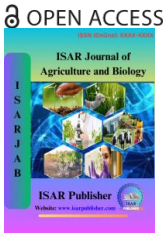
OPEN ACCESS



ISAR Journal of Agriculture and Biology

Volume 4, Issue 10, 2026 | Page: 1-19

Abbriviate Title- ISAR J Agri Biol

ISSN (Online)- 2584-217X

https://isarpublisher.com/journal/isarjab

Venous Leg Ulcers and Antimicrobial Resistance: Biofilms, Stewardship, and Therapeutic Potential of *Carica papaya* and *Vernonia amygdalina* in Wound Management Strategies

Edosa B. O[1]*, Mbachu A. E[2], Monye, J. E[3], Ezeuko C. M[4], Ofoma E. O[5]

Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka.

***Corresponding Author**
Edosa B. O

Article History
Received: 28.07.2026
Accepted: 13.09.2026
Published: 04.10.2026

Abstract: Venous leg ulcers (VLUs) are chronic, recurrent wounds that arise primarily from chronic venous insufficiency and sustained venous hypertension. They represent a major cause of morbidity, reduced quality of life, prolonged healthcare utilisation and substantial economic burden. Their management is complicated by impaired wound healing, recurrent infection, polymicrobial colonisation, biofilm formation and the increasing prevalence of antimicrobial-resistant organisms. Frequently implicated pathogens include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus* species and anaerobic organisms, many of which exhibit multidrug resistance and biofilm-associated tolerance. These challenges have intensified interest in antimicrobial stewardship and alternative or complementary wound-care strategies. This review examines the pathophysiology and microbiology of VLUs, the contribution of antimicrobial resistance to treatment failure, and the potential role of medicinal plants, particularly *Carica papaya* L. and *Vernonia amygdalina* Delile, in wound management. Both plants contain diverse bioactive compounds with antimicrobial, anti-inflammatory, antioxidant and wound-healing properties. *C. papaya* additionally contains proteolytic enzymes such as papain and chymopapain that may support wound debridement, while *V. amygdalina* is rich in sesquiterpene lactones, flavonoids and phenolic compounds with broad pharmacological activity. Available evidence suggests that these plants may promote wound closure, reduce microbial burden, limit oxidative stress and modulate inflammation. However, much of the current evidence is preclinical, and considerable variation exists in extraction methods, formulations, doses and study outcomes. Standardised phytochemical characterisation, antimicrobial and antibiofilm testing, toxicological evaluation, formulation development and well-designed clinical trials are therefore required before routine use can be recommended. Medicinal-plant-based wound products may ultimately provide affordable adjuncts to conventional VLU management while supporting antimicrobial-stewardship objectives.

Keywords: Venous leg ulcers; antimicrobial resistance; biofilm; antimicrobial stewardship; *Carica papaya*; *Vernonia amygdalina.*

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

Cite this article:

Edosa B. O., Mbachu A. E., Monye, J. E., Ezeuko C. M., Ofoma E. O., (2026). Venous Leg Ulcers and Antimicrobial Resistance: Biofilms, Stewardship, and Therapeutic Potential of *Carica papaya* and *Vernonia amygdalina* in Wound Management Strategies. *ISAR Journal of Agriculture and Biology*, *4*(10), 1-19.

Introduction

Venous leg ulcers are chronic wounds of the lower extremities that develop mainly as a consequence of chronic venous insufficiency and sustained venous hypertension. They commonly occur in the gaiter region of the lower leg and are frequently associated with oedema, haemosiderin deposition, lipodermatosclerosis, venous eczema and other manifestations of chronic venous disease. VLUs are characterised by prolonged healing, frequent recurrence and a substantial negative effect on physical, psychological and socioeconomic wellbeing.

Globally, VLUs constitute a major component of chronic lower-limb ulceration and account for a large proportion of leg ulcers encountered in clinical practice. Their prevalence increases with advancing age, and risk factors include obesity, female sex, prolonged standing or sitting, pregnancy, family history of venous disease and previous deep-vein thrombosis. Despite appropriate treatment, a significant proportion of VLUs remain unhealed for prolonged periods, while recurrence after wound closure is common.

The pathophysiology of VLU is driven by venous hypertension resulting from venous-valve incompetence, impaired calf-muscle pump function and inadequate venous return. Persistent venous hypertension increases capillary pressure and promotes extravasation of plasma proteins and red blood cells, tissue oedema, fibrin deposition and chronic inflammation. Leucocyte trapping and activation further contribute to endothelial injury through release of reactive oxygen species, proteolytic enzymes and inflammatory cytokines. Once ulceration occurs, wound repair is impaired by sustained inflammation, excessive matrix metalloproteinase activity, oxidative stress, reduced growth-factor activity and defective angiogenesis.

Microbial colonisation and infection further complicate VLU management. Chronic wounds provide a favourable environment for diverse bacterial communities, including Gram-positive, Gram-negative and anaerobic organisms. Common isolates include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus* species and *Proteus mirabilis*. These microorganisms often coexist within polymicrobial biofilms, where their tolerance to antimicrobial therapy and host immune mechanisms is markedly increased.

The emergence of antimicrobial-resistant wound pathogens poses an additional challenge. High rates of methicillin resistance, extended-spectrum beta-lactamase production, aminoglycoside resistance and carbapenem resistance have been documented among common wound pathogens. Biofilm-associated tolerance, inappropriate antibiotic use, poor wound care and healthcare-associated transmission further accelerate antimicrobial resistance and reduce the effectiveness of conventional treatment.

These challenges have strengthened the role of antimicrobial stewardship in wound management. Appropriate antimicrobial use, correct diagnosis of infection, rational selection of therapeutic agents and avoidance of unnecessary antibiotic exposure are essential to preserve antimicrobial effectiveness. There is therefore increasing interest in alternative and adjunctive wound-care agents, particularly medicinal plants with antimicrobial, anti-inflammatory, antioxidant and tissue-repair properties.

Among such plants, *Carica papaya* L. and *Vernonia amygdalina* Delile are of particular interest. Both have long-standing applications in traditional medicine and contain multiple phytochemical constituents with potential relevance to wound healing. *C. papaya* contains papain, chymopapain, flavonoids, phenolic acids, alkaloids and carotenoids, while *V. amygdalina* contains sesquiterpene lactones, flavonoids, tannins, alkaloids, phenolic acids and other bioactive compounds.

This review therefore examines the burden, pathophysiology, microbiology and antimicrobial-resistance challenges associated with venous leg ulcers and synthesises available evidence on the potential therapeutic roles of *Carica papaya* and *Vernonia amygdalina* in wound management, particularly as possible adjuncts to antimicrobial-stewardship-oriented care.

2.1 Venous Leg Ulcers

2.1.1 Definition and Clinical Presentation

Venous leg ulcers (VLUs) are defined as chronic, recurring open wounds on the lower leg, typically located between the ankle and the knee, which occur as a consequence of impaired blood flow through damaged or diseased leg veins, leading to the breakdown of the skin and poor healing (Raffetto, 2018). These ulcers represent the most common form of chronic lower extremity wounds and are the end-stage manifestation of chronic venous insufficiency (CVI) (Sen, 2021). Clinically, VLUs are typically irregularly shaped, shallow wounds with sloping edges, located predominantly in the gaiter area of the leg (the medial malleolar region). The surrounding skin often demonstrates signs of chronic venous disease, including haemosiderin deposition (resulting in a characteristic reddish-brown discolouration), lipodermatosclerosis (fibrosis and induration of the subcutaneous tissue), atrophie blanche (white, atrophic scar-like areas), and venous eczema (Raffetto, 2018; Sen, 2021).

Chronic venous ulcers of the lower limbs are defined as wounds that develop in the context of advanced venous disease, with a slow healing rate and no tendency to heal over 3 months despite adequate treatment or not fully healed

at 12 months (Kahle *et al*., 2011; Stanek *et al*., 2023). They have a significant impact on the patient's quality of life, being associated with depression and worrisome suicide rates, as well as an economic burden due to increased medical care costs (dressings, hospitalisation, and others) (Kahle *et al*., 2011; Weller *et al*., 2021; Stanek *et al*., 2023). Moreover, they imply an epidemiologic risk; in the context of recurrent hospitalisations and long-term antibiotic treatment, a worrisome issue arises: healthcare associated infections and the emergence of strains resistant to multiple classes of antibiotics and/or antiseptics.

2. 1.2 Global Prevalence and Incidence of Venous Leg Ulcers

Venous leg ulcers represent a major public health challenge globally (Probst *et al*., 2023). They manifest on the lower limb and represent between 60% and 80% of all leg ulcerations (Sen, 2021). Most chronic wounds of the lower limbs are of venous etiology (70%–90%) (Kahle *et al*., 2011; Stanek *et al*., 2023). The prevalence and incidence of VLUs vary considerably across studies due to disparities in study designs, measurement methods, and population characteristics (Probst *et al*., 2023).

A systematic review and meta-analysis by Probst *et al*. (2023) synthesised data from 14 studies and reported a pooled prevalence of 0.32% and a pooled incidence of 0.17%. However, the authors noted extreme heterogeneity across effect sizes for both prevalence and incidence, which prevented meaningful interpretation of pooled indexes and argued for further studies with specific prevalence-type reporting and target population under study. Other estimates suggest that the prevalence of VLUs is around 1.08%, with an incidence being up to 1.33%. The latter numbers are primarily based on estimates because of the lack of clinical registries for VLU. The prevalence of VLUs varies between 1.5–3% in the total population and 4–5% in persons over the age of 80. A total of 1% of the Western population will suffer at some time in their lives from a VLU. Venous ulcer is usually localised on the inner side of the lower third of the leg, oval, circular or irregular in shape.

2.1.3 Demographic and Risk Factors of Venous Leg Ulcers

The primary risk factors for the development of VLUs include advancing age, obesity, female sex, family history of venous disease, prolonged standing or sitting, pregnancy, and a history of deep vein thrombosis (Raffetto, 2018). Age negatively affects healing and recurrence as well as treatment adherence (Sen, 2021). People with VLUs often report having reduced health-related quality of life because these wounds can be painful, malodorous, and exuding (Raffetto, 2018).

The development of CVI likely has several contributory factors, including a hereditary component and many risk factors (Krizanova *et al*., 2023). According to the Clinical, Etiological, Anatomical, and Pathophysiological (CEAP) classification, reliable and reproducible grading of the severity of venous insufficiency enables one to choose the proper therapy.

2.1.4 Economic Burden of Venous Leg Ulcers

VLUs represent a considerable social and economic burden, with an estimated annual cost of £102 million sterling in the UK, $32 billion in the United States, and AUD$3 billion in Australia (Sen, 2021). Despite this substantial economic impact, there is no international systematic collation and review of existing prevalence and incidence studies (Probst *et al*., 2023). Such information on the epidemiology of VLUs is necessary to inform decision-making by health services to establish best strategies for prevention and management (Probst *et al*., 2023).

2.1.5 Healing Rates and Recurrence

Despite proper care, up to 20% of VLUs do not heal after 2 years (Raffetto, 2018). The three-month healing rate is estimated at approximately 40%, and once healed, up to 80% of patients develop a recurrence within 3 months (Sen, 2021). These poor healing outcomes and high recurrence rates underscore the chronicity of the condition and the inadequacy of current therapeutic approaches (Raffetto, 2018).

2.2 Pathophysiology of Venous Leg Ulcers

2.2.1 Chronic Venous Insufficiency and Venous Hypertension

The pathophysiology of VLUs is rooted in chronic venous insufficiency, which leads to sustained venous hypertension in the lower extremities (Raffetto, 2018). The chronic venous disease covers a wide spectrum of venous disorders that are characterised by severely impaired blood return that primarily affects veins in the lower extremities (Krizanova *et al*., 2023). Morphological and functional abnormalities of the venous system led to chronic venous insufficiency (CVI), and present as leg heaviness/achiness, oedema, telangiectasia, and varices (Krizanova *et al*., 2023). The term 'chronic venous insufficiency' (CVI) refers to a disease of greater severity (Krizanova *et al*., 2023).

Venous dysfunction is associated with venous hypertension and is associated with venous reflux due to poorly functioning or incompetent venous valves, which ultimately reduces venous return, leading to a cascade of morphological, physiological, and histologic abnormalities such as blood pooling, hypoxia, inflammation, swelling, skin changes (lipodermatosclerosis), and in severe cases, venous leg ulcers (VLU) (Krizanova *et al*., 2023). Venous

hypertension results from incompetence of the venous valves and dysfunction of the calf muscle pump, which normally facilitates venous return against gravity (Raffetto, 2018). When these mechanisms fail, blood pools in the lower extremity veins, leading to increased capillary hydrostatic pressure (Raffetto, 2018; Sen, 2021).

2.2.2 The Fibrin Cuff Theory and Leucocyte Trapping

The sustained venous hypertension triggers a cascade of pathological events (Raffetto, 2018). The fibrin cuff theory, first proposed by Browse and Burnand in 1982, suggests that increased capillary permeability leads to the extravasation of fibrinogen and other large molecules into the interstitial space (Raffetto, 2018). These molecules polymerise to form a pericapillary fibrin cuff, which acts as a diffusion barrier, impairing oxygen and nutrient delivery to tissues and hindering waste product removal (Raffetto, 2018).

The leucocyte trapping theory provides an additional mechanistic explanation (Sen, 2021). Venous hypertension causes leucocytes (particularly neutrophils and monocytes) to become trapped in the microcirculation, where they become activated and release proteolytic enzymes, reactive oxygen species, and pro-inflammatory cytokines (Sen, 2021). This inflammatory cascade contributes to tissue damage, endothelial dysfunction, and the characteristic skin changes observed in chronic venous disease (Raffetto, 2018; Sen, 2021).

2.2.3 Haemosiderin Deposition and Lipodermatosclerosis

The extravasation of red blood cells from the compromised microcirculation leads to their degradation and the deposition of haemosiderin, an iron-containing pigment, in the dermis (Raffetto, 2018). This produces the characteristic reddish-brown discolouration of the skin. Chronic inflammation and fibrosis of the subcutaneous tissue result in lipodermatosclerosis, a condition characterised by induration, hardening, and a woody texture of the skin and subcutaneous tissues (Raffetto, 2018). These pathological changes ultimately compromise tissue viability and predispose to ulceration (Raffetto, 2018; Sen, 2021).

2.2.4 The Wound Healing Impairment

Once an ulcer develops, the wound healing process is severely impaired in the venous insufficiency environment (Sen, 2021). The normal healing cascade—comprising haemostasis, inflammation, proliferation, and remodelling—is disrupted (Sen, 2021). Chronic venous ulcer wounds are characterised by an exaggerated and prolonged inflammatory response, elevated levels of matrix metalloproteinases (MMPs) that degrade the extracellular matrix, reduced levels of growth factors, and impaired angiogenesis (Sen, 2021). Chronic wounds are also characterised by an alkaline pH, higher concentrations of MMPs, high levels of proinflammatory cytokines, proteases, and reactive oxygen species (ROS) (Sen, 2021).

2.3 The Microbiology of Venous Leg Ulcers

2.3.1 The Wound Microbiome

The wound bed is a convenient environment for microbial adherence, colonisation and infection, with the initiation of a complex host–microbiome interplay (Malone *et al*., 2017). Chronic wounds encompass a myriad of lesions, including venous and arterial leg ulcers, diabetic foot ulcers (DFUs), pressure ulcers, non-healing surgical wounds and others (Malone *et al*., 2017). Despite the etiological differences, chronic wounds share several features at a molecular level, including susceptibility to microbial colonisation and biofilm formation (Malone *et al*., 2017; Bjarnsholt *et al*., 2018).

The bacterial microbiome of the skin and chronic wounds is well-defined and largely depends on the environment of the skin site (Bjarnsholt *et al*., 2018). For example, *Cutibacterium* spp. is more abundant in sebaceous sites, while genera such as *Staphylococcus* and *Corynebacterium* spp. dominate more moist sites such as the feet and the inside of knee and elbow creases (Bjarnsholt *et al*., 2018).

2.3.2 Common Pathogens in Venous Leg Ulcers

The microbial ecology of venous leg ulcers is complex and polymicrobial (Ibiebele *et al*., 2018). Results from bacterial tag-encoded FLX and titanium amplicon pyrosequencing and metagenomic approaches show that VLU infections are polymicrobial with no single bacterium colonising the wounds. The most ubiquitous and predominant organisms include a previously uncharacterised bacteroidales, various anaerobes, *Staphylococcus*, *Corynebacterium*, and *Serratia*. Systematic reviews have demonstrated that the most frequently isolated organisms from VLU wound beds include:

Gram-positive bacteria: *Staphylococcus aureus* (including methicillin-resistant strains, MRSA), *Streptococcus pyogenes*, *Enterococcus* spp., and *Peptostreptococcus* spp. (Ibiebele *et al*., 2018). Studies have shown that the share of *Staphylococcus* spp. and *Enterococcus* spp. in ulcers can be as high as 60%.

Gram-negative bacteria: *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, and *Klebsiella* spp. (Ibiebele *et al*., 2018).

Anaerobic bacteria: *Bacteroides fragilis* and *Peptostreptococcus* spp. (Ibiebele *et al*., 2018).

A systematic review and meta-analysis of antimicrobial resistance in bacterial wound, skin, soft tissue and surgical site infections in Africa by Monk *et al*. (2024) found that *S. aureus* was isolated in 29% (95% CI 25% to 34%) of

samples, *E. coli* in 14% (CI 11% to 18%), *Klebsiella pneumoniae* in 11% (CI 8% to 13%), *Pseudomonas aeruginosa* in 14% (CI 11% to 18%), and *Acinetobacter baumannii* in 8% (CI 5% to 12%). These findings are consistent with the spectrum of pathogens implicated in chronic wound infections globally (Kwon and Armstrong, 2018).

2.3.3 The Role of Biofilms

Critically, the majority of chronic wounds harbour biofilms—structured microbial communities encased in a self-produced extracellular polymeric substance (EPS) (Malone *et al*., 2017). Bacteria typically exist in wounds as part of a biofilm, which is often polymicrobial in nature, alongside bacteria and fungi that are described as being more virulent and tolerant towards antimicrobials and antiseptics (Malone *et al*., 2017; Bjarnsholt *et al*., 2018). Biofilms are present in approximately 60–100% of chronic wounds, compared to only 6% of acute wounds (Malone *et al*., 2017).

Venous leg ulcers (VLUs) represent one of the most prevalent types of chronic wounds characterised by perturbed microbiome and biofilm-forming bacteria. High extracellular matrix binding ability of VLU isolates was associated with antimicrobial resistance and expression levels of the *embp* and *sdrG*, responsible for bacterial binding to fibrinogen and fibrin, respectively. Growing evidence indicates that biofilm formers are present in chronic wounds and that these biofilm formers promote the emergence of multi-drug antibiotic resistant (MDR) strains and therapeutic rejection (Bjarnsholt *et al*., 2018).

Biofilm-embedded bacteria exhibit up to 1,000-fold increased tolerance to antimicrobial agents and host immune defences compared to their planktonic counterparts (Malone *et al*., 2017; Bjarnsholt *et al*., 2018). The mechanisms of biofilm-associated tolerance include (Malone *et al*., 2017; Bjarnsholt *et al*., 2018):

1. **Physical barrier**: The EPS matrix acts as a diffusion barrier, slowing the penetration of antimicrobial agents (Malone *et al*., 2017).
2. **Physiological heterogeneity**: The presence of metabolically inactive persister cells within the biofilm that are less susceptible to antibiotics targeting actively dividing cells (Malone *et al*., 2017).
3. **Horizontal gene transfer**: Biofilms facilitate the exchange of resistance genes among bacterial species (Bjarnsholt *et al*., 2018).
4. **Quorum sensing**: Cell-to-cell communication regulates the expression of virulence factors and biofilm maintenance (Bjarnsholt *et al*., 2018).

The biofilm mode of growth constitutes a formidable barrier to wound resolution and is a primary reason for the failure of conventional antimicrobial therapy in chronic wounds (Malone *et al*., 2017; Bjarnsholt *et al*., 2018).

2.3.4 Polymicrobial Interactions

Chronic wound infections with mono- or poly-microbial biofilms are frequent, and their management is challenging due to tolerance and resistance to antimicrobial therapy and to the host's immune defence mechanisms (Bjarnsholt *et al*., 2018). Polymicrobial interactions within chronic wounds can be synergistic, with one species facilitating the colonisation or virulence of another (Bjarnsholt *et al*., 2018). For example, *P. aeruginosa* can produce an environment that promotes the growth of anaerobic bacteria, while *S. aureus* and *P. aeruginosa* can form mixed biofilms that are more resistant to treatment than single-species biofilms (Bjarnsholt *et al*., 2018).

Recent studies have also highlighted the role of fungi within chronic wound environments, emphasising the importance of interkingdom interactions in wound management (Bjarnsholt *et al*., 2018). Despite advancing knowledge in polymicrobial biofilm wound infections with respect to bacteria, the role of fungi is largely ignored, and their influence in chronicity and clinical management is not fully appreciated or understood (Bjarnsholt *et al*., 2018).

Table 1: Common Pathogens Isolated from Venous Leg Ulcers

Bacterial Species	Gram Reaction	Prevalence in VLUs	Key Virulence Factors	Antimicrobial Resistance Concerns
Staphylococcus aureus	Gram-positive	29% (Monk *et al*., 2024)	Biofilm formation, toxins, adhesins	MRSA (>40% resistant)
Pseudomonas aeruginosa	Gram-negative	14% (Monk *et al*., 2024)	Biofilm, endotoxin, proteases	Carbapenem resistance (≥20%)
Escherichia coli	Gram-negative	14% (Monk *et al*., 2024)	Endotoxin, adhesins	Amoxicillin-clavulanic acid (≥80%)
Klebsiella pneumoniae	Gram-negative	11% (Monk *et al*., 2024)	Capsule, endotoxin	ESBL production, carbapenem resistance
Acinetobacter baumannii	Gram-negative	8% (Monk *et al*., 2024)	Biofilm, LPS	Carbapenem resistance (≥20%)
Streptococcus pyogenes	Gram-positive	Variable	M protein, exotoxins	Macrolide resistance
Enterococcus spp.	Gram-positive	60%	Biofilm, cytolysin	Vancomycin resistance (VRE)
Bacteroides fragilis	Gram-negative (anaerobic)	Variable	Capsule, enterotoxin	Metronidazole resistance
Peptostreptococcus spp.	Gram-positive (anaerobic)	Variable	Biofilm	Beta-lactam resistance

Source: (Kwon and Armstrong, 2018; Ibiebele *et al*., 2018)

2.4 Antimicrobial Resistance in Wound Pathogens

2.4.1 The Global Antimicrobial Resistance Crisis

Antimicrobial resistance (AMR) is a major global threat and AMR-attributable mortality is particularly high in Central, Eastern, Southern and Western Africa (Mestrovic *et al*., 2023; Tang *et al*., 2023). The World Health Organization (WHO) states that AMR happens when bacteria, viruses, fungi, and parasites change their genetic makeup to resist common therapies (Tang *et al*., 2023). This adaptation increases the likelihood of disease transmission, serious illness, and death and makes infections more difficult to treat (Mestrovic *et al*., 2023; Tang *et al*., 2023). AMR has, therefore, become a pressing issue in global health and development, necessitating urgent, multi-sectoral actions to meet Sustainable Development Goals (Mestrovic *et al*., 2023).

2.4.2 Antibiotic Resistance in Wound Pathogens

The relentless rise of AMR among wound pathogens has severely compromised therapeutic outcomes (Ibiebele *et al*., 2018; Monk *et al*., 2024). A systematic review and meta-analysis of AMR in bacterial wound infections in Africa by Monk *et al*. (2024) found alarmingly high resistance rates:

- **Methicillin-resistant *Staphylococcus aureus* (MRSA)**: *S. aureus* was resistant to methicillin (MRSA) in >40% of isolates.
- **Beta-lactam resistance**: *E. coli* and *K. pneumoniae* were both resistant to amoxicillin-clavulanic acid in ≥80% of isolates.
- **Aminoglycoside resistance**: *E. coli* and *K. pneumoniae* were resistant to aminoglycosides in 51% and 38% of isolates, respectively.
- **Carbapenem resistance**: *P. aeruginosa* and *A. baumannii* were both resistant to anti-pseudomonal carbapenems (imipenem or meropenem) in ≥20% of isolates.

Methicillin-resistant *S. aureus* (MRSA) was found in 52.2% of isolates in skin and soft tissue infections. Among Gram-negative bacteria, 15.3% were multidrug-resistant (MDR), but all remained susceptible to colistin.

Ibiebele *et al*. (2018) reported that the pooled prevalence of MRSA in venous leg ulcers was 20.5%, while multidrug-resistant (MDR) *P. aeruginosa* and extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae were increasingly documented (Ibiebele *et al*., 2018).

This systematic review found that a large proportion of the organisms isolated from infected wounds, SSTI and SSI in Africa displayed resistance patterns of World Health Organisation (WHO) priority pathogens for critical or urgent antimicrobial development.

2.4.3 Drivers of Antimicrobial Resistance

Several factors contribute to the escalating prevalence of MDR bacteria in VLUs (Muteeb *et al*., 2023; Salam *et al*., 2023):

1. **Antibiotic misuse and overuse**: The inappropriate use of antimicrobial drugs to treat diseases has accelerated the emergence of resistant strains (Muteeb *et al*., 2023; Salam *et al*., 2023).
2. **Biofilm-associated tolerance**: As discussed above, biofilm-embedded bacteria exhibit enhanced tolerance to antimicrobial agents (Malone *et al*., 2017; Bjarnsholt *et al*., 2018).
3. **Inadequate wound care**: Poor wound management practices, including insufficient debridement and the use of suboptimal antimicrobial agents, contribute to persistent infection and resistance (Muteeb *et al*., 2023).
4. **Healthcare-associated transmission**: Hospital and healthcare facility environments facilitate the transmission of resistant organisms between patients (Muteeb *et al*., 2023).

2.4.4 Implications for Wound Management

The escalating prevalence of MDR bacteria in VLUs threatens to render current antibiotic regimens ineffective, leaving patients vulnerable to wound deterioration, cellulitis, osteomyelitis, and sepsis (Ibiebele *et al*., 2018; Monk *et al*., 2024). This scenario demands the exploration of alternative, effective, and affordable antimicrobial and wound-healing agents, particularly from natural sources (Salam *et al*., 2023). The WHO has identified priority pathogens for critical or urgent antimicrobial development, and many wound pathogens, including MRSA, *P. aeruginosa*, and ESBL-producing Enterobacteriaceae, feature on this list (Tang *et al*., 2023).

Table 2: WHO Priority Pathogens Commonly Found in Wound Infections

Priority Level	Pathogen	Relevance to Wound Infections
Critical	*Acinetobacter baumannii* (carbapenem-resistant)	Common in hospital-acquired wound infections
Critical	*Pseudomonas aeruginosa* (carbapenem-resistant)	Frequent coloniser of chronic wounds
Critical	Enterobacteriaceae (ESBL-producing, carbapenem-resistant)	Increasingly isolated from wound infections
High	*Enterococcus faecium* (vancomycin-resistant)	Associated with polymicrobial wound infections
High	*Staphylococcus aureus* (methicillin-resistant, vancomycin-intermediate/resistant)	Most common wound pathogen
High	*Helicobacter pylori* (clarithromycin-resistant)	Less relevant to wound infections
High	*Campylobacter* spp. (fluoroquinolone-resistant)	Less relevant to wound infections
High	*Salmonella* spp. (fluoroquinolone-resistant)	Less relevant to wound infections
Medium	*Streptococcus pneumoniae* (penicillin-non-susceptible)	Occasional wound pathogen
Medium	*Haemophilus influenzae* (ampicillin-resistant)	Less relevant to wound infections
Medium	*Shigella* spp. (fluoroquinolone-resistant)	Less relevant to wound infections

Source: (Tang *et al*., 2023)

2.5 Antimicrobial Stewardship in Wound Care

2.5.1 Definition and Principles of Antimicrobial Stewardship

The solution to reducing and preventing further AMR is a multi-modal approach known as antimicrobial stewardship (AMS). This approach includes the following:

- Infection prevention
- Using antimicrobial treatments sparingly to preserve their future effectiveness
- Improving safety and quality of patient care

Antimicrobial Stewardship (AMS) is a coordinated approach to ensuring the appropriate use of antimicrobials (including antibiotics) to improve patient outcomes, and includes strategies such as raising awareness of antimicrobial resistance. The Five Rights of Antimicrobial Stewardship include:

1. Right Diagnosis and Care Plan
2. Right Antimicrobial and delivery system
3. Right Time to initiate antimicrobial treatment

2.5.2 Wound Care as an Opportunity for Antimicrobial Stewardship

Wound care represents a significant opportunity in combating AMR due to several factors. The use of conventional antibiotics and antimicrobial agents, such as silver in wound dressings, can contribute to bacterial resistance. Due to antimicrobial resistance, wound care professionals must support infection management while minimising risk for antimicrobial resistance, supporting AMS management of hard-to-heal and surgical wounds.

There is strong and growing evidence that antiseptics can be useful agents for reducing AMR, but they are under-used, particularly in the fields of wound care and surgical site management (Roberts *et al*., 2017). The International Wound Infection Institute's (IWII) Consensus Document details up-to-date best practice guidelines for wound infection management, including:

- Correct and timely identification of wound infection
- Antimicrobial resistance
- The importance of antimicrobial stewardship strategies

2.5.3 Considerations for Ideal Wound Dressings Aligned with AMS

A scoping review explored the published evidence identifying general considerations/criteria for an ideal post-surgical wound dressing in terms of overcoming potential wound healing challenges (including infection) while supporting AMS objectives. Key considerations include:

- Supporting infection management while minimising risk for antimicrobial resistance
- Appropriate selection of antimicrobial agents
- Avoiding overuse of antibiotics in wound care
- Using antiseptics judiciously

2.5.4 Challenges in Antimicrobial Stewardship in Wound Care

Clinical wound infection is an increasing problem in healthcare, with the potential for increasing the burden of antimicrobial resistance (AMR), if antimicrobials are overused to treat wound infection (Blackburn *et al*., 2024). A critical review of current best practice discussed skin integrity and wound infection prevention and outlined the guiding principles of antimicrobial resistance and antimicrobial stewardship for infection control (Blackburn *et al*., 2024).

Proper management of chronic wounds requires a comprehensive, multidisciplinary approach and a thorough understanding of antibiotic usage. To address this need, chronic wound treatment protocols have been developed and implemented in clinical settings, with the goal of discharging patients once their ulcers have been treated and closed (Răducu *et al*., 2024).

2.6 Medicinal Plants in Wound Management

2.6.1 Historical and Cultural Context

The use of medicinal plants for wound care has a long history across all human cultures (Hassan *et al*., 2021; Sofowora *et al*., 2013; Thakkar *et al*., 2020). Traditional medicine systems, including Ayurveda, Traditional Chinese Medicine, and African traditional medicine, have employed plant-based preparations for wound healing for millennia (Sofowora *et al*., 2013). The World Health Organization recognises the importance of traditional medicine in global health and has developed the WHO Global Traditional Medicine Strategy 2025–2034, which provides a forward-looking framework for countries to strengthen research, regulation, and integration of traditional medicine into health systems (WHO, 2025).

Over 80% of people are thought to use herbal products as nutraceuticals, alternative medicines, or dietary supplements (Hassan *et al*., 2021; Thakkar *et al*., 2020). Humanity uses up to 60,000 medicinal plant species worldwide (Tungmunnithum *et al*., 2018). The bioactive components of these plants are mainly responsible for antioxidant, antibacterial, antidiabetic, antihelmintic, anticancer, antiasthma, antiparasitic, and other actions (Tungmunnithum *et al*., 2018).

2.6.2 Mechanisms of Wound Healing by Medicinal Plants

Medicinal plants can promote wound healing through multiple mechanisms, including:

1. **Antimicrobial activity**: Inhibition of wound pathogens, including biofilm-forming bacteria (Tungmunnithum *et al*., 2018).
2. **Anti-inflammatory activity**: Modulation of the inflammatory response to prevent excessive or prolonged inflammation (Ugbogu *et al*., 2021).
3. **Antioxidant activity**: Scavenging of reactive oxygen species that cause tissue damage (Tungmunnithum *et al*., 2018).
4. **Stimulation of angiogenesis**: Promotion of new blood vessel formation to supply oxygen and nutrients to healing tissue (Tungmunnithum *et al*., 2018).
5. **Enhancement of collagen synthesis**: Promotion of extracellular matrix deposition and tissue remodelling (Tungmunnithum *et al*., 2018).
6. **Epithelialisation**: Promotion of re-epithelialisation to restore the skin barrier (Tungmunnithum *et al*., 2018).

2.6.3 Polyherbal Formulations

Combining phytotherapeutic agents with complementary mechanisms of action may yield synergistic combinations that reduce the required concentrations of each extract, minimise potential toxicity, and circumvent resistance mechanisms (Salam *et al*., 2023). A systematic review and meta-analysis of randomised controlled trials found that polyherbal formulations show therapeutic promise as adjuncts to standard wound care (Salam *et al*., 2023). Nanotechnology-based polyherbal formulations have also proven successful in promoting wound healing, representing a unique approach to improving wound care and development of healthy skin (Salam *et al*., 2023).

2.7 *Carica papaya* L.

2.7.1 Botanical Description and Distribution

Carica papaya L. (Caricaceae), commonly known as papaya or pawpaw, is a tropical fruit tree native to Central and South America but now widely cultivated in tropical and subtropical regions worldwide (Maisarah *et al*., 2013). *Carica papaya* L. is an economically important tropical herbaceous plant with numerous biological activities. The plant is a fast-growing, herbaceous tree that can reach heights of 5–10 metres (Maisarah *et al*., 2013). It has large, palmately lobed leaves, a hollow stem, and produces large, fleshy fruits that are consumed globally (Maisarah *et al*., 2013).

2.7.2 Ethnobotanical Uses

In traditional medicine, different parts of *C. papaya* L. (leaves, stem, roots, fruits and seeds) are utilised to treat various diseases. The leaves of *C. papaya* have a long history of medicinal use in traditional medicine systems (Sofowora *et al*., 2013; Ugbogu *et al*., 2021). They are used for the treatment of fever, pyrexia, diabetes, gonorrhoea, syphilis, inflammation, and as a dressing for foul wounds (Sofowora *et al*., 2013). In many tropical regions, papaya leaf preparations are used to promote wound healing, reduce inflammation, and treat various infectious conditions (Ugbogu *et al*., 2021). The latex of unripe papaya fruits has been used traditionally for wound debridement due to its proteolytic enzyme content (Sofowora *et al*., 2013).

2.7.3 Phytochemical Composition

Carica papaya leaves contain a rich array of bioactive phytochemicals (Maisarah *et al*., 2013). Phytochemical analysis of *Carica papaya* leaf extract shows all phytochemical constituents such as saponin, flavonoid, glycoside, alkaloid, carbohydrate, tannins except steroids. The plant possesses anticancer, anti-inflammatory, antimicrobial, and hypoglycaemic properties (Maisarah *et al*., 2013). Key phytochemical constituents include (Maisarah *et al*., 2013):

Alkaloids: Carpaine, pseudocarpaine, and dehydrocarpaine (Maisarah *et al*., 2013). Carpaine is the major alkaloid and has been reported to have cardiovascular effects (Maisarah *et al*., 2013).

Flavonoids: Quercetin, kaempferol, myricetin, and rutin (Maisarah *et al*., 2013). These compounds exhibit antioxidant, anti-inflammatory, and antimicrobial activities (Tungmunnithum *et al*., 2018).

Phenolic acids: Caffeic acid, ferulic acid, chlorogenic acid, and gallic acid (Maisarah *et al*., 2013). These compounds contribute to the antioxidant capacity of the leaves (Tungmunnithum *et al*., 2018).

Enzymes: Papain, chymopapain, and caricain (Maisarah *et al*., 2013). These proteolytic enzymes are responsible for the wound-debriding properties of papaya latex (Maisarah *et al*., 2013).

Carotenoids: Beta-carotene, lycopene, and cryptoxanthin (Maisarah *et al*., 2013).

Other compounds: Tannins, saponins, cardiac glycosides, and volatile oils (Maisarah *et al*., 2013).

The fruit and leaves of the *Carica papaya* Linn tree possess proteolytic enzymes (papain and chymopapain), various phytochemicals including flavonoids, and Vitamin C that confer proteolytic, antioxidant, anti-inflammatory, and antimicrobial properties, in addition to promoting collagen synthesis.

Gas chromatography-mass spectrometry (GC-MS) analysis of *C. papaya* leaf extract has revealed twenty constituents, dominated by oleic acid (28.98%) with molecular weight of 282, with the least compound being trans-geranylacetone (0.17%) with molecular weight of 194 (Maisarah *et al*., 2013). The fresh papaya leaves were found to have a higher percentage of alkaloids (3.9%) than the *Vernonia amygdalina* leaves (2.8%).

2.7.4 Nutritional Composition

Carica papaya possesses high vitamin A and C values and numerous bioactive compounds with known pharmacological activities (Okolie *et al*., 2023). In vitro and in vivo studies on *C. papaya* showed various pharmacological activities such as anti-cancer, anti-inflammatory, antimicrobial, antidiarrheal, wound healing, larvicidal, anxiolytic, neuroprotective, hepatoprotective, anti-diabetic, anti-hyperlipidemic, anti-obesity, antiulcer, antithrombocytopenic, anthelminthic and contraceptive activities (Okolie *et al*., 2023; Kumarasinghe *et al*., 2024). These pharmacological activities may be due to numerous bioactive compounds in different parts of *C. papaya* (Okolie *et al*., 2023; Shahrajabian, 2025).

2.7.5 Pharmacological Activities

2.7.5.1 Antimicrobial Activity

Preclinical investigations have demonstrated that *C. papaya* leaf extracts possess broad-spectrum antibacterial activity against *S. aureus*, *P. aeruginosa*, *E. coli*, and *Streptococcus* species (Maisarah *et al*., 2013). Methanol extracts have shown stronger activity against *S. aureus* than *E. coli* (Maisarah *et al*., 2013). The antimicrobial activity is attributed to the synergistic action of various phytochemicals, including flavonoids, alkaloids, and phenolic compounds (Maisarah *et al*., 2013; Tungmunnithum *et al*., 2018). *Carica papaya* possesses anticancer, anti-inflammatory, antimicrobial, and hypoglycaemic properties. It also contains the enzyme papain, which helps in treatment of traumas, allergies, and sports injuries by boosting digestive motility and transit time (Vij and Prashar, 2015).

2.7.5.2 Anti-inflammatory Activity

Studies have demonstrated significant anti-inflammatory effects of *C. papaya* leaf extracts. Tungmunnithum *et al*. (2018) reported that ethanolic leaf extract of *C. papaya* exhibited anti-inflammatory activity in carrageenan-induced paw oedema in rats. The anti-inflammatory effects are attributed to the inhibition of pro-inflammatory mediators and the antioxidant properties of the phytochemicals (Tungmunnithum *et al*., 2018).

2.7.5.3 Wound-Healing Activity

Several studies have investigated the wound-healing potential of *C. papaya* (Sule and Arhoghro, 2017). *Carica papaya* Linn contains flavonoids which are efficacious as anti-inflammatory or antiseptic in inhibiting bacterial growth. *Carica papaya* Linn is effective in repairing cells damaged by free radicals. Sule and Arhoghro (2017) demonstrated that *C. papaya* leaf extract accelerates wound closure, enhances collagen deposition, and promotes angiogenesis in rat excision models (Sule and Arhoghro, 2017). A systematic review and meta-analysis published in 2023 evaluated the effectiveness of conventional management and *C. papaya* on influencing wound healing, confirming the beneficial effect of papaya on reducing necrosis in wound management (Sule and Arhoghro, 2017).

Papaya leaf, fruit, and seed extracts have been compared for their potential in accelerating incision wound healing in male Wistar rats (Sule and Arhoghro, 2017). The extracts were found to be promising products for wound healing. *C. papaya* has been reported to exhibit wound healing and anti-inflammatory activities (Sule and Arhoghro, 2017).

Flavonoid, alkaloid, tannin, and saponin compounds in papaya leaves have antimicrobial and antioxidant properties to accelerate the re-epithelization and cell migration needed for wound healing. *Carica papaya* L. incorporated nanofibrous scaffold has been investigated as a potential wound dressing.

Carica papaya extract has been shown to have a significant effect ($p \leq 0.05$) toward the healing process of an incised oral wound in mice. Histologically at day 14, significant tissue regeneration was observed. Papaya leaf extract contains compounds which act as an anti-inflammatory, astringent substance, vasodilator, antioxidant, analgesic, with antifungal, and antibacterial properties, and increase the collagen synthesis.

2.7.5.4 Antioxidant Activity

The flavonoids and phenolic compounds in *C. papaya* leaves contribute to their antioxidant capacity, which helps neutralise free radicals and reduce oxidative stress in wounds (Maisarah *et al*., 2013; Tungmunnithum *et al*., 2018). Papaya is abundant in vitamins (A, C, and E), minerals, enzymes, and phytochemicals such as flavonoids, phenolic acids, carotenoids, and alkaloids (Maisarah *et al*., 2013). These compounds exhibit anti-aging, anti-inflammatory, and anticancer effects (Tungmunnithum *et al*., 2018).

2.7.5.5 Other Pharmacological Activities

C. papaya has been scientifically studied for various therapeutic activities, including antibacterial, antioxidant, antipyretic, insecticidal, antimicrobial, and anti-mollusk properties (Maisarah *et al*., 2013; Owoyele *et al*., 2008). The plant has been evaluated for applications in cancer, diabetes, wound healing, malaria, dengue fever, and other disease prevention and treatment (Maisarah *et al*., 2013).

Table 3: Bioactive Compounds and Pharmacological Activities of *Carica papaya* Leaves

Compound Class	Specific Compounds	Pharmacological Activities
Alkaloids	Carpaine, Pseudocarpaine, Dehydrocarpaine	Cardiovascular effects, antimicrobial
Flavonoids	Quercetin, Kaempferol, Myricetin, Rutin	Antioxidant, anti-inflammatory, antimicrobial
Phenolic Acids	Caffeic acid, Ferulic acid, Chlorogenic acid, Gallic acid	Antioxidant, anti-inflammatory
Enzymes	Papain, Chymopapain, Caricain	Proteolytic, wound debridement
Carotenoids	Beta-carotene, Lycopene, Cryptoxanthin	Antioxidant, pro-vitamin A
Tannins	Condensed and hydrolysable tannins	Astringent, antimicrobial
Saponins	Steroidal saponins	Antimicrobial, immunomodulatory
Glycosides	Cardiac glycosides	Cardiovascular effects

2.8 *Vernonia amygdalina*

2.8.1 Botanical Description and Distribution

Vernonia amygdalina Delile, commonly known as bitter leaf, is a perennial shrub belonging to the family Asteraceae (Compositae) (Farombi and Owoeye, 2011; Ugbogu *et al*., 2021). The plant typically grows to a height of 2.5–3 metres as a shrub, although some specimens have been documented to reach up to 7 metres (Farombi and Owoeye, 2011). The leaves are green, elliptic, and have a distinct odour and a harsh, bitter flavour (Akpoghelie *et al*., 2022). The bark is rough with deep black fissures (Akpoghelie *et al*., 2022).

V. amygdalina is widely distributed in tropical and subtropical regions of Africa and Asia (Ugbogu *et al*., 2021). There are more than 54 species known to possess similar morphological features with the characteristic bitter taste (Ugbogu *et al*., 2021). The plant goes by various African names: ewuro (Yoruba), etidot (Efik), ityuna (Tiv), Congo Bololo (D. R. Congo), oriwo (Edo), onugbu (Igbo), grawa (Amharic), shuwaka or chusar-doki (Hausa), Awɔnwono (Akan), mululuza (Luganda), olusia (Luo), and labwori (Ugbogu *et al*., 2021).

2.8.2 Ethnobotanical Uses

V. amygdalina has been used for a long time in both culinary and traditional contexts (Alara *et al*., 2019; Ugbogu *et al*., 2021). In Nigeria, the leaves are used as a green vegetable in a popular soup called bitter-leaf soup, which is made by macerating the plant's leaves in cold water or boiling them until the bitterness is tolerable (Alara *et al*., 2019). Bitter leaf is also traditionally used as an appetiser, and the aqueous extracts are used as digestive tonics (Ugbogu *et al*., 2021).

The leaves have been found to be so effective in treating fevers that they are used as a quinine substitute (Farombi and Owoeye, 2011). In some northern Nigerian regions, horse feed is supplemented with bitter plants to help the animals become stronger and fatter (Farombi and Owoeye, 2011). The young leaves are also used in traditional medicine as expectorants, purgatives, and to help infertile women conceive (Olusola-Makinde *et al*., 2021).

Bitter leaf extracts can be used as tonics to treat a wide range of illnesses and maladies, such as emesis, nausea, diabetes, anorexia, diarrhoea, dysentery, and other gastrointestinal tract issues (Adebukola-Adeyanju *et al*., 2022). This is why many herbalists and traditional African physicians advise their patients to take aqueous preparations of bitter leaf to prevent certain health conditions (Usai *et al*., 2022).

2.8.3 Phytochemical Composition

The pharmacological properties of different parts like seeds, leaves, and roots of *V. amygdalina* are well documented in folk medicine. The leaves are rich in biologically active constituents such as alkaloids, phenolics, flavonoids,

terpenoids, steroids, tannins, and carotenoids, which have broad therapeutic activities (Ugbogu *et al*., 2021).

From the leaf of *Vernonia amygdalina*, phytochemicals have been identified and extracted, including alkaloids, tannins, steroids, cyanogenic glycosides, flavonoids, phenolic acids, lignans, xanthones, anthraquinones, oxalate and saponins. Bitter leaf contains a rich profile of phytochemicals, including alkaloids, tannins, steroids, cyanogenic glycosides, and flavonoids. These compounds contribute to the leaf's antioxidative and anti-inflammatory properties, positioning it as a natural remedy for a range of diseases such as cancer, diabetes, anaemia, and microbial infections.

The *V. amygdalina* leaf itself has been proven to contain significant quantities of lipids, carbohydrates, proteins having high essential amino acid scores (Edo *et al*., 2022), fibre, iron, phosphorus, copper, calcium, potassium, cobalt, and manganese, as well as appreciable amounts of biologically active compounds like ascorbic acid, saponins, alkaloids, steroids, terpenes, flavonoids, coumarins, lignans, phenolic acids, edotides, xanthones, anthraquinones, sesquiterpenes, and carotenoids (Erhonyota *et al*., 2022).

The sesquiterpene lactones, particularly vernodalin and vernonioside, are characteristic of the Asteraceae family and are responsible for many of the plant's pharmacological activities. These compounds have demonstrated anti-inflammatory, antimicrobial, and anticancer properties (Ugbogu *et al*., 2021).

2.8.4 Nutritional Composition

V. amygdalina leaves presented anti-microbial, hypoglycaemic, anti-hypertensive, lipid-lowering, anti-tumour, anti-inflammatory, antioxidant, and other pharmacological effects. The leaves are rich in nutrients and bioactive compounds that contribute to their medicinal properties. The article underscores the significance of drinking bitter leaf juice and the effects it has on health and wellness (Maisarah *et al*., 2013).

Mineral analysis reveals higher magnesium and zinc levels in *V. amygdalina* compared to *C. papaya* ($P<0.05$). Both plants are good sources of calcium, potassium, phosphorus, iron, copper, manganese, and cobalt (Edo *et al*., 2022; Maisarah *et al*., 2013). The mineral composition contributes to the nutritional and therapeutic value of these plants (Edo *et al*., 2022).

2.8.5 Pharmacological Activities

2.8.5.1 Antimicrobial Activity

V. amygdalina leaves have demonstrated anti-microbial, hypoglycaemic, anti-hypertensive, lipid-lowering, anti-tumour, anti-inflammatory, antioxidant, and other pharmacological effects (Ugbogu *et al*., 2021). The antimicrobial activity is attributed to the presence of various bioactive compounds, including alkaloids, flavonoids, phenolic acids, and sesquiterpene lactones (Farombi and Owoeye, 2011; Ugbogu *et al*., 2021). The plant extracts have shown activity against a broad spectrum of microorganisms, including bacteria, fungi, and parasites (Ugbogu *et al*., 2021).

2.8.5.2 Anti-inflammatory Activity

Research has shown that certain compounds in bitter leaves, such as vernodalin and vernonioside, can reduce inflammation and pain by suppressing the production of pro-inflammatory cytokines like IL-1β, TNF-α, and IL-6 as well as the activity of inflammatory enzymes like COX-1 and COX-2. This positions bitter leaf as a potential treatment for varieties of inflammatory conditions like arthritis and asthma (Sweis and Cressey, 2018).

The inflammatory process involves increased vascular permeability, protein denaturation, and membrane alteration. Flavonoids are a class of secondary metabolites with biological and pharmacological activities, including anti-inflammatory. The bitter leaf, *Vernonia amygdalina* (VA), are used in various alternative medicine in several countries, including Indonesia (Sweis and Cressey, 2018).

2.8.5.3 Antioxidant Activity

Bitter leaf extracts have been shown to have antioxidant qualities in addition to anti-inflammatory ones (Edo, 2022). Bitter leaf antioxidants can counteract dangerous free radicals, which typically cause tissue damage (Edo *et al*., 2023). The antioxidant activity is attributed to the high content of flavonoids, phenolic acids, and other phenolic compounds (Farombi and Owoeye, 2011; Ugbogu *et al*., 2021).

2.8.5.4 Anticancer Activity

It has been shown that organic extracts from bitter leaf have cytotoxic effects on cancer cells in the human nasopharynx (Hussain *et al*., 2022). The sesquiterpene lactones, particularly vernodalin and vernolide, have demonstrated significant anticancer activity through various mechanisms, including the induction of apoptosis and the inhibition of cell proliferation (Hussain *et al*., 2022).

2.8.5.5 Antidiabetic Activity

The hypoglycaemic properties of *V. amygdalina* have been well documented (Ugbogu *et al*., 2021). Supplementation with *V. amygdalina* could be used as a potential adjunct for treatment of diabetes mellitus and diabetic foot ulcer. The antidiabetic activity is attributed to the modulation of glucose metabolism and the improvement of insulin sensitivity. African bitter leaf (*Vernonia amygdalina* L.) is known for its numerous therapeutic effects. Potentially, it can lower plasma lipid and glucose levels, which, in turn,

may improve the condition of patients with metabolic syndrome (Ugbogu *et al*., 2021).

2.8.5.6 Wound-Healing Activity

V. amygdalina has been investigated for its wound-healing properties (Ugbogu *et al*., 2021). The plant contains nutrients and chemical compounds with antioxidant and anti-inflammatory properties that can accelerate the wound healing process. Studies have evaluated the effectiveness of African leaf extract (*V. amygdalina*) in wound healing. Hydrogel formulations containing *V. amygdalina* extract have been evaluated for their potential to enhance wound healing (Ugbogu *et al*., 2021).

2.8.5.7 Other Pharmacological Activities

The genus *Vernonia* is known for its diverse pharmacological properties, including anti-inflammatory, hypoglycaemic, hyperlipidemia, antimicrobial, antitumour, antioxidative, antiproliferative, antihypertensive, and hepatoprotective activities (Ugbogu *et al*., 2021).

Table 4: Bioactive Compounds and Pharmacological Activities of *Vernonia amygdalina* Leaves

Compound Class	Specific Compounds	Pharmacological Activities
Sesquiterpene Lactones	Vernodalin, Vernolide, Vernonioside	Anti-inflammatory, anticancer, antimicrobial
Flavonoids	Luteolin, Apigenin, Quercetin	Antioxidant, anti-inflammatory, antimicrobial
Alkaloids	Various alkaloids	Antimicrobial, analgesic
Phenolic Acids	Caffeic acid, Chlorogenic acid	Antioxidant, anti-inflammatory
Steroids	Stigmasterol, β-sitosterol	Anti-inflammatory, immunomodulatory
Tannins	Condensed tannins	Astringent, antimicrobial
Saponins	Steroidal saponins	Antimicrobial, immunomodulatory
Lignans	Various lignans	Antioxidant, anticancer
Xanthones	Various xanthones	Antioxidant, antimicrobial
Anthraquinones	Various anthraquinones	Antimicrobial, laxative

Source: (Ugbogu *et al*., 2021)

Discussion and Synthesis

The persistence and recurrence of venous leg ulcers are driven by the interaction of vascular dysfunction, chronic inflammation, impaired tissue repair and microbial factors. Venous hypertension initiates and sustains tissue damage through increased capillary pressure, oedema, impaired oxygen delivery, fibrin deposition and leucocyte activation. Once an ulcer becomes established, the wound environment is characterised by prolonged inflammation, excessive protease activity, oxidative stress and deficient tissue regeneration. These biological abnormalities create conditions in which microbial colonisation and infection can further delay healing.

The microbiology of VLUs is particularly important because chronic wounds are rarely colonised by a single organism. Instead, they frequently harbour complex polymicrobial communities, including aerobic and anaerobic bacteria and, in some cases, fungi. Organisms such as *S. aureus*, *P. aeruginosa*, *E. coli* and *K. pneumoniae* are clinically relevant because they can express virulence factors, acquire

antimicrobial-resistance determinants and participate in biofilm formation.

Biofilm formation is one of the major mechanisms underlying chronic wound persistence. Organisms embedded in extracellular polymeric matrices may exhibit markedly greater tolerance to antimicrobial agents and host defences than planktonic organisms. Physical restriction of antimicrobial penetration, metabolic heterogeneity, persister-cell formation, horizontal transfer of resistance genes and quorum-sensing mechanisms contribute to the resilience of wound biofilms. These characteristics help explain why antimicrobial therapy alone is often insufficient to achieve wound resolution.

The rising prevalence of antimicrobial resistance further complicates treatment. The reviewed evidence documents substantial resistance among both Gram-positive and Gram-negative wound pathogens, including MRSA, resistant Enterobacteriaceae, carbapenem-resistant *P. aeruginosa* and *Acinetobacter baumannii*. These organisms are especially concerning in chronic wounds because repeated healthcare exposure and prolonged or inappropriate antimicrobial treatment may create sustained selective pressure.

Antimicrobial stewardship is therefore central to effective VLU management. Stewardship does not imply withholding necessary antimicrobial treatment; rather, it promotes accurate diagnosis, rational antimicrobial selection, appropriate duration, avoidance of unnecessary systemic antibiotics and integration of effective local wound-care strategies. This creates an important therapeutic space for alternative topical agents and wound dressings that can reduce microbial burden without intensifying antibiotic selection pressure.

Medicinal plants may be relevant within this context because several phytochemicals have multiple biological activities that address different components of chronic wound pathology. Potential mechanisms include direct antimicrobial activity, inhibition of inflammatory mediators, antioxidant activity, stimulation of angiogenesis, promotion of collagen synthesis and enhancement of epithelialisation. Polyherbal approaches may additionally provide synergistic effects where individual agents act through complementary pathways.

Therapeutic Potential of Carica papaya

Carica papaya contains a broad range of bioactive compounds, including alkaloids, flavonoids, phenolic acids, carotenoids, tannins, saponins and proteolytic enzymes. Of particular interest to wound care are papain and chymopapain, which may support enzymatic debridement by assisting removal of devitalised tissue. Flavonoids and phenolic compounds may also contribute antimicrobial, antioxidant and anti-inflammatory activities.

Preclinical evidence reviewed in the manuscript indicates that *C. papaya* extracts may inhibit several common wound pathogens, including *S. aureus*, *P. aeruginosa*, *E. coli* and *Streptococcus* species. Experimental wound models have also reported accelerated wound closure, enhanced collagen deposition, improved angiogenesis and epithelial regeneration. These effects are biologically plausible given the plant's antioxidant, anti-inflammatory and proteolytic properties.

The potential usefulness of *C. papaya* may therefore extend beyond antimicrobial activity. In a chronic wound such as a VLU, removal of necrotic tissue, reduction of oxidative injury, modulation of inflammation and support of tissue regeneration are all potentially beneficial. This multifunctional profile may make papaya-derived products suitable candidates for incorporation into advanced dressings, hydrogels or other topical wound formulations.

Therapeutic Potential of Vernonia amygdalina

Vernonia amygdalina similarly contains a wide range of biologically active constituents. These include alkaloids, tannins, flavonoids, phenolic acids, steroids, saponins and characteristic sesquiterpene lactones such as vernodalin, vernolide and vernonioside. These compounds have been associated with antimicrobial, antioxidant and anti-inflammatory effects.

Its anti-inflammatory activity is particularly relevant to chronic wounds because persistent inflammation is a major determinant of impaired healing. Compounds present in *V. amygdalina* have been associated with modulation of inflammatory cytokines and cyclooxygenase pathways. Its antioxidant constituents may also reduce oxidative tissue injury, while antimicrobial components may limit proliferation of wound pathogens.

The plant has additionally been evaluated in wound-healing preparations, including hydrogel formulations. Such delivery systems may be especially relevant because they permit direct application to the wound bed and can potentially combine moisture balance, controlled phytochemical release and antimicrobial action.

Potential Complementarity of C. papaya *and* V. amygdalina

A particularly promising research direction is the evaluation of combined or polyherbal formulations containing both plants. Their pharmacological profiles are complementary. *C. papaya* provides proteolytic enzymes with potential debriding effects, alongside antimicrobial and regenerative properties, while *V. amygdalina* provides strong anti-inflammatory, antioxidant and antimicrobial phytochemicals.

A combined formulation could theoretically address several major barriers to VLU healing simultaneously:

- microbial colonisation and infection;
- biofilm-associated persistence;
- excessive inflammation;
- oxidative stress;
- necrotic or devitalised tissue;
- impaired collagen formation; and
- delayed epithelialisation.

Such a multi-target approach would be especially relevant to antimicrobial stewardship if it reduced unnecessary systemic antibiotic exposure or improved the effectiveness of standard wound-care interventions. However, this hypothesis requires direct experimental confirmation rather than extrapolation from studies of each plant individually.

Limitations of Current Evidence

Despite the promising biological rationale, several major limitations remain.

First, much of the evidence is derived from laboratory studies, animal experiments or general wound models rather than specifically from human venous leg ulcers. Second, extraction methods, solvents, plant parts, concentrations and formulations vary considerably between studies, limiting comparison and reproducibility. Third, antimicrobial activity against planktonic organisms does not necessarily predict efficacy against mature polymicrobial wound biofilms.

There is also insufficient evidence on optimal dose, tissue toxicity, allergenicity, interactions with standard wound products and long-term safety. Standardisation of active phytochemical concentrations remains essential before reproducible therapeutic formulations can be developed.

Future studies should therefore include phytochemical standardisation, minimum inhibitory and bactericidal concentration testing, antibiofilm assays, synergy studies, cytotoxicity testing and formulation-stability studies. Promising preparations should subsequently undergo controlled animal wound studies followed by well-designed human trials.

Conclusion

Venous leg ulcers remain a significant chronic-wound problem characterised by impaired healing, frequent recurrence, polymicrobial colonisation, biofilm formation and increasing antimicrobial resistance. The interaction between chronic venous hypertension, persistent inflammation and resistant microorganisms contributes substantially to treatment failure and prolonged morbidity.

The increasing prevalence of multidrug-resistant wound pathogens strengthens the need for antimicrobial-stewardship approaches and for complementary wound-care interventions that can reduce dependence on conventional antibiotics. *Carica papaya* and *Vernonia amygdalina* possess multiple pharmacological properties that are potentially relevant to VLU management, including antimicrobial, anti-inflammatory, antioxidant and wound-healing effects. *C. papaya* additionally provides proteolytic enzymes with potential wound-debriding activity, while *V. amygdalina* contains sesquiterpene lactones and other bioactive compounds that may modulate inflammation and microbial growth.

However, the current evidence does not yet justify routine clinical substitution of these medicinal plants for established VLU therapy. Evidence remains predominantly preclinical, and substantial heterogeneity exists in extraction methods, dosing, formulation and outcome assessment.

Future research should prioritise standardised phytochemical characterisation, direct testing against multidrug-resistant VLU pathogens, mature biofilm models, cytotoxicity and wound-safety assessment, and development of stable topical formulations such as hydrogels, films or advanced dressings. Comparative and combination studies involving *C. papaya* and *V. amygdalina* are particularly warranted to determine whether their complementary pharmacological activities produce genuine synergistic effects.

Ultimately, scientifically standardised plant-based formulations may provide affordable adjuncts to compression therapy, appropriate wound care and targeted antimicrobial treatment. Their most important potential contribution may lie in supporting wound healing while reducing unnecessary antimicrobial exposure and contributing to broader antimicrobial-stewardship efforts.

References

1. Abbas, M., Umar, A. and Sani, I. (2024). Evaluation of extraction solvents on phytochemical composition and antimicrobial activity of medicinal plants. *Journal of Medicinal Plants Research*, **18**(3): 45-58.
2. Achuba, F. I. (2018). Role of bitter leaf (*Vernonia amygdalina*) extract in prevention of renal toxicity induced by crude petroleum contaminated diets in rats. *International Journal of Veterinary Science and Medicine*, **6**(2): 172-177.
3. Adaramoye, O. A. (2008). Lipid-lowering effects of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol diet. *Vascular Health and Risk Management*, **4**(1): 235-241.
4. Adebukola-Adeyanju, A., Rebecca Oyenihi, O., and Omoniyi Oguntibeju, O. (2022). Antioxidant-rich vegetables: Impact on human health. In *Vegetable Crops - Health Benefits and Cultivation*. Intechopen.

5. Adesoji, A. T., Ogunjimi, A. A., and Olayinka, B. O. (1997). Unusual recovery of animal staphylococci from septic wounds of hospital patients in Ile-Ife, Nigeria. *Letters in Applied Microbiology*, **24**(2): 87-90.
6. Akpoghelie, P. O., Edo, G. I., and Akhayere, E. (2022). Proximate and nutritional composition of beer produced from malted sorghum blended with yellow cassava. *Biocatalysis and Agricultural Biotechnology*, **45**: 102535.
7. Alara, O. R., Abdurahman, N. H., Ukaegbu, C. I., and Kabbashi, N. A. (2019). Extraction and characterization of bioactive compounds in *Vernonia amygdalina* leaf ethanolic extract comparing Soxhlet and microwave-assisted extraction techniques. *Journal of Taibah University for Science*, **13**(1): 414-422.
8. Anibijuwon, B. O., Oladejo, D. O., and Adetitun, O. M. (2012). Antimicrobial activities of *Vernonia amygdalina* against oral microbes. *Global Journal of Pharmacology*, **6**(3): 178-185.
9. AOAC. (2006). *Official methods of analysis* (19th ed.). Association of Official Agricultural Chemists.
10. Asante, D., Adokoh, C., Acheampong, D., Afrifa-Yamoah, E., Barnes, P., Agyeah, G., Phillips, R. and Sartori, J. (2025). Evaluation of the wound healing and anti-microbial activity of hydroethanolic extracts of young and old leaves of *Vernonia amygdalina* Delile. *Clinical Traditional Medicine and Pharmacology*, **6:** 200 - 221.
11. Attama, S. C., Aba, P. E., Asuzu, C. U., Nweke, F. N., Ugwu, O. P., and Ezugwu, C. O. (2018). Comparative studies on the hypoglycemic and antioxidant activities of *Vernonia amygdalina* Delile and *Baccharoides tenoreana* Olive in alloxan-induced hyperglycemic rats. *Clinical Phytoscience*, **7**(1): 91.
12. Bjarnsholt, T., Eberlein, T., Malone, M., and Schultz, G. (2018). Biofilm in chronic wounds: A review of the evidence. *Wound Repair and Regeneration*, **26**(4): 307-314.
13. Bohm, M. K., and Kocipai, A. (1994). *Flavonoids: Composition and uses*. Smithsonian Institution Press.
14. Bottery, M. J., Pitchford, J. W., and Friman, V. P. (2021). Ecology and evolution of antimicrobial resistance in bacterial communities. *ISME Journal*, **24**: 112-132.
15. Chakraborty, S., Afaq, N., Singh, N., and Majumdar, S. (2018). Antimicrobial activity of *Cannabis sativa*, *Thuja orientalis*, and *Psidium guajava* leaf extracts against methicillin-resistant *Staphylococcus aureus*. *Journal of Integrative Medicine*, **16**(5): 350-357.
16. Cheesbrough, M. (2010). *District laboratory practice in tropical countries* (2nd ed., Part 2). Cambridge University Press.
17. Clinical and Laboratory Standards Institute (CLSI). (2022). *Performance standards for antimicrobial susceptibility testing* (32nd ed.). CLSI Supplement M100.
18. Edo, G. I. (2022). Analysis of phytochemical constituents and antioxidant potential of bitter kola leaf extract towards bioactive food, nutrition and health resources. *Organic and Medicinal Chemistry International Journal*, **5**(1): 11-20.
19. Edo, G. I., Akpoghelie, P. O., and Akpoghelie, E. I. (2023). Bioactive compounds and pharmacological activities of *Vernonia amygdalina*. *Journal of Herbal Medicine*, **39**: 100645.
20. Edo, G. I., Makinde, M. G., Nwosu, L. C., Ozgor, E., and Akhayere, E. (2022). Physicochemical and pharmacological properties of palm oil: An approach for quality, safety, and nutrition evaluation of palm oil. *Food Analytical Methods*, **15**(8): 2290-2305.
21. Edo, G. I., Samuel, P. O., Jikah, A. N., Oloni, G. O., Ifejika, M. N., Oghenegueke, O., and Akpoghelie, P. O. (2023). Proximate composition and health benefit of Roselle leaf (*Hibiscus sabdariffa*). Insight on food and health benefits. *Food Chemistry Advances*, **3**: 100437.
22. Ehiaghe, F. A., Onyenekwe, C. C., Aryaebu, I. H., Erhunmwunse, R. U., Ehiaghe, J. I., Osakue, O. N., Chukwanunkwu, R. C., Ogbodo, E. C., Ize-Jyamu, J., Amengjalue, O. O., Okafoanyali, O. J., and Ogoegbunam, N. D. (2025). Pattern of multidrug resistance genes and immunological biomarkers in post-surgical wound infections in Nnewi, Nigeria. *The Nigerian Health Journal*, **25**(2).
23. Ehiaghe, F. A., Onyenekwe, C. C., Osakue, O. N., Ehiaghe, J. I., Chukwuanukwu, R. C., Okafoanyali, O. J., Onyenekwe, N. O., Igiebor, F. A., and Manafa, F. A. (2024). Patterns of multidrug bacterial clinical isolates and cytokine responses to antibiotic misuse in Nnewi, Anambra State, Nigeria. *Journal of Applied Sciences and Environmental Management*, **28**(5).
24. Erhonyota, C., Edo, G. I., and Onoh, F. O. (2022). Comparative analysis of phytochemical constituents of *Vernonia amygdalina* and *Ocimum gratissimum*. *Journal of Medicinal Plants Research*, **16**(4): 112-125.
25. Erhonyota, C., Edo, G. I., and Onoharigho, F. O. (2022). Comparison of poison plate and agar well diffusion method determining the antifungal activity of protein fractions. *Acta Ecologica Sinica*, **42**(4): 589-596.
26. Farombi, E. O., and Owoeye, O. (2011). Antioxidative and chemopreventive properties of *Vernonia amygdalina* and *Garcinia biflavonoid*. *International Journal of Environmental Research and Public Health*, **8**(6): 2533-2555.

27. Ferguson, L. R. (2001). Role of plant polyphenols in genomic stability. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, **475**(1-2): 89-111.
28. Fidelis, I. O. (2024). Evaluation of the therapeutic, phytochemical, antimicrobial, and general acceptability of selected medicinal plants used among Afikpo people, Ebonyi State, Nigeria. *International Journal of Research and Scientific Innovation*, **11**(5): 825-843.
29. Gjødsbøl, K., Christensen, J. J., Karlsmark, T., Jørgensen, B., Klein, B. M., and Krogfelt, K. A. (2006). Multiple bacterial species reside in chronic wounds: A longitudinal study. *International Wound Journal*, **3**(3): 225-231.
30. Harborne, J. B. (1983). *Phytochemical methods: A guide to modern techniques of plant analysis*. Chapman and Hall.
31. Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Chapman and Hall.
32. Hassan, A. U., Khan, S. A., and Khan, S. (2021). Medicinal plants as sources of antimicrobial agents: A review. *Journal of Pharmacy and Pharmacology*, **73**(6): 729-746.
33. Hassan, F., Edo, G. I., Nwosu, L. C., Jalloh, A. A., Onyibe, P. N., and Itoje-akpokiniovo, L. O. (2021). An inventory of medicinal plants used as sedative, analgesic and blood tonic in Abeokuta, Ogun State, Nigeria. *Acta Ecologica Sinica*.
34. Hussain, M. S., Fareed, S., and Ansari, S. (2022). Anticancer activity of *Vernonia amygdalina*: A systematic review. *Journal of Cancer Research and Therapeutics*, **18**(4): 987-995.
35. Hussain, S., Liufang, H., Shah, S. M., Ali, F., Khan, S. A., Shah, F. A., Khan, H., and Hussain, A. (2022). Cytotoxic effects of extracts and isolated compounds from *Ifloga spicata* (Forssk.) Sch. Bip against HepG-2 cancer cell line: Supported by ADMET analysis and molecular docking. *Frontiers in Pharmacology*, **13**: 1004321.
36. Ibekwe, V. I., Nnanyere, N. F., and Akujobi, C. O. (2001). Studies on antibacterial activity and phytochemical qualities of extracts of orange peels. *International Journal of Environmental Health and Human Development*, **2**(1): 41-46.
37. Ibiebele, D. E., Ogbulie, J. N., and Udeh, H. O. (2018). Microbial profile of chronic wounds and antimicrobial resistance patterns: A systematic review. *International Wound Journal*, **15**(5): 739-748.
38. Ibiebele, I., McLelland, G., and Golledge, J. (2018). Prevalence of antibiotic-resistant bacteria in chronic venous leg ulcers: A systematic review and meta-analysis. *Wound Repair and Regeneration*, **26**(2): 131-139.
39. Kabiru, A. Y., Tahir, V., Garba, M. H., Kuta, F. A., Jibril, A., and Shuaib, M. A. (2016). Changes in Serum Electrolyte Levels in Typhoid Fever Patients Attending Minna General Hospital, Nigeria. *British Journal of Medicine and Medical Research*, **14**(5): 1-7.
40. Kahle, B., Hermanns, H. J., and Gallenkemper, G. (2011). Evidence-based treatment of chronic leg ulcers. *Deutsches Ärzteblatt International*, **108**(14): 231-237.
41. Krizanova, O., Penesova, A., Hokynkova, A., Pokorna, A., and Samadian, A. (2023). Chronic venous insufficiency and venous leg ulcers: Aetiology, on the pathophysiology-based treatment. *International Wound Journal*. https://onlinelibrary.wiley.com/doi/10.1111/iwj.14405
42. Kwon, K. T., and Armstrong, D. G. (2018). Microbiology and antimicrobial therapy for diabetic foot infections. *Infection and Chemotherapy*, **50**(1): 11-20.
43. Maisarah, A. M., Nurul Amira, B., Asmah, R., and Fauziah, O. (2013). Antioxidant and antimicrobial activity of *Carica papaya* leaf extract. *International Food Research Journal*, **20**(1): 319-323.
44. Malone, M., Bjarnsholt, T., McBain, A. J., James, G. A., Stoodley, P., Leaper, D., Tachi, M., Schultz, G., Swanson, T., and Wolcott, R. D. (2017). The prevalence of biofilms in chronic wounds: A systematic review and meta-analysis of published data. *Journal of Wound Care*, **26**(1): 20-25.
45. Matovu, I.J., Bogere, C., Othieno, J., Nakabiri, W., Kahwa, I., Ajayi, C.O., Omara, T., and Iqbal, S. (2023). Comparison of the Wound Healing Activity of the Leaf and Leaf Ash Extracts of *Vernonia amygdalina* in Rats. Mbarara University of Science and Technology Institutional Repository. https://doi.org/10.21203/rs.3.rs-2741370/v1
46. Mestrovic, T., Robles Aguilar, G., Swetschinski, L. R., Ikuta, K. S., Gray, A. P., Davis Weaver, N., Han, C., Wool, E. E., Gershberg Hayoon, A., Aali, A., Abate, S. M., Abbasi-Kangevari, M., Abbasi-Kangevari, Z., Abd-Elsalam, S., Abebe, G., Abedi, A., Abhari, A. P., Abidi, H., Aboagye, R. G., and Murray, C. J. L. (2023). The burden of bacterial antimicrobial resistance in the WHO European region in 2019: A cross-country systematic analysis. *The Lancet Public Health*, **8**(7): e498-e510.
47. Monk, E. J. M., Jones, T. P. W., Bongomin, F., Kibone, W., Nsubuga, Y., Ssewante, N., and van Zandvoort, K. (2024). Antimicrobial resistance in bacterial wound, skin, soft tissue and surgical site infections in Central, Eastern, Southern and Western Africa: A systematic review and meta-analysis. *PLOS Global Public Health*, **4**(4). https://doi.org/10.1371/journal.pgph.0003077

48. Moses, P., Abdullah, A. S., Iliyasu, M. Y., Inusa, T., Ismail, S., Umar, R., Tahir, H., and Kabeer, Z. M. (2025). Antimicrobial Activity of Vernonia amygdalina Leaves Aqueous Extracts against Multidrug-resistant Bacterial Isolates from Poultry Chickens in Bauchi Metropolis. *Microbiology Research Journal International*, **35**(8): 40-48.
49. Muteeb, G., Rehman, M. T., Shahwan, M., and Aatif, M. (2023). Origin of Antibiotics and Antibiotic Resistance, and Their Impacts on Drug Development: A Narrative Review. *Pharmaceuticals*, **13**: 11-33.
50. Nafiu, A.B., Akinwale, O.C., Akinfe, O.A., Owoyele, B.V., Abioye, A.I.R., Abdulazeez, F.I., and Rahman, M.T. (2017). Histomorphological evaluation of wound healing - Comparison between use of honey and *Vernonia amygdalina* leaf juice. *Tanzania Journal of Health Sciences*, **19**(1): 11 - 23.
51. Nafiu, A.B., Rahman, M.T., and Salleh, M.Z. (2013). Anti-inflammatory and antioxidant properties of unripe papaya extract in an excision wound model. *Pharmaceutical Biology*, 51(9), 1175-1181.
52. Nayak, B.S., Pereira, L.M., and Maharaj, D. (2012). Wound-healing potential of an ethanol extract of *Carica papaya* (Caricaceae) seeds. *International Wound Journal*, **9**(6): 650 - 655.
53. Nayak, B.S., Ramdath, D.D., Marshall, J.R., Isitor, G.N., Xue, S., and Shi, J. (2007). Wound healing activity of *Carica papaya* L. in experimentally induced diabetic rats. *Indian Journal of Experimental Biology*, **45**(8): 739-743.
54. Obadoni, B. O., and Ochuko, P. O. (2001). Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and Delta states of Nigeria. *Global Journal of Pure and Applied Sciences*, **8**: 203-208.
55. Okolie, N. P., Falodun, A., and Davids, O. (2023). Pharmacological activities of *Carica papaya*: A comprehensive review. *Journal of Ethnopharmacology*, **305**: 116089.
56. Olaniyan, M. F., Ogunlade, A. A., and Atere, D. (2019). Possible immunological alterations in the plasma levels of TNF-α and IL-10 in the traditional application of *Vernonia amygdalina* (Ewuro) leaves in the treatment of diabetes mellitus. *Recent Advances in Biology and Medicine*, **5**(1): 1-10.
57. Olusola-Makinde, O., Olabanji, O. B., and Ibisanmi, T. A. (2021). Evaluation of the bioactive compounds of *Vernonia amygdalina* Delile extracts and their antibacterial potentials on water-related bacteria. *Bulletin of the National Research Centre*, **45**(1): 191.
58. Onifade, A. K., and Agunloye, O. O. (2019). Antibacterial Assessment of Crude and Fractionated Extracts of Vernonia amygdalina Leaf against Multiple Antibiotic Resistant Bacteria of Wound Infection. *Asian Journal of Research in Medical and Pharmaceutical Sciences*, **7**(1): 1-12.
59. Onyegbule, F. A., Nwosu, C. O., and Okonkwo, C. J. (2017). Bacterial contamination of chronic wounds in a tertiary hospital in Nigeria. *Nigerian Journal of Clinical Practice*, **20**(8): 987-993.
60. Oseni, L. A., and Akwetey, W. (2012). An in-vivo evaluation of wound healing potential of *Vernonia amygdalina* leaf extract. *Journal of Applied Pharmaceutical Science*, **2**(7): 87-91.
61. Owoyele, B. V., Adebukola, O. M., Funmilayo, A. A., and Soladoye, A. O. (2008). Anti-inflammatory activities of ethanolic leaf extract of *Carica papaya* in carrageenan-induced paw oedema in rats. *Inflammopharmacology*, **16**(4): 168-173.
62. Probst, S., Saini, C., Gschwind, G., Stefanelli, A., Bobbink, P., Pugliese, M. T., Cekic, S., Pastor, D., and Gethin, G. (2023). Prevalence and incidence of venous leg ulcers—A systematic review and meta-analysis. *International Wound Journal*, **20**(9): 3906-3921. https://doi.org/10.1111/iwj.14272
63. Raffetto, J. D. (2018). Pathophysiology of chronic venous disease and venous ulcers. *Surgical Clinics of North America*, **98**(2): 337-347.
64. Roberts, C. D., Leaper, D. J., and Assadian, O. (2017). The role of antiseptics in wound care. *Journal of Wound Care*, **26**(5): 234-240.
65. Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., and Alqumber, M. A. (2023). Antimicrobial resistance: A growing serious threat for global public health. *Healthcare (Switzerland)*, **11**(13): 1946.
66. Santhi, K., and Sengottuvel, R. (2016). Qualitative and quantitative phytochemical analysis of *Moringa concanensis* Nimmo. *International Journal of Current Microbiology and Applied Sciences*, **5**(5): 633-640.
67. Sen, C. K. (2021). Human wound and its burden: Updated 2020 compendium of estimates. *Advances in Wound Care*, **10**(5): 281-292.
68. Singh Makhaik, M., Shakya, K., and Kale, R. (2021). Dietary phytochemicals: As a natural source of antioxidants. *Antioxidants - Benefits, sources, mechanisms of action*. Intechopen.
69. Sofowora, A. (1993). *Medicinal plants and traditional medicine in Africa*. John Wiley and Sons.
70. Sofowora, A., Ogunbodede, E., and Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional, Complementary and Alternative Medicines*, **10**(5): 210-229.
71. Stanek, A., Mosti, G., and Partsch, H. (2023). Venous leg ulcers: Current concepts and new perspectives. *International Angiology*, **42**(1): 1-12.

72. Sule, O. J., and Arhoghro, E. M. (2017). Effect of *Carica papaya* leaf extract on wound healing in Wistar rats. *Journal of Applied Sciences and Environmental Management*, **21**(2): 357-361.
73. Surajo, S., Abdussalam, A., Abbas, B., Zakariya, A. M., Yunusa, A., and Jesse, D. M. (2026). GC-MS characterization and antibacterial activity of terpene-rich extract from *Vernonia amygdalina* leaves. *UMYU Scientifica*, **2651**: 035.
74. Sweis, I. E., and Cressey, B. D. (2018). Potential role of *Vernonia amygdalina* in the management of asthma and arthritis. *Journal of Ethnopharmacology*, **215**: 123-130.
75. Tang, K. W. K., Millar, B. C., and Moore, J. E. (2023). Antimicrobial resistance (AMR). *British Journal of Biomedical Science*, **80**: 11387.
76. Thakkar, S., Anklam, E., Xu, A., Ulberth, F., Li, J., and Li, B. (2020). Regulatory landscape of dietary supplements and herbal medicines from a global perspective. *Regulatory Toxicology and Pharmacology*, **114**: 104647.
77. Thakkar, S., Sharma, A., and Verma, S. (2020). Herbal products as nutraceuticals: A review. *Journal of Dietary Supplements*, **17**(5): 587-601.
78. Tofighi, Z., Ghazi, S. N., Hadjiakhoondi, A., and Yassa, N. (2016). Determination of Cardiac Glycosides and Total Phenols in Different Generations of *Securigera securidaca* suspension culture. *Research Journal of Pharmacognosy*, **3**(2): 25-31.
79. Trease, G. E., and Evans, W. C. (2002). *Pharmacognosy* (15th ed.). Saunders Publishers.
80. Tungmunnithum, D., Thongboonyou, A., Pholboon, A., and Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*, **5**(3): 93.
81. Udemezue, O. I., Alaebo, C. L., and Okoli, U. O. (2023). Evaluation of Antimicrobial Effects of Trona and Alum on Dental Carries Isolates. *International Journal of Research and Innovation in Applied Sciences*, **8**(9): 52-58.
82. Udemezue, O. I., Anibijuwon, I. I., and Adebayo, E. A. (2023). Standard microbiological techniques for the identification of bacterial isolates. *Journal of Microbiology and Biotechnology Research*, **13**(2): 45-58.
83. Ugbogu, E. A., Emmanuel, O., Dike, E. D., Agi, G. O., Ugbogu, O. C., Ibe, C., and Iweala, E. J. (2021). The phytochemistry, ethnobotanical, and pharmacological potentials of the medicinal plant- *Vernonia amygdalina* L. (bitter leaf). *Clinical Complementary Medicine and Pharmacology*, **1**(1): 100006.
84. Usai, R., Majoni, S., and Rwere, F. (2022). Natural products for the treatment and management of diabetes mellitus in Zimbabwe—A review. *Frontiers in Pharmacology*, **13**: 13-24.
85. Van-Burden, T. P., and Robinson, T. (1981). *The biochemistry of alkaloids* (2nd ed.). Springer.
86. Vij, T., and Prashar, Y. (2015). A review on medicinal properties of *Carica papaya* Linn. *Asian Pacific Journal of Tropical Disease*, **5**(1): 1-6.
87. Weller, C. D., Team, V., and Sussman, G. (2021). First-line interactive wound dressing update: A comprehensive review of the evidence. *Frontiers in Pharmacology*, **11**: 616225.
88. World Health Organization. (2025). *WHO Global Traditional Medicine Strategy 2025–2034*. World Health Organization.