In-vitro Phytochemical and Antimicrobial Activity of Selected Parts of *Securidaca longipendunculata* Extracts on Clinical Wound Isolates from Diabetic Foot Ulcers

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Certification

This is to certify that this project was carried out and submitted by **Olubunmi Afolake AYOOLA** with matriculation number **LCU/PG/002720** carried out this research work titled ''***In-vitro* Antimicrobial and Phytochemical Activity of Selected Parts of *Securidaca longipedunculata* Extracts on Wound Clinical Isolates from Diabetic Foot Ulcers**' 'in the Department of Biological sciences, Faculty of Natural and Applied Sciences, Lead City University, Ibadan, Oyo State, for the award of Master's Degree (M.Sc.) in Environmental Microbiology and that this has not been previously submitted.

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Dedication

This research project is dedicated to Almighty God and also to everyone who has contributed to the success of this work.

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Even though the above-mentioned institutions and persons have assisted in the process of this research work, I alone stand responsible for the errors, if any, found in the work.

Abstract

Plant derived compound offer an alternative additional potential source of new antimicrobial, *Securidaca longipedunculata* is one such medicinal plant with a long history of use in Nigeria and African. Diabetic foot ulcers (DFUs) are a significant complication of diabetes mellitus, often leading to chronic infections due to antibiotic-resistant. This study aimed at investigating the *in-vitro phytochemical* and antimicrobial activities of ethanol, n- hexane, chloroform, and aqueous extracts from selected parts of *S. longipendunculata* (root bark, stem bark, and leaves) against wound isolates from DFUs. The tested microorganisms included *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter cloacae, Streptococcus pyogenes*, *Acinetobacter baumannii* and *Candida albicans*. The *In-vitro* phytochemical and antimicrobial activities of the plant extract were determined by agar well diffusion method and phytochemical analysis of the plant extract was carried out using standard method. The phytochemical analysis results of the extracts shown the presence of alkaloids, flavonoids, saponins, tannins, and terpenoid. The *In-vitro phytochemical and* antimicrobial activities results of the extracts using different solvent shown that at 150 mg/mL ethanolic extract had highest zone of inhibition 22.50± 0.5mm against (*E. coli*), 21.00mm ±1.40mm against (*K. pneumonia)* followed by *S. longipendun cu lata* chloroform extract at 18 ± 0.5 mm (*A. baumannii)* and aqueous extract has lowest value of 0.6 ± 0.0 mm (*K. pneumonia)*. The result of ethanolic extract were compared with standard antibiotic of Amikacin ≥ 20mm and Ceftaxidime ≥ 21 mm, revealed that ethanolic extracts had a greater inhibition zone of 22.5 ± 0.5 mm and 21.0 mm ±1.40mm against *E. coli* and K. *pneumonia* respectively. Also, the ethanolic had greater zone of inhibition of 22 mm against *C. albicans* when compared with standard antifungi(fluconazole). The study concludes that S. *longipendunculata* had antimicrobial activity which can be used to develop new therapies for managing diabetic foot ulcers

Keywords: *Securidaca longipedunculata*, Antimicrobial Activity, Phytochemical Analysis.

Word count: 300

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Abbreviation	Meaning
DFU	Diabetic Foot Ulcers
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
CFU	Colony Forming Units
DMSO	Dimethyl Sulfoxide
RBC	Red Blood Cell
CLSI	Clinical and Laboratory Standards Institute
AST	Antimicrobial Susceptibility Testing
PCR	Polymerase Chain Reaction
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High-Performance Liquid Chromatography
NA	Nutrient Agar
TSA	Tryptic Soy Agar
NIST	National Institute of Standards and Technology
SDA	Sabouraud Dextrose Agar
UV	Ultraviolet
FRAP	Ferric Reducing Antioxidant Power
ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid
LD50	Lethal Dose 50
PMNs	Polymorphonuclear Neutrophils

MRSA Methicillin-resistant Staphylococcus aureus

HBOT Hyperbaric oxygen therapy

CRKP Carbapenem resistance *Klebsiella pneumoni*

Chapter One

Introduction

1.1 Background to the Study

Plants have recently become a vital source of natural products for improving human health, especially in underdeveloped nations where traditional medicine continues to be the major source of healthcare for a large proportion of the population[1]. Using plants to treat a variety of illnesses is not a recent development; primary healthcare has long made use of them. The discovery of these medicinal plants' therapeutic qualities has been made possible in large part by observations of their use and efficacy[2]. Several African nations, along with other global regions, have pushed for the testing of plants used in traditional medicine to confirm their antibacterial qualities and explore incorporating them into primary healthcare. It has been claimed that several plants around the world contain antibacterial properties for example, *S. longipedunculata.*[3] Also *Securidacalongipedunculata,* is commonly known as the violet tree. The plant is well known for having therapeutic, pharmacological and medical benefits[4] Nearly every part of the plant is further utilized by humans to treat variety of illness such as couch, constipation, pneumonia, diabetes. It is an african medicinal plant belongs to the polygalaceae family[5]. The plant plays a significant role in both conventional and alternative medicine. In Hausa, it is referred to as Uwarmagunguna, which means "the mother of all drugs" because of its plethora of therapeutic applications[5]. In Igbo, its name, "ezeogwu," which

translates to "king of medicines" because of its great medicinal power in treating illnesses, particularly inflammatory diseases such as asthma, tuberculosis, chronic peptic ulcer, and active hepatitis where it is widely used. In Yoruba, it is called Ipeta[5]. This plant's root has long been used to treat a variety of conditions, including fungal infections, fever, headaches, cancer, rheumatism, tuberculosis (TB), diabetes, venereal diseases, syphilis, sexual impotence, toothaches, pains, epilepsy, convulsions, constipation, pneumonia, backaches, blood purification, skin infections, and to treat wound infection, e.g., diabetes foot ulcer (DFU)[5].

Furthermore, antimicrobial drug resistance is a constant global concern, even with pharmaceutical companies' best efforts to create new and more potent therapies [3]. Pathogens often colonize specific areas and displace some native flora but rarely lead to infection or an immune response. However, any microbe that colonizes the skin or enters a wound can potentially cause an infection if the skin barrier is compromised or the immune system is weakened. The likelihood of infection depends largely on the bacteria present on the skin, as well as the depth and location of the wound[4]. Wound is a localized defect or excavation of the skin or underlying soft tissue where pathogenic organisms have infiltrated into the surrounding viable tissue is referred to as an infected wound. When a wound becomes infected, the body's immune system is triggered, which slows the healing process and causes inflammation and tissue damage[7]. Many infections, such as an infection from a scratch or hair follicle, are commensal and go away on their own. If such infections are not treated; they may worsen and necessitate medical attention[7]. When a wound becomes infected, the body's immune

system is triggered, which slows the healing process and causes inflammation and tissue damage. A scrape or an infected hair follicle are examples of infections that are self-contained and go away on their own. If neglected, some infections might worsen and necessitate medical attention [8]. Wound is considered infected when bacteria are present and multiply, causing an individual's body to react locally or systemically. Infections in wounds are linked to chronic wounds, delayed healing, higher risk of hospitalization, amputation of a limb or finger, and higher healthcare expenses[5].It is acknowledged that the existence of biofilm poses a problem in an infected wounds and is linked to chronicity and delay healing, example; diabetes foot ulcer. Early detection and treatment of wound infection can speed up the healing process and lower the chance of complications[5].

The side effect of diabetes mellitus is diabetic foot ulcers (DFUs), which can have a serious negative impact on morbidity and mortality. In their lifetime, foot ulcers affect 15–25% of diabetes people[11]. These ulcers can lead to extended hospital stays, amputations, and a marked decline in quality of life. The main causes of DFUs' persistent nature are their poor wound healing and high susceptibility to infections, which makes the hunt for potent antibacterial drugs necessary[12].

A variety of aerobic and anaerobic bacteria, such as *S. aureus*, *P. aeruginosa*, *E. coli*, and other anaerobic species, are frequently involved in infections linked to DFUs[13]. The therapy of these diseases is severely hampered by the rising incidence of bacteria that are resistant to antibiotics. As a result, the

search for substitute treatment medicines capable of successfully combating these resistant strains is urgent.

1.2 Statement of the Problem

The rising prevalence of antimicrobial resistance has rendered many conventional antibiotics ineffective, particularly in managing infections in DFUs. Infections caused by pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Candida albicans* are increasingly resistant to commonly used antibiotics and antifungal agents. This resistance prolongs treatment duration, increases healthcare costs, and escalates the risk of amputations or death. Despite advances in synthetic drug development, plant-derived compounds remain a vital source of new antimicrobial agents. However, there is limited scientific data on the antimicrobial efficacy of *S. longipendunculata* against clinical isolates from DFUs. This study aims to fill this gap by evaluating the in-vitro antimicrobial activities of ethanol, n-hexane, chloroform, and aqueous extracts from selected parts of the plant against wound pathogens, providing evidence for its potential use in managing resistant infections.

1.3 Justification of the Study

Medicinal plants such as *S. longipendunculata* are abundant, affordable, and culturally accepted in African communities. Their bioactive compounds represent a natural, sustainable alternative to synthetic antibiotics[5]. Previous studies have demonstrated the antimicrobial potential of *S. longipendunculata*, but its application in treating infections related to DFUs

remains underexplored. By investigating the antimicrobial activities of the plant against clinical isolates from DFUs, this study contributes to the search for novel, plant-based therapies for managing antibiotic-resistant infections. The findings may provide a scientific basis for developing new drugs or enhancing existing treatments for DFU-associated pathogens.

1.4 Aim and Objective(s) of the Study

To evaluate the *in-vitro* phytochemical and antimicrobial activity of selected parts of *S. longipedunculata* extracts on wound clinical isolates from diabetic foot ulcers.

Specific Objectives

1. To identify and collect different parts of *S. longipedunculata* used for treating infections.
2. To prepare various extracts from these plant parts using different solvents ethanol, n- hexane, chloroforom and distilled water.
3. To determine Phytochemical analysis for *S. longipedunculata* root bark, stem and leaves extracts, for qualitative and quantitative, using.
4. To determine the antimicrobial activities of these extracts against clinical wound isolates from DFUs.
5. To determine the combination of a plant extracts against wound clinical isolates from DFUs.
6. To compare the effectiveness of the plant extracts with standard antibiotics.

1.5 Research Questions

i. What part *S. longepedunculata* exhibit antimicrobial activity against clinical wound isolate from diabetic foot ulcers?
ii. How effective are the extracts of these parts of *S. longepedunculata* in inhibiting the growth of DFU-associated with clinical isolates?
iii. What are the phytochemical constituents of these plant parts?
iv. How do the antimicrobial activities of these extracts compare with those of standard antibiotics?
v. What are the MIC and MBC values of the extracts against the targeted clinical isolates?

1.6 Significance of the Study

This study has the potential of providing novel antimicrobial compound that can be developed into therapeutic agents especially with the growing concern of antibiotic resistance. It will provide scientific validation for the traditional use of *S. longipedunculata* in treating infections diabetic wound ulcer, contributing to the body of knowledge on medicinal plants. The findings may lead to the development of new, plant-based antimicrobial agents that can be used as alternatives or adjuncts to conventional antibiotics, particularly for treating antibiotic-resistant infections wound ulcer. Effective management of DFUs can significantly reduce morbidity, healthcare costs, and improve the quality of life for diabetic patients. Highlighting the medicinal value of *S. longipedunculata* may promote its conservation and sustainable use.

1.7 Scope of the Study

This study is limited to the investigation of the effectiveness of the plants stem, root bark, and leaf extracts against wound clinical isolates microorganisms by evaluating the antimicrobial potential of *S. longipedunculata* plant.

The study involves in collection of the stem bark, root and leaf of *S .longipedunculata* from selected location (Ijaye, Akinyele Local Government Area, Oyo State). The plants material will be properly identified and authenticated.

Antimicrobial assays will also be performed to determine the susceptibility of the selected microorganisms to the plant extracts. Efficacy of the plant extracts will be done by comparing with standard antibiotics. Appropriate statistical method will be used to analyze the result obtained from the antimicrobial assays. The inhibitory zone or minimum inhibitory concentrations MIC will be calculated to determine the antimicrobial activity of the extracts.

Phytochemical analysis of the plant extracts will be carried out to identify the various bioactive compounds.

1.8 Limitations of the Study

The study will not cover the *in-vivo* effects of the plant extracts. The study will be limited to bacteria and fungi isolates and may not cover viral pathogens.

1.9 Operational Definition of Terms

i. **Antimicrobial Activity**: The ability of a substance to kill or inhibit the growth of microorganisms.

ii. ***Securidaca longipedunculata***: A medicinal plant native to Africa, known for its various pharmacological properties.

iii. ***In-vitro***: Refers to the technique of performing a given procedure in a controlled environment outside of a living organism.

iv. **Diabetic Foot Ulcer (DFU)**: An open sore or wound that occurs in approximately 15% of patients with diabetes, usually located on the bottom of the foot.

v. **Clinical Isolates**: Microorganisms isolated from clinical specimens for the purpose of identifying pathogens and testing their susceptibilities to antimicrobial agents.

Endnotes

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Chapter Two

Literature Review

2.1 *Securidaca longipedunculata*

Securidaca longipedunculata is known as the violet tree, a tiny or perennial tree that grows in tropical Africa. It is well-known for being widely used in conventional medicine to treat a wide range of illnesses, like antibacterial applied to wounds and infections[1]. Anti-inflammatory, used for inflammatory disorders, antipyretics are used to lower fever. Because of its many medicinal qualities, this plant, which belongs to the *Polygalaceae* family, is renowned for being widely used in traditional African medicine[2]. The violet tree is a tiny, deciduous tree that can reach a height of ten meters. Its tall, pendulous fruit and purple flowers set it apart. The leaves are simple, alternating, and have a leathery feel, while the bark is tough and grayish-brown, widely utilized in ancient medicinal treatments[2]. *Securidaca longipedunculata* is widely distributed and thrives in rocky hillsides, open forests, throughout sub-Saharan African and savannas[3]. It is a hardy plant in its natural habitat because of its excellent adaptability to different soil types and ability to resist dry circumstances. The plant has been used in traditional African medicine for ages. The plant's roots, bark, leaves, and seeds are among its elements that have many medical uses[4].

Bark and roots of *Securidaca longipedunculata* are utilized as anti-inflammatory and analgesics. Bark is used to treat respiratory conditions like coughs and bronchitis, while root preparations are traditionally used to treat rheumatism, arthritis, and headachess[4]. Seeds of *S. longipendunculata* are

used as laxatives to treat constipation and other digestive problems, while leaves, recognized for their antibacterial and antifungal qualities, are used to treat skin infections, wounds, and ulcers, as well as gastrointestinal problems like diarrhoea and dysentery[5]. In addition to its medical properties, the plant has cultural value in many African traditions and is employed in pest management.

2.2 Scientific Classification

Kingdom: Plantae

Phylum: Streptophyta

Class: Equisetopsida

Order: Fables

Family: Polygalaceae

Genius: *Securidaca*

Species: *Securidaca longipedunculata*(Fersen.)

Figure 2:2: ***Securidaca longepedunculata***

Source[5].

2.3 Phytochemical Constituents of *Securidaca longipedunculata*

The medicinal properties of *S. longipedunculata* attributed to its rich phytochemical profile. The compounds include:

i. Flavonoids: Possess antioxidant, anti-inflammatory, and antimicrobial activities.
ii. Alkaloids: Exhibit a wide range of biological activities, including analgesic and antimicrobial activities.
iii. Saponins: They exhibit antifungal and antibacterial effects.
iv. Tannins: Known for their astringent properties, useful in treating wounds and ulcers[6] .

2.4 Antimicrobial Activity of *Securidaca longipedunculata*

Securidaca longipedunculata, is a violet tree, or plant that is well-known for its therapeutic uses in traditional African medicine. It belongs to the family Polygalaceae and is native to several parts of Africa, particularly West Africa[7]. Traditional medicine has employed a number of plant parts, including the roots, leaves, and bark, to treat a range of conditions, including infections. Validating these traditional uses and comprehending the plant's potential as a source of novel antimicrobial compounds have been the main goals of recent scientific studies. The high phytochemical makeup of *S. longipedunculata* is ascribed to its antibacterial action[8]. Numerous bioactive substances, including saponins, flavonoids, alkaloids, and tannins, have been found in the plant through studies[8]. These substances have a well-known capacity to impede the growth of bacteria, fungus, and viruses, among other

antimicrobial qualities. These compounds' existence in *S. longipediculata* highlights the plant's potential as a source of antibacterial agents[9].

Several researchers have examined the antimicrobial characteristics of *S. longipediculata.* For example, their studies have demonstrated that the plant's roots and leaves have strong antibacterial action against a variety of harmful bacteria, such *as Pseudomonas aeruginosa, Escherichia coli,* and *Staphylococcus aureus*[10]. These microorganisms are frequently linked to illnesses, including respiratory, urinary tract, and wound infections. The presence of saponins and flavonoids, which have been demonstrated to damage bacterial cell membranes and stop bacterial development, is frequently credited with the antibacterial activity[11].

2.4.1 Antifungal Activity

Securidaca longipedunculata has antifungal action in addition to antibacterial qualities. Research has indicated that the plant's extracts had the ability to proficiently suppress the growth of multiple fungal infections, such as *Aspergillus niger* and *Candida albicans*[12]. These fungi cause diseases including *Aspergillosis* and *Candidiasis*, which can be especially harmful to people with weakened immune systems. Alkaloids and tannins found in the *S. longipedunculata* are thought to be responsible for the antifungal activity of the plant by interfering with the formation and function of fungal cell walls[13].

2.4.2 Mechanisms of Action

There are numerous and intricate ways *S. longipedunculata* carries out its antibacterial activities. One theory puts it that saponins break down the membranes of microorganisms, causing cell lysis and death[14]. On the other side, flavonoids are believed to block important enzymes needed for microbial metabolism and replication. Furthermore, it has been demonstrated that tannins bind to proteins and other molecules on the surface of microorganisms, inhibiting pathogen adherence and colonization. The broad-spectrum antibacterial action seen in the extracts is a result of these complex processes[13].

2.4.3 Applications in Modern Medicine

The antibacterial activities of *S. longipedunculata* have major significance for modern medicine. New antimicrobial drugs are desperately needed as

antibiotic resistance keeps growing on a global scale. The bioactive substances in the plant present a viable path for the creation of brand-new antibiotics and antifungal medications. Furthermore, the plant's historical use as an infection remedy raises the possibility that it could be a useful tool for creating supplemental treatments to cure microbial infections[16].

The plant has strong antibacterial qualities, which have been confirmed by both scientific studies and traditional usage. Its potent phytochemical makeup, which includes tannins, alkaloids, flavonoids, and saponins, is what makes it effective against a variety of bacterial and fungal infections. These chemicals' various modes of action demonstrate the plant's promise as a source of cutting-edge antibacterial agents.

2.4.4 Pharmacological Activities

Antimicrobial intensity research has indicated that plant extracts possess noteworthy antibacterial activity against a range of pathogens, encompassing bacteria such as *P. aeruginosa, E. coli,* and *S. aureus*, as well as fungi like *C. albicans*[17]. This raises the possibility of creating novel antibiotic treatments, particularly in light of the growing prevalence of antibiotic resistance[17].

Effects of anti-Inflammation and analgesia strong analgesic and anti-inflammatory effects have been shown in research, primarily as a result of the presence of alkaloids and flavonoids. Because of these characteristics, the herb can help treat inflammatory diseases like rheumatism and arthritis[18].

The plant has strong antioxidant activity, which is attributed to its high flavonoid content. Free radicals, which can harm cells and play a role in the development of chronic illnesses including cancer and heart disease, are

mostly countered by antioxidants[19].

Potential anticancer effects according to preliminary research, plant extracts may possess anticancer capabilities, it has been discovered that some substances cause cancer cells to undergo apoptosis, or programmed cell death, which stops the cells from proliferating and growing. This region is under intensive research, with the possibility for producing new anticancer therapies[20].

2.4.5 Toxicity and Safety

Securidaca longipendunculata has several therapeutic uses. According to some research, if ingested in excessive amounts, several parts especially the seeds may be poisonous and resulted in neurological and gastrointestinal problems. As a result, the plant must be under the supervision of a licensed healthcare provider is crucial[21].

2.4.6 Conservation Status

Securidaca longipendunculata is not considered as an endangered plant. Its population in the wild could be threatened by habitat degradation and overharvesting for medical uses. In order to guarantee the long-term availability of this priceless medicinal plant, conservation initiatives and sustainable harvesting methods are essential[22].

The amazing plant has a long history of use in traditional African medicine. Its broad therapeutic characteristics, supplemented by a variety of phytochemicals, making it a great resource for treating numerous illnesses. Its pharmacological properties are still being studied, and this leads to the

discovery of new possible uses, especially in antibacterial and anticancer treatments. To guarantee its survival for future generations, it is crucial to strike a balance between its use and conservation initiatives. *S. longipendunculata* can continue to play a significant role in healthcare by combining traditional knowledge with cutting-edge scientific research to provide natural therapies for a range of medical conditions[23].

2.5 Diabetic Foot Ulcer Wound Infections

Diabetes mellitus-related plantar ulcers are prone to infection because of the increased frequency of mixed wound microbiota and the polymorphonuclear neutrophils (PMNs) poor defense against invasive microorganisms[24]. However, wound infection can be reduced with the best care, which includes pressure release, adequate dressings, and debridement of the damaged tissue. Diabetes wounds treated with a moisture-retentive hydrocolloid dressing had a 2.5% infection rate as opposed to a 6% infection rate when covered with a conventional gauze dressing. In diabetic foot ulcers treated with hydrocolloid dressing, Laing also noted a comparable infection incidence of 2%, even though it was noted that the number of species increased throughout therapy[25].

Staphylococcus aureus, along with other aerobes such *S. epidermidis, Streptococcus sp., P. aeruginosa, Enterococcus sp.,* and coliform bacteria, is a common isolation in diabetic foot ulcers, as it is in most wound types[26]. Anaerobes have been isolated from up to 95% of diabetic wounds using effective microbiological techniques; *Streptococcus, Bacteroides*, and *Prevotella sp*. were the most frequently isolated microorganisms. The necessity to identify the exact microorganism(s) causing diabetic foot ulcers

was questioned in light of the polymicrobial character of these wounds, and it was recommended that a deeper comprehension of the general microbiology of these wounds should serve as the basis for infection treatment[27]. The statement that repeated cultures after the initial culture and subsequent therapy do not confirm or rule out the presence of infection supported this point of view. As a result, the foot infection must be diagnosed predominantly on the basis of clinical findings[27].

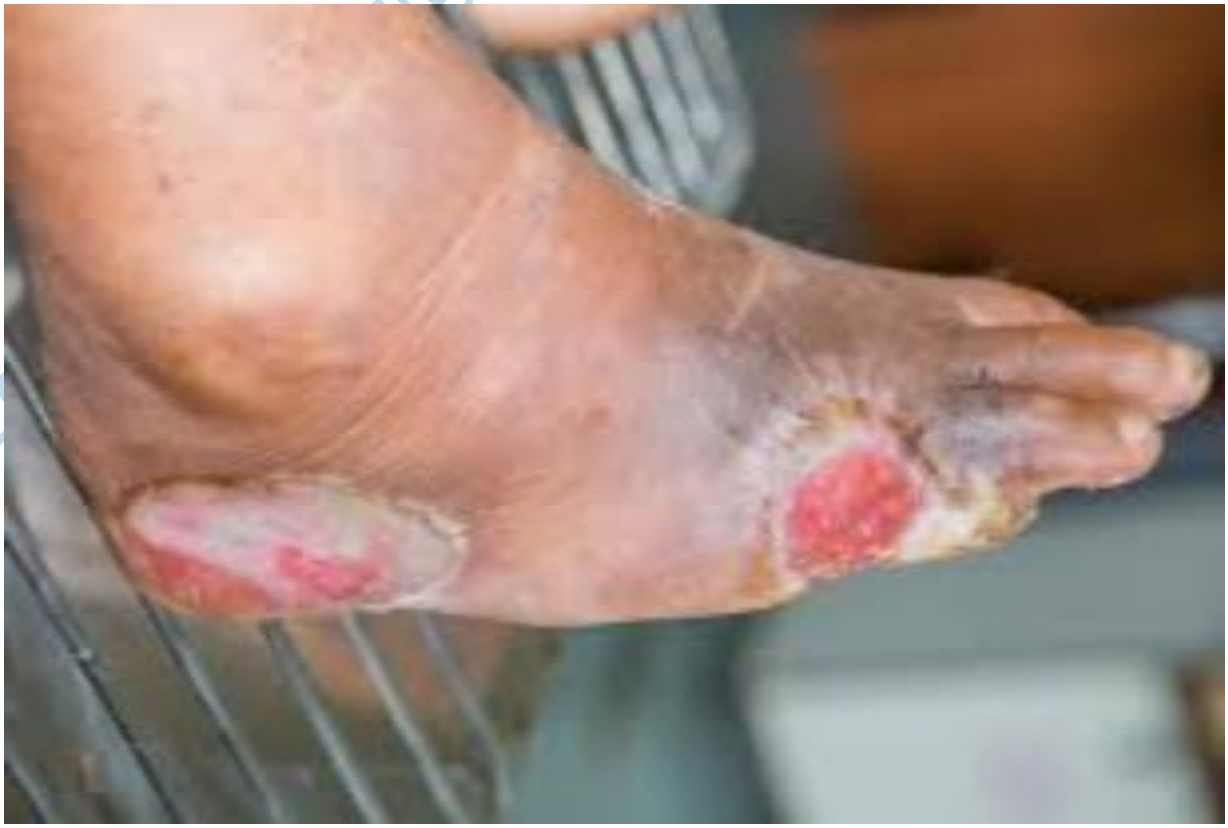

Figure 2.6: Diabetic Foot Ulcer

Source[25].

2.6.1 Causes of Wound Infection

Wound infection can arise from major tissue trauma from surgery, Also, it can also be from a minor cut, bite, or skin puncture from everyday activities[28]. The causes of wounds are infinite, the sources of infection are most commonly two-fold which are bacteria and fungi. The skin's colonization by its natural biome, or flora, includes bacteria that inhabit its surface. These bacteria can contaminate injured areas, potentially leading to wound infections. For instance, an infection might occur after stepping on shells at the beach or cleaning a fish tank with an open cut. Even routine tasks like these can result in infected wounds[29].

More severe but less common infections can arise from surgical site wounds after medical procedures. These infections are typically caused by bacteria present on the patient's skin at the time of the surgical incision. Whether the procedure involves joint replacement or abdominal surgery, the exposure and damage to tissue, even in sterile environments, carry a slight risk of infection.

Wounds have diverse causes, and consequently, infections also stem from various sources, primarily bacterial and fungal microorganisms. Common pathogens that cause wound infections include: Bacterial such as; *Staphylococcus aureus*, *Streptococcus pyogenes*, *Methicillin-Resistant Staphylococcus aureus (MRSA), Clostridium* and others causing conditions like cellulitis.

Fungi such as yeast and molds, including *Candida, Cladosporium, and Aspergillus*.

Furthermore, some conditions can mimic wounds without being actual injuries. For example, viral infections like shingles can present as oozing rashes that resemble wounds. Diabetic foot ulcers (DFUs) are among the most common and severe complications of type 2 diabetes. These chronic wounds result from a combination of factors, including peripheral neuropathy, peripheral arterial disease, and immune deficiencies associated with diabetes[28]. DFUs pose a significant health challenge due to their high risk of infection and the complexity of their treatment[31].

2.6.1 Historical Perspective

Diabetes-related foot problems have long been known about. Foot ulcers and their remedies are mentioned in early medical books, including those written by the Greeks and Egyptians. constituted a critical turning point in the treatment of diabetes, but a better knowledge of the connection between diabetes and foot ulcers didn't start to develop until the late 19th and early 20th centuries[32]. This discovery decreased the frequency of acute complications and enhanced the prognosis for diabetic patients. On the other hand, persistent issues such as DFUs persisted to provide noteworthy difficulties[33]. Research on the particular consequences of diabetes, such as neuropathy and peripheral artery disease, which are both important contributors to the development of diabetic foot ulcers, started to pick up steam in the middle of the 20th century. Research conducted in the 1950s and 60s demonstrated how crucial blood glucose management is to averting diabetes complications[34].

By the end of the 20th century, DFU management had changed dramatically. The introduction of interdisciplinary approaches combining endocrinologists,

podiatrists, vascular surgeons, and wound care specialists improved patient outcomes. Treatment for DFUs has evolved significantly with the advent of enhanced wound care products, including as dressings, growth factors, and skin substitutes, along with the use of hyperbaric oxygen therapy[35].

2.6.2 Pathophysiology of DFUs

One of the main contributing causes to the development of DFUs is peripheral neuropathy. It is the outcome of chronic hyperglycemia, which destroys the nerves and causes the feet to lose feeling[26]. Due to this lack of feeling, patients may not notice small wounds or pressure points, which could cause them to develop into ulcers without the patient's knowledge[36]. Peripheral Artery Disease (PAD), is another important component. It is characterized by artery narrowing or blockage, which lowers blood flow to the lower extremities. As a result of the reduced circulation, wounds and ulcers heal more slowly and are more prone to infection[37]. Diabetes also impairs immunological function, reducing its ability to fight off infections. Due to this immunodeficiency, ulcers can be difficult to cure if they become infected, frequently needing lengthy antibiotic therapy and occasionally culminating in sepsis[38].

2.6.3 Clinical Features and Diagnosis

Diabetes Foot Ulcer (DFU) symptoms can vary, but they frequently involve the following: continuous foot discomfort or tenderness; swelling and redness around the wound; pus or discharge from the ulcer; an unpleasant stench; and black tissue surrounding the ulcer, which indicates necrosis[39]. A comprehensive clinical examination, evaluation of the feet's blood flow and nerve function, and occasionally imaging tests to gauge the degree of tissue

involvement are all part of the diagnosis process[40]. A number of risk factors, such as inadequate glycemic management, diabetes duration, smoking, elevated cholesterol, obesity, a history of foot ulcers or amputations, and inadequate footwear, raise the chance of developing diabetic foot ulcers. The key to preventing DFUs is recognizing and controlling these risk factors [41].

2.6.4 Treatment Approaches

Debridement (removal of necrotic tissue to promote healing), the use of specialty wound dressings to maintain a moist environment and protect against infection, the use of orthotic devices or special footwear to reduce pressure on the ulcer, and the use of antibiotics to treat and prevent infections are all important components of effective management of DFUs[42]. Utilizing bio-engineered skin to cover and treat the ulcer, applying growth factors to promote tissue repair, and using hyperbaric oxygen therapy (HBOT) to speed up wound healing and fight infection are examples of advanced therapies. Surgical intervention may be required in extreme situations. This can involve angioplasty or bypass surgery, which are methods to increase blood flow, as well as partial or total amputations in situations where the infection is uncontrollable[43].

2.6.5 Prevention

Preventing diabetic foot ulcers is critical and includes having healthcare professionals examine patients' feet on a regular basis, educating patients about the value of blood glucose management and adequate foot care, and

making sure patients wear shoes that fit properly and don't rub against their skin or generate pressure points[44].

2.7 *Klebsiella pneumoniae* in Diabetic Wound Ulcers

One prevalent and dangerous side effect of diabetes that can result in significant morbidity and medical expenses is diabetic wound ulcers[45]. Patients with diabetes have poor blood circulation and weakened immune systems, which makes these ulcers more prone to infection. *Klebsiella pneumoniae* is one of the bacteria linked to diabetic wound infections that is particularly noteworthy because of its rising incidence and resistance to several antibiotics [46].

2.7.1 Epidemiology

Klebsiella pneumoniae is frequently isolated from diabetic wound ulcers. Research has indicated that individuals with diabetes have an increased vulnerability to gram-negative bacterial infections, such as *K. pneumoniae. K. pneumoniae* was found to be one of the most common pathogens recovered from diabetic foot infections in a study, showing its importance in this patient population. The bacteria's capacity to create biofilms on the surfaces of wounds makes therapy and elimination even more challenging[47].

2.7.2 Pathogenesis

Numerous virulence factors contribute to the pathogenesis of *K. pneumoniae* in diabetic wound ulcers. Among these is a polysaccharide capsule that shields the bacterium from the host immune system and phagocytosis[48]. Furthermore, the synthesis of siderophores—molecules that take iron from the host—contributes to the development and survival of bacteria. Some researchers' studies claim that *K. pneumoniae* adheres to host tissues through

the use of fimbriae and adhesins, which promotes colonization and infection. These virulence factors cause persistent infections that are challenging to treat, especially in diabetes individuals who have weakened immune responses[49].

2.7.3 Antibiotic Resistance

Antibiotic resistance is a major problem for treating infections in diabetic wound ulcers. Numerous medicines, including carbapenems, which are frequently used as last-resort therapies, have been shown to be resistant to *K. pneumoniae*[50]. The emergence of carbapenem-resistant *K. pneumoniae* (CRKP), which generates carbapenems enzymes that hydrolyse carbapenems and make them useless, was reported by certain researchers. The use of more toxic or less effective antibiotics is required as a result of this resistance, which complicates treatment plans[51].

2.7.4 Clinical Manifestations

Klebsiella pneumoniae can lead to serious infections in diabetic wound ulcers that cause significant tissue damage and slow recovery[52]. The bacterium's capacity to create biofilms on the surface of the wound aggravates the infection and increases its resistance to antibiotic therapy and the immune system's reaction. According to a study conducted by certain researchers, sepsis, a potentially fatal illness, can arise from bloodstream infections caused by *K. pneumoniae* in diabetic patients[53].

2.7.5 Treatment and Management

Antibiotic medication and strict wound care are combined to address *K. pneumoniae* infections in diabetic wound ulcers. Broad-spectrum antibiotics are frequently used in empirical therapy until susceptibility testing allows for the direction of more specialized care[54]. Researchers have undertaken

studies that suggest treating MDR and extensively drug-resistant (XDR*) K. pneumoniae* infections may require combination therapy with polymyxins, tigecycline, and aminoglycosides[55]. Additionally, to control biofilm formation and encourage healing, regular debridement and appropriate wound care are necessary[56].

Because of its resistance to antibiotics, *K. pneumoniae* is a prominent pathogen in diabetic wound ulcers, increasing the risk of serious infections and making treatment more difficult[57]. The bacterium poses a serious threat in clinical settings because of its virulence characteristics and the weakened immune system in diabetic patients. Maintaining *K. pneumoniae* infections in diabetic wound ulcers and reducing their negative effects on patient outcomes require ongoing study and enhanced infection control procedures[47].

2.8 *Escherichia coli* (*E. coli*)

Gram-negative, rod-shaped *E. coli* bacteria are a typical component of the human gut flora[59]. It can, however, turn pathogenic and result in a variety of illnesses, such as wound infections, sepsis, and urinary tract infections[60]. In diabetic patients, wound ulcers are a common and dangerous consequence, and *E. coli* is frequently isolated from these infections. Because *E. coli* can develop biofilms and becomes increasingly resistant to antibiotics, its presence in diabetic wound ulcers poses a serious concern[48].

2.8.1 Epidemiology

Diabetic wound ulcers are particularly susceptible to infections by various bacterial pathogens, including *E. coli*. A study by some researchers highlighted that *E. coli* is one of the common pathogens isolated from diabetic foot infections. The study underscored the bacterium's role in

polymicrobial infections, which are common in diabetic ulcers. The presence of *E. coli* in these ulcers is associated with prolonged healing times and increased risk of complications[49].

2.8.2 Pathogenesis

Multiple virulence factors are involved in the pathogenicity of *E. coli* in diabetic wound ulcers. These include adhesins, which enable the bacteria to stick to the surface of the wound, and biofilm formation, which shields the bacteria from antibiotic therapy and the human immunological reaction[64]. Because they create a persistent reservoir of infection that is challenging to eliminate, biofilms are important in the development of chronic wound infections. Additionally, *E. coli* produces a variety of toxins and enzymes that contribute to tissue damage and inflammation[50].

2.8.3 Antibiotic Resistance

Antibiotic resistance is a major problem for treating *E. coli* infections in diabetic wound ulcers. Numerous antibiotics, such as aminoglycosides, beta-lactams, and fluoroquinolones, have been shown to cause resistance in *E. coli*. According to certain studies, the frequency of *E. coli* that produces extended-spectrum beta-lactamases (ESBLs) is rising, making it more challenging to treat these infections with conventional antibiotics[66]. Treatment attempts are made more difficult by the propagation of resistance genes via plasmids and other mobile genetic components[51].

2.8.4 Clinical Manifestations

In diabetic wound ulcers, *E. coli* can cause severe infections characterized by increased inflammation, tissue necrosis, and delayed healing. Group of some researchers described the clinical presentation of infected diabetic foot ulcers, noting that infections often manifest with signs of local inflammation, purulent discharge, and sometimes systemic symptoms such as fever. The presence of *E. coli* in these wounds is associated with a higher risk of systemic infections, including sepsis, particularly in immunocompromised diabetic patients[52].

2.8.5 Treatment and Management

One of the main issues with treating *E. coli* infections in diabetic wound ulcers is antibiotic resistance. Numerous antibiotics, such as aminoglycosides, beta-lactams, and fluoroquinolones, have been proven to cause resistance in *E. coli*[70]. Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* is becoming more common, according to some research, which makes it harder to treat these infections with traditional antibiotics. Resistance genes spread through plasmids and other mobile genetic components, complicating treatment attempts[53].

2.9*Pseudomonas aeruginosa* in Diabetic Wound Ulcers

The rod-shaped, ram-negative bacteria *Pseudomonas aeruginosa* is a major opportunistic disease in humans. It is well-known for having an innate resistance to numerous antibiotics and for being able to flourish in a variety of contexts, including medical facilities[54]. *Pseudomonas aeruginosa*

infections can cause serious consequences and extended healing times in diabetes patients with wound ulcers[55].

2.9.1 Epidemiology

Diabetic wound ulcers frequently become colonized and infected by a variety of bacterial pathogens, including *Pseudomonas aeruginosa*. Studies have shown that *P. aeruginosa* is a common isolate from chronic wounds, including diabetic foot ulcers[74]. A study conducted by some researcher reveal that *P. aeruginosa* is often associated with high morbidity due to its resistance to multiple antibiotics and its capacity to form biofilms on wound surfaces, which protects it from both the immune system and antimicrobial treatments[56].

2.9.2 Pathogenesis

The pathogenesis of *P. aeruginosa* in diabetic wound ulcers involves several key virulence factors. These include the production of exotoxins such as exotoxin A, which inhibits protein synthesis in host cells, and elastases that degrade host tissues. Furthermore, *P. aeruginosa* has a robust ability to form biofilms, complex communities of bacteria that adhere to surfaces and are embedded in a protective extracellular matrix[76]. With the study conducted some years back, it was revealed that biofilm formation is a critical factor in chronic infections, contributing to the persistence and resistance of *P. aeruginosa* in diabetic wound ulcers[57].

2.9.3 Antibiotic Resistance

One of the major challenges in managing *Pseudomonas aeruginosa* infections is its high level of intrinsic and acquired resistance to multiple antibiotics. *P. aeruginosa* has several mechanisms of resistance, including the production of β-lactamases that degrade β-lactam antibiotics, efflux pumps that expel antibiotics from the bacterial cell, and mutations that alter antibiotic targets[58]. As noted by some researchers, the prevalence of multidrug-resistant (MDR) *P. aeruginosa* strains has been increasing, complicating the treatment of infections in diabetic wound ulcers[59].

2.9.4 Clinical Manifestations

In diabetic wound ulcers, *Pseudomonas aeruginosa* infections can manifest with various clinical features, including increased exudate, a characteristic sweet or fruity odor, and the presence of blue-green pigments (pyocyanin) in the wound. These infections often lead to delayed healing, increased tissue destruction, and a higher risk of systemic complications. With some researcher findings, *P. aeruginosa* infections are associated with a higher incidence of osteomyelitis (bone infection) in diabetic patients, which can lead to severe outcomes and even limb amputation if not adequately managed[60].

2.9.5 Treatment and Management

Antimicrobial medication along with strict wound care are two important components of the multi modal strategy needed to address Pseudomonas aeruginosa infections in diabetic wound ulcers. Empirical antibiotic therapy

often involves drugs having action against *P. aeruginosa*, such as piperacillin-tazobactam, ceftazidime, or carbapenems[61]. However, sensitivity tests and culture should direct final treatment. Treatment with combination antibiotics may be required for MDR strains. Effective wound care techniques, such as routine debridement, appropriate wound dressing, and steps to increase blood flow and lessen pressure on the wound, are crucial for fostering healing and preventing recurrence in addition to antibiotic therapy[62].

2.10 *Streptococcus pyogenes* in Diabetic Wound Ulcers

Streptococcus pyogenes (Group A *Streptococcus,* GAS) is a Gram-positive, beta-hemolytic bacterium that is a major human pathogen. It is responsible for a wide range of infections, from mild superficial skin infections to severe invasive diseases such as necrotizing fasciitis and *streptococcal* toxic shock syndrome. In diabetic patients, wound ulcers are particularly susceptible to infections by *S. pyogenes*, leading to significant complications and healthcare challenges[63].

2.10.1 Epidemiology

Diabetic wound ulcers are prone to infections by various bacterial pathogens, including *S. pyogenes*. Studies have shown that *S. pyogenes* is a common isolate from chronic wounds, including diabetic foot ulcers. A study emphasized the role of *S. pyogenes* in diabetic foot infections, noting its capacity to cause rapid tissue destruction and severe systemic complications.

The prevalence of *S. pyogenes* in these ulcers is associated with increased morbidity and prolonged hospital stays[64].

2.10.2 Pathogenesis

The pathogenesis of *S. pyogenes* in diabetic wound ulcers involves several virulence factors. These include the M protein, which inhibits phagocytosis and helps the bacteria evade the host immune response, and various exotoxins such as streptolysins and pyrogenic exotoxins, which damage host tissues and contribute to the severity of the infection. According to a researcher, the ability of *S. pyogenes* to produce a hyaluronic acid capsule further aids in its evasion from the immune system, enhancing its pathogenic potential in diabetic wound infections[65].

2.10.3 Antibiotic Resistance

Streptococcus pyogenes is generally sensitive to beta-lactam antibiotics, such as penicillin, resistance to other antibiotics used in diabetic wound infections, such as macrolides and clindamycin, has been reported. A researcher conducted a study some years back noted that the emergence of macrolide-resistant *S. pyogenes* strains complicates the treatment of these infections, especially in patients allergic to penicillin. The spread of resistance genes through horizontal gene transfer further exacerbates the issue, making it essential to monitor antibiotic susceptibility patterns in clinical settings[66].

2.10.4 Clinical Manifestations

In diabetic wound ulcers, *S. pyogenes* infections can lead to severe local and systemic manifestations. The bacteria can cause rapid and extensive tissue destruction, known as necrotizing fasciitis, which requires prompt surgical intervention. Additionally, *S. pyogenes* can cause cellulitis, characterized by diffuse inflammation of the skin and subcutaneous tissues, as well as erysipelas, a distinct form of superficial cellulitis with lymphatic involvement. According to some researcher's findings, these infections are often associated with systemic symptoms such as fever, chills, and signs of systemic toxicity, highlighting the aggressive nature of *S. pyogenes* in diabetic wound ulcers[67].

2.10.5 Treatment and Management

Antibiotic medication and diligent wound care are combined to address *Streptococcus pyogenes* infections in diabetic wound ulcers. In order to address any co-infecting pathogens, beta-lactam antibiotics like amoxicillin or penicillin are frequently used in conjunction with beta-lactamase inhibitors in empirical antibiotic therapy. Clindamycin or macrolides may be utilized in penicillin allergy instances, although resistance needs to be taken into account. A study emphasized the significance of supplemental treatments, including intravenous immunoglobulin (IVIG), for severe cases such as toxic shock syndrome caused by streptococcal bacteria[87]. Furthermore, strict wound care procedures are necessary to encourage healing and stop recurrence. These procedures include routine debridement, appropriate wound dressing, and actions to increase blood flow and lessen

pressure on the wound. Because of its virulence features and propensity for antibiotic resistance, *S. pyogenes* is a prominent pathogen in diabetic wound ulcers, increasing the risk of serious infections and making treatment more difficult. Because of the bacterium's capacity to quickly destroy tissue and induce systemic problems, immediate and efficient management is required. To manage these infections and enhance patient outcomes, strict wound care procedures must be combined with tailored antibiotic medication. To battle *S. pyogenes* infections in diabetic wound ulcers, innovative approaches to explore pathogenesis and resistance mechanisms are necessary[68].

2.11 *Staphylococcus aureus* in Diabetic Wound Ulcers

Staphylococcus aureus is a Gram-positive bacterium that is a leading cause of both hospital and community-acquired infections. It is known for its ability to cause a range of infections, from minor skin infections to life-threatening diseases such as sepsis, pneumonia, and endocarditis. In diabetic patients, wound ulcers are particularly susceptible to infections by *S. aureus*, which can significantly hinder the healing process and lead to severe complications[69].

2.11.1 Epidemiology

Diabetic wound ulcers are commonly infected by *S. aureus*. Studies have shown that *S. aureus* is one of the most frequently isolated pathogens from diabetic foot ulcers. According to a study conducted by some researchers, *S. aureus* is responsible for a significant proportion of infections in diabetic foot ulcers, with both methicillin-susceptible *S. aureus* (MSSA) and

methicillin-resistant *S. aureus* (MRSA) being prevalent. The high prevalence of *S. aureus* in diabetic wound ulcers is associated with increased morbidity and healthcare costs due to the need for prolonged treatment and the risk of severe complications[70].

2.11.2 Pathogenesis

The pathogenesis of *Staphylococcus aureus* in diabetic wound ulcers involves several virulence factors. These include surface proteins that promote adhesion to host tissues, enzymes such as coagulase and hyaluronidase that facilitate tissue invasion, and toxins such as alpha-toxin and Panton-Valentine leucocidin (PVL) that damage host tissues and immune cells. A researcher emphasized the role of biofilm formation in the pathogenesis of *S. aureus* infections. Biofilms protect the bacteria from the host immune response and antibiotics, contributing to chronic and recurrent infections[71].

2.11.3 Antibiotic Resistance

A major challenge in treating *Staphylococcus aureus* infections is antibiotic resistance. MRSA, in particular, poses a significant threat due to its resistance to beta-lactam antibiotics, which include methicillin and other penicillin derivatives. Some researcher discussed the mechanisms of resistance in *S. aureus*, highlighting the role of the MRSA gene that encodes a penicillin-binding protein with low affinity for beta-lactams. The emergence of vancomycin-resistant *S. aureus* (VRSA) further complicates

treatment, necessitating the use of alternative and often less effective or more toxic antibiotics[72].

2.11.4 Clinical Manifestations

In diabetic wound ulcers, *Staphylococcus aureus* infections can manifest with a range of clinical features, from mild local inflammation to severe, spreading infections. These infections often present with signs of cellulitis, abscess formation, and purulent discharge. In severe cases, *S. aureus* can cause necrotizing fasciitis and osteomyelitis, which are associated with significant morbidity and require aggressive treatment. According to some researcher's findings, *S. aureus* infections in diabetic foot ulcers are a leading cause of hospital admissions and often necessitate surgical intervention[73].

2.11.5 Treatment and Management

Antibiotic medication along with proactive wound care are combined to address *Staphylococcus aureus* infections in diabetic wound ulcers. Empirical antibiotic therapy frequently uses medications like clindamycin, trimethoprim-sulfamethoxazole, doxycycline, or linezolid to treat both MSSA and MRSA. However, the results of the culture and sensitivity tests should direct definite therapy. A few research groups emphasized the significance of complete wound care, which includes regular debridement, suitable dressings, and techniques to enhance blood flow and lessen pressure on the wound. Vancomycin or daptomycin are frequently used to treat MRSA infections; however, depending on susceptibility patterns, other

drugs such ceftaroline or delafloxacin may be taken into consideration. Because of its virulence characteristics and antibiotic resistance, *Staphylococcus aureus* is a prominent pathogen in diabetic wound ulcers, increasing the risk of serious infections and making treatment more difficult. The bacterium's capacity to create toxins and form biofilms worsens the infection and slows the healing process. Effective management involves a mix of targeted antibiotic therapy and intensive wound care practices. Further investigation into the pathophysiology and mechanisms of resistance is essential to creating novel approaches to treat *S. aureus* infections in diabetic wound ulcers[74].

2.12 *Acinetobacter baumannii* in Diabetic Wound Ulcers

Acinetobacter baumannii is a Gram-negative, opportunistic pathogen that has become a significant cause of nosocomial infections worldwide. It is known for its remarkable ability to acquire resistance to multiple antibiotics and to survive in various environmental conditions. In diabetic patients, wound ulcers are particularly susceptible to infections by *A. baumannii*, which can lead to severe complications and present considerable treatment challenges[75].

2.12.1 Epidemiology

Diabetic wound ulcers are highly susceptible to infections by various bacterial pathogens, including *A.baumannii*. Studies have shown that *A. baumannii* is increasingly isolated from chronic wounds and diabetic foot ulcers. According to the findings of some researcher's, the prevalence of *A.*

baumannii in diabetic wound infections has been rising, particularly in healthcare settings where it can easily spread and persist. The bacterium's ability to survive on surfaces and medical equipment contributes to its role in hospital-acquired infections[76].

2.12.2 Pathogenesis

The pathogenesis of *Acinetobacter baumannii* in diabetic wound ulcers involves several virulence factors. These include outer membrane proteins and lipopolysaccharides that facilitate adhesion and invasion of host tissues, biofilm formation that protects the bacteria from the immune system and antibiotics, and the production of enzymes and toxins that cause tissue damage. A study by some researchers, biofilm formation is particularly important in the chronic nature of *A. baumannii* infections, as it allows the bacteria to persist in the wound environment and evade host defenses[77].

2.12.3 Antibiotic Resistance

One of the major challenges in treating *Acinetobacter baumannii* infections is its extensive antibiotic resistance. *A. baumannii* has developed resistance to a wide range of antibiotics, including beta-lactams, aminoglycosides, and fluoroquinonin, through mechanisms such as the production of beta-lactamases, efflux pumps, and modification of antibiotic targets. Some researchers discussed the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *A. baumannii* strains, which significantly complicates the treatment of infections in diabetic wound ulcers. The ability

of *A. baumannii* to acquire resistance genes through horizontal gene transfer further exacerbates this issue[78].

2.12.4 Clinical Manifestations

In diabetic wound ulcers, *Acinetobacter baumannii* infections can lead to severe local and systemic manifestations. These infections are often characterized by increased inflammation, purulent discharge, and delayed healing. In severe cases, *A. baumannii* can cause necrotizing infections and osteomyelitis, leading to significant tissue destruction and a higher risk of systemic complications such as sepsis. According to discovery by some researchers, infections caused by MDR *A. baumannii* are associated with higher morbidity and mortality rates, particularly in immunocompromised diabetic patients[79].

2.13 *Enterobacter cloacae* in Diabetic Wound Ulcers

Enterobacter cloacae is a gram-negative bacterium belonging to the Enterobacteriaceae family, known for its potential to cause nosocomial infections, particularly in immunocompromised patients. In diabetic individuals, wound ulcers are susceptible to infections by *E. cloacae*, which can complicate healing and lead to severe clinical outcomes[80].

2.13.1 Epidemiology

Diabetic wound ulcers frequently harbor *Enterobacter cloacae* as a causative agent of infections. Studies have highlighted the presence of *E. cloacae* in diabetic foot ulcers and other chronic wounds, often as part of

polymicrobial infections. According to findings by some researchers, *E. cloacae* infections in diabetic patients are associated with prolonged hospital stays and increased healthcare costs due to the need for extensive treatment and management[101].

2.13.2 Pathogenesis

The pathogenesis of *Enterobacter cloacae* in diabetic wound ulcers involves several virulence factors. These include adhesins that facilitate attachment to host tissues, lipopolysaccharides (LPS) that induce inflammation and immune responses, and the production of beta-lactamases that confer resistance to beta-lactam antibiotics. *E. cloacae* can also form biofilms on wound surfaces, which protect it from host defenses and antimicrobial agents, contributing to chronic infections[81].

2.13.3Antibiotic Resistance

Antibiotic resistance is a significant concern in the management of *Enterobacter cloacae* infections. *E. cloacae* strains frequently exhibit resistance to multiple antibiotics, including beta-lactams, fluoroquinolones, and aminoglycosides. The production of extended-spectrum beta-lactamases (ESBLs) and Amp beta-lactamases is common among clinical isolates, limiting treatment options. Surveillance of antibiotic resistance patterns and susceptibility testing are crucial for guiding empirical and targeted therapy[82].

2.13.4 Clinical Manifestations

In diabetic wound ulcers, *Enterobacter cloacae* infections can present with various clinical manifestations, ranging from mild localized inflammation to severe soft tissue infections. These infections may lead to increased wound exudate, delayed healing, and systemic symptoms such as fever and malaise. In immunocompromised diabetic patients, *E. cloacae* infections can progress to bloodstream infections and sepsis, necessitating prompt intervention and comprehensive management[83].

2.14 *Candida albicans* in Diabetic Wound Ulcers

Candida albicans is a yeast-like fungus that is part of the normal human microbiota but can become pathogenic under certain conditions, including in diabetic patients with compromised immune function. In diabetic wound ulcers, *C. albicans* infections can occur as secondary infections, complicating wound healing and leading to persistent and recurrent infections[84].

2.14.1 Epidemiology

While *Candida albicans* primarily colonizes mucosal surfaces such as the oral cavity and gastrointestinal tract, it can also infect skin and soft tissues, particularly in moist and warm environments like diabetic foot ulcers. Diabetic patients are predisposed to *C. albicans* infections due to hyperglycemia, impaired wound healing, and altered immune responses. *C. albicans* is one of the most common causes of fungal infections in hospitalized diabetic patients, including those with chronic wounds[85].

2.14.2 Pathogenesis

The pathogenesis of *Candida albicans* in diabetic wound ulcers involves several virulence factors. These include adhesins that facilitate attachment to host tissues, secreted hydrolytic enzymes (e.g., proteases and phospholipases) that degrade host proteins and lipids, and the ability to form biofilms on wound surfaces. Biofilm formation protects *C. albicans* from host immune responses and antifungal agents, contributing to persistent infections and treatment challenges[86].

2.14.3 Antifungal Resistance

In clinical settings, antifungal resistance of *Candida albicans* has become a major concern. Antifungal drugs like fluconazole and echinocandins usually work on *Candida albicans*, however overuse and chronic exposure to these drugs can cause resistance. Changes in drug targets (such as the ergosterol production pathway) and efflux pumps, which extract antifungal drugs from fungal cells, are examples of resistance mechanisms. In order to determine the best course of treatment for diabetic individuals with *Candida* infections, some studies stressed the significance of monitoring for antifungal resistance[87].

2.14.4 Clinical Manifestations

In diabetic wound ulcers, *Candida albicans* infections can present with various clinical manifestations, depending on the severity and depth of the infection. Superficial infections may manifest as erythematous and pruritic

lesions, whereas deeper infections can lead to cellulitis, abscess formation, and tissue necrosis. Systemic dissemination of *C. albicans* can occur in immunocompromised diabetic patients, leading to invasive *candidiasis* with septicemia and organ involvement[88].

2.14.5 Treatment and Management

The management of *Candida albicans* infections in diabetic wound ulcers requires a tailored approach based on the severity of infection and antifungal susceptibility testing. Topical antifungal agents such as clotrimazole or miconazole may be used for superficial infections, while systemic therapy with fluconazole, echinocandins (e.g., caspofungin), or amphotericin B is warranted for deeper or systemic infections. Combination therapy and prolonged treatment courses may be necessary in cases of persistent or resistant infections. Additionally, optimizing glycemic control and implementing effective wound care practices, including debridement and appropriate dressing, are essential for promoting wound healing and preventing recurrence[89].

Candida albicans infections in diabetic wound ulcers pose significant challenges due to their ability to form biofilms, develop resistance to antifungal agents, and cause both localized and systemic infections. Effective management requires a multidisciplinary approach, integrating antifungal therapy with meticulous wound care and management of underlying diabetes. Continued research into the mechanisms of antifungal resistance and new treatment strategies is essential for improving outcomes in diabetic patients with *Candida* infections[90].

2.15 Antimicrobial Effects of *Securidaca longipendunculata* Parts

Research on *Securidaca longipendunculata* highlights its potent antimicrobial properties against a spectrum of pathogens. Root extracts have shown significant activity against gram-negative bacteria such as *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *E. cloacae*, and *A. baumannii*, as well as gram-positive bacteria including *S. aureus* and *S. pyogenes*. Leaf extracts also exhibit strong antimicrobial effects against these bacteria, supported by studies indicating their efficacy in inhibiting *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *S. aureus*, *S. pyogenes*, *E. cloacae*, and *A. baumannii*. Additionally, seed extracts have demonstrated promising antimicrobial activity, particularly against *S. aureus*, *E. coli*, and *P. aeruginosa*. These antimicrobial effects are attributed to the diverse phytochemical composition of *S. longipendunculata*, including alkaloids, flavonoids, and tannins, which disrupt microbial cell membranes and inhibit vital enzymatic processes crucial for microbial survival[91].

Research on *S. longipendunculata* highlights its potent antimicrobial properties against a spectrum of pathogens[92]. Root extracts have shown significant activity against gram-negative bacteria such as *K. pneumoniae*, *E. coli*, *Pseudomonas aeruginosa*, *E. cloacae*, and *A. baumannii*, as well as gram-positive bacteria including *Staphylococcus aureus* and *S. pyogenes*. Leaf extracts also exhibit strong antimicrobial effects against these bacteria, supported by studies indicating their efficacy in inhibiting *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *S. aureus*, *S. pyogenes*, *E. cloacae*, and *A. baumannii*. Additionally, seed extracts have demonstrated promising antimicrobial

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2.16 Standard Antibiotics

a. Levofloxacin: Levofloxacin belongs to the fluoroquinolone class and is widely used for its broad-spectrum activity against both gram-positive and gram-negative bacteria. It inhibits bacterial DNA gyrase and topoisomerase IV enzymes, essential for DNA replication and repair. Some authors have extensively studied its pharmacokinetics, efficacy, and resistance mechanisms[94].

b. Amikacin: Amikacin is an aminoglycoside antibiotic effective against aerobic gram-negative bacteria like *Pseudomonas aeruginosa* and *E. coli*. Its mechanism involves binding to the bacterial 30S ribosomal sub unit, thereby inhibiting protein synthesis. Studies by some researchers have contributed to understanding its clinical applications and resistance patterns[95].

c. Gentamicin: Another aminoglycoside, gentamicin acts similarly to amikacin by binding to the bacterial 30S ribosomal subunit. It is used for severe gram-negative infections and has been extensively researched for its pharmacokinetics and efficacy. Some Authors have contributed to the literature on its clinical use and resistance mechanisms[96].

d. Cefuroxime: Cefuroxime is a second-generation cephalosporin antibiotic effective against a wide range of gram-positive and gram-negative bacteria. It inhibits bacterial cell wall synthesis by binding to penicillin-binding

proteins. Another Authors have explored its pharmacokinetics, efficacy in various infections, and resistance patterns[97].

e. Ceftriaxone: Ceftriaxone is a third-generation cephalosporin known for its broad-spectrum activity against gram-positive and gram-negative bacteria. It inhibits bacterial cell wall synthesis. Research has contributed to understanding its clinical applications, pharmacokinetics, and resistance mechanism[98].

f. Augmentin (Amoxicillin/Clavulanate): Augmentin is a combination antibiotic consisting of amoxicillin (a penicillin derivative) and clavulanate (a beta-lactamase inhibitor). It is effective against a broad spectrum of bacteria, including beta-lactamase-producing strains. Some Authors have studied its efficacy, pharmacokinetics, and resistance patterns[99].

g. Imipenem: Imipenem is a carbapenem antibiotic with broad-spectrum activity against gram-positive and gram-negative bacteria, including multi-drug resistant strains. It inhibits bacterial cell wall synthesis. Studies by some authors have contributed to understanding its clinical use, pharmacokinetics, and resistance mechanisms[100].

h. Streptomycin: Streptomycin is an aminoglycoside antibiotic effective against tuberculosis and certain gram-negative bacteria. It binds to the bacterial 30S ribosomal subunit, inhibiting protein synthesis. Some authors have explored its pharmacokinetics, clinical efficacy, and resistance[101].

i. Ciprofloxacin: Ciprofloxacin is a fluoroquinolone antibiotic used to treat a wide range of infections, particularly gram-negative bacteria. It inhibits bacterial DNA gyrase and topoisomerase IV enzymes. Research by some

researcher has contributed to understanding its pharmacokinetics, efficacy, and resistance mechanisms[102].

j. Erythromycin: Erythromycin is a macrolide antibiotic effective against gram-positive bacteria and some gram-negative bacteria. It inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit. Some Authors have studied its clinical use, resistance patterns, and pharmacokinetics[102].

Endnotes

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Chapter Three

Methodology

3.1 Collection and Processing of Plant Materials

Fresh roots bark, stem bark and leaf of *S. longipedunculata* was collected from Ijaye, Akinyele Local Government Area in Oyo state. Identification was done in the taxonomy department of the Forestry Research Institute of Nigeria (FRIN). The plant was verified before depositing in the herbarium under voucher number FHI. 113621. The plant materials were properly washed under tap water, rinsed with distilled water, air-dried, blended and kept in a clean container for subsequent use.

3.2 Collection of the Test Organisms

Pure clinical isolates of bacterial strains of *E. coli, K. pneumonia, S. aureus, S. pyogenes, A. baumannii, P. aeruginosa, E. cloaca* and *C. albican* collected from the Microbiology Laboratory Department in the University College Hospital, Ibadan. Eight clinical isolates were collected maintained on nutrient agar slant at 4ºC and sub cultured on nutrient agar plate for 24hr prior to testing. Similarly, the fungal isolate was maintained on potato dextrose slant and sub cultured on Potato dextrose agar plate. The Enterobacteriaceae were identified by using (API)20E kit while *Streptococcus pyogenes* was identified by using (API) 20 Strep kit. and *Staphylococcus aureus.* Also identified by using (API)20 staph kit.

3.3 Preparation of Plant Extracts

Preparation of Extract

Fifteen grams (15 g) each of the powdered plant material was weighed using electronic weighing scale (a Metler Toledo FA21041A) and soaked individually in 100 ml of ethanol, hexane, chloroform and aqueous (solvent) measured in a 1000 ml capacity conical flask. The mixture was covered with aluminum foil and allowed to stay for1 24 hours (h) at room temperature with optical shaker for homogeneous mixture. Thereafter, the solvents were concentrated and evaporated on a rotary evaporator at 400^{o}C to produce the extracts, which were then filtered using Whatman N0 1 filter paper with a pore size[1].

3.4 Phytochemical Screening of the Extracts of the Seed, Leaf and Root of *S. longipedunculata*

3.4.1 Qualitative Determinations Procedure

Phytochemical investigation was carried out on the plant extracts for both qualitative and quantitative testing with the solvent extract of the plant using standard procedures to identify the bio-active constituents in which described[3].

a. Test for Tannins

About 0.5 g of the dried powdered sample was be boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were be added and observed for brownish green or a blue-black coloration[11].

b. Test for Phlobatannins

Deposition of a red precipitate when an aqueous extract of mixture of each plant sample was boiled with 1% aqueous hydrochloric acid taken as evidence for the presence of Phlotannins.

c. Test for Saponin

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

d. Test for Flavonoids

Three methods were used to determine the presence of flavonoids in the plant sample[4]. 5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2S0_4. A yellow coloration observed in each extract was indicated in the presence of flavonoids. The yellow coloration disappeared on standing. Few drops of 1 % aluminum solution were added to a portion of each filtrate. A yellow coloration observed indicated the presence of flavonoids.

A portion of the mixture powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia

solution. A yellow coloration observed indicated a positive test for flavonoids.

e. Test for Steroids

About 2 ml of acetic anhydride was added to 0.5 g Ethanolic extract of each sample with 2 ml H_2S0_4. The color changed from violet to blue or green in some samples indicates the presence of steroids.

f. Test for Terpenoids (Salkowski Test)

About 5 ml of each extract was mixed in 2 ml of chloroform and concentrated H_2S0_4 (3ml) was carefully added to form a layer. A reddish-brown coloration of the interface was formed to show positive results for the presence of terpenoids.

g. Test for Anthraquinones

Two hundred milligrams of the mixture samples were boiled with 6 ml of 1% HCl and filtered. The filtrate was shaken with 5 ml of benzene, filtered and 2 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, violet or red color in the ammoniacal phase indicated the presence of free hydroxyl anthraquinones.

h. Test for Carbohydrates

Few drops (3ml) of mixture sample solution were taken in a clean and dry test tube. Then a few drop of α- naphthol solution was added to it and shaken carefully. Finally, 1ml H_2SO_4 concentration was be added to the test tube slowly and the solution was allowed to stay for few mins The presence

of carbohydrate was indicated by the formation of a violet ring or purple violet coloration at the junction of the two liquids.

3.4.2 Quantitative Determinations Procedure

a. Tannin

Sample of 0.20g was measured into a 50 mL beaker 20mL of 50% methanol was added and covered with Para film and placed in a water bath at 77-80oC for 1 hour. It was shaking thoroughly to ensure a uniform mixing. The extract was quantitatively filtered using a double layered Whatman No 41 filter paper into a 100 mL volumetric flask, 20 mL water added, 2.5 mL folin-Denis reagent and 10 mL of 17% $Na2CO_3$ was added and mixed properly6. The mixture was made up to mark with water mixed well and allow to stand for 20min, the bluish –green color was developed at the end of 20 min Working standard solutions of Tannin of range 0-10ppm was treated similarly as 1mL sample. The absorbance of the Tannic acid standard solutions as well as samples was read after color development on a Spectronic 21D spectrophotometer at a wavelength of 760 nm. % Tannin was calculated using the formula[7].

$$\%\text{TANNIN} = \frac{\text{absorbance of sample X average gradient factor X Dilution factor}}{\text{Wt. of Sample X } 10{,}000}$$

b. Alkaloids Procedure: This is a distillation and titrimetric procedure 2g of finely ground sample was weighed into a 100 mL beaker and 20 mL of 80% absolute alcohol added to give a smooth paste. The mixture was transferred to a 250mL flask and more alcohol added to make up to 100 mL and 1g magnesium oxide added. The mixture was digested in a boiling water bath for 1.5 hr under a reflux air condenser with occasional shaking. The

mixture was filtered while hot through a small Buchner funnel. The residue was returned to the flask and re-digested for 30 min with 50 mL alcohol after which the alcohol was evaporated, adding hot water to replace the alcohol lost. When all the alcohol has been removed, 3 drops of 10% HCl was added. The whole solution was later transferred into a 250 mL volumetric flask 5mL of zinc acetate solution and 5ml of potassium ferrocyanide solution was added, thoroughly mixed to give a homogenous solution.

The flask was allowed to stand for a few minutes, filtered through a dry filter paper and 10 mL of the filtrate was transferred into a separator funnel and the alkaloids present was extracted vigorously by shaking with five successive portions of chloroform. The residue obtained was dissolved in 10 mL hot distilled water and transferred into a kjeldahl tube with the addition of 0.20g sucrose and 10 mL Conc.H2SO4 and 0.02 g selenium for digestion to a colorless solution to determine %N by Kjeldahl distillation method9. %Nitrogen got was converted to % total alkaloid by multiplying by a factor of 3.26 i.e % Total alkaloid = %N X 3.26

% Alkaloids = %N X 3.26

c. Flavonoids Determination

Finely of 0.50 g ground sample was weighed into a 100 mL beaker and 80 mL of 95%, Ethanol added and stirred with a glass rod to prevent lumping. The mixture was filtered through a Whatman No.1. filter into a 100 mL volumetric flask and made up to mark with Ethanol. 1 mL of the extract was pipetted into 50 mL volumetric flask, four drops of Conc. HCL was added via a dropping pipette after which 0.5 g of magnesium turnings added to develop a magenta red coloration. Standard flavonoid solution of range 0-5 ppm was prepared from 100 ppm stock solution and treated in a similar way with HCl and magnesium turnings like sample. The absorbance of magenta red coloration of sample and standard solutions was read on a digital.

d. Saponin

Finely of 1 g ground sample was weighed into a 250 mL beaker and 100 mL of isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure uniform mixing.

Thereafter the mixture was filtered through a whatman No1 filter paper into a 100 mL beaker and 20 mL of 40% saturated solution of maganesium carbonate was added. The mixture obtained with saturated $MgCO_3$ was again filtered through a Whatman No1 filter paper to obtain a clear colorless solution. 1 mL of the colorless solution was pipetted into 50 mL volumetric flask and 2 mL of 5% $FeCl_3$ solution was added and made up to mark with distilled water. It was allowed to stand for 30min for blood red color to develop. 0-10 ppm standard Saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly with 2 mL of 5% $FeCl_3$ solution was done for 1 mL sample above Jenway V6300 Spectrophotometer at a wavelength of 520 nm. The percentage flavonoid was calculated using the formula.

$$\frac{\text{Absorbance of sample x average gradient factor x dilution factor}}{\text{Wt. sample x 10,000}}$$

The absorbance of the sample as well as standard saponin solutions was read after color development in a Jenway V6300 Spectrophotometer at a wavelength of 380min.

$$\%\text{Saponin} = \frac{\text{Absorbance of Sample x Gradient Factor x Dilution Factor}}{\text{Wt. of sample x10,000}}$$

e. Determination of Glycoside

10 mL of extract was pipetted into a 250 mL Conical Flask. 50ml Chloroform was added and shaken on a Vortex Mixer for 1 hr. The mixture was filtered into 100 mL Conical flask and 10 mL pyridine, 2 mL of 2% sodium nitroprusside was added, shaken thoroughly for 10 minutes. 3 mL of 20% NaOH was later add to develop a brownish yellow colour[4].

Glycoside standard of concentrations, which range from 0-5mg /mL was prepared from 100 mg/mL stock Glycoside standard. The series of standards 0-5 mg/mL was treated similarly like sample above.

The absorbance's of sample as well as standards were read on a Spectronic 21D Digital Spectrophotometer at a wavelength of 510 nm. % Glucoside was calculated using the formula:

$$\frac{\text{Absorbance of sample x gradient factor x dilution factor}}{\text{Wt. of sample x10,000}}$$

f. Determination of Steroids

Sample of 0.50 g extract was weighed into a 100 mL beaker 20 mL of Chloroform-Methanol (2:1) mixture was added to dissolve the extract upon shaking for 30 minutes on a shaker. The whole mixture will later filter through a Whatman No.1filter paper into another dry clean 100 mL Conical Flask.

The resultant residue was repeatedly treated with Chloroform-Methanol mixture until free of Steroids. 1mL of the filtrate was pipetted into a 30 mL test tube and 5 mL of alcoholic KOH was added and shaken thoroughly to obtain a homogenous mixture. The mixture was later placed in a water bath set at 37^0C-40^0C for 90 minutes. It was cooled to room temperature and 10 mL of petroleum ether added followed by the addition of 5 mL distilled water. This was evaporated to dryness on the water bath. 6 mL of Liebermann Burchard reagent was added to the residue in dry bottle and absorbance taken at a wavelength of 620 nm on a Spectronic digital Spectrophotometer[5].

Standard Steroids of concentration of 0-4 mg/mL was prepared from 100 mg/mL stock steroid solution and was treated similarly like sample as above. % Steroid was calculated using the formula:

$$\frac{\text{Absorbance of Sample x Gradient x Dilution Factor}}{\text{Wt of sample x 10,000}}$$

g. Determination of Cardenolides

Extract of 0.50 g was accurately weighed into 100 mL beaker followed by the addition of 50 mL of Chloroform to dissolve the extract.0.20 of Sodium bicarbonate powder ($NaHCO_3$) was added after complete dissolution of extract in Chloroform to remove any free acid. The mixture was later transferred into a 250 mL Separatory funnel and thoroughly shaken to allow the two layers to separate. 5 drops of acetic anhydride were added to allow the mixture to be cleared and free of cloudy suspensions11. This was filtered through a Whatman No 1 Filter paper into 100 mL Volumetric Flask and make up to mark with chloroform. Standard cardenolides solutions of concentration 0-10 mg/mL was prepared from 100 mg/mL stock cardenolide solution and treated similarly as sample above to obtain the gradient factor. % Cardenolide was calculated using the formula:

$$\frac{\text{Absorbance of Sample x Gradient Factor x Dilution Factor}}{\text{Wt. of sample x 10,000}}$$

h. Determination of Phlobatannin

Sample of 0.50 g extract was weighed into 50 mL beaker. 20 mL of 50% Methanol was added and covered with parafilm and placed in a water bath set at 77-80^{0}C for 1 hour. The mixture was properly shaken to ensure uniform mixing and later filtered through a Whatman No 1 Filter paper into

a 50 mL volumetric flask using aqueous methanol to rinse, and make up to mark with distilled water. 1ml of the sample extract was pipetted into a 50 mL volumetric flask, 20 mL water 2.5 mL Folin-Dennis reagent and 10 mL of 17% Sodium carbonate was added to the solution in the 50 mL Flask. This mixture was homogenized thoroughly for 20 mins.0-5 mg/mL of Phlobatannin standard concentration will be prepared from100 mg/mL phlobatannin stock solution and treated like sample above[6]. The absorbance of standard solutions as well as sample was read on a Spectronic 21D spectrophotometer at a wavelength of 550 nm. % Phlobatannin was calculated using the formula:

$$\frac{\text{Absorbance of Sample x Gradient Factor x Dilution Factor}}{\text{Wt. of sample x 10,000}}$$

i. Determination of Anthraquinones

Sample of 0.50 g was weighed into 250 mL beaker and 60 mL benzene added and stirred with a glass rod to prevent lumping. This was filtered into 100 mL volumetric flask using Whatman No.1 filter paper.10 mL of filterate was pipetted into another 100 mL volumetric flask and 0.2% Zinc dust was added followed by the addition of 50 mL hot 5% NaOH solution. The mixture was heated just below boiling point for five minutes and then rapidly filtered and wash once in water. The filtrate was again heat with another 50 mL of 5% NaOH to develop a red colour[12]. Standard Anthraquinone solution of range 0-5 mg/l was prepared from 100 mg/l stock Anthraquinone and treated in a similar way with 0.2% Zinc dust and NaOH like sample. The absorbance of sample as well as that of standard concentrations was read on a Digital Spectrophotometer at a wavelength of 640 nm. The percentage anthraquinone was calculated using the formula:

$$\frac{\text{Absorbance of Sample x Gradient Factor x Dilution Factor}}{\text{Wt. of sample x 10,000}}$$

j. Determination of Terpenoid

Sample of 0.50 g was weighed into a 50 mL Conical Flask, 20 mL of 2:1 Chloroform-Methanol mixture was added, shake thoroughly and allow to stand for 15 minutes. The mixture was later centrifuge for another 15 minutes. Supernatant obtain was discarded, and the precipitate was re-washed with another 20 mL chloroform-methanol mixture for re-centrifugation[13].

The resultant precipitate was dissolved in 40ml of 10% Sodium Deodocyl Sulphate solution. 1ml of 0.01 M Ferric Chloride solution was added to the above at 30s interval shake well, and allows to stand 30 minutes. Standard Terpenoid of concentration range 0-5 mg/ml was prepared from 100 mg/l stock Terpenes solution from Sigma-Aldrich chemicals, U.S.A. The absorbances of sample as well as that of standard concentrations of Terpenes was read on a Digital Spectrophotometer at a wavelength of 510 nm. The percentage Terpene is calculated using the formula:

$$\frac{\text{Absorbance of Sample x Gradient Factor x Dilution Factor}}{\text{Wt. of sample x 10,000}}$$

k. Determination of Phenol

Sample of 0.20 g was weighed into a 50 mL beaker, 20 mL of acetone was added and homogenize properly for 1 hr to prevent lumping. The mixture was filtered through a Whatman No.1 filter paper into a 100 mL Volumetric Flask using acetone to rinse and make up to mark with distilled water with thorough mixing. 1 mL of sample extract was pipetted into 50 mL

Volumetric flask, 20 mL water added, 3mL of phosphomolybdic acid was added followed by the addition of 5 mL of 23% Na2CO3 and mix thoroughly, make up to mark with distilled water and allowed to stand for 10 min to develop bluish-green colour.

Standard Phenol of concentration range 0-10 mg/mL was prepared from 100 mg/L stock Phenol solution from Sigma-Aldrich chemicals, U.S.A. The absorbances of sample as well as that of 105 standard concentrations of Phenol was read on a Digital Spectrophotometer at a wavelength of 510 nm. The percentage Phenol was calculated using the formula:

$$\frac{\text{Absorbance of sample x gradient factor x dilution factor}}{\text{Wt. of sample x 10,000}}$$

l. Determination of Chalcones

Sample of 0.50 g extract was weighed into a 100 mL beaker 20 mL of Chloroform-Methanol (2:1) mixture was added to dissolve the extract upon shaking for 30minutes on a shaker. The whole mixture was later filtered through a Whatman No.1filter paper into another dry clean 100 mL Conical Flask/Beaker. The resultant residue was repeatedly treated with Chloroform-Methanol mixture until free of Chalcones. 1mL of the filtrate was pipetted into a 30ml test tube and 5ml of alcoholic KOH was added and shaken thoroughly to obtain homogenous mixture. The mixture was later placed in a water bath set at 37 0C – 40 0C for 90 minutes. It was cooled to room temperature and 10 mL of petroleum ether added followed by the addition of 5 mL distilled water. This was evaporated to dryness on the water bath. 6 mL of Liebermann Burchard reagent was added to the residue in dry bottle

and absorbance taken at a wavelength of 620 nm on a Spectronic digital Spectrophotometer.

Standard Chalcones of concentration of 0-4 mg/mL was prepared from 100 mg/mL stock steroid solution and treated similarly like sample as above. % Chalcones was calculated using the formula:

$$\frac{\text{Absorbance of Sample x Gradient x Dilution Factor}}{\text{Wt of sample x 10,000}}$$

3.5 Preparation of Extract

Fifteen grams (15 g) each of the powdered plant material was weighed using electronic weighing balance (a Metler Toledo FA21041A) and soaked individually in 100 ml of ethanol, n-hexane, chloroform and aqueous (solvent) measured in a 1000 ml capacity conical flask. The mixture was covered with aluminum foil and allowed to stay for 24 hours at room temperature with optical shaker for homogeneous mixture. Thereafter, the solvents were concentrated and evaporated on a rotary evaporator at 400°C to produce the extracts, which were then filtered using Whatman N0 1 filter paper with a pore size.

3.6 Validity of Test Organisms

The organisms was maintained in nutrient agar slants for 48 hours in a refrigerator at about 40C before they are further sub-cultured into freshly prepared petri dishes using streak plate method. Gram staining procedure and other relevant biochemical tests such as catalase, starch hydrolysis, oxidase, urease, indole and citrate was carried out on each of the isolates to establish the validity and viability of the test bacteria[7].

3.7 Biochemical Tests Identification of Tested Microorganisms

3.7.1 Gram's Staining Procedure

Thin smear of pure bacterial culture was made on clean glass slide air dried and heat fixed. Smear was covered with crystal violet for 30 seconds, washed with distilled water and smear was flooded with Grams iodine solution for 60 seconds, washed with 95% ethyl alcohol and then distilled water again, the smear will be covered with safranin for 30 seconds, wash with distilled water and blot dried. Air dried and observed under microscope[8].

3.7.2 Catalase Test

Catalase test is used to identify microorganisms that produce the enzyme catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into oxygen and water $2H_2O_2 \rightarrow 2H_2O+O_2$. This test was carried out in a lamina flow hood. Small inoculum of bacteria isolate was mixed into two to three drops of hydrogen peroxide solution (3%) and observed for the rapid elaboration of oxygen bubbles occurs. The lack of catalase was observed by a lack or weak bubbles production[9].

3.7.3 Indole Test

This test is important in the grouping and identification of anaerobic bacteria. The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. Tryptophan is an amino acid that can undergo deamination and hydrolysis by bacteria that express tryptophanase enzyme.

Twenty-four hours young culture media was inoculated into 10 mL of tryptophan broth which was already sterilizes at 121 °C for 15 minutes and allow cooling before the inoculation, incubated at 37°C for 24-48 hours. After 48 hours of incubation, 5 drops of 0.5 mL of Kovac's reagent were added to the broth culture, shaken gently allow to stand for 20 minutes. Formation of a deep red color at the top layer indicates a positive and yellow coloration indicates negative result[4].

3.7.4 Citrate Test

This test was carried out to study the ability of an organism to use citrate as a sole source of carbon and energy. 24g of citrate agar was dissolved in 500 mL of distilled water followed by sterilization in autoclave. Dispense into the petri dishes allow to cool, aseptically inoculate by streaking the organisms once across the surface. The inoculated medium was incubated for 24 to 48 hours, the color of the medium indicates the result. A change from green to blue indicates utilization of the citrate which is positive but if the media retain the green color after incubation period then the bacteria is citrate negative[4].

3.7.5 Hydrolysis of Starch Test

This test was done to identify bacteria that can hydrolyze starch (amylose and amylopectin). Starch agar plates were inoculated with the selected isolates and incubated at 35°C for 2 days. After incubation each plate was flooded with aqueous iodine for 30 seconds. The iodine reacts with the starch to give a dark brown color. Hence a clear zone around the bacteria growth indicates positive results, while blue black coloration indicates a negative result[4].

3.7.6 Oxidase Test

Impregnated oxidase strip was put on clean Petri dish, using a sterile inoculating loop to pick up a well-isolated colony of test bacteria from fresh culture and make a smear on the oxidase strip. Observation for color change from initial color to purple was noted within 60 seconds, this indicates oxidase positive, but with no color change indicate negative[10].

3.8 Preparation of Media

3.8.1 Preparation of Nutrient Agar (NA)

Nutrient agar (NA) was prepared in line with the manufactures instructions and following the method described by a study[2]. Briefly, 28 g of NA was dissolved in 1000 ml of distilled water, the suspension was mixed until completely homogenized. The conical flask containing the media was plugged with cotton wool and capped with aluminum foil. The flask was sterilized using autoclave at 121^{o}C for 15 minutes, cooled to 30^{o}C and poured into sterile plates[10].

3.8.2 Chocolate Agar

Chocolate agar was prepared by adding defibrinated blood to the warm base media (>60 °C) and gently shaking the mixture for 15–20 min, promoting the hemolysis of erythrocytes and the release of hemin (X factor) and nicotinamide adenine dinucleotide (NAD) which are particularly required for the growth of microorganism. Suspend 20 g in 460 ml of distilled water and heated with frequent agitation until the medium boils well. Sterilize by autoclaving at 121 °C for 15 minutes. Cool to 50 °C and add aseptically 35

ml of sterile defibrinated blood and chocolate by heating at 80 °C for 10 min[3].

3.8.3 Chromogenic Agar

Chromogenic agar for isolation and differentiation of major clinical-significant *Candida* species. About 47.7 g of powder base was slowly dispersed in 1 liter of purified water. Stir until agar is well thickened. Heat and bring to boiling (100 °C) while swirling or stirring regularly. Do not heat more than100 °C[11].

3.9 Anti-bacterial Activities of the Plant Extract against Test Pathogen

Using inoculating wire loop, the test organisms was streaked on the surface of the solidified nutrient agar plates. A sterile 6 mm cork-borer was used to make a uniform deep well into the agar gel. Each of the well was filled with 1 mL of the extracts prepared in different solvents. The Petri plates were allowed to stand for 30minutes at room temperature to allow the proper diffusion of extract. The controls were then set up using the solvents alone, without the extracts. Sterilized distilled water was used as control for the aqueous sample. The plates were then incubated at 37℃ for 24hours after which the zone of inhibition was measured with the transparent ruler.

3.9.1 Determination of Different Concentrations of the Leaf, Root Bark, And Stem Bark Extract Used

Stock solution of the plant fractions were prepared by mixing 15 g of each extract in 100ml of solvent (150 mg/ml). Then other concentrations were

prepared from the stock solutions by using law of equivalence proportion formula[12]. $C_1V_1 = C_2V_2$

3.9.2 Antibiotic Susceptibility Test

Antibiotic susceptibility testing was carried out with the use of antibiotic discs (Gram positive and Gram negative) by the disk diffusion method following the method of a researcher[3]. Before each antibiotic disc was placed on each of the media surface, the pathogenic bacteria isolates were streaked on each of the nutrient agar plates after which the antibiotic discs were aseptically placed on each of the agar plates using sterile forceps. The agar plates were incubated at 37°C for 24 hrs. Afterwards, the plates were examined for zones of inhibition. The zones of inhibition around each antibiotic disc were measured in millimeters[13].

3.9.3 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined on the tested organism. Minimum inhibitory concentration is the lowest concentration of extract required to completely inhibit test organism up to 48 hours incubation or lowest concentration that can result to significant decrease in inoculum's viability greater than 90%[14].

3.9.4 Minimum Bacteria Concentration (MBC)

This is the minimum concentration that is required to completely destroy over 75% viable inoculum. This is practically determined on Agar well diffusion (AWD) technique by a measure of > 10mm diameter of zone of inhibition. It determines the effectiveness or potency of an extract[14].

3.10 Method of Data Analysis

All collected data were entered into a computer, and analyzed using the statistical package for the social sciences (SPSS) version 23. The data were subjected to descriptive (i.e. frequency distribution tables, percentages and proportions, mean and standard deviations) and inferential statistics (i.e. t-test and F-test) statistical treatment. Descriptive statistics was employed to describe characteristics of the study variables; t-test was used to compare the means of extract concentrations; and F-test was also used to compare the means within the extract concentrations and the various solvents for extraction. The level of statistical significance was set at $p \leq 0.05$ for all analysis in this study. The results were displayed in tables and percentages[15].

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Chapter Four

4.1 Result of Findings

The rising prevalence of bacterial diseases in Africa, particularly in Nigeria, is primarily attributed to the growing resistance to antibacterial drugs, often stemming from the misuse and overuse of antibiotics. As a result, the quest for alternative sources of chemotherapeutic agents to combat this challenge has emerged as a global priority. From the research finding of this work the antimicrobial activity of the root bark of *S. longipedunculata* against selected pathogenic microorganisms was evaluated using various solvent extracts at concentrations of 150 mg/mL, 100 mg/mL, 75 mg/mL, and 60 mg/mL. The MIC (Minimum Inhibitory Concentration) is identified as the lowest concentration at which growth is inhibited, while the MBC (Minimum Bactericidal Concentration) would indicate the concentration at which the pathogen is effectively killed.

Tables 4.1 and 4.2 revealed the phytochemical screening of *S. longipedunculata* with a range of bioactive compounds in the stem bark, leaf, and root bark, both in qualitative and quantitative terms. Qualitative analysis showed the presence of terpenoids, flavonoids, alkaloids, tannins, and saponins in all plant parts, with varying intensities. Terpenoids were found in moderate to high concentrations in ethanol extracts of all parts, while

flavonoids were present at low intensities across all extracts. Alkaloids were abundant in the leaf, showing a high intensity in ethanol and chloroform extracts, while tannins and saponins appeared with moderate presence in most extracts. Interestingly, anthraquinones, steroids, and phlobatannins were absent in all parts of the plant, both qualitatively and quantitatively.

In terms of quantitative analysis, the root showed the highest concentration of terpenoids (12.235 mg/mL), while the leaf had the highest concentration of alkaloids (7.033 mg/mL). Saponins were found to have similar concentrations in all plant parts, with the root bark showing the highest value (1.321 mg/mL). Flavonoid concentrations were highest in the root (1.884 mg/mL), while tannins were most concentrated in the leaf (2.594 mg/mL). These results suggest that different parts of *S. longipedunculata* are rich in different phytochemicals, with the root bark being particularly abundant in terpenoids and saponins, while the leaf is a good source of alkaloids. The stem bark, although positive for many compounds, generally had lower concentrations of phytochemicals compared to the other parts.

Table 4.1: Quantitative Phytochemical screening of Stem Bark, Leaf and Root bark (*S. Longipedunculata*)

Phytochemical	Ethanol			Hexane			Chloroform			Aqueous		
	S	L	R	S	L	R	S	L	R	S	L	R
Terpenoid	9.001	6.026	12.235	-	-	8.021	-	-	-	-		-
Flavonoid	1.130	1.048	1.884	-	-	0.2765	-	-	-	-		-
Alkaloid	2.729	7.033	5.19	0.014	0.249	0.2685	-	-	-	-		-
Tannins	1.457	2.594	2.407	0.19	0.283	0.2605	-	-	-	-		-
Saponin	1.139	1.130	1.321	1.010	1.012	0.028	-	-	-	-		-
Anthraquinones	-	-	-	-	-	-	-	-	-	-		-
Steroids	-	-	-	-	-	-	-	-	-	-		-
Phlobatannins	-	-	-	-	-	-	-	-	-	-		-

Source: Author work, 2024

Key:--= negative S-Stem Bark L-Leaf R-Root Bark

Table 4.2: Qualitative Phytochemical screening of Stem Bark, Leaf and Root Bark (*S. Longipedunculata)*

Phytochemical	Ethanol			Hexane			Chloroform			Aqueous		
	S	L	R	S	L	R	S	L	R	S	R	L
Terpenoid	+++	++	++	-	-	+	-	-	-	-	-	-
Flavonoid	+	+	+	-	-	+	--	-	-	-	-	-
Alkaloid	+++	+++	++	+	+	+	-	-	-	-	-	-
Tannins	+	+	+	+	+	+	-	-	_	-	-	-
Saponin	+	+	+	+	+	+	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-		-	-	-	-	-	-
Steroids	-	-	-	-	-	-	-	-	-	-	-	-
Phlobatannins	_	-		-	-	-	-	-	-	-	-	-

Source: Author`s field Work, 2024

KEY: - =Negative, + = positive, ++ =Moderate, +++ =Heavy

S-Stem Bark L-Leaf R-Root Bark

The antimicrobial activities of the root bark of *S. longipedunculata*in table 4.3 were evaluated against several pathogenic microorganisms using different solvent extractions: ethanol, hexane, chloroform, and aqueous. The results demonstrate varying degrees of inhibition depending on the microorganism and the concentration of the solvent extract. For *Klebsiella pneumoniae*, the ethanolic extract exhibited the most notable antimicrobial activity, with an inhibition zone of 15.0±1.4 mm at 150 mg/mL, and a reduced activity of 11.0±1.41 mm at 100 mg/ml. Hexane and chloroform extracts showed weaker or no activity, while the aqueous extract had a smaller inhibition zone of 12.50±0.71 mm at 150 mg/mL.

In the case of *Escherichia coli*, the ethanolic extract displayed moderate inhibition, with a zone of 13.50±0.71 mm at 150 mg/mL, decreasing slightly at 100 mg/mL (12.80±0.71 mm), and further reducing to 7.5±0.70 mm at 75 mg/mL. Hexane showed some activity (10.5±0.70 mm at 150 mg/mL), but no activity was observed with the chloroform and aqueous extracts. While *Staphylococcus aureus,* was showed effective inhibition zone of 19.50±0.71 mm at 150 mg/ml with ethanol extract and no activity was observed from the hexane, chloroform, or aqueous extracts. In *Acinetobacter baumannii*, the ethanol extract also showed intermediate activity with an inhibition zone of 17.50±1.41 mm at 150 mg/mL, which dropped to 6.00±0.10 mm at 100

mg/mL. Hexane, chloroform, and aqueous extracts showed no antimicrobial effect.

For *Pseudomonas aeruginosa*, the ethanolic extract demonstrated the strongest activity, with a zone of 22.0±1.41 mm at 150 mg/mL and 20.0±1.40 mm at 100 mg/mL. The hexane extract had a lower but notable activity of 10.50±0.71 mm at 150 mg/mL, while chloroform showed a smaller inhibition (14.0±0.00 mm). No activity was observed in the aqueous extract.

In the case of *Enterobacter cloacae*, the ethanolic extract showed good activity with a zone of 20.0±0.71 mm at 150 mg/mL, while the hexane extract also exhibited moderate activity, showing 14.0±0.83 mm at 150 mg/mL and 10.0±0.70 mm at 100 mg/ml. No activity was detected in the chloroform and aqueous extracts for *Streptococcus pyogenes*, the ethanol extract showed mild inhibition (12.0±0.50 mm at 150 mg/mL), which decreased at lower concentrations. The chloroform extract exhibited activity (15.0±0.51 mm at 150 mg/ml, 13.0±0.49 mm at 100 mg/mL), but the hexane and aqueous extracts showed no activity. Finally, for *Candida albicans*, the ethanol extract exhibited mild inhibition (10.0±0.70 mm at 150 mg/mL) and decreased at lower concentrations. No activity was observed from hexane, chloroform, or aqueous extracts.

Table4.3: Antimicrobial Activities of Root Bark of *S. Longipedunculata* (mm)/ Concentration (mg/mL) against Selected Pathogenic Microorganisms

Solvent Extraction	Isolates	150	100	75	60	Solvent Negative Control
Ethanol	***K. pneumonia***	15.0±1.4	11.0±1.41	-	-	-
Hexane		14.0±0.90	-	-	-	-
Chloroform		18.0±0.00	-	-	-	-
Aqueous		12.50±0.71	-	-	-	-
Ethanol	***E. coli***	13.50±0.71	12.80±0.71	7.5±0.70	-	-
Hexane		10.5±0.70.1	-	-	-	-
Chloroform		-	-	-	-	
Aqueous		-	-	-	-	
Ethanol	**S. aureus**	19.50±0.71	-	-	-	-
Hexane		-	-	-	-	-
Chloroform		-	-	-	-	
Aqueous		-	-	-	-	
Ethanol	***A. baumannii***	17.50±1.41	5.00±010	-	-	
Hexane		-	-	-	-	-
Chloroform		-	-	-		
Aqueous		-	-	-		
Ethanol	***P. aeruginosa***	22.0±1.41	20.0±1.40	-	-	-
Hexane		10.50±0.71	8.50±0.71	-	-	
Chloroform		14.0±0.00	-	-	-	-
Aqueous		-	-	-	-	
Ethanol	***E. cloacae***	20.0±0.71	-	-	-	
Hexane		14.0±0.83	10.0±0.70	-	-	
Chloroform		-	-	-	-	-
Aqueous		-	-	-	-	
Ethanol	***S. pyogenes***	12.0±0.50	10.0±0.48	-	-	-
Hexane		-	-	-	-	-
Chlorofor		15.0±0.51	13.0±0.49	-	-	-
Aqueous		-	-	-	-	-
Ethanol	***C. albicans***	10±0.70	8±0.69	-	-	-
Hexane		-	-	-	-	-
Chloroform		-	-	-	-	-
Aqueous		-	-	-	-	

Source: Authors Field Work, 2024

Key: mean ± (standard error)

- = negative control

The antimicrobial activities of *Securidaca longipedunculata* extracts from its leaf, stem bark, and a mixture of root bark, leaf, and stem bark were tested against several pathogenic microorganisms at varying concentrations (150 mg/mL, 100 mg/mL, 75 mg/mL, 60 mg/mL). The results reveal notable trends in antimicrobial effectiveness, particularly for ethanol extracts.

Leaf Extract (Table 4.4) The ethanol extract from the leaf exhibited significant antimicrobial activity against a range of microorganisms. The highest inhibition was observed for *Klebsiella pneumoniae* (13.5 ± 0.71 mm at 150 mg/mL), *Escherichia coli* (15.5 ± 0.71 mm at 150 mg/mL), and *Acinetobacter baumannii* (18.0 ± 1.41 mm at 150 mg/mL). For *Staphylococcus aureus*, ethanol at 150 mg/mL produced a zone of 13.0 ± 1.41 mm, which decreased with lower concentrations. The *Pseudomonas aeruginosa* inhibition was 15.5 ± 0.71 mm at 150 mg/mL, reducing to 4.0 ± 0.00 mm at 60 mg/mL. For *Enterobacter cloacae* and *Streptococcus pyogenes*, ethanol extract also showed activity, but the inhibition zones were smaller, with values ranging from 13.5 ± 0.71 mm to 16.0 ± 2.83 mm for *S. pyogenes*. However, hexane, chloroform, and aqueous extracts were ineffective across all microorganisms.

Stem Bark Extract (Table 4.5): The ethanol extract from the stem bark showed significant inhibition against *Escherichia coli* (13.5 ± 0.5 mm at 150 mg/mL), but weaker activity was observed for *Acinetobacter baumannii* (12.0 ± 1.41 mm at 150 mg/mL) and *Pseudomonas aeruginosa* (11.0 ± 1.0 mm at 150 mg/mL). The hexane extract exhibited stronger activity against *E. coli* (15.5 ± 0.5 mm at 150 mg/mL), but the other solvents (chloroform and aqueous) showed no significant inhibition. *Enterobacter cloacae* was

weakly inhibited by hexane (9.5 ± 0.5 mm at 150 mg/mL), while no activity was observed for other extracts. The stem bark's ethanolic extract demonstrated only limited activity against *Streptococcus pyogenes* (12.5 ± 0.5 mm at 150 mg/mL), and no significant inhibition was seen for Candida albicans. Mixture of Root Bark, Leaf, and Stem Bark Extract (Table 4.6): The mixture of root bark, leaf, and stem bark extracts demonstrated the strongest antimicrobial activity, particularly with the ethanolic extract. For *Klebsiella pneumoniae*, inhibition was 21.0 ± 1.40 mm at 150 mg/mL, and for *Escherichia coli*, it was 22.5 ± 0.5 mm at 150 mg/mL. *Staphylococcus aureus* showed inhibition of 20.5 ± 0.05 mm at 150 mg/mL, while *Acinetobacter baumannii* was also effectively inhibited (22.0 ± 1.42 mm at 150 mg/mL). For *Pseudomonas aeruginosa*, the ethanolic extract produced inhibition of 20.0 ± 1.0 mm at 150 mg/mL. The mixture's ethanolic extract was also effective against *Enterobacter cloacae* (22.0 ± 1.42 mm at 150 mg/mL) and *Streptococcus pyogenes* (19.5 ± 1.5 mm at 150 mg/mL). *Candida albicans* was inhibited by the ethanol extract with a zone of 22.0 ± 1.50 mm at 150 mg/mL. While hexane and chloroform extracts demonstrated some activity, they were generally less potent than the ethanolic extract. Aqueous extracts were largely ineffective.

The ethanol extract of *Securidaca longipedunculata* from both the leaf and the mixture of root bark, leaf, and stem bark exhibited the strongest antimicrobial activities across a wide range of microorganisms, with significant inhibition zones observed at 150 mg/mL concentrations. The leaf extract showed consistent activity, particularly against *K. pneumoniae, E. coli,* and *A. baumannii*, while the mixture of all three plant parts was the

most potent, particularly against *K. pneumoniae, E. coli, S. aureus, and P. aeruginosa.* In contrast, the stem bark extract was less potent, showing weaker inhibition overall. Hexane, chloroform, and aqueous extracts displayed minimal or no antimicrobial activity across the tested pathogens, confirming that ethanol is the most effective solvent for extracting antimicrobial compounds from *S. longipedunculata*.

Table 4.4: Antimicrobial Activities of Leaf of *S. longipedunculata* (mm)/Concentration(mg/mL) against Selected Pathogenic Microorganisms

Solvent Extraction	Isolates	150	100	75	60	Solvent Negative Control
Ethanol	***K. pneumonia***	13.5±0.71	12.5±071	10.5±0.71	8.5±0.71	-
Hexane		-	-	-	-	-
Chloroform		-	-	-	-	-
Aqueous		-	-	-	-	-
Ethanol	***E. coli***	15.50±0.71	-	-	-	-
Hexane		-	-	-	-	-
Chloroform		-	-	-	-	
Aqueous		-	-	-	-	
Ethanol	***S. aureus***	13.0±1.41	11.5±0.71	11.0±1.410	7.5±0.71	-
Hexane		-	-	-	-	-
Chloroform		-	-	-	-	
Aqueous		-	-	-	-	
Ethanol	***A. baumannii***	18.0±1.41	-	-	-	
Hexane		-	-	-	-	-
Chloroform		-	-	-		
Aqueous		-	-	-	-	
Ethanol	***P. aeruginosa***	15.5±0.71	9.5±0.71	9.5±0.71	4.0±0.00	-
Hexane		10.50±0.71	8.50±0.71	-	-	
Chloroform		14.0±0.00	-	-	-	-
Aqueous		-	-	-	-	
Ethanol	***E. cloacae***	13.5±0.71	11.0±1.41	9.5±0.71	7.5±0.71	
Hexane		-	-	-	-	
Chloroform		-	-	-	-	-
Aqueous		-	-	-	-	
Ethanol	***S.pyogenes***	16.0±2.83	15.0±1.41	11.5±0.71	6.5±0.12	-
Hexane		-	-	-	-	-
Chloroform		-	-	-	-	-
Aqueous		-	-	-	-	-
Ethanol	***C. albicans***	18.0±1.4	10±0.70	-	-	-
Hexane		-	-	-	-	-
Chloroform			-	-	-	-
Aqueous			-	-	-	-

Source: Author's Field Work, 2024

Key: mean ± (standard error)

- = negative control

Table 4.5: Antimicrobial Activities of Stem Bark of *Securidaca longipedunculata* (mm)/Concentration (mg/mL) against Selected Pathogenic Microorganisms

Solvent Extraction	Isolates	150	100	75	100	Solvent Negative Control
Ethanol	*K. pneumonia*	-	-	-	-	-
Hexane		-	-	-	-	-
Chloroform		-	-	-	-	-
Aqueous		-	-	-	-	-
Ethanol	*E. coli*	13.5±0.5	11.5±0.5	-	-	-
Hexane		15.5±0.5	14.0±0.00	-	-	-
Chloroform		-	-	-	-	
Aqueous		-	-	-	-	
Ethanol	*S. aureus*	-	-	-	-	-
Hexane		-	-	-	-	-
Chloroform		-	-	-	-	
Aqueous		-	-	-	-	
Ethanol	*A.baumannii*	12.0±1.41	-	-	-	
Hexane		-	-	-	-	-
Chloroform		-	-	-		
Aqueous		-	-	-	-	
Ethanol	*P. aeruginosa*	11.0±1.0	9.5±0.5	7.0±0.71	8.5±0.5	-
Hexane		10.50±0.71	8.50±0.71	-	-	
Chloroform		20.0±1.41	18.0±1.39	16.0±0.15	-	-
Aqueous		-	-	-	-	
Ethanol	*E. cloacae*	7.0±0.71	-	-	-	
Hexane		9.5±0.5	-	-	-	
Chloroform		-	-	-	-	-
Aqueous		-	-	-	-	
Ethanol	*S. pyogenes*	12.5±0.5	10.0±0.00	-	-	-
Hexane		-	-	-	-	-
Chloroform		-	-	-	-	-
Aqueous		-	-	-	-	-
Ethanol	*C. albicans*	12.0±0.05	-	-	-	-
Hexane		-	-	-	-	-
Chloroform			-		-	-
Aqueous			-	-	-	-

Source: Author's Field Work, 2024

Key: mean ± (standard error)

- = negative control

Table 4.6: Antimicrobial Activities of Mixture (Root Bark, leaf and Stem bark) of *Securidaca longipedunculata* (mm)/ Concentration (mg/mL) against Selected Pathogenic Microorganisms

Solvent Extraction	Isolates	150	100	75	60	Solvent Negative Control
Ethanol	*K. pneumonia*	21.0±1.40	19.5±0.5	7.0±0.00	-	-
Hexane		12.5±0.71	10.5±0.5	-	-	-
Chloroform		18.0±1.42	-	-	-	-
Aqueous		-	-	-	6.0±0.00	-
Ethanol	*E. coli*	22.5±0.5	18.0±0.5	-	-	-
hexane		12.0±0.5	-	-	-	-
Chloroform		18±1.42	16±1.40	-	-	
Aqueous		-	-	-	-	
Ethanol	*S. aureus*	20.5±0.05	11.0±0.00	6.5±0.5	5.5±0.5	-
hexane		-	-	-	-	-
Chloroform		-	-	-	-	
Aqueous		-	-	-	-	
Ethanol	*A.baumannii*	22±1.42	18.0±1.40	-	-	
Hexane		-	-	-	-	-
Chloroform		18.0±1.40	16.0±1.30	-		
Aqueous		-	-	-	-	
Ethanol	*P. aeruginosa*	20.0±1.0	16.5±0.5	11.0±0.01	-	-
Hexane		10.50±0.71	8.50±0.71	-	-	
Chloroform		-	-	-	-	-
Aqueous		-	-	-	-	
Ethanol	*E. cloacae*	22±1.42	19.0±1.39	-	-	
Hexane		-	-	-	-	
Chloroform		18.0±1.40	10.0±0.70	-	-	-
Aqueous		-	-	-	-	
Ethanol	*S. pyogenes*	19.5±1.5	17.5±1.40	9.0±0.5	-	-
Hexane		-	-	-	-	-
Chloroform		12.0±0.5	-	-	-	-
Aqueous		-	-	-	-	-
Ethanol	*C. albicans*	22.0±1.50	20±1.00	12.0±0.14	-	-
Hexane		-	-	-	-	-
Chloroform			-	-	-	-
Aqueous		-	-	-	-	-

Source: Author's Field Work, 2024

Key: mean ± (standard error)

- = negative control

Table 4.7 showed the antimicrobial susceptibility testing results compared with Clinical and Laboratory Standard Institute (CLSI) standards reveal distinct patterns of antibiotic efficacy against the selected pathogens. Gentamycin (10 µg) demonstrated sensitivity against *K. pneumoniae* (18 mm) and *E. cloacae* (19 mm), both meeting the CLSI threshold for sensitivity (≥18 mm). However, it was only partially effective against *E. coli* (16 mm) and *S. aureus* (16 mm), within the intermediate range (15-17 mm). *A. baumannii*, *S. pyogenes*, and *P. aeruginosa* were resistant, with inhibition zones far below the sensitivity level. Amikacin (30 µg) showed high efficacy against *K. pneumoniae*, *E. coli*, *E. cloacae*, and *S. aureus*, with inhibition zones above or meeting the 20 mm threshold, indicating sensitivity. For *S. pyogenes* (17 mm), It was within the intermediate range. (17-19 mm), and it was less effective against *A. baumannii* and *P. aeruginosa*, which showed resistance. Augmentin (30 µg) was effective against *K. pneumoniae*, *E. coli*, *S. aureus*, and *Streptococcus pyogenes*, showing susceptibility with inhibition zones of 17 mm or higher. However, it showed intermediate effectiveness against *E. cloacae* (16 mm) and *A. baumannii* (15 mm), indicating only partial effectiveness, while *Pseudomonas aeruginosa* was resistant. Fluconazole displayed 20 mm inhibition zone against *Candida albicans*, suggesting a possible susceptibility despite no direct CLSI values for reference. Ciprofloxacin (5 µg) was largely ineffective, with most microorganisms showing resistance, including *K. pneumoniae* (20 mm), *E. coli* (21 mm), *Enterobacter cloacae* (16 mm), *Acinetobacter baumannii* (15 mm), *Streptococcus pyogenes* (14 mm), *S. aureus* (6 mm), and *Pseudomonas aeruginosa* (6 mm), all below the

CLSI sensitivity threshold of ≥26 mm. Ceftriaxone (30 µg) displayed intermediate activity, with *K. pneumoniae*, *E. coli*, and *Enterobacter cloacae* showing inhibition zones within 15-22 mm, while *Streptococcus pyogenes* (19 mm) met the sensitivity threshold. However, it was ineffective against *Acinetobacter baumannii*, *S. aureus*, and *Pseudomonas aeruginosa*, all showing resistance with zones below 15 mm. Ceftazidime (30 µg) also exhibited moderate efficacy across *K. pneumoniae*, *E. coli*, *Enterobacter cloacae*, and *S. pyogenes*, each showing intermediate susceptibility with inhibition zones around 17-20 mm. However, it was ineffective against *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, both resistant with low inhibition zones. Lastly, Cefoxitin (30 µg) displayed moderate effectiveness, showing inhibition zones of 18-20 mm for *K. pneumoniae*, *E. coli*, *Enterobacter cloacae*, *Acinetobacter baumannii*, *Streptococcus pyogenes*, and *S. aureus*, indicating some degree of susceptibility, though no CLSI standard was provided. *Pseudomonas aeruginosa*, however, was resistant with an inhibition zone of just 6 mm. In summary, *S. aureus*, *K. pneumoniae*, and *E. coli* were notably susceptible to Amikacin and Gentamycin, while Augmentin and Cefoxitin displayed moderate efficacy across several strains. Ciprofloxacin and Ceftriaxone were less effective, with most pathogens resistant, especially *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.

Table 4.7: Antibiotic Susceptibility (mm) Test against Selected Microorganism

Antibiotic Disc	Code	Conc.	*Klebsiella pneumonia*	*Escherichia coli*	*Enterobacter cloacae*	*Acinetobacter baumannii*	*Streptococcus pyogenes*	*Candida albicans*	*Staph aureus*	*Pseudomon aeruginosa*
Gentamycin	CN	10ug	18(S)	16(I)	19(S)	-	10(R)	-	16(I)	-
Amikacin	AK	30ug	21(S)	20(S)	20(S)	10(R)	17(I)	-	20(S)	18(I)
Augmentin	Aug	30ug	18(S)	20(S)	16(I)	15(I)	18(S)	-	17(S)	-
Fluconazole	FLX	-	-	-	-	-	-	20(S)	-	-
Ciprofloxacin	Cip	10ug	20(R)	21(R)	16(R)	15(R)	14(R)	-	-	-
Ceftriaxone	Cro	10ug	18(R)	18(R)	17(R)	14(R)	19(R)	-	-	-
Ceftazidime	CAZ	30ug	20(I)	19(I)	20(I)	14(R)	17(R)	-	17(R)	19(I)
Cefoxitin	Fox	30ug	20(S)	18(I)	19(I)	19(I)	18(I)	-	20(S)	-

Source: Author's Field Work, 2024

Key: S: Sensitive I: Intermediate R: Resistant

4.2 Discussion of Findings

In this study, Table 4.1 highlights the significant antimicrobial activity of root bark extracts, with ethanolic extract showing strong inhibition against *K. pneumoniae* (15.0 ±1.4 mm), *S. aureus* (19.5± 0.71 mm), and *P. aeruginosa* (22.0 ± 1.41mm) at 150 mg/mL. These findings align with prior studies that have reported on the high antimicrobial potential of root bark extracts, attributed to bioactive compounds like flavonoids, tannins, and saponins, which have been found to disrupt bacterial cell membranes[2]. Hexane and chloroform extracts also exhibited activity against some organisms, though generally lower than ethanolic extract. Notably, chloroform showed inhibition against *K. pneumoniae* and *S. pyogenes*, indicating that non-polar compounds within the root bark also possess antimicrobial properties. In table 4.1 ethanolic extracts of the root bark which has zone of inhibition of 22.0 ± 1.41 mm displayed notable activity, particularly against *P. aeruginosa* at 150mg/mL with the MIC of 100mg/mL and MBC of 150mg/mL . While *E. cloacae* inhibited MBC at150mg/mL and *S. aureus* shows MIC inhibition at 150mg/mL . These findings align with prior studies that have reported on the high antimicrobial potential of root bark extracts, attributed to bioactive compounds like flavonoids, tannins, and saponins, which have been found to disrupt bacterial cell membranes[3]. Hexane and chloroform extracts showed moderate activity against some isolates, but their efficacy was generally lower than ethanol extracts indicating that nonpolar compounds within the root bark also possess antimicrobial properties,

compared with leaf ethanol extract presented in Table 4.2, the leaf extracts showed high to moderate activity, with ethanolic extracts again proving most effective. The highest inhibition was observed against *A. baumannii* (18.0±1.4mm) and *S. pyogenes* (16.0±0.15) at 150 mg/mL. The effectiveness of the leaf extracts could be linked to the presence of phenolic compounds, known for their antimicrobial properties[4]. N-hexane and chloroform extracts were generally less effective, though hexane displayed low activity against *P. aeruginosa* at higher concentrations. The low activity with hexane and chloroform is consistent with findings in other studies, which suggest that polar compounds in the leaves might be more responsible for the antimicrobial effects[4].

Table 4.3 showed that stem bark extracts had limited activity compared to root bark and leaf extracts, although ethanolic extracts did inhibit *E. coli* (13.5±0.02mm) and *A. baumannii* (12.0±0.01 mm) at 150 mg/mL. N-hexane extracts displayed significant activity against *E. coli* (15.5 ± 0.5mm), which is consistent with findings from studies that attribute the antimicrobial properties of stem bark to terpenoids and alkaloids[5]. Chloroform exhibited moderate inhibition against *P. aeruginosa* (20.0±1.0 mm) and *E. cloacae* (9.5 ± 1.05 mm). These results support the idea that stem bark might contain specific non polar compounds that are selectively effective against some gram-negative bacteria[5].

Furthermore, the combination of root bark, leaf, and stem bark extracts revealed enhanced antimicrobial activity, particularly with ethanolic extracts, which showed significant inhibition zones for *K. pneumoniae* (21.0±1.50 mm), *E. coli* (22.5±0.5mm), and *C. albicans* (22.0±1.50 mm) at 150 mg/mL

as shown in Table 4.4. The mixture's superior efficacy aligns with current literature on the synergistic effect of combining different plant parts, as the varied phytochemical profiles enhance antimicrobial potency[6]. This synergy is attributed to multiple compounds working in concert to inhibit bacterial growth through diverse mechanisms, such as disrupting cell walls, inhibiting enzyme activities, and reducing protein synthesis.

Table 4.5 provides insights into the antibiotic susceptibility testing of the selected microorganisms. using convectional antibiotics, However, the ethanolic extracts of *S. longipendunculata* exhibited comparable or greater inhibition zones for certain pathogens than conventional antibiotics like gentamicin and ciprofloxacin, particularly against *K. pneumoniae*, *E. coli*, and *S. aureus*. This observation aligns with findings in recent literature where plant-based antimicrobials are shown to be effective, particularly against multidrug-resistant pathogens[7]. Studies emphasize that phytochemicals in plants such as alkaloids and saponins can enhance antibiotic activity or even overcome some resistance mechanisms, making them valuable in the fight against antimicrobial resistance[8].The observed antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as fungi (*C. albicans*), highlights *S. longipedunculata's* potential as a broad-spectrum antimicrobial agent. This efficacy is particularly notable in the mixed extracts, which achieved larger inhibition zones for certain organisms compared to the individual plant parts. Literature supports the clinical relevance of such findings, with many studies indicating that traditional medicinal plants can serve as a source of effective, natural alternatives to synthetic antibiotics[9].

The phytochemical screening revealed that the ethanolic extracts of *S. longipedunculata* contained the highest concentrations of bioactive compounds, followed by hexane, and chloroform and aqueous extracts generally showing no detectable levels of the tested phytochemicals. In this work revealed the presence of tannins, saponins. terpenoids, alkaloids and phlobatannins. The presence of these phytochemicals in this cannot be emphasized. This agrees with findings that discovered that phytochemicals in medicinal plants are the active content responsible for the pharmacological potentials of medicinal plants[3].

The root bark contained the highest concentration of terpenoids (12.235±1.08 mg/mL) and a moderate value of alkaloids (5.19±0.03 mg/mL) and tannins (2.407±0.04 mg/ mL) in the ethanol extract. Hexane extracts also contained these compounds but in significantly lower quantity.

The leaf extracts contained a notable value of alkaloids (7.033±0.03 mg/ mL) and tannins (2.594±0.08 mg/ mL) in ethanolic extracts, indicating its potential as a rich source of these bioactive compounds.

The stem bark was found to have lower concentrations of phytochemicals compared to the root bark and leaf. Terpenoids (9.001±0.01 mg/ mL) and alkaloids (2.729±0.03 mg/ mL) were the most abundant compounds in the ethanolic extract.

The results suggest that *S. longipedunculata* contains a range of bioactive phytochemicals, which contribute to its antimicrobial properties. Ethanol is the most effective solvent for extracting these compounds, highlighting the importance of solvent choice in phytochemical and antimicrobial studies. The root bark appears to be the most potent part of the plant, particularly in

ethanolic extracts, due to its higher concentration of terpenoids, alkaloids, and tannins, which are likely responsible for its strong antimicrobial activity. The synergistic effect observed in the mixture of different plant parts suggests that a combination of extracts may enhance the overall antimicrobial efficacy.

From the finding of this study the ethanolic extracts of *S. longipedunculata* showed significant antimicrobial activity against various pathogens, including *K. pneumoniae*, *E. coli*, and *S. aureus*. Previous studies, reported the efficacy of ethanolic extracts in disrupting bacterial cell walls due to the presence of bioactive compounds like flavonoids and alkaloids[10]. The moderate antimicrobial activity observed with hexane and chloroform extracts can be compared with findings from researchers, who reported that non-polar solvents like hexane and chloroform are effective in extracting terpenoids and alkaloids, which are known to have antimicrobial properties[11].

The significant presence of terpenoids in the root bark, especially in ethanol and hexane extracts, aligns with findings from studies conducted last year, who reported that *S. longipedunculata* is rich in terpenoids, contributing to its antimicrobial efficacy[12].

The presence of flavonoids and tannins, particularly in ethanol extracts, is consistent with the literature, where these compounds are frequently cited for their antioxidant and antimicrobial properties. In accordance with a study by some researchers support the role of flavonoids in enhancing the antibacterial activity against Gram-positive and Gram-negative bacteria [12].

The significant alkaloid content in ethanolic extracts, which is higher in the root bark and leaves, is corroborated with the studies that are conducted

some years back, which highlight alkaloids' role in disrupting microbial cell functions[14].

The combined and the separate plant crude extracts showed great activity against most microorganisms tested. The combined root, leave and stem ethanol and chloroform extracts showed sensitivity against all the microorganisms tested, follow by ethanolic root extract that showed sensitivity against *S. aureus*, *P. aeruginosa* and *E. cloacae*. However, leave ethanolic extracts showed sensitivity against *A. baumannii* and *Candida albicans* at the highest concentration and stem bark chloroform extracts shown highest sensitivity inhibition zone against *P. aeruginosa.* This is probably due to the solvent used and phytochemical contents present at each part of the plant contributed to its effectiveness against selected microorganism present. The antimicrobial activities of different parts of *S. longipedunculata* were assessed against several pathogenic microorganisms, including *K. pneumoniae*, *E. coli*, *S. aureus*, *A. baumannii*, *P. aeruginosa*, *E. cloacae*, *S. pyogenes*, and *C. albicans*. These results indicated that ethanol extracts generally exhibited the highest antimicrobial activity across all tested microorganisms, followed by N- hexane and chloroform extracts, with aqueous extracts showing little to no activity. The results, displayed in Tables 4.1 through 4.4, revealed that ethanol extracts exhibited the most consistent antimicrobial activity across the different plant parts, followed by n-hexane and chloroform. This trend is supported by existing literature, where ethanolic extracts is often found to be an effective solvent for extracting bioactive compounds due to its ability to dissolve a wide range of polar and nonpolar compounds, thus maximizing the antimicrobial efficacy of plant extracts[12].

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Chapter Five

Conclusion

5.1 Summary of Findings

The findings from the antimicrobial activity tests on *Securidaca longipedunculata* extracts reveal that ethanol is the most effective solvent for extracting antimicrobial compounds from the plant. Ethanol extracts from both the leaf and the mixture of root bark, leaf, and stem bark showed the strongest inhibitory effects against a wide range of pathogenic microorganisms, including *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Streptococcus pyogenes*, and *Candida albicans*, particularly at higher concentrations (150 mg/mL). The leaf extract exhibited significant activity against *K. pneumoniae*, *E. coli*, and *A. baumannii*, while the mixture of the three plant parts was the most potent, with substantial inhibition against several pathogens, including *P. aeruginosa* and *S. aureus*. The stem bark extract was less effective overall, with weaker activity primarily against *E. coli* and *P. aeruginosa*. In contrast, hexane, chloroform, and aqueous extracts demonstrated minimal or no antimicrobial activity. These results highlight the potential of *Securidaca longipedunculata* as a source of natural antimicrobial agents, particularly through its ethanol extract.

5.2 Conclusion

The study concludes that *Securidaca longipendunculata* possesses diverse phytochemicals, with its ethanolic extracts demonstrating significant antimicrobial activity against pathogens like *Escherichia coli, Klebsiella*

pneumoniae, Staphylococcus aureus, Acinetobacter baumannii, and *Candida albicans*. Ethanolic extracts of the plant's root bark, stem bark, and leaves showed the highest potency, often comparable to standard antibiotics like Amikacin and Ciprofloxacin, supporting its traditional use in treating infections. The antimicrobial activity varied with the extraction solvent, with ethanol consistently being the most effective. This highlights its potential as a natural source of antimicrobial agents.

5.3 Recommendations

Based on the findings of this research, the following recommendations are proposed:

1. Further Phytochemical Research: More detailed phytochemical analysis is necessary to isolate and identify the specific active compounds responsible for the antimicrobial activity observed in *S. longipedunculata* extracts. This will help in developing new, targeted antimicrobial agents.
2. *In Vivo* Studies: Future studies should focus on conducting *In Vivo* experiments to evaluate the safety, efficacy, and pharmacokinetics of the plant extracts in animal models and eventually in humans.
3. Broader Solvent Testing: In addition to ethanol, hexane, chloroform, and aqueous extractions, other solvents should be explored to determine if they can yield even more potent antimicrobial compounds from the plant.
4. Development of Pharmaceutical Formulations: Efforts should be made to develop pharmaceutical formulations (such as ointments, creams, or capsules) using the extracts, particularly for treating

infections like wound infections, where microbial resistance to synthetic antibiotics is a growing concern.

5. Cytotoxicity and Toxicology Studies: To ensure the safe use of *Securidaca longipendunculata* extracts, toxicity and cytotoxicity studies should be conducted to determine safe dosages and rule out harmful side effects.
6. Exploration of Synergistic Effects: Future research should explore the synergistic potential between *S. longipedunculata* extracts and conventional antibiotics, as this could provide new solutions to combat multi-drug-resistant infections.
7. Consideration of Regional Variability: Given that the phytochemical composition of plants can vary based on environmental factors, the antimicrobial properties of *S. longipedunculata* from different geographic regions should be compared to ensure consistent effectiveness in various settings.

5.4 Contribution to Knowledge

This study contributes to the field of phytochemistry and microbiology by providing new insight into the phytochemical composition and antimicrobial activity of *S. longipedunculata extracts.* The research highlights the potential of *S. longipedunculata* as a source of novel antimicrobial compounds which can be used to develop new therapies for managing diabetic foot ulcer.

5.5 Suggested Areas for Further Research

While this study has made important contributions, it also opens several avenues

for future research.

I. Mechanism of Action: Further studies are needed to understand the specific mechanisms by which the plant extracts inhibit microbial growth. This could involve biochemical and molecular-level investigations.

II. Phytochemical Analysis: More in-depth phytochemical analysis should be conducted to isolate and identify the active compounds responsible for the antimicrobial activity, which could help in the formulation of new drugs.

III. *In Vivo* Studies: While the in vitro results are promising, in vivo studies are necessary to evaluate the safety, efficacy, and pharmacokinetics of the plant extracts in living organisms.

IV. Synergistic Effects: Research into the synergistic effects between *S. longipedunculata* extracts and standard antibiotics could provide new strategies for treating infections, particularly those caused by multi-drug-resistant pathogens.

V. Formulation and Application: Investigations into developing pharmaceutical formulations (e.g., creams, ointments, or capsules) from the extracts could explore the potential for clinical use in treating infections, especially wound infections like those from diabetic foot ulcers.

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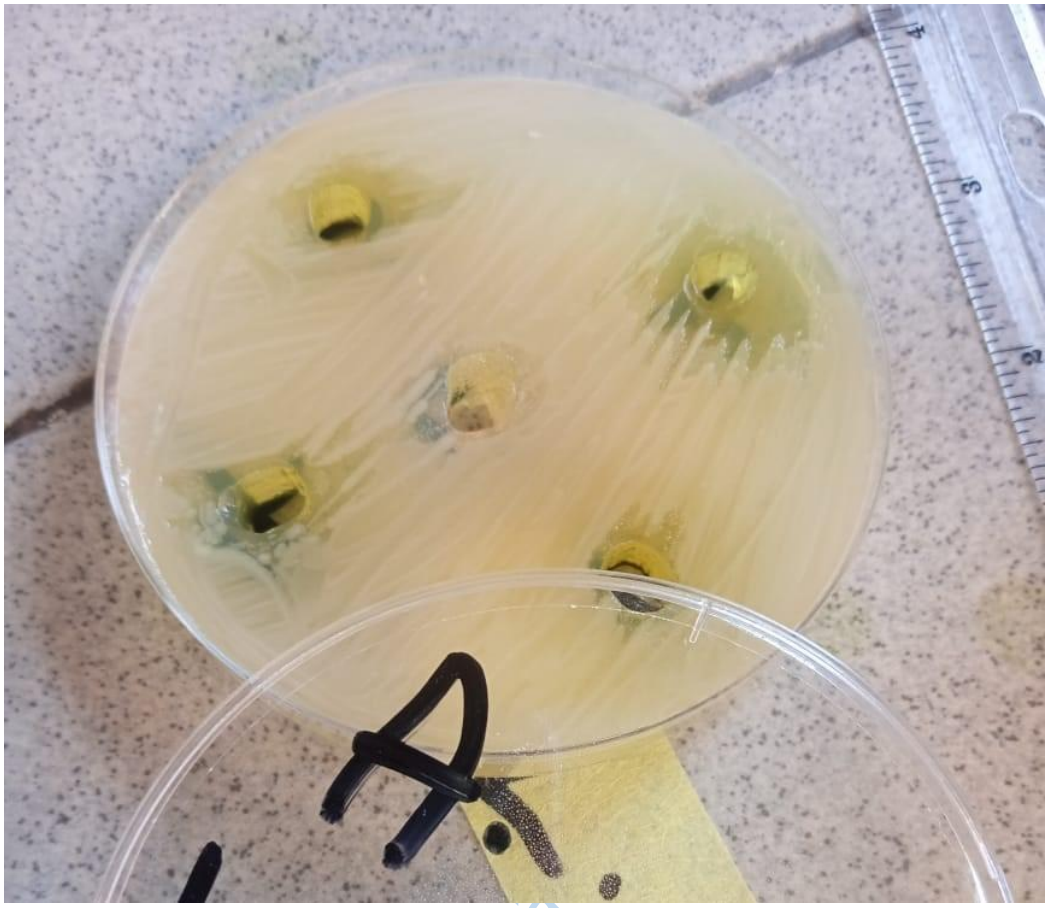
Appendices

Appendix i

In-vitro Antimicrobial Activity of Ethanolic Extract of the Root Bark of *Securidaca longipeduculata* on *Pseudomonas aeruginosa* Showing the Zone of Inhibition

Source: Author's Laboratory Work 2024



Appendix ii

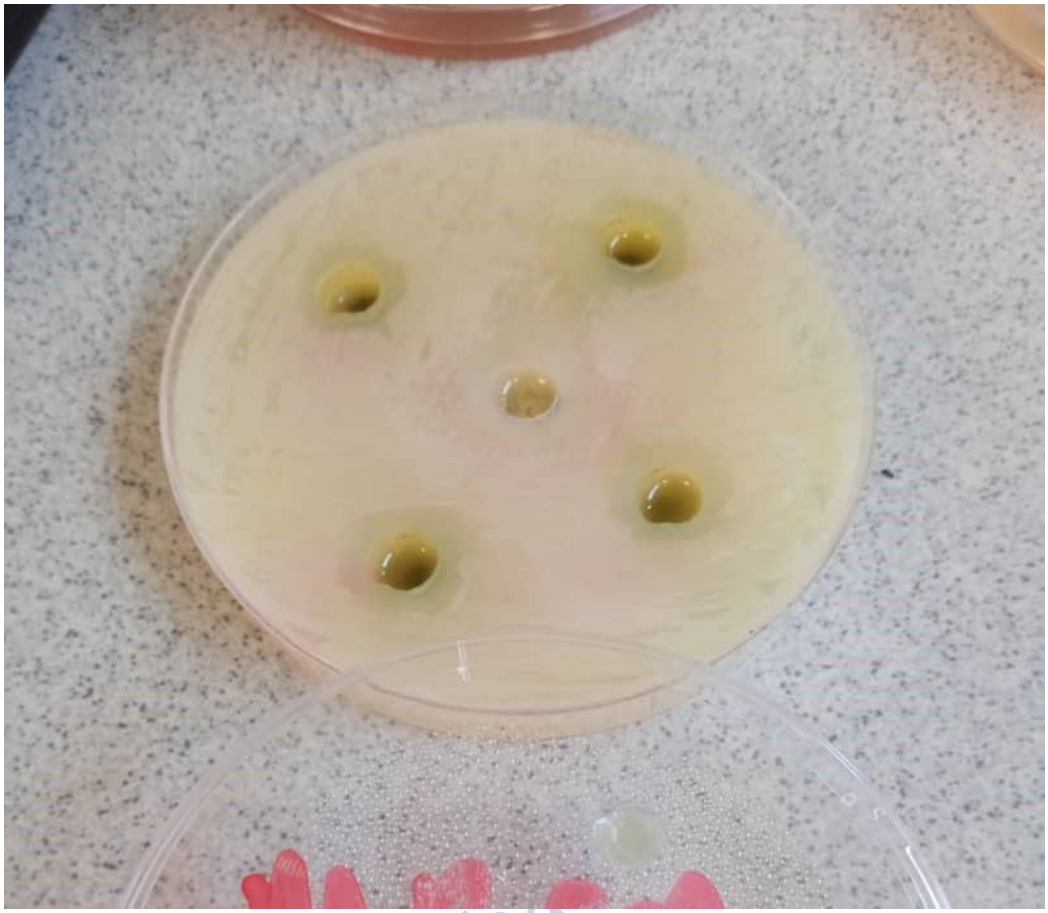
In-vitro Antimicrobial Activity of Ethanolic Extract of the Leaf of *Securidaca longipediunculata* on *Acinetobacter baumannii* Showing the Zone of Inhibition

Source: Author's Laboratory Work 2024

Appendix iii

In-vitro Antimicrobial Activity of Ethanolic Extract of the Stem Bark of *Securidaca longipendunculata* on *Klebsiella pneumonia* Showing the Zone of Inhibition

Source: Author's Laboratory Work 2024



Appendix iv

In-vitro Antimicrobial Activity of Mixture of Ethanolic Root, Bark, Leaf, Stem of *Securidaca longipendunculata* on *Staphylococcus aureus* Showing the Zone of Inhibition.

Source: Author's Laboratory Work 2024

Appendix v

In-vitro Antimicrobial Activity of Mixture of EthanolicExtract of *Securidaca longipendunculata on Escherichia coli* showing the Zone of Inhibition.

Source: Author's Laboratory Work, 2024

Bio-Data

A. Personal Data

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Name of next of Kin: AYOOLA Oludayo Michael

B. Educational Background

Educational Institutions Attended with Dates and Qualifications

School Attended	Dates	Qualifications
Olabisi Onabanjo University, Ogun State	2005-2010	B.Sc.
Lead city University, Ibadan	2021-2024	MSc in view
African Church Grammar School	1988	(WASSCE)

C. Working Experience with Dates

Forestry Research Institute of Nigeria (2001 till dates)

D. Awards

Membership of Academic Professional Bodies.

Nigerian Institute of Laboratory Science Technology (NISLT)

E. Publications

G.T ANGURUWA.; C.A ODEGA.; I.T ADEMOLA.; A.A ADEBISI.; O. AAYOOLA. AND M.O KUPOLUYI: Evaluation of briquettes produced from extractive-free residues of hardwood species using Gum-Arabic as binder. Journal of the Faculty of Agriculture, J. Volume 3, Issue 1, page 59 - 68 **(2024)**

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AYOOLA. O.A. ADEDIRAN.K, ADESINA A.F AND BAMKEFA.B: In-Vitro Antimicrobial and Phytochemical Activity of Selected Parts of *Securidaca longipedunculata* Extracts on Wound Clinical Isolates from Diabetic Foot Ulcers. A paper presented at 4th International Conference at Lead City University, (FASCON 2024)

F. Training

Research Training for All Postgraduate Students, Lead city University, Ibadan Postgraduate College.

Theme: AI, Research and Grant Writing Training November 2024

_____ _____

Signature Date

University Compliance Certification

This is to certify that, this dissertation written by AYOOLA Olubunmi Afolake with matriculation number LCU/PG/002720 in the department of Biological Sciences, Faculty of Natural and Applied Sciences, Lead City University, Ibadan is in full compliance with the approved format and style.

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Signature Date