Antibacterial Effect of Vigna Subterranean (Bambara Nut) Leaf Extract on Bacteria Isolated from Wound Swab

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Abstract- Wound infections pose a significant global health challenge, exacerbated by the alarming rise of multidrug-resistant (MDR) bacteria. The increasing ineffectiveness of conventional antibiotics necessitates the exploration of alternative therapeutic strategies, particularly those derived from natural sources. This research investigates the antibacterial properties of Vigna subterranea (Bambara nut) leaf extract against common wound-infecting bacteria, focusing on MDR strains. The study employed a comprehensive approach, including phytochemical analysis, antibacterial testing using the disk diffusion and microdilution methods, and a comparative analysis against standard antibiotics. Vigna subterranea leaf extract demonstrated moderate antibacterial activity against Staphylococcus aureus and Escherichia coli, with MIC values ranging from 500 µg/mL to 600 µg/mL. The extract exhibited limited effectiveness against Pseudomonas aeruginosa. Phytochemical analysis revealed the presence of bioactive compounds such as flavonoids, tannins, saponins, and alkaloids, suggesting their potential contribution to the observed antibacterial properties. These findings highlight the potential of Vigna subterranea leaf extract as a natural antibacterial agent for wound management, particularly against Staphylococcus aureus. The extract's safe profile, broad availability, and potential synergistic effects with existing treatments make it a promising candidate for development as a topical wound treatment. Further research is crucial to elucidate the mechanisms of action, optimize its therapeutic potential, and establish its clinical relevance through in vivo and clinical trials. This research contributes to the growing body of knowledge on plant-based antimicrobial agents, providing a potential solution to the critical challenge of antibiotic resistance in wound infections. The exploration of Vigna subterranea leaf extract offers a promising avenue for developing sustainable and accessible treatment options for wound management, particularly in resource-limited settings.

I. INTRODUCTION

1.1 Background

Wound infections are a significant global health challenge, primarily due to their high incidence and the growing problem of antibiotic resistance. Open wounds, whether from surgeries, injuries, or chronic conditions like diabetic foot ulcers, are particularly vulnerable to bacterial infections when hygiene practices are inadequate. Common pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* are frequently involved, leading to complications ranging from localized inflammation to severe systemic infections like sepsis. These infections can result in prolonged hospital stays and higher healthcare costs (Ding *et al*., 2022; Ilyas *et al*., 2024).

Wound infections are among the most frequent hospital-acquired infections, with *Staphylococcus aureus* being a major pathogen, especially its methicillin-resistant strain, MRSA. The treatment of MRSA-related infections is challenging due to its resistance to multiple antibiotics, resulting in higher morbidity and mortality rates, particularly among vulnerable populations such as diabetic patients and post-surgical individuals (John *et al*., 2003; Hassoun *et al*., 2017). Similarly, *Pseudomonas aeruginosa* is known for its ability to form biofilms, which significantly increases its resistance to both the immune system and antibiotics (Diggle *et al*., 2020; Qin *et al*., 2022). This biofilm formation is a key factor in chronic infections, as it provides a physical barrier that prevents antibiotics from effectively penetrating the infection site (Costerton *et al*., 1999; Hall-Stoodley *et al*., 2004).

The rising prevalence of multidrug-resistant (MDR) bacteria, including MRSA, MDR *Pseudomonas* *aeruginosa*, and extended-spectrum beta-lactamase (ESBL)-producing *E. coli*, represents one of the most critical challenges in wound infection management today. These pathogens are increasingly resistant to multiple antibiotics, complicating treatment and heightening the risk of chronic infections, sepsis, and even death (Rossolini *et al*., 2014; Mirghani *et al*., 2022). Biofilms, which are highly resistant to both antibiotics and immune responses, further complicate treatment efforts, emphasizing the urgent need for alternative therapeutic strategies (Lewis, 2001; Roy *et al*., 2018).

Antibiotic resistance is a pressing issue in modern healthcare. Bacteria evolve and develop resistance mechanisms—such as biofilm formation, altering drug targets, or producing enzymes that neutralize antibiotics—rendering many conventional treatments less effective. This crisis is exacerbated by the slow pace of new antibiotic development, driven by high costs, long approval times, and the rapid emergence of resistance to new drugs (Gould & Bal, 2013; Ventola, 2015). As a result, alternative treatment approaches are being actively explored, including the use of antimicrobial peptides, bacteriophages, and natural products.

Of particular interest is the potential of medicinal plants, which have been used for centuries in traditional medicine and are now being revisited for their antimicrobial properties. These plants contain bioactive compounds like alkaloids, flavonoids, tannins, and saponins, which exhibit antimicrobial effects through mechanisms such as disrupting bacterial cell walls, inhibiting biofilm formation, and interfering with key metabolic pathways (Cowan, 1999; Silva & Júnior, 2009). Medicinal plants are especially promising due to their complex chemical composition and multifaceted modes of action, which make it difficult for bacteria to develop resistance. Unlike synthetic antibiotics that typically target a single bacterial function, the bioactive compounds in plants often act synergistically, increasing their potency and effectiveness against a broader range of pathogens (Burt, 2004).

One plant with promising antimicrobial activity is *Vigna subterranea*, commonly known as the Bambara nut. Native to Africa, this leguminous plant has long been used for both its nutritional value and its medicinal properties. The seeds are rich in proteins, carbohydrates, and essential vitamins, while the leaves have been used in traditional medicine to treat various ailments (Udeh *et al*., 2020; Jideani & Jideani, 2021). Recent studies have identified key phytochemicals in *Vigna subterranea*—including flavonoids, tannins, and saponins—that exhibit significant antimicrobial effects against common wound pathogens like *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Wanyama *et al*., 2017; Adedayo *et al*., 2021). However, there is a significant research gap regarding the antimicrobial properties of *Vigna subterranea* against multidrug-resistant strains. Specifically, the effectiveness of its extracts against MDR bacteria and the mechanisms underlying its antimicrobial activity remain largely unexplored.

This study aims to fill this gap by systematically investigating the antimicrobial efficacy of *Vigna subterranea* leaf extract against various MDR bacteria, examining its phytochemical composition, mechanisms of action, and potential synergistic effects. Given the increasing threat of MDR bacteria and the limitations of conventional antibiotic treatments, further investigation into the antimicrobial properties of *Vigna subterranea* is warranted. The potential for this plant to provide a natural, accessible, and effective alternative for managing wound infections, particularly in resource-limited settings, makes it a compelling subject for research. By exploring its phytochemical composition and mechanisms of action, this study aims to contribute to the growing body of knowledge on plant-based antimicrobial agents and their potential role in combating antibiotic resistance.

1.2 Justification for the Research

The alarming rise of multidrug-resistant (MDR) bacteria, particularly in the context of wound infections, necessitates urgent exploration of alternative treatment strategies. Traditional antibiotics, once the cornerstone of infection management, are increasingly ineffective due to bacteria evolving resistance mechanisms, such as biofilm formation and the production of enzymes that degrade antibiotics (Gould & Bal, 2013; Ventola, 2015). This critical challenge posed by antibiotic resistance, especially in pathogens like

Staphylococcus aureus (MRSA), *Pseudomonas aeruginosa*, and *Escherichia coli*, underscores the need for innovative therapeutic approaches (Garoy *et al*., 2019; Romero *et al*., 2021).

Given this backdrop, medicinal plants offer a promising, cost-effective, and accessible alternative. Many medicinal plants have been traditionally utilized for their antimicrobial properties and are now being rediscovered for modern therapeutic applications. These plants contain bioactive phytochemicals that can disrupt bacterial growth and biofilm formation, often acting through multiple mechanisms that make it more difficult for bacteria to develop resistance (Cowan, 1999; Burt, 2004). Furthermore, the ability of plant-derived compounds to act synergistically enhances their potency and reduces the likelihood of resistance development, providing a significant advantage over conventional antibiotics (Silva & Júnior, 2009).

One such plant, *Vigna subterranea* (Bambara nut), has demonstrated potential antimicrobial activity against common wound pathogens, including *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Wanyama *et al*., 2017; Adedayo *et al*., 2021). Despite its traditional use and initial studies highlighting its antimicrobial efficacy, comprehensive research into the phytochemical properties of *Vigna subterranea* and its application in treating MDR infections remains limited. This study aims to systematically investigate the antimicrobial efficacy of *Vigna subterranea* leaf extract against various MDR bacteria, examining its phytochemical composition, mechanisms of action, and potential synergistic effects.

Investigating the effectiveness of *Vigna subterranea* could provide a novel, natural alternative for managing wound infections, particularly in resource-limited settings where access to conventional treatments is often restricted. This research is justified by the growing global health threat posed by MDR bacterial infections, the limitations of current antibiotic treatments, and the promising antimicrobial potential of medicinal plants like *Vigna subterranea*. By exploring plant-based remedies, this study addresses the pressing issue of antibiotic resistance and contributes to the development of accessible, sustainable, and effective treatment options for wound infections.

1.3 Aims and Objectives

This study aims to investigate the antibacterial properties of *Vigna subterranea* leaf extract against bacteria isolated from wound swabs. The specific objectives of the study include:

- Identifying the phytochemical constituents of *Vigna subterranea* leaf extract responsible for its antibacterial activity.
- Evaluating the efficacy of the extract against common wound-infecting bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli*.
- Comparing the antibacterial activity of the plant extract with standard antibiotics used in clinical settings.
- Exploring the potential of *Vigna subterranea* leaf extract as a natural remedy for managing antibiotic-resistant wound infections.

1.4 Research Questions

Based on the specific objectives, the following research questions are formulated:

1. What are the phytochemical constituents of *Vigna subterranea* leaf extract that contribute to its antibacterial activity?
2. How effective is *Vigna subterranea* leaf extract in inhibiting the growth of common wound-infecting bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*?
3. How does the antibacterial activity of *Vigna subterranea* leaf extract compare with standard antibiotics commonly used in clinical settings?
4. Can *Vigna subterranea* leaf extract be a potential natural remedy for managing antibiotic-resistant wound infections?

1.5 Hypotheses

The leaf extract of Vigna subterranea exhibits significant antibacterial activity against multidrug-resistant bacteria, such as Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli, due to its phytochemical constituents like flavonoids, tannins, and saponins, which act synergistically through mechanisms including cell wall disruption and biofilm inhibition, providing efficacy comparable to or exceeding that of standard antibiotics while reducing resistance development.

II. LITERATURE REVIEW

2.1 Wound Infections

Wound infections arise when bacteria invade damaged tissue, impairing the healing process and potentially leading to severe complications if untreated. These infections can occur in any wound, from minor cuts to surgical incisions, and are most common when the wound is contaminated by bacteria such as *Staphylococcus aureus*, *Escherichia coli*, or *Pseudomonas aeruginosa*. The impact of wound infections is significant, contributing to increased morbidity, prolonged hospital stays, and higher healthcare costs (van Walraven & Musselman, 2013; Kołpa *et al*., 2020).

Epidemiology of Wound Infections

Wound infections are prevalent in both community and hospital settings, with surgical site infections (SSIs) being among the most common healthcare-associated infections (HAIs). The global incidence of SSIs varies but can affect up to 20% of post-surgical patients depending on the type of surgery and patient demographics. In regions with limited healthcare resources, the burden of wound infections is exacerbated by factors like poor hygiene, lack of access to antibiotics, and substandard wound care practices (Bassetti *et al*., 2023)

The incidence of chronic wounds, particularly those associated with diabetes, pressure ulcers, and venous leg ulcers, has been rising. Chronic wounds often involve complicated microbial ecosystems that persist due to factors like reduced blood flow, immune system suppression, and prolonged inflammation. Approximately 1-2% of the population in developed countries suffer from chronic wounds at any given time, with elderly patients being particularly vulnerable (Ding *et al*., 2022).

Types of Wound Infections: Acute vs. Chronic

Wound infections are generally classified as either acute or chronic, depending on the nature and duration of the infection. Acute wound infections typically result from traumatic injuries, burns, or surgical procedures, where bacteria enter the wound, leading to local inflammation, pus formation, and delayed healing. These infections tend to develop rapidly and can often be resolved with timely antibiotic treatment or wound management.

In contrast, chronic wound infections develop slowly and are usually associated with long-standing conditions such as diabetic ulcers, venous leg ulcers, or pressure sores. Chronic wounds can persist for months or years and are often characterized by the formation of biofilms, a protective layer of bacteria that makes the infection resistant to antibiotics and the body's immune defenses (Ding *et al*., 2022). The chronic nature of these infections often requires more advanced therapeutic strategies, including debridement (removal of dead tissue) and long-term antibiotic therapy.

Bacterial Involvement in Wound Infections and Impact of Biofilms on Healing

Bacterial colonization is a key factor in wound infections, with common pathogens like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* frequently involved. Methicillin-resistant *Staphylococcus aureus* (MRSA) is especially problematic in hospital environments, leading to more severe outcomes and higher mortality rates (Ding *et al*., 2022). Wound infections occur when bacterial loads surpass the body's ability to fight them off. This begins with bacterial attachment to the wound surface, followed by proliferation, which can lead to biofilm formation. The severity of infection often depends on the type of bacteria, as pathogens like *Pseudomonas aeruginosa* are known for their robust biofilm formation, making treatment more challenging (Bassetti *et al*., 2023).

Biofilms play a crucial role in chronic wound infections, complicating the healing process. A biofilm is a structured community of bacteria encased in a self-produced polymeric matrix that adheres to the wound surface. This matrix shields the bacteria from external threats, including antibiotics and immune cells, which makes biofilm-associated infections notoriously hard to treat. As a result, biofilms lead to prolonged inflammation, delayed wound healing, and an increased risk of systemic infection (Ding *et al*., 2022; Bassetti *et al*., 2023).

In acute wounds, the absence of biofilms usually allows for faster recovery, assuming appropriate wound care is provided. However, chronic wounds that harbor biofilms may persist for months or years, often requiring more aggressive treatments such as antimicrobial dressings, physical debridement, or newer therapies like ultrasound-assisted wound cleaning.

Complications of Wound Infections

The complications arising from wound infections can be severe and may include delayed healing, abscess formation, and the spread of infection to deeper tissues, leading to conditions like cellulitis, osteomyelitis, or sepsis. In chronic wounds, the prolonged presence of bacteria and biofilms can exacerbate inflammation and prevent healing, often leading to significant morbidity and healthcare costs.

One of the most concerning complications is the development of antibiotic-resistant infections, which are becoming increasingly common in both hospital and community settings. These infections are more difficult to treat, often requiring longer hospital stays, more aggressive treatments, and higher healthcare costs. In extreme cases, multidrug-resistant infections can be life-threatening (Ding *et al*., 2022; Bassetti *et al*., 2023).

Clinical Significance of Wound Swabs

Wound swabs are a critical tool in the diagnosis and management of wound infections. The primary purpose of a wound swab is to identify the type of bacteria present in a wound, allowing for targeted antimicrobial therapy. The clinical significance of wound swabs lies in their ability to guide treatment decisions, particularly when multidrug-resistant organisms (MDROs) are involved (Ding *et al*., 2022)

Swabs are typically obtained from the wound surface and sent to a microbiology laboratory for culture. Modern molecular techniques, such as polymerase chain reaction (PCR), have improved the sensitivity of detecting pathogens in wound swabs, allowing for more accurate and rapid diagnosis of infections. In cases where biofilms are suspected, swabs can also help identify the presence of these bacterial communities, which may influence treatment strategies (Bassetti *et al*., 2023).

However, it is important to note that not all wounds that contain bacteria are infected. The presence of bacteria in a wound, known as colonization, does not necessarily indicate infection unless there are clinical signs of infection such as redness, swelling, pain, or purulent discharge. This distinction is crucial for avoiding the unnecessary use of antibiotics, which can contribute to the development of antibiotic-resistant bacteria (Ding *et al*., 2022).

Wound Management Strategies

Effective management of wound infections relies on multiple strategies aimed at reducing bacterial burden, promoting healing, and addressing any complications that may arise. The key strategies involved in the management of wound infections include:

1. Debridement

Debridement is a critical step in managing wound infections, especially when there is necrotic (dead) tissue or debris present in the wound. By removing necrotic tissue, the microbial load is reduced, and biofilms that protect bacteria from treatments can be disrupted, allowing the wound to heal more effectively. Several studies emphasize the importance of debridement in improving wound outcomes, highlighting it as a first-line intervention for infected or non-healing wounds (Chen *et al*., 2018; Swanson *et al*., 2020). Various debridement techniques include surgical, autolytic, mechanical, and enzymatic methods, all of which are selected based on the wound's condition and patient needs.

2. Antimicrobial Therapy

Antimicrobial therapy is often required to address infections, especially when bacterial involvement is significant. Topical antimicrobials are used for localized infections, while systemic antibiotics are recommended for more severe or spreading infections. The choice of antimicrobial agents should be guided by wound culture results to ensure the treatment targets the specific pathogens involved (Chen *et al*., 2018; Swanson *et al*., 2020). In some cases, if resistant bacteria are identified, antimicrobial therapy must be adjusted accordingly to prevent further complications and ensure effective treatment.

3. Moisture Balance

Maintaining the right moisture balance in a wound is essential for promoting optimal healing. A wound that is too dry can impede healing, while excessive moisture can increase the risk of maceration and infection. The use of advanced wound dressings that maintain a moist environment, while also managing exudate (fluid produced by the wound), is crucial. This ensures that cells responsible for healing can function effectively in a balanced environment. Moisture-retentive dressings, such as hydrogels and foam dressings, are commonly used to achieve this balance (Chen *et al*., 2018; Swanson *et al*., 2020).

4. Monitoring and Reassessment

Constant monitoring of the wound's healing progress is essential to evaluate the effectiveness of treatments. If a wound shows signs of delayed healing or worsening infection, it is necessary to reassess for the potential presence of biofilms or resistant pathogens that may require a change in treatment strategies. Regular wound reassessment ensures that interventions such as debridement, dressing changes, and antimicrobial therapy are adjusted as needed to facilitate healing (Chen *et al*., 2018; Swanson *et al*., 2020).

2.2 Antibiotic Resistance

Antibiotic resistance occurs when bacteria evolve mechanisms to withstand the effects of drugs designed to kill or inhibit their growth. This phenomenon renders conventional antibiotic treatments ineffective, leading to persistent infections and increased morbidity and mortality. According to the World Health Organization (WHO), antibiotic resistance is one of the top ten global public health threats. The situation is particularly alarming in wound infections, where the presence of MDR pathogens can lead to delayed healing, higher risks of systemic infections, and increased healthcare costs (Monk *et al*., 2024).

The development of antibiotics in the 20th century revolutionized medicine, significantly reducing mortality rates from bacterial infections. However, the widespread use—and often misuse—of antibiotics has accelerated the emergence of resistant strains. As early as the 1940s, resistance to penicillin was observed, and today, many of the antibiotics developed in the mid-20th century are losing their efficacy due to bacterial adaptation. The evolution of resistance is an inevitable consequence of natural selection, but human behavior—such as over-prescription and incomplete treatment courses—has exacerbated the problem (Thapa *et al*., 2023; Ilyas *et al*., 2024).

Mechanisms of Resistance

Bacteria have developed a variety of mechanisms to resist antibiotic action. Understanding these mechanisms is crucial in developing strategies to counteract resistance.

Some bacteria produce enzymes that can break down antibiotics. A well-known example is the production of beta-lactamases, which destroy the beta-lactam ring in penicillins and cephalosporins, rendering these antibiotics ineffective (Ding *et al*., 2022). Certain bacteria possess efflux pumps that actively transport antibiotics out of the bacterial cell before they can exert their effects. This mechanism is common in Pseudomonas aeruginosa and Escherichia coli and can result in resistance to multiple antibiotics simultaneously (Qin *et al*., 2022). Bacteria can alter the molecular targets of antibiotics, such as modifying the binding sites of drugs like vancomycin and fluoroquinolones, thus preventing the drug from binding and exerting its bactericidal or bacteriostatic effects. .

In Gram-negative bacteria, the outer membrane serves as a barrier that limits antibiotic penetration. Changes in porin proteins in the outer membrane can further reduce drug entry, contributing to resistance.

One of the most significant mechanisms in wound care is the ability of bacteria to form biofilms. These are protective communities of bacteria that adhere to surfaces and secrete a matrix that shields them from antibiotics and the immune system. Biofilms are a common cause of chronic wound infections and are particularly difficult to eradicate.

Importance of Alternatives

Given the alarming rise of antibiotic resistance, there is an urgent need to explore alternative therapies. These therapies can work either alone or in conjunction with traditional antibiotics to enhance efficacy and prevent further resistance development.

1. Phage Therapy: Bacteriophages (viruses that infect bacteria) offer a promising alternative to antibiotics. Phages are highly specific to their bacterial hosts and can lyse bacterial cells, reducing infection. Recent studies have demonstrated the effectiveness of phage therapy in treating MDR wound infections (Lin *et al*., 2017).

2. Antimicrobial Peptides: These occurring peptides are part of the innate immune system in many organisms and have broad-spectrum activity against bacteria, fungi, and viruses. Antimicrobial peptides are less likely to induce resistance because they target bacterial membranes rather than specific metabolic processes (Lei *et al*., 2019; Zhang *et al*., 2021).
3. Natural Plant Extracts: Medicinal plant *Vigna subterranea* and *Curcuma longa* have been shown to possess antimicrobial properties. These extracts contain phytochemicals that can disrupt bacterial cell walls or interfere with biofilm formation. Their use in wound care has shown promise, particularly in resource-limited settings where access to antibiotics may be restricted (Gupta *et al*., 2015; Udeh *et al*., 2020; Jideani & Jideani, 2021).
4. Probiotics: The application of beneficial bacteria at wound sites can help outcompete pathogenic bacteria, reducing the likelihood of infection and promoting healing. Probiotic therapy is an emerging field in wound care, with studies showing that certain strains of *Lactobacillus* and *Bifidobacterium* can inhibit the growth of harmful bacteria like *Pseudomonas aeruginosa* (Bădăluță *et al*., 2024).
5. Silver and Iodine-Based Dressings: These dressings release antibacterial agents that can prevent bacterial colonization and biofilm formation. Silver dressings, in particular, are widely used in wound care and have been shown to be effective against both Gram-positive and Gram-negative bacteria, including MDR strains (Yousefian *et al*., 2023).

The Role of Multidrug-Resistant Pathogens in Wound Care

MDR pathogens are frequently isolated from wound infections, particularly in hospital settings. These pathogens include methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and carbapenem-resistant *Pseudomonas aeruginosa*. The presence of these pathogens in wounds significantly complicates treatment, often requiring the use of last-resort antibiotics such as colistin or tigecycline, which may have severe side effects (Monk *et al*., 2024).

In chronic wounds, biofilm formation is a major contributor to the persistence of infection. Biofilms protect bacteria from both the host immune system and antibiotic treatment, making infections difficult to treat. Studies have shown that up to 90% of chronic wound infections involve biofilms, which can contain multiple species of bacteria, including MDR strains (Guo *et al*., 2020).

Patients with diabetes are particularly vulnerable to wound infections, as impaired blood circulation and neuropathy can delay wound healing. Diabetic foot ulcers (DFUs) are often complicated by MDR bacteria, leading to prolonged infection, increased risk of amputation, and higher mortality rates. Addressing these infections requires a multifaceted approach, including; debridement, targeted antibiotic therapy, and alternative treatments such as phage therapy or antimicrobial dressings (Sisay *et al*., 2024).

2.3 Medicinal Plants and Wound Healing: A Review of Plants with Proven Antibacterial Properties

1. Azadirachta indica (Neem) and Its Role in Wound Healing

Azadirachta indica, commonly known as neem, has been used for centuries in traditional medicine for its therapeutic properties. Neem is widely known for its antimicrobial, anti-inflammatory, and immunomodulatory activities, making it an effective agent in wound healing. The plant contains bioactive compounds such as nimbidin, nimbolide, quercetin, and azadirachtin, which contribute to its medicinal properties.

Antibacterial Properties

Neem exhibits potent antibacterial activity against a wide range of pathogens, including both Gram-positive and Gram-negative bacteria. Several studies have demonstrated the effectiveness of neem extracts in inhibiting the growth of bacteria commonly associated with wound infections, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. These bacteria are known to cause chronic wounds and delayed healing. The antibacterial properties of neem are attributed to its ability to disrupt bacterial cell walls, inhibit biofilm formation, and prevent bacterial adhesion to wound surfaces (Alzohairy, 2016; Biswas *et al*., 2002).

Wound Healing Properties

The wound healing potential of neem has been extensively studied in both in vitro and in vivo models.

Neem oil, in particular, has been shown to promote wound healing by accelerating the proliferation of fibroblasts, enhancing collagen synthesis, and reducing inflammation at the wound site (Subapriya & Nagini, 2005). In animal studies, neem extract-treated wounds demonstrated faster healing rates compared to untreated wounds, with significant reductions in wound size and enhanced tissue regeneration (Chundran *et al*., 2015; Maan *et al*., 2017; Nasrine *et al*., 2023). Neem's anti-inflammatory effects also contribute to its ability to modulate the inflammatory phase of wound healing, thus preventing excessive inflammation and promoting tissue repair (Arshad, 2018).

Mechanisms of Action

The wound healing properties of neem are mediated through several mechanisms. Neem's antibacterial action helps prevent wound infections and reduces the microbial burden at the wound site, creating an environment conducive to healing. Additionally, neem's antioxidant properties protect tissues from oxidative damage, which can delay wound healing (Nahak *et al*., 2010). By scavenging free radicals, neem helps maintain cellular integrity and supports the formation of new tissue. Furthermore, neem's ability to enhance collagen synthesis and fibroblast proliferation plays a crucial role in the remodeling phase of wound healing (Nasrine *et al*., 2023).

2. Curcuma longa (Turmeric) and Its Role in Wound Healing

Curcuma longa, commonly known as turmeric, is a medicinal plant widely used in traditional medicine for its anti-inflammatory, antioxidant, and antimicrobial properties. The active compound in turmeric, curcumin, is responsible for its therapeutic effects. Curcumin has gained significant attention in recent years for its potential role in wound healing and its ability to fight bacterial infections (Hewlings & Kalman, 2017; Deepak *et al*., 2023).

Antibacterial Properties

Turmeric has been shown to exhibit broad-spectrum antibacterial activity against various wound pathogens, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Studies have demonstrated that curcumin disrupts bacterial cell membranes, inhibits bacterial biofilm formation, and reduces bacterial virulence, making it a potent agent in preventing and treating wound infections (Gunes *et al*., 2016). In particular, curcumin has shown efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA), a common cause of chronic wound infections (Mun *et al*., 2013).

Wound Healing Properties

Curcumin has been extensively studied for its role in promoting wound healing. It has been shown to modulate various phases of the wound healing process, including inflammation, proliferation, and remodeling. Curcumin's anti-inflammatory properties help reduce excessive inflammation, which can impair wound healing. By downregulating pro-inflammatory cytokines such as TNF-α and IL-6, curcumin helps create an environment conducive to tissue repair (Akbik *et al*., 2014).

In addition to its anti-inflammatory effects, curcumin enhances collagen synthesis and promotes the proliferation of fibroblasts and keratinocytes, which are essential for wound closure and tissue regeneration (Raina *et al*., 2008). Animal studies have shown that curcumin-treated wounds exhibit faster healing rates, reduced wound size, and improved tensile strength compared to untreated wounds (Sidhu *et al*., 1999). Moreover, curcumin's antioxidant properties protect tissues from oxidative stress, which can delay wound healing by causing cellular damage (Tomeh *et al*., 2019).

Mechanisms of Action

Curcumin exerts its wound healing effects through multiple mechanisms. Its antibacterial action helps prevent wound infections and reduces the microbial burden at the wound site, while its anti-inflammatory properties modulate the immune response to prevent chronic inflammation (Hewlings & Kalman, 2017). Curcumin also promotes angiogenesis, the formation of new blood vessels, which is essential for supplying oxygen and nutrients to the healing wound (Akbik *et al*., 2014). Furthermore, curcumin enhances the deposition of collagen, a key structural protein involved in wound healing, thereby improving the strength and integrity of the newly formed tissue (Phan *et al*., 2003).

3. Moringa oleifera (Moringa) and Its Role in Wound Healing

Moringa oleifera, commonly known as moringa, is a medicinal plant that has gained attention for its numerous health benefits, including its antimicrobial, anti-inflammatory, and wound healing properties. Moringa contains bioactive compounds such as flavonoids, alkaloids, and phenolic acids, which contribute to its therapeutic effects (Mbikay, 2012; Rockwood *et al*., 2013).

Antibacterial Properties

Moringa has been shown to exhibit antibacterial activity against a wide range of bacteria, including those commonly associated with wound infections. Studies have demonstrated that moringa leaf extracts inhibit the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, among others (Cáceres *et al*., 1991). The antibacterial properties of moringa are attributed to its ability to disrupt bacterial cell walls, inhibit biofilm formation, and reduce bacterial adhesion to wound surfaces (Bukar *et al*., 2010).

Wound Healing Properties

Moringa has been traditionally used in various cultures for its wound healing properties. The leaves, bark, and seeds of the moringa plant have been used to treat cuts, burns, and infections. Moringa leaf extract has been shown to enhance wound healing by promoting the proliferation of fibroblasts, increasing collagen deposition, and reducing inflammation at the wound site (Rathi *et al*., 2006). In animal studies, wounds treated with moringa extract demonstrated faster healing rates, reduced wound size, and improved tissue regeneration compared to untreated wounds (Gopalakrishnan *et al*., 2016).

Mechanisms of Action

The wound healing properties of moringa are mediated through several mechanisms. Its antibacterial action helps prevent wound infections and reduces the microbial burden at the wound site, creating a favorable environment for healing (Anwar *et al*., 2007). Moringa's anti-inflammatory properties help modulate the immune response and prevent excessive inflammation, which can impair wound healing. Additionally, moringa's antioxidant properties protect tissues from oxidative damage, which can delay wound healing by causing cellular damage (Verma *et al*., 2009). Moringa also promotes angiogenesis, which is essential for supplying oxygen and nutrients to the healing wound (Tiloke *et al*., 2013).

2.4 Bioactive Compounds in Vigna subterranea: In-depth Analysis of Phytochemicals and Their Relevance in Antimicrobial Activity

2.4.1 In-depth Analysis of the Phytochemicals of *Vigna subterranea*

Figure 1: Picture of *Vigna subterranea* plant

Vigna subterranea, commonly known as Bambara groundnut, is a legume that has gained attention for its nutritional and medicinal properties. The seeds and leaves of *Vigna subterranea* are rich in bioactive compounds, including flavonoids, phenols, saponins, tannins and alkaloids, which are known for their therapeutic potential, especially in antimicrobial activities.

1. Flavonoids

Flavonoids are a major group of polyphenolic compounds found in *Vigna subterranea*. These compounds are known for their antioxidant properties and their role in the plant's defense against pathogens. Flavonoids can inhibit bacterial growth by disrupting cell wall synthesis and bacterial enzyme activities. Studies have highlighted the antimicrobial potency of flavonoid-rich extracts from *Vigna subterranea* against a wide spectrum of bacterial strains, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Harris *et al*., 2018; Adedayo *et al*., 2021; Adebowale *et al*., 2020; Ramatsetse *et al*., 2022).

2. Phenols

Phenolic compounds, another group of phytochemicals present in *Vigna subterranea*, are renowned for their ability to donate hydrogen atoms, acting as free radical scavengers. This antioxidant activity indirectly supports antimicrobial effects by protecting the plant's tissues from oxidative damage, which could make them vulnerable to infections. Additionally, phenols can exert direct antimicrobial activity by damaging bacterial cell membranes, leading to leakage of cellular contents (Jideani *et al*., 2014; Okafor *et al.,* 2022).

3. Saponins

Saponins are glycosidic compounds that exhibit surface-active properties and are found abundantly in Vigna subterranea. They are known for their antimicrobial activity due to their ability to form complexes with sterols in microbial cell membranes, causing increased membrane permeability and eventual cell lysis. Saponins in *Vigna subterranea* have shown significant antibacterial effects, particularly against Gram-positive bacteria (Wanyama, 2018). The membranolytic effects of saponins make them a promising bioactive compound in the fight against antibiotic-resistant strains.

4. Tannins

Tannins are a class of polyphenolic compounds found in significant amounts in *Vigna subterranea*. These compounds are well-known for their astringent properties and their ability to precipitate proteins. Tannins exert antimicrobial effects by binding to bacterial cell walls and precipitating microbial proteins, which inhibits microbial growth and metabolism. Additionally, tannins can disrupt biofilm formation, a key factor in bacterial resistance, thus making them effective against biofilm-forming bacteria. Their role in controlling infections, especially in wound healing and digestive disorders, has been extensively studied (Romani *et al,* 2006; Harris, 2017).

5. Alkaloids

Alkaloids are nitrogen-containing compounds that have potent biological activities, including antimicrobial properties. Alkaloids in *Vigna subterranea* interfere with microbial DNA replication and protein synthesis, which inhibits bacterial growth. Alkaloid-rich extracts from *Vigna subterranea* have been shown to be particularly effective against fungal pathogens, offering potential use in the treatment of fungal infections (Harris, 2017).

2.4.2 Relevance in Antimicrobial Activity

The combination of flavonoids, phenols, saponins, tannins and alkaloids in *Vigna subterranea* creates a multifaceted antimicrobial defense. These bioactive compounds work synergistically to inhibit microbial growth, disrupt cell membranes, and prevent the proliferation of pathogens. The antimicrobial potential of these compounds is crucial in the development of natural alternatives to synthetic antibiotics, especially in an era where antibiotic resistance is becoming a major global health issue. The antimicrobial activity of *Vigna subterranea* extracts has been validated in several in vitro studies, showing its effectiveness against both bacterial and fungal pathogens (Oyeyinka *et al*., 2021; Wanyama, 2018; Harris, 2017; Wanyama *et al*., 2017; Ajiboye & Oyejobi, 2017; Klompong & Benjakul, 2015).

2.5 Previous Research on *Vigna subterranea*: Antibacterial Activity and Applications in Infections

Vigna subterranea (Bambara nut) has been the focus of several studies exploring its potential as a natural antibacterial agent. The seeds of this legume are rich in bioactive compounds, such as flavonoids, tannins, phenols, and alkaloids, which have shown significant antimicrobial properties. Key studies have highlighted its effectiveness against a variety of bacterial infections, positioning Bambara nut as a promising candidate for developing natural antibacterial therapies.

1. Antibacterial Activity Against Common Pathogens

Several studies have confirmed the broad-spectrum antibacterial activity of *Vigna subterranea* extracts. For instance, research by Wanyama (2018) demonstrated that extracts from Bambara nut seeds effectively inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. These bacterial species are commonly associated with skin infections, food poisoning, and wound infections. The study highlighted that the flavonoids and alkaloids in the extracts contributed to their bactericidal effects by disrupting bacterial cell walls and inhibiting enzyme function.

Similarly, Taahir (2017) investigated the antibacterial activity of Bambara nut against *Klebsiella pneumoniae,* a pathogen linked to respiratory infections. The findings showed that the phenolic compounds present in the seed extracts disrupted the bacterial membrane integrity, causing cell lysis. This points to Bambara nut's potential for treating respiratory and gastrointestinal infections.

2. Application in Wound Infections

The application of *Vigna subterranea* in treating wound infections has been another significant area of research. Jideani et al. (2014) evaluated the efficacy of

Bambara nut extracts in managing wound infections caused by multi-drug-resistant bacteria, including *Methicillin-resistant Staphylococcus aureus* (MRSA). The study demonstrated that the tannins and phenols in the extracts exhibited potent antibacterial activity, reducing bacterial colonization in infected wounds. The bioactive compounds also accelerated wound healing by reducing inflammation and promoting collagen deposition at the wound site.

3. Treatment of Foodborne Infections

In addition to its role in wound infections, *Vigna subterranea* has been studied for its application in preventing foodborne bacterial infections. Okafor et al. (2022) explored the antibacterial activity of Bambara nut extracts against foodborne pathogens like *Salmonella typhi* and *Listeria monocytogenes*. The study found that the extracts were effective in inhibiting bacterial growth, suggesting potential uses in food preservation and safety, as well as in the treatment of foodborne illnesses. This aligns with the traditional use of Bambara nut in various African cultures to treat digestive issues and food-related infections.

III. RESEARCH METHODOLOGY

3.1 Research Design

This study followed an experimental research design to assess the antibacterial activity of *Vigna subterranea* leaf extracts. The experiment was conducted in a controlled laboratory environment. The design involved collecting plant materials, preparing extracts, isolating bacteria from clinical wound samples, and testing the antibacterial activity of the extracts. The primary focus was on the disk diffusion method and determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts against isolated bacterial strains.

3.2 Collection of Plant Material

The leaves of *Vigna subterranea* (Bambara groundnut) were collected from a farm in Owerri known for the cultivation of Bambara groundnut. Once collected, the plant material was authenticated by a botanist from the Department of Botany in Imo State University to ensure proper identification. The leaves thereafter were washed, air-dried at room temperature, and then pulverized into a fine powder for extraction purposes. This powdered material was stored in airtight containers to maintain its phytochemical integrity until extraction.

3.3 Extraction Process

The extraction process was carried out using ethanol as the solvent. For ethanol extraction, 50 g of the powdered leaf sample was mixed with 500 mL of 70% ethanol and left to macerate for 72 hours at room temperature. The mixture was then filtered using Whatman filter paper, and the filtrate was evaporated under reduced pressure in a rotary evaporator until a semi-solid residue was obtained.

3.4 Isolation of Bacteria

Bacteria was isolated from clinical wound swabs obtained from patients with bacterial infections, especially those caused by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The samples were cultured on selective media, and colonies were identified based on morphological, biochemical, and molecular methods to confirm the bacterial species. Once identified, pure colonies were isolated and maintained on nutrient agar slants for further antibacterial testing.

3.5 Antibacterial Testing Methods

1. Disk Diffusion Method: This method was used to evaluate the antibacterial activity of the leaf extracts. Sterile filter paper disks (6 mm in diameter) was impregnated with varying concentrations of the extracts and placed on agar plates previously inoculated with the bacterial isolates. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition were measured. Larger zones of inhibition indicated stronger antibacterial activity.
2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): MIC is the lowest concentration of the extract that inhibits bacterial growth, while MBC is the lowest concentration that kills the bacteria. A broth microdilution method was used to determine these values. Serial dilutions of the extract were prepared in 96-well microplates, and bacterial suspensions were added. After incubation, the wells were examined for bacterial growth, with MIC being determined by the lowest concentration that shows no visible growth. MBC was determined by subculturing the contents of wells showing no growth onto agar plates and identifying the lowest concentration that resulted in no bacterial colonies.

3.6 Controls and Comparison with Standard Antibiotics
Positive and negative controls were incorporated into the study. The positive control consisted of standard antibiotics such as ciprofloxacin and amoxicillin, which are known for their antibacterial properties. Sterile distilled water and ethanol served as negative controls for the aqueous and ethanol extracts, respectively. The comparison of the extract's antibacterial efficacy with these antibiotics helped to evaluate the potential of *Vigna subterranea* as an alternative treatment for bacterial infections.

3.7 Phytochemical Composition Analysis Using Gas Chromatography-Mass Spectroscopy (GC-MS)
Gas Chromatography-Mass Spectroscopy (GC-MS) is a highly effective analytical technique used to identify and quantify volatile and semi-volatile organic compounds present in the leaf extract of *Vigna subterranea*. The process begins with sample preparation, where the leaf extract is obtained using solvents such as ethanol or methanol. After extraction, the sample is typically concentrated using a rotary evaporator to remove excess solvent. In some cases, certain phytochemicals may require derivatization to enhance their volatility and thermal stability for GC-MS analysis. For instance, silylation can be employed to modify phenolic compounds and improve their performance during analysis.

The GC-MS analytical procedure itself involves several key steps. First, a small volume of the prepared extract, often about 1 µL, is injected into the GC-MS system. The sample is then vaporized and passed through a chromatographic column where the individual components are separated based on their volatility and interaction with the stationary phase of the column. As these separated compounds exit the column, they enter the mass spectrometer, where they are ionized. The mass-to-charge ratio (m/z) of the ionized fragments is detected, generating a mass spectrum that is subsequently compared to reference spectra from databases such as the National Institute of Standards and Technology (NIST) to identify the compounds present in the extract. This method allowed for a detailed analysis of the phytochemical composition of *Vigna subterranea* leaf extract.

3.8 Ethical Consideration
Ethical approval for this study was obtained from the Imo State University Ethical Committee and the management of the Federal University Teaching Hospital Owerri. Informed consent was secured from all patients whose clinical wound swabs were used, ensuring voluntary participation, and maintaining the confidentiality and privacy of their information. The study strictly followed ethical guidelines for research involving human participants and adhered to biosafety protocols to prevent contamination or harm. Additionally, plant material was collected responsibly to ensure no adverse impact on local biodiversity.

3.9 Statistical Analysis
Data generated from the study was analyzed statistically using SPSS software version 23. The data were computed using means and standard deviations and visualized through tables and bar graphs.

IV. RESULT

4.1 Presentation of Results
Table 4.1: Comparative analysis of the effects of antibacterial activity of *Vigna subterranea* Leaf extract and standard antibiotics against three isolated bacteria strains from wound swabs.

Bacterial Strains	Concentration and Zones of Inhibition (mm) of extract				Ciprofloxacin (100 µg/mL)	Amoxicillin (100 µg/mL)	Minimum Inhibitory Concentration (MIC, µg/mL)	Minimum Bactericidal Concentration (MBC, µg/mL)	Comparison with Standard Antibiotics
	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL					

Escherichia coli	12.5 ± 0.1	16.0 ± 0.2	20.2 ± 0.3	23.0 ± 0.5	25.0 ± 0.4	13.0 ± 0.2	600 ± 5.0	1200 ± 10.0	Moderate activity
Pseudomonas aeruginosa	11.0 ± 0.5	14.5 ± 0.4	18.0 ± 0.2	21.0 ± 0.6	22.0 ± 0.4	5.0 ± 0.1	800 ± 8.0	1600 ± 20.0	Limited effectiveness
Staphylococcus aureus	14.0 ± 0.3	18.5 ± 0.5	22.0 ± 0.4	25.0 ± 0.4	23.0 ± 0.2	16.0 ± 0.3	500 ± 7.0	1000 ± 15.0	Moderate activity

The presented data highlights the effects of antibacterial activity of *Vigna subterranea* leaf extract and standard antibiotics against three isolated bacteria strains: *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* from wound swabs. The results indicate a nuanced effectiveness of the extract, particularly in relation to the established standard antibiotics, amoxicillin and ciprofloxacin.

Starting with *Escherichia coli*, the extract demonstrated a clear dose-dependent antibacterial effect. At a concentration of 25 µg/mL, the extract produced a zone of inhibition of 12.5 mm, which increased to 16.0 mm at 50 µg/mL, 20.2 mm at 75 µg/mL, and reached 23.0 mm at the highest concentration of 100 µg/mL. This trend signifies that higher concentrations significantly enhance the extract's ability to inhibit bacterial growth. When compared to ciprofloxacin, which achieved a zone of inhibition of 25.0 mm, the extract showed moderate activity, indicating it is effective but not as potent as ciprofloxacin. In contrast, amoxicillin only generated a zone of 13.0 mm, suggesting that ciprofloxacin is the superior antibiotic for treating *E. coli* infections. The Minimum Inhibitory Concentration (MIC) for the extract was determined to be 600 µg/mL, meaning this is the lowest concentration needed to halt bacterial growth, while the Minimum Bactericidal Concentration (MBC) was found to be 1200 µg/mL, indicating the minimum level required to kill the bacteria.

Turning to *Pseudomonas aeruginosa*, the extract's antibacterial efficacy was less pronounced. The zones of inhibition recorded were lower, with measurements of 11.0 mm at 25 µg/mL, 14.5 mm at 50 µg/mL, 18.0 mm at 75 µg/mL, and 21.0 mm at 100 µg/mL. These values suggest that *P. aeruginosa* presents a greater challenge due to its known resistance to many antibiotics. Ciprofloxacin achieved a higher zone of inhibition at 22.0 mm, while amoxicillin was substantially less effective, yielding only 5.0 mm. The MIC for the extract against *P. aeruginosa* was determined to be 800 µg/mL, and the MBC was 1600 µg/mL, reinforcing the observation of limited effectiveness against this strain.

In contrast, the extract showed promising results against *Staphylococcus aureus*. The zones of inhibition ranged from 14.0 mm at 25 µg/mL to 25.0 mm at 100 µg/mL, reflecting a strong antibacterial effect that suggests the extract's effectiveness increases with higher concentrations. When compared to ciprofloxacin, which showed a zone of 23.0 mm, and amoxicillin at 16.0 mm, the extract performed well, indicating moderate activity against *S. aureus*. The MIC for this strain was recorded at 500 µg/mL, with an MBC of 1000 µg/mL, suggesting that the extract may be a viable option for treatment against this particular bacterial infection.

Overall, the findings indicate that the extract possesses varying degrees of antibacterial activity across different strains, with moderate effectiveness against *Escherichia coli* and *Staphylococcus aureus*, and limited effectiveness against *Pseudomonas aeruginosa*. While the extract demonstrates potential as an antibacterial agent, especially against *S. aureus*, further studies are warranted to explore its mechanisms of action and to enhance its effectiveness, particularly against resistant strains like *P. aeruginosa*.

Table 4.2: Results from Phytochemical Screening and Quantitative Analysis.

Phytochemicals	Qualitative Screening (Presence/Absence)	Quantitative Analysis (% Composition)
Flavonoids	Present	8.75%
Tannins	Present	6.50%
Saponins	Present	5.20%
Alkaloids	Present	7.10%
Phenols	Present	4.95%
Steroids	Absent	-
Glycosides	Present	3.40%
Terpenoids	Present	2.80%
Reducing Sugars	Absent	-
Proteins	Present	4.20%

The table presents a detailed analysis of the phytochemical composition of the *Vigna subterranea* leaf extract, showing both the presence or absence of specific bioactive compounds and their respective percentage compositions where applicable.

Starting with flavonoids, the table indicates their presence with a relatively high percentage composition of 8.75%. Flavonoids are widely recognized for their potent antioxidant, anti-inflammatory, and antimicrobial properties, suggesting that this compound could play a significant role in the biological activities of the plant extract, particularly in promoting health and preventing oxidative damage.

Tannins are also present in the extract, with a composition of 6.50%. Tannins are known for their astringent, antimicrobial, and antioxidant properties. Their ability to inhibit bacterial growth, especially in wound infections, underscores their therapeutic potential.

The presence of saponins, with a 5.20% composition, is noteworthy as these compounds are known for their detergent-like properties that can disrupt cell membranes, particularly in pathogens. Saponins also exhibit antimicrobial and immune-boosting activities, making them valuable in medical applications.

Alkaloids were detected at 7.10%, suggesting another key bioactive component. Alkaloids possess diverse therapeutic properties, including antimicrobial, analgesic, and anti-inflammatory effects. The relatively high percentage indicates their possible contribution to the overall pharmacological effects of the extract.

Phenols, present at 4.95%, are essential for their antioxidant capacity, helping to neutralize free radicals. Their antimicrobial properties also contribute to the overall health benefits of the plant extract, particularly in preventing microbial infections and supporting the immune system.

Steroids were absent from the extract, meaning these compounds do not contribute to its biological activity. Steroids are typically associated with anti-inflammatory properties, but their absence suggests that the other phytochemicals compensate for these effects.

Glycosides were present at 3.40%. These compounds are known for their role in plant defense and their potential impact on cardiovascular health and metabolism. They also contribute to antimicrobial activity and may have a synergistic effect with other phytochemicals.

Terpenoids, present at 2.80%, are known for their diverse biological activities, including antimicrobial, anti-inflammatory, and antioxidant properties. Although present at a lower concentration, terpenoids are important in modulating the activity of other compounds within the extract.

Reducing sugars were absent from the extract, indicating that the sample lacks simple sugars that typically play roles in energy provision and metabolic processes.

Finally, proteins were found in the extract, with a composition of 4.20%. Proteins are crucial for various biological functions, including enzymatic activities and structural roles. Their presence suggests that the extract could contribute to cellular functions and repair processes.

In summary, the phytochemical screening reveals a rich presence of bioactive compounds such as flavonoids, tannins, saponins, alkaloids, phenols, glycosides, and terpenoids, all of which likely work in synergy to confer a wide range of therapeutic benefits, especially in antimicrobial, antioxidant, and anti-inflammatory applications. The absence of steroids and reducing sugars does not detract from the potential potency of the extract, which remains robust due to the high concentrations of other active compounds.

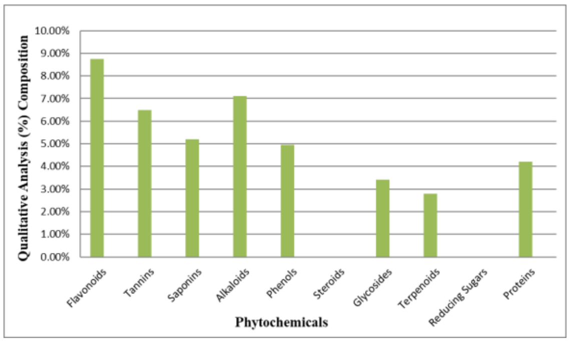

Figure 2: Percentage Composition of the Quantitative Analysis of the Phytochemicals of *Vigna subterranea*.

From the bar graph, flavonoids was the highest composition of the *Vigna subterranea* leaf extract, followed by alkaloids, tannins, phenols and saponins while Terpenoids was the lowest composition, followed by glycosides and proteins.

V. DISCUSSION AND CONCLUSION

5.1 Discussion

The findings of this study indicate that *Vigna subterranea* leaf extract exhibits varying degrees of antibacterial activity against three prevalent wound pathogens: *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. These results align with earlier studies that reported the antimicrobial properties of *Vigna subterranea* extracts, particularly those derived from its seeds. Research by Wanyama (2018) and Taahir (2017) demonstrated the broad-spectrum activity of *Vigna subterranea* against various bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. This consistency reinforces the suggestion that the antibacterial effects of this plant are not limited to specific strains but extend to a wide range of pathogens. Similar findings were reported by Okafor et al. (2022), who observed significant antibacterial activity of Bambara nut extracts against foodborne pathogens, further emphasizing its broad-spectrum efficacy.

The phytochemical composition of the leaf extract, which includes flavonoids, tannins, phenols, saponins, and alkaloids, is also in agreement with previous analyses of *Vigna subterranea* seeds. These bioactive compounds are well-known for their antimicrobial properties, which likely contribute to the antibacterial efficacy observed in this study. Jideani et al. (2014) emphasized the role of tannins and phenols in reducing bacterial colonization and promoting wound healing, supporting the outcomes of this research. Furthermore, a study by Harris et al. (2018) highlighted the synergistic effects of flavonoids and alkaloids in disrupting bacterial cell walls, thus enhancing the overall antibacterial action.

Moreover, while much of the prior research has focused on the antibacterial potential of *Vigna subterranea* seed extracts, this study shifts the focus to leaf extracts. This exploration broadens our understanding of the plant's therapeutic capabilities, highlighting that not only the seeds but also the leaves of *Vigna subterranea* possess significant antibacterial properties. This is consistent with findings by Adebowale et al. (2020), which noted that both the leaves and seeds contain varying concentrations of bioactive compounds that contribute to antimicrobial activity.

A key aspect of this study is its focus on pathogens commonly associated with wound infections, making the findings particularly relevant for wound healing applications. In contrast, previous studies often evaluated the broader antibacterial properties of the plant extract. This targeted approach provides clinically meaningful insights, suggesting that *Vigna subterranea* leaf extract could have potential applications in treating wound-related bacterial infections. This is corroborated by research conducted by Oyeyinka et al. (2021), which documented the efficacy of *Vigna subterranea* extracts in managing infections caused by multi-drug-resistant bacteria, reinforcing the potential of this plant as a natural alternative to synthetic antibiotics.

5.2 Implications for Potential Therapies

The findings from this study suggest that Vigna subterranea leaf extract holds significant potential as a natural antibacterial agent for wound management, especially against *Staphylococcus aureus*. The extract's moderate activity against this pathogen, coupled with its safe profile and widespread availability in various regions, makes it a strong candidate for topical wound treatment.

Incorporating Vigna subterranea leaf extract into topical wound dressings could serve as an effective and natural method for controlling bacterial infections and enhancing wound healing. The antimicrobial properties demonstrated in this study position the extract as a promising option for treating wound-related infections, particularly those caused by *Staphylococcus aureus* and *Escherichia coli*.

Moreover, formulating the extract into antimicrobial solutions or antiseptic washes presents an alternative to conventional antiseptic agents. This could be particularly useful in situations where antibiotic resistance poses a major challenge, as the natural antibacterial properties of the extract may help manage resistant strains without contributing to the further development of resistance.

Furthermore, the extract has potential to serve as a complementary therapy when used alongside standard treatments for wound infections. By boosting the effectiveness of antibiotics, Vigna subterranea leaf extract could play an important role in reducing the risk of antibiotic resistance and improving overall clinical outcomes in the treatment of wound infections.

5.3 Future Research Directions

This study provides a preliminary assessment of Vigna subterranea leaf extract's antibacterial potential, revealing promising results. However, further research is necessary to fully explore its therapeutic value and address potential limitations.

Understanding the mechanism of action is crucial for optimizing the extract's therapeutic potential. It is essential to elucidate the specific pathways through which the extract inhibits bacterial growth to enhance its efficacy in wound management.

In vivo studies on animal models are also needed to validate the extract’s effectiveness under realistic conditions. These studies would provide insights into its wound-healing properties and assess its safety profile in a biological system.

To ensure its relevance in clinical settings, clinical trials involving human subjects are vital. These trials would confirm the extract’s safety and effectiveness in treating wound infections, offering a clearer understanding of its potential in medical applications.
Additionally, investigating potential synergistic effects with other natural antibacterial agents or existing antibiotics could lead to more potent treatment strategies. Such combinations may enhance the extract’s antibacterial activity and reduce the development of antibiotic resistance.

Lastly, the development of practical formulations for topical use, such as creams, gels, or wound dressings, is essential. These formulations would allow for safe and effective application, making the extract a viable option for wound care treatments.

5.4 Limitations

This study presents valuable insights into the antibacterial potential of Vigna subterranea leaf extract; however, several limitations warrant further investigation.

First, the scope of this research was limited, focusing on only a select number of bacterial strains and extract concentrations. To gain a more comprehensive understanding of the extract's efficacy, it is crucial to explore a wider range of pathogens and varying concentrations.

Additionally, the findings were derived from in vitro studies, which do not fully capture the complex biological environment of a wound. While these preliminary results are promising, it is important to conduct further research that reflects the dynamics of wound healing in a more realistic setting.

Furthermore, although the phytochemical analysis through GC-MS provided valuable insights into the extract's composition, a more comprehensive evaluation is necessary. This should include an analysis of all bioactive compounds present in the extract to deepen our understanding of its therapeutic potential and to identify the specific components responsible for its antibacterial activity. By addressing these limitations, future research can better establish the clinical relevance of Vigna subterranea leaf extract in wound management.

5.5 Conclusion

The study's findings provide a compelling foundation for exploring *Vigna subterranea* leaf extract as a potential natural antibacterial agent for wound healing. The extract's moderate activity against *Staphylococcus aureus* highlights its potential for development as a topical treatment. However, further research is necessary to validate its efficacy and safety in a clinical setting.

This study underscores the importance of investigating natural sources for antibacterial agents in the face of rising antibiotic resistance. *Vigna subterranea*, a readily available and safe plant, holds potential for development as a natural solution for wound care, contributing to a more sustainable and effective approach to managing wound infections.

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