1 CONCLUSION.

The study demonstrates that the banana stem possesses antibacterial potential, primarily in its ethanolic extract, due to the presence of bioactive phytochemicals, emphasizing their potential as suitable alternatives to synthetic antibiotics as Antimicrobial resistance, remains on the rise. This study establishes a basis for the use of traditional plants as medicine for microbial infections. Therefore, further investigations on the mechanism of action and toxicity level is essential to fully optimize the potential of *Musa spp* stem as a therapeutic agent in the treatment of infections caused by microorganism.

5.2 RECOMMENDATIONS.

1. **The use of synergistic antibiotics:** Plant-based antibiotics should be in synergy with conventional antibiotics due to their ability to resensitize resistant bacteria to conventional antibiotics and also due to their multi- target mechanism making it harder to develop resistance against these synergistic antibiotics.
2. **Increased healthcare attention and use of Plant-based antibiotics:** Plants like the banana plant and a lot of others are rich in phytochemicals, such as flavonoids and terpenoids and hold a great deal of antimicrobial properties while having little or no side effect unlike some conventional antibiotics.
3. **More Studies and research on Medicinal plants:** Plants with medicinal properties should be studied a lot more, seeking the best way to maximize their medicinal properties and different application methods as an alternative to conventional antibiotics.
4. **Combating Resistance:** Plant-based antibiotics due to their multi-target mechanism are not easily resisted by bacteria. The rise of Anti-Microbial Resistance gives plant- based antibiotics a great opportunity due to its multi-target mechanism and local availability.

5.3 CONTRIBUTION TO KNOWLEDGE

It contributes to knowledge by providing scientific evidence that plant-based antibiotics have the ability to combat bacteria. It also adds to local data to support the use of plant-based antibiotics especially against resistant bacteria and encourages the rise of synergistic antibiotics as against conventional antibiotics.

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