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Diabetic Foot Ulcers in the Era of Antimicrobial Resistance: Biofilms, Stewardship, and Therapeutic Potential of *Azadirachta indica* and *Moringa oleifera*

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Abstract: Diabetic foot ulcers (DFUs) are among the most debilitating complications of diabetes mellitus and are associated with recurrent infection, prolonged hospitalization, lower-limb amputation, substantial healthcare expenditure, and increased mortality. Their management is increasingly complicated by polymicrobial infection, microbial biofilm formation, and the emergence of multidrug-resistant organisms, including methicillin-resistant *Staphylococcus aureus*, extended-spectrum β-lactamase-producing Enterobacterales, carbapenem-resistant organisms, and multidrug-resistant *Pseudomonas aeruginosa*. Biofilm formation further promotes chronicity by limiting antimicrobial penetration, facilitating bacterial persistence and horizontal gene transfer, and protecting microorganisms from host immune responses. These challenges underscore the importance of antimicrobial stewardship, including appropriate wound sampling, culture-directed treatment, de-escalation of broad-spectrum antibiotics, avoidance of antimicrobial therapy in clinically uninfected ulcers, and optimization of treatment duration. This review synthesizes current evidence on the pathogenesis and microbiology of DFUs, mechanisms of biofilm-associated antimicrobial resistance, antimicrobial stewardship strategies, and the potential role of medicinal plants as complementary wound-management agents. Particular attention is given to *Azadirachta indica* (neem) and *Moringa oleifera*, whose phytochemical constituents demonstrate antimicrobial, antibiofilm, antioxidant, anti-inflammatory, and wound-healing activities. Neem may inhibit bacterial adhesion, quorum sensing, efflux mechanisms, and biofilm development, while moringa-derived compounds may disrupt microbial membranes, inhibit bacterial enzymes and biofilm formation, and promote tissue regeneration. Despite promising experimental evidence, clinical translation remains constrained by limited standardization of plant extracts, insufficient topical safety data, variability in extraction methods and concentrations, and a paucity of rigorous clinical trials in patients with DFUs. Neem and moringa therefore represent promising adjunctive candidates rather than established replacements for conventional DFU management. Future research should prioritize standardized formulations, toxicity assessment, mechanistic antibiofilm studies, dose optimization, and well-designed clinical trials to establish their efficacy and safety.

Keywords: Diabetic foot ulcer; antimicrobial resistance; biofilm; antimicrobial stewardship; *Azadirachta indica*; *Moringa oleifera*.

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1. INTRODUCTION

Diabetes mellitus constitutes a major and rapidly expanding global public health challenge. The increasing prevalence of diabetes has been accompanied by a corresponding rise in chronic complications, particularly diabetic foot disease. Diabetic foot ulcers are full-thickness wounds occurring below the ankle in individuals with diabetes and are among the most clinically important complications of the disease. They result from the complex interaction of peripheral neuropathy, peripheral arterial disease, impaired immunity, abnormal biomechanics, repeated trauma, poor glycaemic control and defective wound healing. Once established, DFUs may become chronic, infected and difficult to heal, substantially increasing the risk of hospitalization, amputation and death.

The clinical burden of DFUs extends beyond the wound itself. Peripheral neuropathy reduces protective sensation and permits repetitive unnoticed injury, while vascular insufficiency compromises tissue perfusion and oxygen delivery. Hyperglycaemia further impairs neutrophil function, macrophage activity, angiogenesis and growth-factor production. These abnormalities create a wound environment characterized by impaired host defence, tissue hypoxia, chronic inflammation and delayed regeneration, thereby facilitating microbial colonization and infection.

Diabetic foot infections are microbiologically complex. Although Gram-positive organisms such as *Staphylococcus aureus* remain important pathogens, chronic and previously treated ulcers frequently harbour Gram-negative organisms including *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter* spp. and *Acinetobacter baumannii*. Anaerobic organisms and fungi may also contribute, particularly in deep, necrotic and long-standing ulcers. The polymicrobial character of these infections complicates antimicrobial selection and may contribute to treatment failure.

An additional therapeutic challenge is microbial biofilm formation. Biofilms are organized microbial communities embedded within a self-produced extracellular polymeric matrix. Within chronic diabetic wounds, biofilms promote microbial persistence by reducing antimicrobial penetration, creating metabolically inactive persister populations, facilitating quorum sensing and horizontal gene transfer, and protecting microorganisms from host immune mechanisms. Consequently, biofilm-associated infections are frequently refractory to conventional antimicrobial treatment and are associated with delayed wound healing and recurrent infection.

The emergence of antimicrobial resistance further compounds these difficulties. Methicillin-resistant *S. aureus*, extended-spectrum β-lactamase-producing Enterobacterales, carbapenem-resistant organisms and multidrug-resistant *P. aeruginosa* are increasingly reported in diabetic foot infections. The problem is particularly concerning in resource-limited settings, where microbiological diagnostics and antimicrobial susceptibility testing may be unavailable and empirical broad-spectrum antimicrobial use is common. These circumstances make antimicrobial stewardship an essential component of DFU management.

Current stewardship principles emphasize treating only clinically infected wounds, obtaining appropriate microbiological samples, selecting antibiotics according to culture and susceptibility findings, narrowing antimicrobial therapy whenever possible, and avoiding unnecessarily prolonged treatment. However, the persistence of biofilms and increasing antimicrobial resistance have stimulated interest in alternative and complementary therapeutic approaches.

Medicinal plants represent one potential source of novel wound-management agents because their phytochemicals may exert antimicrobial effects through multiple mechanisms. Plant-derived compounds can disrupt microbial membranes, interfere with cell-wall and nucleic-acid synthesis, inhibit efflux pumps, attenuate quorum sensing and interfere with biofilm formation. In addition, some medicinal plants possess antioxidant, anti-inflammatory and tissue-regenerative properties that may be particularly advantageous in the impaired wound-healing environment associated with diabetes.

Among the medicinal plants of interest are *Azadirachta indica* (neem) and *Moringa oleifera*. Neem contains bioactive compounds including azadirachtin, nimbin, nimbolide, nimbidin, quercetin, rutin and kaempferol, whereas moringa contains isothiocyanates, flavonoids, phenolic acids, glucosinolates and other biologically active constituents. Experimental evidence suggests that both plants possess antimicrobial and antibiofilm activities in addition to anti-inflammatory, antioxidant and wound-healing properties.

This review therefore examines the pathogenesis and microbiology of diabetic foot ulcers, the roles of biofilms and antimicrobial resistance in treatment failure, contemporary principles of antimicrobial stewardship, and the therapeutic potential of *A. indica* and *M. oleifera* as complementary agents in diabetic wound management. It also highlights current evidence gaps, safety considerations and priorities for future translational research.

2.1 Diabetes Mellitus: A Global Health Crisis

2.1.1 Epidemiology and Burden of Diabetes

Diabetes mellitus (DM) represents one of the most significant public health challenges of the 21st century. According to the International Diabetes Federation (IDF)

Diabetes Atlas 11th Edition, an estimated 589 million adults aged 20–79 years were living with diabetes in 2024, representing 11.11% of the global adult population (Sun *et al*., 2022; International Diabetes Federation, 2024). This figure is projected to rise to 853 million by 2050, affecting approximately 12.96% of the global adult population. The IDF further estimates that diabetes directly caused 3.4 million deaths in 2024, with almost 40% of these deaths occurring in individuals under 60 years of age (International Diabetes Federation, 2024).

The economic burden of diabetes is equally staggering. The global healthcare expenditure on diabetes was estimated at $966 billion in 2021 and is projected to reach $1,054 billion by 2045 (Sun *et al*., 2022). In the United States alone, the direct costs associated with diabetes care amount to approximately $273 billion annually, accompanied by an additional $90 billion in indirect costs (Centers for Disease Control and Prevention, 2023). The global diabetic foot ulcer treatment market was valued at USD 7 billion in 2024 and is expected to grow from USD 7.4 billion in 2025 to USD 12.8 billion in 2034 at a compound annual growth rate of 6.3% (Diabetic Foot Ulcer Treatment Market Report, 2025).

The majority of people with diabetes (approximately 80%) reside in low- and middle-income countries (LMICs), where healthcare infrastructure is often inadequate to manage the growing burden of the disease and its complications (Sun *et al*., 2022). In sub-Saharan Africa, the prevalence of diabetes is rising rapidly, driven by urbanization, dietary changes, and increasing sedentary lifestyles. The African region is projected to experience the highest proportional increase in diabetes prevalence of any world region over the coming decades. The cost of diabetic foot care is 50% to 200% higher than that of primary diabetes care, placing an enormous strain on already resource-constrained healthcare systems in these regions (International Diabetes Federation, 2024).

2.1.2 Pathophysiology of Diabetes Mellitus

Diabetes mellitus is a chronic metabolic disorder characterized by sustained hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The two main types of diabetes are:

Type 1 Diabetes Mellitus (T1DM): This form results from autoimmune destruction of the pancreatic β-cells, leading to absolute insulin deficiency. T1DM accounts for approximately 5-10% of all diabetes cases and typically presents in childhood or adolescence. Over 9.5 million people had type 1 diabetes in 2024, of whom 1.9 million were children and adolescents under the age of 20 (American Diabetes Association, 2024).

Type 2 Diabetes Mellitus (T2DM): This form accounts for 90-95% of diabetes cases and is characterized by insulin resistance coupled with relative insulin deficiency. T2DM is strongly associated with obesity, physical inactivity, and genetic predisposition. The rising prevalence of T2DM parallels the global epidemic of obesity (American Diabetes Association, 2024).

The pathophysiology of both types of diabetes involves multiple interconnected mechanisms. In T1DM, the autoimmune destruction of β-cells is mediated by T lymphocytes, with genetic susceptibility (HLA-DR3 and HLA-DR4) and environmental triggers (viral infections, dietary factors) playing important roles (Atkinson *et al*., 2014). In T2DM, the development of insulin resistance in peripheral tissues (muscle, adipose tissue, liver) is accompanied by progressive β-cell dysfunction, leading to relative insulin deficiency (DeFronzo *et al*., 2015).

Chronic hyperglycemia in both types of diabetes leads to the development of microvascular complications (retinopathy, nephropathy, and neuropathy) and macrovascular complications (cardiovascular disease, cerebrovascular disease, and peripheral arterial disease). These complications are the primary drivers of morbidity and mortality in the diabetic population (Armstrong *et al*., 2023). The pathophysiology of diabetic complications involves multiple pathways, including increased flux through the polyol pathway, accumulation of advanced glycation end-products (AGEs), activation of protein kinase C, and increased oxidative stress (Brownlee, 2015).

2.2 Diabetic Foot Ulcers: Definition and Clinical Significance

Diabetic foot ulcers (DFUs) are defined as full-thickness wounds below the ankle in a person with diabetes, irrespective of duration (Schaper *et al*., 2023). They represent one of the most devastating and costly complications of diabetes, occurring in approximately 15-25% of diabetic patients during their lifetime (Armstrong *et al*., 2023). The lifetime risk of developing a foot ulcer in diabetic patients is 34%, and more than 50% of diabetic foot ulcers will become infected (Villa *et al*., 2024).

The clinical significance of DFUs cannot be overstated. These ulcers precede 80% of lower extremity amputations in individuals diagnosed with diabetes and are correlated with heightened mortality rates. Approximately 18.6 million individuals worldwide experience DFUs annually, with 1.6 million cases reported in the United States alone. Furthermore, more than 60% of non-traumatic lower-limb amputations occur within the diabetic population (Armstrong *et al*., 2023). A European study group found that up to 5% of diabetic patients with a DFU required a major amputation within one year (Armstrong *et al*., 2023).

The prognosis for patients with DFUs is poor, with a five-year survival rate of approximately 50% even after amputation. Five-year mortality rates are as high as 45% for neuropathic ulcers and 55% for ischemic ulcers (Armstrong *et al*., 2023). These mortality rates are comparable to or exceed those of many common cancers, including prostate, breast, and colon cancer (Armstrong *et al*., 2023). The recurrence rate of DFUs is exceptionally high, even after effective wound healing, with recurrence rates of up to 65% over 5 years. Nearly 20% of affected individuals eventually require lower extremity amputation (Santos *et al*., 2025).

Table 1: Global Epidemiology of Diabetes and Diabetic Foot Ulcers

Parameter	Estimate	Reference
Adults with diabetes (2024)	589 million	International Diabetes Federation (2024)
Projected adults with diabetes (2050)	853 million	International Diabetes Federation (2024)
Global diabetes prevalence (2024)	11.11%	International Diabetes Federation (2024)
Annual diabetes-related deaths	3.4 million	International Diabetes Federation (2024)
Lifetime risk of DFU in diabetics	15-34%	Armstrong *et al*. (2023)
DFU prevalence in diabetics	6.3%	Global DFU Prevalence Study (2024)
Annual DFU cases (global)	18.6 million	Global DFU Prevalence Study (2024)
DFU cases annually (USA)	1.6 million	Armstrong *et al*. (2023)
Lower extremity amputations preceded by DFU	80%	Armstrong *et al*. (2023)
5-year survival post-amputation	~50%	Republication of DFU Evaluation and Treatment (2023)
DFU recurrence rate over 5 years	Up to 65%	Santos *et al*. (2025)

Source: International Diabetes Federation (2024), Armstrong *et al*. (2023)

2.2.1 Pathogenesis of Diabetic Foot Ulcers

The pathogenesis of DFUs is multifactorial, involving a complex interplay of several interconnected factors (Hinchliffe *et al*., 2023). Understanding these factors is essential for developing effective prevention and treatment strategies. Diabetic foot disease arises from chronic pathologic processes such as neuropathy, peripheral artery disease (PAD), biomechanical problems, and impaired wound healing. The pathophysiology of DFUs involves metabolic dysfunction, diabetic immunopathy, diabetic neuropathy, and angiopathy (Hinchliffe *et al*., 2023).

2.2.1.1 Peripheral Neuropathy

Diabetic peripheral neuropathy (DPN) is the most important risk factor for DFU development. DPN affects up to 50% of patients with long-standing diabetes and is characterized by progressive loss of nerve function (Armstrong *et al*., 2023). The pathophysiology of DPN involves multiple mechanisms:

1. **Metabolic factors:** Chronic hyperglycemia leads to increased flux through the polyol pathway, accumulation of advanced glycation end-products (AGEs), and activation of protein kinase C, all of which contribute to neuronal damage (Brownlee, 2015). The processes by which hyperglycemia causes peripheral nerve damage are related to adenosine triphosphate deficiency, the polyol pathway, oxidative stress, protein kinase C activity, and proinflammatory processes (The

Pathophysiology of Diabetic Foot: A Narrative Review, 2023).

2. **Microvascular factors:** Endoneurial hypoxia resulting from microvascular disease impairs nerve function and promotes neuronal death (Hinchliffe *et al*., 2023).
3. **Oxidative stress:** Hyperglycemia-induced oxidative damage to neuronal proteins and lipids contributes to neuropathy progression (Pop-Busui *et al*., 2017).

The clinical consequences of DPN include:

- **Sensory neuropathy:** Loss of protective sensation (LOPS) renders the patient unable to perceive pain, pressure, or temperature changes, allowing minor trauma to go unnoticed and progress to ulceration.
- **Motor neuropathy:** Weakness of intrinsic foot muscles leads to foot deformities (e.g., claw toes, Charcot foot) and altered biomechanics, creating high-pressure areas susceptible to repetitive trauma.
- **Autonomic neuropathy:** Reduced sweating leads to dry, fissured skin that is prone to cracking and infection.

2.2.1.2 Peripheral Arterial Disease

Peripheral arterial disease (PAD) is prevalent in patients with diabetes and affects approximately 20-30% of patients with diabetes. PAD is a major contributor to DFU pathogenesis (Hinchliffe *et al*., 2023). The diabetic vasculopathy involves both macrovascular (atherosclerosis) and microvascular disease (Armstrong *et al*., 2023):

Macrovascular disease: Diabetes accelerates atherosclerosis through multiple mechanisms, including endothelial dysfunction, increased oxidative stress, and enhanced platelet aggregation. Atherosclerotic plaques in the lower extremity arteries cause luminal narrowing, reducing blood flow to the foot and impairing wound healing. Diabetes significantly increases the risk of developing peripheral arterial disease, and vascular event rates are higher in diabetic individuals with PAD than in non-diabetic individuals.

Microvascular disease: Diabetes-induced microangiopathy affects the small vessels that supply the skin and subcutaneous tissues. Capillary basement membrane thickening, endothelial dysfunction, and reduced vasodilatory capacity compromise tissue perfusion and oxygen delivery.

The combination of neuropathy and PAD creates a "perfect storm" for ulcer development: the patient cannot feel injury (neuropathy), and the injured tissue cannot heal adequately due to poor blood supply (PAD). Peripheral vascular disease and neuropathy are significant predisposing factors for DFUs.

2.2.1.3 Immunological Impairments in Diabetes

The hyperglycemic milieu in diabetes impairs both innate and adaptive immunity, creating an immunocompromised environment within the wound bed (Hinchliffe *et al*., 2023). Key immunological defects include:

1. **Neutrophil dysfunction:** Hyperglycemia impairs neutrophil chemotaxis, phagocytosis, and bactericidal activity (Koh *et al*., 2010). The oxidative burst required for intracellular killing of pathogens is compromised in diabetic neutrophils.
2. **Macrophage dysfunction:** Diabetes alters macrophage polarization and function. There is a shift from the pro-inflammatory (M1) to the anti-inflammatory (M2) phenotype, which impairs the initial inflammatory response required for wound debridement and pathogen clearance (Barman *et al*., 2023). Diabetic macrophages also exhibit reduced phagocytic capacity and impaired cytokine production.
3. **Impaired angiogenesis:** The diabetic wound environment is characterized by reduced levels of vascular endothelial growth factor (VEGF) and other angiogenic factors, impairing new blood vessel formation and wound healing.
4. **Reduced growth factor production:** Levels of key growth factors, including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), and epidermal growth factor (EGF), are reduced in diabetic wounds (Falanga, 2005).

These immunological impairments, combined with the open, necrotic, and ischemic nature of DFUs, create a fertile niche for rapid microbial colonization and biofilm formation, transitioning a simple wound into a chronically infected ulcer.

2.2.1.4 Biomechanical Factors and Foot Deformities

Abnormal biomechanics and foot deformities play a crucial role in DFU pathogenesis. Motor neuropathy leads to intrinsic muscle weakness and atrophy, resulting in foot deformities such as claw toes, hammer toes, and Charcot foot. These deformities create high-pressure areas that are susceptible to repetitive trauma and ulceration.

The Charcot foot, characterized by progressive bone destruction, fractures, and joint dislocations, is a particularly severe complication that dramatically increases the risk of ulceration and amputation (Schaper *et al*., 2023). Abnormal biomechanics, combined with neuropathy and PAD, create a

vicious cycle of tissue injury, impaired healing, and progressive foot deformity.

2.2.1.5 Risk Factors for DFU Development

Multiple risk factors contribute to DFU development, including:

- Peripheral neuropathy
- Peripheral arterial disease
- Mild or recurrent foot trauma
- Infection
- Foot abnormalities
- History of diabetic foot ulcers or amputations
- Charcot osteoarthropathy
- Poor glycemic control
- Long duration of diabetes
- Renal disease
- Cardiovascular disease
- Frailty
- Smoking
- Obesity

The risk factors for multidrug-resistant organism (MDRO) infections in DFUs specifically include amputation (odds ratio: 5.92), polymicrobial infections (OR: 7.49), biofilm production (OR: 5.00), recent antibiotic use (OR: 3.97), and an ulcer duration greater than 30 days (OR: 2.23).

2.3 Classification and Staging of Diabetic Foot Ulcers

Several classification systems have been developed to grade DFUs based on their depth, extent of infection, and presence of ischemia. The most commonly used systems include:

2.3.1 Meggitt-Wagner Classification

The Meggitt-Wagner classification is the oldest and most widely used system for grading DFUs. It consists of six grades (Wagner Grading of Diabetic Foot Ulcers, 2023):

- **Grade 0:** Pre-ulcerative/high-risk foot (no break in the skin)
- **Grade 1:** Superficial ulcer (involves epidermis, dermis, or subcutaneous tissue)
- **Grade 2:** Deep ulcer extending to ligament, tendon, joint capsule, bone, or deep fascia without abscess or osteomyelitis
- **Grade 3:** Deep ulcer with abscess, osteomyelitis, or joint sepsis
- **Grade 4:** Localized gangrene (forefoot or heel)
- **Grade 5:** Extensive gangrene (entire foot)

The Wagner system is based mainly on the depth of the wound (grades 1-3) and the severity of gangrene (grades 4-5). The severity of DFUs can be classified into mild-to-moderate (Wagner grade score <3) and severe (Wagner grade score ≥3) (Zhang *et al.*, 2023). Studies have shown a correlation between higher Wagner grades and worse clinical outcomes, with the highest neutrophil-to-lymphocyte ratio observed at Wagner grade 5 (Ulandari *et al*., 2023; Yang *et al*., 2023).

2.3.2 University of Texas Classification System

The University of Texas (UT) classification system evaluates DFUs based on both depth and the presence of infection and ischemia. This system provides a more comprehensive assessment than the Wagner system and has been shown to better predict outcomes (Joy *et al*., 2023).

2.3.3 PEDIS and SINBAD Classification Systems

The PEDIS (Perfusion, Extent, Depth, Infection, Sensation) system was developed by the International Working Group on the Diabetic Foot (IWGDF) to provide a standardized approach to DFU classification. The SINBAD (Site, Ischemia, Neuropathy, Bacterial Infection, Area, Depth) system is recommended for communication between healthcare professionals about the characteristics of an ulcer (IWGDF Guidelines, 2023).

The IWGDF/IDSA (Infectious Diseases Society of America) infection classification system is used to classify the severity of diabetic foot infections (IWGDF Guidelines, 2023):

- **Mild infections:** Local infection involving only the skin and subcutaneous tissue, with erythema <2 cm
- **Moderate infections:** Local infection with erythema >2 cm, or involving deeper structures (tendon, muscle, joint, bone)
- **Severe infections:** Local infection with systemic signs of infection (fever, leukocytosis, etc.)

2.4 Microbiology of Diabetic Foot Ulcers

2.4.1 The Polymicrobial Nature of DFU Infections

Diabetic foot infections (DFIs) are polymicrobial, biofilm-associated infections involving complex bacterial communities organized in functional equivalent pathogroups, all including anaerobes (Villa *et al*., 2024). The microbial landscape of infected DFUs is dynamic and diverse, with both aerobic and anaerobic bacteria frequently present. Multiple pathophysiological factors favor the growth of anaerobes in this context, including tissue hypoxia, necrotic tissue, and impaired host defenses (Villa *et al*., 2024).

The IWGDF classifies DFIs based on severity, with mild infections typically involving only the skin and subcutaneous tissue, moderate infections extending deeper (to tendon, muscle, or bone), and severe infections associated with systemic signs of infection (Schaper *et al*., 2023).

A prospective cohort study of 206 DFU patients found that Wagner's grading of Grade 3 constituted a major proportion (35.43%) of cases, with 57% of patients having monomicrobial infections and 35% having polymicrobial infections (Current Trends in Aerobic and Anaerobic Antibiogram Resistance Pattern, 2024). However, the true prevalence of polymicrobial infections is likely higher, as culture-based methods may underestimate the diversity of microorganisms present.

2.4.2 Aerobic Bacteria in DFUs

Gram-positive bacteria are the most frequently isolated organisms from DFUs, with *Staphylococcus aureus* being the predominant pathogen in both community-acquired and healthcare-associated infections. Other common Gram-positive isolates include:

- *Staphylococcus epidermidis*
- *Enterococcus faecalis* and *Enterococcus faecium*
- *Streptococcus pyogenes* (Group A Streptococcus)
- *Streptococcus agalactiae* (Group B Streptococcus)
- *Streptococcus anginosus* group

Gram-negative bacteria are also frequently isolated, particularly in chronic, previously treated, or more severe infections. Common Gram-negative isolates include:

- *Pseudomonas aeruginosa*
- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Proteus mirabilis*
- *Enterobacter cloacae*
- *Morganella morganii*
- *Acinetobacter baumannii* (Villa *et al*., 2024)

2.4.3 Anaerobic Bacteria in DFUs

The role of anaerobes in DFIs has been historically underappreciated due to challenges in sampling, transport, and laboratory processing. However, recent advances in cultivation-independent techniques have revealed that anaerobes are far more prevalent than previously recognized (Villa *et al*., 2024).

Studies based on conventional culture methods show a weighted average of 17% of patients with anaerobes in DFIs. However, when 16S rRNA gene sequencing is employed, the weighted average increases dramatically to 83.8% (Villa *et al*., 2024). This finding indicates that culture methods significantly underestimate both the presence and diversity of anaerobes in DFIs. Culture largely underestimated not only the presence but also the diversity of anaerobes compared with cultivation-independent approaches, but both methods showed that anaerobic Gram-negative bacilli and Gram-positive cocci were the most commonly identified in DFIs (Villa *et al*., 2024).

Common anaerobic isolates from DFUs include:

- **Gram-negative bacilli:** *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Prevotella* spp., *Porphyromonas* spp., *Fusobacterium* spp.
- **Gram-positive cocci:** *Peptoniphilus* spp., *Finegoldia magna*, *Anaerococcus* spp.
- **Gram-positive bacilli:** *Clostridium* spp., *Actinomyces* spp.

The clinical significance of anaerobes in DFIs is increasingly recognized. Anaerobes are more present in deeper lesions, and their detection was associated with fever, malodorous lesions, and ulcer depth and duration (Villa *et al*., 2024). More specifically, initial abundance of *Peptoniphilus* spp. was associated with ulcer-impaired healing, *Fusobacterium* spp. detection was significantly correlated with the duration of DFI, and the presence of *Bacteroides* spp. was significantly associated with amputation (Villa *et al*., 2024).

The high rate of patients with DFIs involving anaerobes, the increased knowledge on the species identified, their virulence factors, and their potential role in wound evolution support recommendations combining debridement and antibiotic therapy effective on anaerobes in moderate and severe DFIs (Villa *et al*., 2024).

2.4.4 Fungal Pathogens in DFUs

Fungi are increasingly recognized as important pathogens in DFUs, particularly in chronic, non-healing wounds. Common fungal isolates from DFUs include:

- *Candida albicans*
- *Candida parapsilosis*
- *Candida tropicalis*
- *Aspergillus* spp.
- *Fusarium* spp.

Fungal presence in DFUs is associated with prolonged wound duration, previous antibiotic use, and worse clinical outcomes (Sotto *et al*., 2021). The role of fungi in DFUs warrants further investigation, as they may contribute to chronicity and treatment failure.

Table 2: Common Microorganisms Isolated from Diabetic Foot Ulcers

Gram-Positive Aerobes	Gram-Negative Aerobes	Anaerobes	Fungi
Staphylococcus aureus	*Pseudomonas aeruginosa*	*Bacteroides fragilis*	*Candida albicans*
S. epidermidis	*Escherichia coli*	*Peptoniphilus* spp.	*C. parapsilosis*
Enterococcus faecalis	*Klebsiella pneumoniae*	*Fusobacterium* spp.	*Aspergillus* spp.
E. faecium	*Proteus mirabilis*	*Finegoldia magna*	*Fusarium* spp.
Streptococcus pyogenes	*Enterobacter cloacae*	*Prevotella* spp.	
S. agalactiae	*Morganella morganii*	*Clostridium* spp.	
S. anginosus	*Acinetobacter baumannii*	*Actinomyces* spp.	

Source: (Villa *et al*., 2024; Rajha *et al*., 2024).

2.4.5 Geographic Variation in DFU Microbiology

The microbiology of DFUs exhibits notable geographic diversity, with specific species and resistance patterns varying across regions. A systematic review of Diabetic Foot Infections microbiology across different continents revealed significant differences in pathogen distribution and antimicrobial resistance rates (Rajha *et al*., 2024).

In North America and Europe, *S. aureus* remains the predominant pathogen, with MRSA rates typically below 20% in most high-income regions. In contrast, African studies report MRSA rates exceeding 30%, with ESBL detection approaching 50% in Enterobacterales. Asia shows the highest incidence (49.36%) of Gram-negative aerobic bacteria, with *E. Coli* (10.77%), *Enterobacter* spp. (3.95%), and *P. aeruginosa* (11.08%) being the most common isolates. These geographic variations have important implications for empirical antibiotic selection and highlight the need for locally adapted treatment guidelines (Rajha *et al*., 2024).

2.4.6 Isolation and Identification of DFU Pathogens

The accurate isolation and identification of DFU pathogens is essential for appropriate antimicrobial therapy. The IWGDF/IDSA guidelines provide recommendations for (Senneville *et al*., 2023):

1. **Strict indications for sampling wounds:** Only clinically infected wounds should be sampled for culture.
2. **The best way to perform sampling:** Tissue biopsy or deep swab using the Levine technique is recommended.
3. **Avoiding the misuse of broad-spectrum antibiotic regimens:** Targeted therapy based on culture and susceptibility results is preferred.

The Levine technique involves rotating a sterile swab over a 1 cm^3 area of viable tissue with sufficient pressure to express fluid (Haalboom *et al*., 2019). This technique provides a more representative sample of the wound microbiome than superficial swabbing.

2.5 Biofilm in Diabetic Foot Ulcers

2.5.1 Definition and Characteristics of Biofilms

Biofilms are structured communities of microorganisms encased in a self-produced extracellular polymeric substance (EPS) matrix that adheres to biological or abiotic surfaces (Costerton *et al*., 2019). The EPS matrix is composed of polysaccharides, proteins, nucleic acids, and lipids, and provides the bacterial community with structural integrity and protection from environmental stressors.

Key characteristics of biofilms include:

1. **Structural complexity:** Biofilms are highly organized structures with microcolonies, channels, and water-filled pores that facilitate nutrient and waste exchange.

2. **Physiological heterogeneity:** Bacteria within biofilms exhibit differential gene expression, with cells in different regions showing distinct metabolic activities and susceptibility to antimicrobials.
3. **Quorum sensing:** Biofilm bacteria communicate through quorum sensing (QS) systems, coordinating gene expression and behavior in response to population density.
4. **Antimicrobial tolerance:** Biofilm-embedded bacteria are inherently more tolerant to antimicrobial agents and host immune defenses compared to planktonic (free-floating) cells.

2.5.2 Biofilm Formation in DFUs

Biofilm formation in DFUs follows a multi-step process (Pouget *et al*., 2023):

Step 1: Initial Adhesion. Bacteria attach to the wound surface, a process facilitated by surface proteins (adhesins) and host factors such as fibronectin, fibrinogen, and collagen.

Step 2: Irreversible Attachment. Following initial adhesion, bacteria produce EPS, leading to more stable attachment. This stage is associated with increased expression of genes involved in polysaccharide synthesis and biofilm formation.

Step 3: Microcolony Formation. Attached bacteria proliferate and form microcolonies, which grow and merge into larger structures.

Step 4: Biofilm Maturation. The biofilm develops a complex three-dimensional architecture with channels for nutrient and waste exchange. Mature biofilms are characterized by high cell density, EPS production, and the establishment of QS systems.

Step 5: Dispersion. Cells at the periphery of the biofilm detach and disperse to colonize new sites. This stage completes the biofilm life cycle and contributes to the spread of infection.

In diabetic wounds, microorganisms colonize the wound's surface and produce a biofilm that prevents the lesion from healing. These biofilms are extremely challenging to treat because the EPS matrix shields the bacterial cells from antimicrobial agents and host defences.

2.5.3 Prevalence of Biofilms in DFUs

Most bacteria present in chronic ulcers are living in biofilms (Villa *et al*., 2024). A prospective cohort study of 187 DFU patients found that 61.5% of the isolates produced biofilms (Villa *et al*., 2024). The high prevalence of biofilm-producing bacteria in DFUs has significant clinical implications:

1. **Delayed healing:** Biofilms delay wound healing by maintaining a chronic inflammatory state and preventing granulation tissue formation.
2. **Treatment failure:** Biofilm-associated infections are inherently tolerant to antimicrobial agents, leading to frequent treatment failures.
3. **Increased morbidity:** Biofilm presence is associated with longer hospital stays, higher rates of amputation, and increased mortality.

Due to their ability to synthesize biofilms, the wound becomes more chronic and incurable, posing a serious threat to immunocompromised diabetic patients.

2.5.4 Mechanisms of Biofilm-Associated Antimicrobial Resistance

Biofilm-associated antimicrobial resistance involves multiple mechanisms (Pouget *et al*., 2023):

1. **Physical barrier:** The EPS matrix acts as a physical barrier, limiting the penetration of antimicrobial agents into the deeper layers of the biofilm. The matrix binds and sequesters antibiotics, preventing them from reaching bacterial cells.
2. **Reduced growth rate:** Cells within biofilms often exhibit reduced metabolic activity and slow growth, making them less susceptible to antibiotics that target actively dividing cells.
3. **Persister cells:** A small subpopulation of cells within biofilms adopts a dormant or "persister" state, characterized by minimal metabolic activity and high tolerance to antibiotics. These cells can survive antibiotic treatment and repopulate the biofilm once treatment is withdrawn.
4. **Quorum sensing-mediated resistance:** QS systems in biofilms regulate the expression of genes involved in antimicrobial resistance, including efflux pumps and antibiotic-modifying enzymes.
5. **Horizontal gene transfer:** The close proximity of cells within biofilms facilitates horizontal gene transfer (HGT), promoting the spread of resistance genes among bacterial populations.
6. **Biofilm as a favoring factor for anaerobic growth:** The biofilm environment creates anaerobic conditions that favor the growth of anaerobes, which may contribute to polymicrobial infections and increased resistance (Villa *et al*., 2024).

Hyperglycemia promotes specific mechanisms of bacterial virulence known to contribute to infection chronicity, including tissue adherence and biofilm formation.

2.6 Antimicrobial Resistance in Diabetic Foot Ulcers

2.6.1 The Global Antimicrobial Resistance Crisis

Antimicrobial resistance (AMR) is recognized by the World Health Organization (WHO) as one of the top ten global public health threats facing humanity (World Health Organization, 2019). Bacterial illnesses claim the lives of 17 million people annually, making infectious disease the second greatest cause of death worldwide. The emergence and proliferation of AMR among DFU pathogens have rendered conventional antibiotics increasingly ineffective (Martens and Demain, 2017).

The crisis of antibiotic resistance is compounded by the low number of new antibiotics created and approved over the past few decades (Andrei *et al*., 2018). The lack of alternatives to reduce antimicrobial resistance necessitates the urgent exploration of alternative and complementary therapeutic strategies. Numerous bacterial species establish infection in DFUs, and treatment with antibiotics often fails due to widespread antibiotic resistance and biofilm formation.

2.6.2 Major Drug-Resistant Pathogens in DFUs

Methicillin-Resistant *Staphylococcus aureus* (MRSA)

MRSA is one of the most clinically significant drug-resistant pathogens in DFUs. A systematic review and meta-analysis by Zhou *et al*. (2024) revealed a decreasing yet still significant global prevalence of MRSA in DFUs. The prevalence of MRSA in DFUs varies significantly by geographic region:

- Africa: Highest incidence at 26.90%
- North America and Europe: Typically below 20%
- Asia: Intermediate prevalence

A study of 187 DFU patients found that among the 27 MRSA isolates, 81.5% carried the *mecA* gene. MRSA infection in DFUs is associated with increased risk of osteitis and treatment failure. The management of MRSA-infected DFUs is challenging, requiring the use of alternative antibiotics such as vancomycin, linezolid, or daptomycin, which are often more expensive and associated with more adverse effects (Villa *et al*., 2024).

Extended-Spectrum β-Lactamase (ESBL)-Producing Enterobacterales

ESBL-producing Enterobacterales, particularly *E. coli* and *K. pneumoniae*, are increasingly prevalent in DFUs. ESBL enzymes hydrolyze penicillins, cephalosporins, and aztreonam, rendering these antibiotics ineffective. In African DFI studies, ESBL detection approaches 50% in Enterobacterales.

A study found that 45% of the Gram-negative isolates (40 out of 89 isolates) were ESBL producers, and 22.5% of the Gram-negative isolates were carbapenem-resistant Enterobacteriaceae (CRE) (20 out of 89 isolates) (Microbiological Profile of Diabetic Foot Infections, 2024). Another study found that 98.8% of the ESBL-producing organisms harboured at least one ESBL gene, with *blaCTX-M* being the most common (27.2%) (Villa *et al*., 2024).

Carbapenem-Resistant Enterobacteriaceae (CRE)

Carbapenem resistance is a growing concern in DFUs. A study found that carbapenem resistance was confirmed in 25.6% of the isolates, with *blaNDM* being the predominant carbapenemase gene (69.3%). Co-resistance with ESBL genes was detected in 66.7% of the *blaNDM*-producing isolates (Villa *et al*., 2024).

Multidrug-Resistant *Pseudomonas aeruginosa*

P. aeruginosa is intrinsically resistant to many antibiotics and readily acquires additional resistance mechanisms. Multidrug-resistant (MDR), extensively drug-resistant (XDR), and even pandrug-resistant (PDR) strains of *P. aeruginosa* have been isolated from DFUs. The resistance markers observed include ESBL producer, AmpC producer, MBL producer, methicillin resistance, and inducible clindamycin resistance (Villa *et al*., 2024).

Antimicrobial Resistance in Anaerobes

Antimicrobial resistance of anaerobes in DFIs remains slightly studied and warrants more consideration in the context of increasing resistance of the most frequently identified anaerobes in DFIs (Villa *et al*., 2024).

2.6.3 Definition of Multidrug Resistance

The international expert proposal for interim standard definitions for acquired resistance defines:

- **Multidrug-resistant (MDR):** Non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos *et al*., 2012).
- **Extensively drug-resistant (XDR):** Non-susceptibility to at least one agent in all but two or fewer antimicrobial categories.
- **Pandrug-resistant (PDR):** Non-susceptibility to all agents in all antimicrobial categories (Magiorakos *et al*., 2012).

2.6.4 Risk Factors for Multidrug-Resistant Organisms in DFUs

A study of 187 DFU patients identified the following key risk factors for MDR infections (Villa *et al*., 2024):

- Amputation (OR: 5.92)
- Polymicrobial infections (OR: 7.49)

- Biofilm production (OR: 5.00)
- Recent antibiotic use (OR: 3.97)
- Ulcer duration > 30 days (OR: 2.23)

Sex, age, and weight were not significantly associated with MDR infections. The study demonstrated a high prevalence of MDROs in DFUs, primarily driven by biofilm-producing polymicrobial infections and resistance genes like *blaNDM* and *blaCTX-M* (Villa *et al*., 2024).

2.6.5 Antimicrobial Resistance Patterns in African DFUs

The burden of AMR in DFUs is particularly severe in Africa, where healthcare resources are limited and empirical antibiotic use is common. A systematic review and meta-analysis of bacterial profiles and antimicrobial resistance patterns in sub-Saharan African DFIs revealed that both Gram-positive and Gram-negative bacteria were associated with DFUs (Wada *et al*., 2023).

Key findings from African studies include:

- High prevalence of MRSA (>30% in many studies)
- High prevalence of ESBL-producing Enterobacterales (approaching 50%)
- High rates of MDR among *P. aeruginosa* isolates
- Limited availability of antimicrobial susceptibility testing

The prevalence of DFUs in the South African public healthcare sector is estimated at 5.4-6.0% among patients with diabetes, with more recent studies noting that 28% of patients with diabetes presenting to primary healthcare facilities in South Africa had developed DFUs (Wada *et al*., 2023).

2.6.6 Implications of AMR for DFU Management

The high prevalence of AMR in DFU pathogens has profound implications for clinical management:

1. **Empirical antibiotic therapy:** The choice of empirical antibiotics must be guided by local resistance patterns.
2. **Targeted therapy:** Culture and susceptibility results are essential for guiding targeted therapy.
3. **Antibiotic stewardship:** Reducing the duration of antibiotic therapy and avoiding unnecessary antibiotic use are critical for preserving antibiotic efficacy.
4. **Alternative therapies:** The exploration of alternative therapies, including plant-based antimicrobials, is urgently needed.

2.7 Antimicrobial Stewardship in Diabetic Foot Infections

2.7.1 Principles of Antimicrobial Stewardship

Antimicrobial stewardship (AMS) refers to coordinated interventions designed to improve and measure the appropriate use of antimicrobials by promoting the selection of the optimal antimicrobial drug regimen, dose, duration of therapy, and route of administration (Dellit *et al*., 2017). AMS is particularly important in DFIs, where antibiotic overuse and misuse contribute to the development of resistance.

The core principles of AMS in DFIs include:

1. **Diagnostic stewardship:** Ensuring that only clinically infected wounds are sampled for culture.
2. **Targeted therapy:** Using culture and susceptibility results to guide antibiotic selection.
3. **Appropriate duration:** Avoiding prolonged courses of antibiotics.
4. **De-escalation:** Switching from broad-spectrum to narrow-spectrum antibiotics when possible.
5. **Multidisciplinary approach:** Involving specialists in diabetes, podiatry, infection, and vascular surgery.

2.7.2 2023 IWGDF/IDSA Guidelines for DFI Management

The 2023 IWGDF/IDSA guidelines for the diagnosis and treatment of diabetes-related foot infections represent a significant update to previous guidelines (Senneville *et al*., 2023). Key recommendations include:

1. **Reducing the duration of antibiotic therapy:** The guidelines propose reducing the duration of antibiotic therapy to 10 days in patients with severe or moderate soft-tissue infections of the foot following surgical debridement (Senneville *et al*., 2023).
2. **Bedside percutaneous bone biopsy:** Data now support the safety of bedside percutaneous bone biopsy in persons with a suspicion of diabetes-related osteomyelitis of the foot (Senneville *et al*., 2023).
3. **Shorter follow-up period:** The post-end of treatment follow-up necessary to define remission of osteomyelitis has been reduced from 12 to 6 months (Senneville *et al*., 2023).
4. **Avoiding antibiotics in uninfected ulcers:** Systemic or local antibiotics should not be used to treat clinically uninfected foot ulcers with the intent to minimize the risk of infection or

promote the healing of the ulcer (Senneville *et al*., 2023).

2.7.3 Antimicrobial Stewardship Recommendations

The IWGDF/IDSA guidelines contribute to antimicrobial stewardship efforts by providing recommendations for (Senneville *et al*., 2023):

1. Strict indications for sampling wounds
2. The best way to perform sampling to avoid the misuse of broad-spectrum antibiotic regimens
3. Reducing the duration of patient exposure to antibiotics

The 2023 guidelines globally confirm the recommendations made in 2019 with an improved methodology, including applying Grading of Recommendations Assessment, Development and Evaluation (GRADE) principles. New evidence identified was evaluated on the basis of shared outcomes that had been revised to include direct and patient-centered outcomes.

2.7.4 Challenges in AMS Implementation

Despite the availability of evidence-based guidelines, the implementation of AMS in DFIs faces several challenges:

1. **Lack of rapid diagnostics:** Traditional culture methods are slow, leading to the initiation of empirical therapy.
2. **Limited access to susceptibility testing:** In resource-limited settings, susceptibility testing may not be available.
3. **Complexity of DFIs:** The polymicrobial, biofilm-associated nature of DFIs makes antibiotic selection challenging.
4. **Patient-related factors:** Patients may have multiple comorbidities that complicate treatment.
5. **Healthcare system factors:** Fragmented care and lack of multidisciplinary teams may impede AMS implementation (Abbas *et al*., 2024).

2.8 Current Management of Diabetic Foot Ulcers

2.8.1 Standard of Care

The management of DFUs requires a multidisciplinary approach encompassing (Schaper *et al*., 2023):

1. **Wound debridement:** Removal of necrotic tissue, callus, and foreign material to create a clean wound bed conducive to healing.
2. **Pressure offloading:** Reduction of mechanical pressure on the ulcerated area through the use of specialized footwear, total contact casts, or removable walkers.
3. **Revascularization:** Restoration of blood flow through surgical or endovascular interventions in patients with significant peripheral arterial disease.
4. **Infection control:** Appropriate antimicrobial therapy based on culture and susceptibility results, combined with local wound care.
5. **Glycemic control:** Optimization of blood glucose levels to improve immune function and wound healing.
6. **Advanced wound care:** Use of specialized dressings, negative pressure wound therapy, growth factors, or skin substitutes in non-healing wounds.

2.8.2 Limitations of Current Therapies

Despite advances in wound care, the effectiveness of current treatments remains limited, with a significant number of patients requiring amputation. Key limitations include:

1. **Antibiotic resistance:** The emergence of MDR pathogens renders empirical antibiotic therapy increasingly ineffective.
2. **Biofilm persistence:** Conventional antimicrobial therapies often fail to eliminate biofilms, leading to recurrent infections.
3. **Poor wound healing:** The diabetic wound environment is characterized by impaired angiogenesis, reduced growth factor production, and chronic inflammation, all of which impede healing.
4. **High recurrence rate:** Even after successful healing, DFUs recur in up to 40% of patients within one year and up to 65% over 5 years (Santos *et al*., 2025).
5. **Economic burden:** The costs associated with DFU management are substantial, particularly for patients requiring hospitalization or amputation.

2.9 Medicinal Plants in Wound Healing and Infection Management

2.9.1 Historical and Ethnopharmacological Context

Plants have been used to treat and cure a wide range of illnesses for millennia. Traditional medicine systems, including Ayurveda, Traditional Chinese Medicine, and African traditional medicine, have long utilized plant-based preparations for wound care and infection management (Vaou *et al*., 2021).

The use of therapeutic herbs is steadily increasing on a global scale, driven by the emergence of resistant diseases and growing scientific awareness of herbal treatments as important therapeutic alternatives (Vaou *et al*., 2021). The

current common perception that "green medicine" is safer and more reliable than expensive synthetic pharmaceuticals, many of which have negative side effects, is a primary cause of this resurgence of interest in plant-derived medications (Nair and Chanda, 2017).

2.9.2 Rationale for Plant-Based Antimicrobials

The search for novel antimicrobial compounds from natural sources, including plants, has intensified due to the crisis of antibiotic resistance (Balouiri *et al*., 2016). Medicinal plants represent a vast and historically validated reservoir of bioactive compounds.

Key advantages of plant-based antimicrobials include:

1. **Structural diversity:** Plants produce a wide range of secondary metabolites with diverse chemical structures and mechanisms of action.
2. **Multi-target effects:** Plant extracts often contain multiple bioactive compounds that act synergistically on different bacterial targets, potentially reducing the development of resistance.
3. **Combination with conventional antibiotics:** Plant extracts or their bioactive components may have synergistic effects with antibiotics, counteracting bacterial resistance mechanisms.
4. **Traditional knowledge:** Ethnopharmacological knowledge provides a valuable starting point for identifying plants with therapeutic potential.

2.9.3 Mechanisms of Antibacterial Action of Plant Extracts

Plant-derived antibacterial compounds exert their effects through multiple mechanisms:

1. Disruption of cell membrane integrity: Many plant secondary metabolites, including flavonoids and terpenoids, interact with bacterial cell membranes, disrupting their structure and function. This leads to increased membrane permeability, leakage of intracellular contents, and cell death (Quelemes *et al*., 2015).

2. Inhibition of cell wall synthesis: Some plant compounds interfere with bacterial cell wall synthesis, weakening the cell wall and causing osmotic lysis.

3. Inhibition of protein synthesis: Certain phytochemicals bind to bacterial ribosomes, inhibiting protein synthesis and bacterial growth.

4. Inhibition of nucleic acid synthesis: Polyphenols can intercalate into DNA, inhibit topoisomerases, or interfere with DNA replication and transcription.

5. Inhibition of efflux pumps: Some plant compounds inhibit bacterial efflux pumps, increasing the intracellular concentration of antibiotics and restoring their activity.

6. Quorum sensing inhibition: Many plant extracts interfere with bacterial QS systems, reducing the expression of virulence factors and preventing biofilm formation.

7. Biofilm disruption: Plant extracts can prevent biofilm formation or disrupt existing biofilms by interfering with EPS production, adhesion, or QS.

8. Inhibition of enzyme activity: Plant compounds can inhibit key bacterial enzymes, including those involved in metabolism, cell wall synthesis, and virulence factor production (Quelemes *et al*., 2015).

2.10 *Azadirachta indica* (Neem)

2.10.1 Botanical Description and Distribution

Azadirachta indica A. Juss., commonly known as neem, is a fast-growing, evergreen tree belonging to the family Meliaceae (mahogany family). The tree is native to the Indian subcontinent but has been widely naturalized in tropical and subtropical regions across Africa, the Americas, and Southeast Asia (Saleem *et al*., 2023).

The tree can reach heights of 15-20 meters and has a dense, rounded crown with compound, pinnate leaves. The leaves are dark green, 20-40 cm long, with 8-19 leaflets. The small, white, fragrant flowers are arranged in axillary panicles, and the fruit is a smooth, yellow-green drupe with a sweet, oily pulp. Since ancient times, *Azadirachta indica*, or neem, has been a well-known species of plant that produces a broad range of bioactive terpenoid chemicals that are involved in a variety of biological functions (Saleem *et al*., 2023).

2.10.2 Traditional Uses

Neem has been used in traditional medicine for over 4,000 years and is often referred to as the "village pharmacy" or "wonder tree" in India. In Ayurvedic medicine, neem is used for a wide range of conditions, including:

- Skin disorders (wounds, ulcers, eczema, psoriasis)
- Infections (bacterial, fungal, viral)
- Gastrointestinal disorders
- Fever and malaria
- Diabetes mellitus
- Respiratory conditions

Traditionally, leaf pastes or extracts of neem have been applied directly to wounds for their antiseptic, anti-inflammatory, and wound-healing properties (Rahman *et al*., 2024).

2.10.3 Phytochemical Composition

Phytochemical investigations have identified over 140 distinct compounds in *A. indica*, distributed across all plant parts. The phytochemical composition, antimicrobial efficacy, and potential applications of *A. indica*, particularly in combating MDR pathogens, have been extensively reviewed. Key phytochemicals include:

Terpenoids and Limonoids: Neem is particularly rich in limonoids, a class of highly oxygenated triterpenoids. Major limonoids include:

- **Azadirachtin:** The most well-known neem compound, with potent insecticidal, antibacterial, and antiviral properties.
- **Nimbin:** A triterpenoid with anti-inflammatory and antimicrobial activity.
- **Nimbolide:** A limonoid with potent antibacterial, anticancer, and anti-inflammatory properties.
- **Gedunin:** A limonoid with antimalarial and anticancer activity.
- **Nimbidin:** A tetranortriterpenoid with antimicrobial and anti-inflammatory properties.

Flavonoids: Neem leaves contain various flavonoids, including quercetin, rutin, and kaempferol. These compounds have antioxidant, anti-inflammatory, and antimicrobial properties.

Phenolic Acids: Neem contains phenolic acids such as gallic acid, caffeic acid, and chlorogenic acid, which contribute to its antioxidant and antimicrobial activities.

Other Compounds: Neem also contains alkaloids (including azadirachtine), saponins, tannins, and various volatile oils.

The GC-MS characterization for *A. indica* showed the presence of phenols, organic acids, and carbohydrates with cannabidiol as the most abundant (Bolade *et al*., 2021; Nagini *et al*., 2024)

Table 3: Major Phytochemicals in *Azadirachta indica* Leaves

Class	Compound	Key Activities
Limonoids	Azadirachtin	Insecticidal, antibacterial, antiviral
Limonoids	Nimbin	Anti-inflammatory, antimicrobial
Limonoids	Nimbolide	Antibacterial, anticancer, anti-inflammatory
Limonoids	Gedunin	Antimalarial, anticancer
Limonoids	Nimbidin	Antimicrobial, anti-inflammatory
Flavonoids	Quercetin	Antioxidant, anti-inflammatory, antimicrobial
Flavonoids	Rutin	Antioxidant, anti-inflammatory
Flavonoids	Kaempferol	Antioxidant, antimicrobial
Phenolic acids	Gallic acid	Antioxidant, antimicrobial
Phenolic acids	Caffeic acid	Antioxidant, anti-inflammatory

Source: (Alzohairy, 2016; Sarkar *et al*., 2021).

2.10.4 Mechanisms of Antibacterial Action

The antibacterial mechanisms of neem extracts have been attributed to multiple pathways (Alzohairy, 2016):

1. **Disruption of bacterial cell membrane integrity:** Neem compounds interact with bacterial cell membranes, disrupting their structure and leading to leakage of intracellular contents.
2. **Inhibition of efflux pumps:** Neem components inhibit bacterial efflux pumps, enhancing the intracellular concentration of antibiotics.
3. **Attenuation of quorum sensing and biofilm formation:** Neem extracts interfere with QS systems, reducing the expression of virulence factors and preventing biofilm formation (Quelemes *et al*., 2015).
4. **Inhibition of cell wall synthesis:** Some neem compounds interfere with bacterial cell wall synthesis.
5. **Anti-adhesive properties:** Neem components reduce bacterial adhesion to surfaces, preventing initial biofilm formation.

2.10.5 Antibiofilm Activity

Neem leaf extracts have demonstrated potent antibiofilm activity against various pathogens. Quelemes *et al*. (2015) showed that neem leaf extract effectively inhibited biofilm formation and disrupted pre-formed biofilms of resistant *S. aureus*. Neem oil prevents the formation of biofilms and causes morphological alterations in *P. aeruginosa* and *S. aureus*. Natural antibacterial agents could be a safe solution for controlling bacterial biofilms without affecting human health and environmental safety.

The antibiofilm mechanisms of neem include:

1. Inhibition of bacterial adhesion to surfaces
2. Interference with EPS production
3. Disruption of QS systems
4. Dispersion of mature biofilms

2.10.6 Anti-inflammatory and Wound-Healing Properties

Neem possesses significant anti-inflammatory and wound-healing properties that are particularly relevant to DFU management (Sarkar *et al*., 2021):

- **Anti-inflammatory:** Neem compounds inhibit the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), reduce oxidative stress, and modulate the inflammatory response.
- **Wound healing:** Neem extracts promote wound healing by enhancing granulation tissue formation, increasing collagen deposition, and stimulating angiogenesis (Rahman *et al*., 2024).
- **Antioxidant:** The flavonoids and phenolic acids in neem provide potent antioxidant activity, protecting tissues from oxidative damage.

2.10.7 Safety Evaluation of Neem

Despite its long history of traditional use, systematic safety evaluations of neem for topical application are limited. Some studies have suggested that neem extracts may cause skin irritation or allergic reactions in sensitive individuals. The safety evaluation of neem extracts is an important area for further research, particularly for formulations intended for topical application on compromised skin (Sarkar *et al*., 2021).

2.11 *Moringa oleifera*

2.11.1 Botanical Description and Distribution

Moringa oleifera Lam., commonly known as moringa, drumstick tree, or miracle tree, is a fast-growing, deciduous tree belonging to the family Moringaceae. The tree is native to the foothills of the Himalayas in northwestern India but is now widely cultivated throughout the tropics and subtropics (Jikah and Edo, 2023).

The tree can reach heights of 5-12 meters and has a slender trunk with soft, corky bark. The leaves are bipinnately compound, 20-70 cm long, with numerous small leaflets. The flowers are fragrant, white or cream-colored, and borne in axillary panicles. The fruit is a long, slender, three-sided pod (drumstick) containing numerous winged seeds (Jikah and Edo, 2023).

2.11.2 Traditional Uses

Moringa oleifera is often dubbed the "miracle tree" due to its remarkable nutritional and medicinal properties. Traditionally, various parts of the tree have been used for:

- Nutritional supplementation (leaves are rich in proteins, vitamins, and minerals)
- Wound healing (leaf pastes applied topically)
- Infections (bacterial, fungal, and parasitic) treeatment
- Diabetes management
- Cardiovascular health
- Anti-inflammatory conditions

The leaves are particularly valued for their high nutritional content and medicinal properties (Kou *et al*., 2018). *Moringa oleifera* leaves are acknowledged to decline

blood glucose concentration, modeled in type 2 diabetics control.

2.11.3 Phytochemical Composition

Moringa oleifera leaves are rich in a diverse array of phytochemicals, including (Vergara-Jimenez *et al*., 2017:

Isothiocyanates: These are the most characteristic compounds of moringa, including 4-(α-L-rhamnosyloxy) benzyl isothiocyanate, which has potent antimicrobial and anticancer properties.

Flavonoids: Moringa leaves contain significant amounts of flavonoids, including kaempferol, quercetin, and isorhamnetin. These compounds provide antioxidant, anti-inflammatory, and antimicrobial activities.

Phenolic Acids: Various phenolic acids, including chlorogenic acid, gallic acid, and ferulic acid, contribute to the antioxidant and antimicrobial properties.

Glucosinolates: These sulfur-containing compounds are precursors to isothiocyanates and have antimicrobial, anticancer, and anti-inflammatory activities.

Other Compounds: Moringa also contains alkaloids, saponins, sterols, and unique proteins.

Nutritional Composition: Moringa leaves are exceptionally nutritious, containing:

- Proteins (all essential amino acids)
- Vitamins (A, B-complex, C, E, K)
- Minerals (calcium, potassium, iron, magnesium, zinc)
- Carotenoids (β-carotene, lutein, zeaxanthin)

Moringa oleifera is very rich in vitamins, minerals, fatty acids, and phytochemical compounds like quercetin, kaempferol, and vicenin-2, that play a role in the wound healing process.

Table 4: Major Phytochemicals in *Moringa oleifera* Leaves

Class	Compound	Key Activities
Isothiocyanates	4-(α-L-rhamnosyloxy) benzyl isothiocyanate	Antimicrobial, anticancer, anti-inflammatory
Flavonoids	Kaempferol	Antioxidant, anti-inflammatory, antimicrobial
Flavonoids	Quercetin	Antioxidant, anti-inflammatory, antimicrobial
Flavonoids	Isorhamnetin	Antioxidant, anti-inflammatory
Phenolic acids	Chlorogenic acid	Antioxidant, antimicrobial
Phenolic acids	Gallic acid	Antioxidant, antimicrobial
Glucosinolates	Various	Antimicrobial, anticancer
Sterols	β-sitosterol	Anti-inflammatory, cholesterol-lowering

Source: (Kou *et al*., 2018; Vergara-Jimenez *et al*., 2017).

2.11.4 Antimicrobial Activity

Moringa oleifera demonstrates broad-spectrum antimicrobial activity against bacteria, fungi, and viruses. Studies have shown moringa leaf extracts to be effective against:

- Gram-positive bacteria: *S. aureus* (including MRSA), *S. pneumoniae*, *Bacillus* spp.
- Gram-negative bacteria: *P. aeruginosa*, *E. coli*, *Salmonella* spp., *K. pneumoniae*
- Fungi: *Candida albicans*, *Aspergillus* spp.

A hydro-alcoholic bark extract of *Moringa oleifera* (MOHE) exhibited significant antibacterial activity against pathogenic bacteria species, efficiently reduced heat-induced RBC

hemolysis, with anti-inflammatory effect of 73% at 0.2 mg/mL, and promoted fibroblast proliferation (148.83%) and improved migration, resulting in a wound closure rate of 46.3%. *Moringa oleifera* extracted at 35 MPa reflected highest inhibition zones of 27 ± 0.1 mm, 30 ± 0.2 mm, and 30 ± 0.1 mm against *C. glabrata*, *C. tropicalis*, and *C. albicans* (Manhas and Malairaman, 2026).

2.11.5 Antibiofilm Activity

Moringa leaf extracts demonstrate significant antibiofilm activity against various pathogens (Lee *et al*., 2017; Al-Malki *et al*., 2023). Studies have shown that moringa leaf extracts:

- Inhibit biofilm formation by *S. aureus* and *P. aeruginosa*
- Prevent cariogenic biofilm formation by *S. mutans*
- Reduce biofilm development by >70% against test pathogens
- Disrupt pre-formed biofilms and reduce biofilm biomass

The antibiofilm mechanisms of moringa include:

1. Interference with bacterial QS systems that regulate biofilm formation
2. Inhibition of bacterial adhesion to surfaces
3. Disruption of EPS production
4. Reduction of virulence factor expression

2.11.6 Mechanisms of Antibacterial Action

The antibacterial mechanisms of moringa involve multiple pathways (Kou *et al*., 2018; Vergara-Jimenez *et al*., 2017):

1. **Disruption of cell membrane integrity:** Moringa compounds (particularly flavonoids and phenolic acids) interact with bacterial cell membranes, increasing permeability and causing cell death.
2. **Inhibition of enzyme activity:** Moringa compounds inhibit key bacterial enzymes, including those involved in metabolism and virulence.
3. **DNA interaction:** Polyphenols can intercalate into DNA or interact with DNA gyrase/topoisomerase, inhibiting DNA replication and transcription.
4. **Inhibition of biofilm formation:** Moringa compounds interfere with QS systems and biofilm formation.

2.11.7 Anti-inflammatory, Antioxidant, and Wound-Healing Properties

Moringa oleifera possesses properties that are particularly relevant to DFU management (Kou *et al*., 2018):

- **Anti-inflammatory:** Moringa extracts inhibit the production of pro-inflammatory cytokines, reduce oxidative stress, and modulate inflammatory pathways.
- **Antioxidant:** The high content of flavonoids and phenolic acids provides potent antioxidant activity, protecting tissues from oxidative damage.
- **Wound healing:** Moringa extracts promote wound healing by enhancing granulation tissue formation, increasing collagen deposition, and stimulating angiogenesis. TGF-β and VEGF are vital in cell proliferation and regeneration, as evidenced in processes like wound healing. The leaves of *Moringa oleifera* Lam exhibit anti-inflammatory and cell regenerative properties that may facilitate the transition from the inflammatory to the proliferative phase of wound healing.
- **Immunomodulatory:** Moringa compounds modulate immune function, enhancing the body's ability to fight infections.

Numerous studies show the antioxidant and anti-inflammatory effects of *Moringa oleifera* leaves decrease the levels of AGEs in individuals with hyperglycemia and promote unimpeded wound healing.

2.11.8 Safety Evaluation of Moringa

Moringa extracts are generally well-tolerated but may cause mild irritation in some cases. The safety of combination extracts has not been adequately evaluated. As with neem, systematic safety evaluations of moringa for topical application are limited, and further research is needed to establish the safety profile of moringa-based topical formulations.

2.12 Safety Assessment of Medicinal Plant Extracts

2.12.1 Importance of Safety Evaluation

The assertion that "natural" equates to "safe" is a fallacy. Phytochemicals can be potent skin irritants, sensitizers, or cytotoxic agents. A comprehensive, multi-level safety evaluation is essential before plant extracts can be recommended for therapeutic use.

Key safety concerns for topical plant extracts include:

1. **Dermal irritation:** Local irritation, redness, swelling, or pain at the application site.
2. **Skin sensitization:** Allergic contact dermatitis resulting from repeated exposure.
3. **Photoirritation/photosensitization:** Irritation or sensitization triggered by light exposure.
4. **Cytotoxicity:** Damage to skin cells (keratinocytes, fibroblasts).

5. **Systemic toxicity:** Absorption of toxic compounds through the skin.

2.12.2 Regulatory Guidelines for Safety Assessment

The Organisation for Economic Co-operation and Development (OECD) provides guidelines for the safety assessment of chemicals, including plant extracts. Key guidelines relevant to dermal safety include:

- **OECD 404: Acute Dermal Irritation/Corrosion:** This test evaluates the irritant or corrosive potential of a substance on intact and abraded skin. The substance is applied to the skin of rabbits, and reactions are scored at specified intervals.
- **OECD 406: Skin Sensitisation (Guinea Pig Maximisation Test):** This test evaluates the potential of a substance to cause allergic contact dermatitis. Guinea pigs are exposed to the substance during an induction phase, and a challenge phase assesses the sensitization response.
- **OECD 402: Acute Dermal Toxicity:** This test evaluates the systemic toxicity of a substance following a single dermal application.
- **OECD 439: *In Vitro* Skin Irritation:** This test uses reconstructed human epidermis to assess skin irritation potential.

DISCUSSION AND SYNTHESIS

The evidence reviewed demonstrates that the difficulty in treating DFUs arises from the convergence of host-related, microbial and healthcare-system factors rather than infection alone. Neuropathy, vascular insufficiency, immune dysfunction and poor tissue regeneration establish a biological environment in which wounds form readily but heal poorly. Microbial colonization subsequently introduces additional barriers to healing, particularly when infection becomes polymicrobial and biofilm-associated.

Biofilms as a Bridge between Chronicity and Antimicrobial Resistance

Biofilm formation represents a central mechanism linking persistent infection with antimicrobial treatment failure. The extracellular polymeric matrix restricts antimicrobial penetration, while physiological heterogeneity within the biofilm produces microorganisms with varying metabolic states and antimicrobial susceptibility. Slowly growing organisms and persister cells may survive antimicrobial concentrations that would eradicate planktonic organisms. Following discontinuation of therapy, surviving organisms can repopulate the wound and contribute to recurrent infection.

Biofilms additionally facilitate quorum-sensing communication and horizontal gene transfer. These mechanisms may enhance virulence and promote dissemination of antimicrobial-resistance determinants within polymicrobial communities. The hypoxic microenvironment within mature biofilms may also favour anaerobic organisms, further increasing microbial complexity. Thus, successful treatment cannot rely exclusively on systemic antimicrobials; wound debridement and other measures directed at disrupting the physical and biological structure of biofilms remain important.

Antimicrobial Resistance and the Need for Stewardship

Increasing antimicrobial resistance significantly narrows therapeutic options in DFIs. MRSA, ESBL-producing Enterobacterales, carbapenem-resistant organisms and resistant *P. aeruginosa* have all been identified as clinically important pathogens. The reviewed evidence indicates that biofilm production, polymicrobial infection, previous antibiotic exposure and prolonged ulcer duration are important predictors of multidrug-resistant infection.

These findings support a stewardship-based approach rather than routine broad-spectrum antimicrobial administration. Antibiotics should not be prescribed simply because an ulcer is present. Clinically uninfected ulcers should not receive systemic or topical antibiotics solely to prevent infection or accelerate healing. When infection is present, appropriate deep-tissue sampling and antimicrobial susceptibility testing permit more rational selection of therapy. Empirical treatment should reflect local microbiological patterns, followed by de-escalation once microbiological results become available.

Nevertheless, effective stewardship is more difficult in resource-constrained settings where rapid diagnostic tests, microbiology laboratories and antimicrobial susceptibility testing may be unavailable. Delayed results encourage prolonged empirical treatment, while limited multidisciplinary services further compromise wound management. Strengthening diagnostic capacity is therefore integral to antimicrobial stewardship in DFUs.

Why Medicinal Plants Merit Investigation

The increasing limitations of conventional antimicrobials provide a scientific rationale for investigating medicinal plants, but plant-derived therapies should be regarded as potential adjuncts rather than substitutes for evidence-based DFU care. Their principal attraction lies in their chemical diversity and multi-target mechanisms.

Unlike conventional antimicrobials that often act against a limited molecular target, crude or standardized plant preparations may contain numerous biologically active molecules capable of simultaneously influencing microbial membranes, efflux systems, enzymes, oxidative stress,

inflammation, quorum sensing and biofilm development. This multimodal activity could theoretically reduce bacterial persistence while simultaneously improving the wound microenvironment.

However, such complexity also presents a major methodological challenge. Extract composition may vary according to plant source, geographical location, plant part, harvesting conditions, extraction solvent, temperature, storage conditions and formulation. Consequently, apparently contradictory experimental findings may reflect differences in preparation rather than genuine biological inconsistency.

Therapeutic Potential of *Azadirachta indica*

Neem possesses several characteristics relevant to infected diabetic wounds. Its limonoids, flavonoids and phenolic compounds demonstrate antimicrobial, antioxidant and anti-inflammatory activity. Proposed antimicrobial mechanisms include disruption of microbial membranes, interference with cell-wall synthesis, inhibition of efflux pumps, reduction of bacterial adherence and attenuation of quorum-sensing mechanisms.

Particularly important is the reported ability of neem preparations to inhibit initial biofilm formation and disrupt established biofilms. Interference with extracellular polymeric substance production, bacterial adhesion and quorum sensing suggests that neem may have utility against microbial phenotypes that are poorly responsive to conventional antibiotics.

Neem also demonstrates biological effects that extend beyond antimicrobial activity. Its reported antioxidant and anti-inflammatory properties may reduce excessive oxidative and inflammatory damage, while enhancement of granulation tissue, collagen formation and angiogenesis could favour tissue repair. These combined activities make neem biologically attractive as a potential topical adjunct for chronic diabetic wounds.

However, available evidence does not yet establish neem as an approved treatment for DFUs. Topical application to compromised skin raises concerns regarding irritation, sensitization, cytotoxicity and systemic absorption. Standardized safety evaluation and clinical trials are required before routine therapeutic use can be recommended.

Therapeutic Potential of *Moringa oleifera*

Moringa oleifera similarly contains numerous compounds of potential relevance to chronic wound treatment, including isothiocyanates, quercetin, kaempferol, phenolic acids and glucosinolates. Reported antimicrobial activity includes effects against *S. aureus*, *P. aeruginosa*, *E. coli*, *K. pneumoniae* and fungal organisms.

The antibiofilm properties of moringa are particularly relevant to DFUs. Moringa extracts have been reported to interfere with quorum sensing, bacterial adhesion and extracellular polymeric substance production and to reduce established biofilm biomass. Its phytochemicals may additionally disrupt microbial membranes, inhibit enzymes and interfere with nucleic-acid-related processes.

Moringa also appears to influence several pathways involved in tissue repair. Anti-inflammatory and antioxidant activity may reduce persistent wound inflammation and oxidative injury, while effects on fibroblast activity, collagen deposition, granulation tissue and angiogenic pathways may promote progression from inflammatory to proliferative phases of healing.

Despite this potential, evidence for topical use specifically in human DFUs remains insufficient. Formulation, effective concentration, dosing frequency, duration of therapy and interaction with conventional wound-care products remain inadequately defined.

Safety as a Prerequisite for Translation

An important conclusion from the reviewed evidence is that biological activity cannot be equated with clinical safety. The assumption that naturally derived products are inherently safe is scientifically inappropriate. Extracts intended for topical application to diabetic wounds must undergo systematic evaluation for irritation, sensitization, cytotoxicity, phototoxicity and potential systemic toxicity.

This consideration is especially important in diabetes because the skin and underlying tissues may already be ischemic, inflamed and immunologically compromised. A preparation that demonstrates strong antimicrobial activity but damages keratinocytes or fibroblasts could ultimately delay wound healing.

Consequently, therapeutic development should progress from phytochemical standardization and in-vitro antimicrobial testing to antibiofilm assays, cytotoxicity testing, appropriate preclinical wound models and eventually controlled human studies.

Integrating Plant-Based Therapies With Antimicrobial Stewardship

The potential role of neem and moringa is best conceptualized within an integrated DFU-management framework. Standard treatment—including debridement, pressure offloading, revascularization where necessary, glycaemic optimization, microbiologically guided antimicrobial therapy and multidisciplinary wound care—remains fundamental.

Plant-derived interventions may eventually complement these measures by reducing microbial burden, inhibiting biofilm formation, promoting wound repair or enhancing the

activity of conventional antimicrobials. Demonstration of synergy with antibiotics would be particularly valuable because an adjunct capable of lowering required antibiotic exposure could potentially contribute to antimicrobial stewardship.

However, claims of antimicrobial synergy require rigorous experimental confirmation using standardized methodologies such as minimum inhibitory concentration testing, fractional inhibitory concentration indices, time-kill assays and biofilm-specific models before clinical recommendations can be made.

3. CONCLUSION

Diabetic foot ulcers remain a major complication of diabetes and are characterized by impaired healing, recurrent infection, substantial morbidity, high rates of amputation and considerable healthcare costs. Their treatment is increasingly complicated by polymicrobial infection, microbial biofilms and the emergence of multidrug-resistant pathogens. Biofilms contribute substantially to persistence by restricting antimicrobial penetration, facilitating microbial tolerance and resistance, promoting horizontal gene transfer and enabling recurrent infection.

Antimicrobial stewardship is therefore central to contemporary DFU management. Appropriate microbiological sampling, susceptibility-guided therapy, avoidance of antibiotics for clinically uninfected ulcers, de-escalation from broad-spectrum agents and rational treatment duration are essential for preserving antimicrobial effectiveness. These measures must operate alongside debridement, offloading, vascular assessment and intervention, glycaemic optimization and multidisciplinary wound care.

The reviewed evidence suggests that *Azadirachta indica* and *Moringa oleifera* possess biologically plausible therapeutic properties relevant to DFUs. Their phytochemicals demonstrate antimicrobial and antibiofilm effects through mechanisms including membrane disruption, inhibition of adhesion and quorum sensing, interference with extracellular polymeric substance production and modulation of microbial enzymes. Their antioxidant, anti-inflammatory and wound-healing properties may provide additional benefits within the metabolically compromised diabetic wound environment.

Nevertheless, most evidence supporting these medicinal plants remains preclinical or derived from in-vitro investigations, and direct evidence from well-designed clinical trials in patients with diabetic foot ulcers is limited. Important unresolved issues include variability in phytochemical composition, absence of standardized extraction and formulation procedures, uncertain optimal concentrations, incomplete dermal safety data and insufficient knowledge of interactions with conventional antimicrobial and wound-care therapies.

Future research should therefore focus on phytochemical standardization, dose-response studies, antimicrobial and antibiofilm mechanisms against clinically relevant multidrug-resistant DFU isolates, cytotoxicity and dermal safety assessment, pharmaceutical formulation development, antibiotic-plant synergy studies and appropriately powered randomized clinical trials. Studies conducted in regions with a high burden of antimicrobial resistance, particularly sub-Saharan Africa, would be especially valuable.

Accordingly, neem and moringa should presently be regarded as promising candidates for development as adjunctive therapies rather than established replacements for conventional DFU treatment. Their successful translation into clinical practice will depend on rigorous demonstration of efficacy, safety, reproducibility and added benefit within an antimicrobial-stewardship framework.

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