ORIGINAL ARTICLE

ISSN: 0331 - 670X

https://doi.org/10.51412/psnnjp.2025.27

The Nigerian Journal of PHARMACY

AS MEN OF HONOUR WE JOIN HANDS

THE OFFICIAL ORGAN OF THE PHARMACEUTICAL SOCIETY OF NIGERIA FOUNDED 1927

Evaluation of Antimicrobial Activity of Methanol Extracts of three Selected Lower Plants Against Wound Pathogens

[1]Osungunna Michael Oluwole, [2]Odediran Samuel Akintunde, [3]Bamgboye Rachael Adebola [4]Akinwumi Florence Olubunmi, [5]Onifade Deborah Ifeoluwa, [6]Mowobi Dorcas Oluwabusayo, [7]Ojo Deborah Toluwalope, [8]Akosile Oluwayomi Rebecca

[1,4,5,6,7]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

[2,8]Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria

[3]Natural History Museum, Obafemi Awolowo University, Ile Ife

ARTICLE INFO

Article history:

Received 15th December 2024
Revised 20th April 2025
Accepted 27th April 2025
Online Published

Keywords:

Lower plants,

Wound,

Pathogens

*Corresponding Author:
Dr. Osungunna Michael Oluwole
E-mail: mowole@oauife.edu.ng
Tel: +2348051542596

ABSTRACT

Background: The resistance of bacteria to conventional antibiotics has been of great concern worldwide. This has prompted the need to explore alternative natural sources, such as plants, for safer, cheaper and more effective therapies. The use of medicinal plants in wound care is a common practice in traditional medicine. Therefore, the methanol extracts of three lower plants (*Nephrolepis biserrata (*Fam: Nephrolepidaceae*), Platycerium stemaria (*Fam: Polypodiaceae*)*, and *Platycerium angolense (*Fam: Polypodiaceae*)*) were assessed for their antimicrobial activities against some wound pathogens.
Methods: Wound swabs were collected from twenty patients presenting with wounds at the University Health Centre in Ile-Ife, cultured and the bacteria isolated were identified using conventional biochemical tests. Antimicrobial Susceptibility Test (AST) was carried out on Mueller-Hinton agar using the disk-diffusion method. The Minimum Inhibitory Concentrations (MIC) of methanol plants' extracts against the identified bacteria were determined using the broth micro-dilution technique.
Results: Among the 20 bacteria isolated, 55% and 45% were Gram-positive and Gram-negative, respectively. The most common was *Micrococcus luteus* (25%), then *Staphylococcus aureus* (20%), *Pseudomonas aeruginosa* (15%), *Bacillus spp.* (10%), *Enterobacter aerogenes* (10%), *Proteus mirabilis* (10%), *Escherichia coli* (5%), and *Klebsiella pneumoniae* (5%). The methanol extract of *N. biserrata* showed the highest antimicrobial activity. However, all the isolates that were resistant to azithromycin antibiotic were not susceptible to all the extracts of the 3 selected plants.
Conclusion: *N. biserrata* and *Platycerium spp*. leaves could be useful in the management of wound infections as they exhibited antimicrobial activity against isolated wound pathogens. The results from this study support the ethnobotanical use of the plants in wound treatment

INTRODUCTION: A wound is any physical harm to the body, usually resulting from a blow, cut, or other impact that interferes with the skin's natural structure and function[1]. Wound can be acute or chronic; closed or open depending on the underlying reason for wound formation and the physiology of wound healing[2]. Unlike the acute wound which usually results from sudden trauma or surgery, heals in a timely and orderly manner, and progress through the expected stages of wound healing, the most common aetiology of chronic wounds include diabetes mellitus, autoimmune illnesses, hypoxia, trauma, and poor care during the early phases of wounding[3]. Impaired wound healing is a common and important problem in individuals with diabetes mellitus because it can result in pressure,

venous, and foot ulcers. As a result, there is a higher chance of serious consequences, including infection and amputation with diabetic wounds[4]. According to studies, wound infections account for 70–80% of surgical patients' deaths and constitute one-third of nosocomial infections. These infections are linked to high patient morbidity and mortality, particularly in low and middle-income countries (LMICs)[5]. The most common Gram-positive bacteria known to cause wound infections are *Enterococcus*, *Coagulase-negative Staphylococci* (CoNS), and *Staphylococcus aureus*. Examples of Gram-negative bacteria are Acinetobacter, *Proteus spp.*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*[6]. Some fungi, such as *Candida albicans*, *Candida parapsilosis*, *Malasezzia restricta,* and *Curvularia lunata,* are also involved[7]. Infected wounds may require the use of antibiotics for treatment. However, the use of antibiotics in wound treatment has been correlated with increased antibiotic resistance in hospitals and communities[8]. This has necessitated the use of medicinal plants in the treatment of wounds. However, some lower plants such as *Nephrolepis biserrata, Platycerium stemaria* and *Platycerium angolense* (Figure 1) have been used traditionally for their wound healing property while scientific reports supporting these reported ethnobotanical uses are limited. This study therefore aimed at evaluating the antimicrobial activity of these selected lower plants against some wound pathogens.

Figure 1: Pictures of *Nephrolepis biserrata, Platycerium stemaria* and *Platycerium angolense*

Methods:

Ethics approval and consent to participate: Ethical clearance with HREC No: IPH/OAU/12/1738 was obtained from the Health Research Ethics Committee of Obafemi Awolowo University, Ile-Ife, Nigeria before the commencement of this work.

Study Area

The study was carried out at the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Obafemi Awolowo University (O.A.U), Ile-Ife.

Collection of Samples

Twenty wound swabs were collected from patients presenting with wounds that had been judged as infected by the presence of purulent material at the O.A.U health centre after obtaining ethical clearance from the Health Research Ethics Committee of the University. Patients that were visiting the health centre for the first time with fresh wounds were excluded from the study. The swabs were suspended in 5 mL peptone water and incubated for 24 h at 37°C. The overnight grown cultures were streaked on sterile nutrient agar plates and incubated at 37°C for 24h. The distinct colonies obtained were characterized using conventional biochemical tests[9]. The identified bacterial isolates were stored on agar slopes and stored at 4°C until needed.

Antibiotic susceptibility

This was done according to Kirby-Bauer disk diffusion method using the Clinical and Standard Laboratory Institute guidelines[10]. Briefly, about 4 colonies of the bacterial cells were dissolved in 10mL sterile distilled water and the turbidity adjusted to 0.5 McFarland standard. The resulting bacterial suspension was spread onto the oven-dried Mueller-Hinton agar surface contained in a petri dish using a sterile swab stick. The antibiotic disc was placed onto the Mueller-Hinton surface to make contact with the bacterial cells with the aid of a sterile forceps. The plates were incubated at 37°C for 24h after which the zone of inhibition to each antibiotic was measured and recorded. Based on the value, each bacterium was classified as sensitive, intermediate or resistant according to the Clinical

and Standard Laboratory Institute guidelines[10]. Each antibiotic disc was composed of AS: Ampicillin/Sulbactam (20µg); BA: Co-trimoxazole (25µg), C: Cefotaxime (30µg); PT: Piperacillin/Tazobactam (110µg); C: Chloramphenicol (30µg); CP: Ciprofloxacin (30µg); CR: Ceftriaxone (30µg); TE: Tetracycline (30µg); OF: Ofloxacin (5µg); GM: Gentamicin (10µg); AT: Azithromycin (1µg); LE: Levofloxacin (5µg).

Plant Collection and Authentication

The leaves (fronds) of *Nephrolepis bisserata* were collected at the Oil Palm plantation before Opa Dam, along Road 7, Obafemi Awolowo University. The leaves of *Platycerium stemaria* and *Platycerium angolense* were collected behind Oduduwa Hall Lecture Theatre, Obafemi Awolowo University. The plants were identified by Dr. (Mrs.) R.A. Bamigboye and authenticated at the Faculty of Pharmacy herbarium by Mr. I.I. Ogunlowo of the Pharmacognosy Department, Obafemi Awolowo University. Specimens with Voucher numbers FPI 2320, FPI 2319 and FPI 2321 were deposited for *Nephrolepis biserrata, Platycerium stemaria* and *Platycerium angolense,* respectively.

Plant Preparation and Extraction

Briefly, 200 g each of the plant species (*Nephrolepis biserrata, Platycerium stemaria* and *Platycerium angolense*) were separately collected, air-dried, powdered and macerated in 2 L of absolute (99.8%) methanol each for 72 hours with intermittent shaking. The resultant extract was filtered using a whatmann no 1 filter paper and the filtrate was concentrated *in-vacuo* using a rotary evaporator and weighed.

Determination of the minimum inhibitory concentrations (MICs) of the extracts

The minimum inhibitory concentrations (MIC) of the plant extracts were determined using the microdilution method[10]. Briefly, the bacterial inoculum of 5 x 10^5 colony forming units per mL (CFU/mL) was added to a microplate containing dilutions of the extract solution (0.1953 - 100 mg/mL), absolute (99.8%) methanol (vehicle control), or streptomycin (1 mg/mL; positive control). Bacterial growth was assessed using 0.2 mg/mL resazurin solution and the microplates were incubated at 37°C for 1 hour. MIC was defined as the lowest concentration of a given treatment that inhibits bacterial growth.

Phytochemical Screening

Each plant extract was analysed for the presence of phytochemicals such as alkaloids, flavonoids, glycosides, tannins, saponins, phenols, terpenoids using standard procedures[11].

Test for Tannins

About 200 mg of the crude plant extract was boiled with 10 mL of distilled water; and 0.1% Ferric chloride was added to the mixture; which was then observed for blue-black coloration indicating the presence of tannins.

Test for Alkaloids

The plant extract was dissolved in 100 mL of water, filtered, and heated in steam with 2 mL of the filtrate and three drops of 1% HCl. Then, 1 mL of the heated mixture was combined with 6 mL of the Mayer-Wagner reagent. The appearance of a cream or brown-red colored precipitate indicated the presence of alkaloids.

Test for Saponins

About 0.5 milliliters of the extract and 5 mL of distilled water were combined and agitated. Then, the formation of foam confirmed the presence of saponins.

Test for Flavonoids and Glycosides

200 mg of the plant extract was mixed with 10 mL of ethanol and filtrated. Two mL of the filtrate, concentrated HCl, and magnesium ribbon were mixed. The formation of a pink or red color indicates the presence of flavonoids. The formation of a yellowish colour upon adding 1mL of distilled water and NaOH to 0.5mL of the crude plant extract indicated the presence of glycosides.

Test for Steroids

About 1 mL of the crude extract was mixed with 10 mL of chloroform and 10 mL of sulfuric acid, and the formation of the bilayer (red top layer and greenish bottom layer) revealed the presence of steroids.

Test for Terpenoids

The presence of terpenoids was determined by the formation of a reddish-brown colour in the test for terpenoids, which included mixing of 0.5 mL of crude extract with 2 mL of chloroform and 3 mL of sulfuric acid.

Test for Phenols

About three drops of $FeCl_3$, and 1 mL of K_2Fe (CN_6) were added to 1 mL of the extract. The formation of greenish blue colour confirmed the presence of phenol.

Results

Table 1: The percentage distribution of bacterial isolates from wound swabs

Organisms	Number of Isolates	Percentage distribution (%)
Bacillus subtilis	2	10%
Enterobacter aerogenes	2	10%
Escherichia coli	1	5%
Klebsiella pneumo niae	1	5%
Micrococcus luteus	5	25%
Proteus mirabilis	2	10%
Pseudomonas aeruginosa	3	15%
Staphylococcus aureus	4	20%
TOTAL	**20**	**100%**

The percentage distribution of bacterial isolates associated with wound swab is as shown in Table 1. *Micrococcus luteus* and *Staphylococcus aureus* were the predominant bacterial species with percentage occurrence of 25 and 20%, respectively. However, *Escherichia coli* and *Klebsiella pneumoniae* had the least percentage of 5% each.

Table 2: Percentage distribution of antibiotic susceptibility profiles of isolated wound pathogens

Organisms	AS (%)	BA (%)	CF (%)	PT (%)	C (%)	CP (%)	CR (%)	TE (%)	OF (%)	GM (%)	AT (%)	LE (%)
Bacillus spp. (n = 2)	50	50	50	50	100	50	100	100	100	100	50	100
Enterobacter aerogenes (n = 2)	50	100	50	100	100	100	0	100	100	100	50	100
Escherichia coli (n = 1)	100	100	100	100	100	100	100	0	100	100	100	100
Klebsiella pneumoniae (n = 1)	100	0	0	100	100	100	0	100	100	100	100	100

Micrococcus luteus (n = 5)	100	100	60	100	100	40	80	100	100	60	80	100
Proteus mirabilis (n = 2)	0	50	50	100	50	100	100	50	100	100	0	100
Pseudomonas aeruginosa (n = 3)	100	100	0	100	100	66.6	66.6	100	100	100	100	100
Staphylococcus aureus (n = 4)	75	100	75	75	50	50	100	100	75	100	50	75

KEYS: AS: Ampicillin/Sulbactam (20µg); BA: Co-trimoxazole (25µg), CF: Cefotaxime (30µg); PT: Piperacillin/Tazobactam (110µg); C: Chloramphenicol (30µg); CP: Ciprofloxacin (30µg); CR: Ceftriaxone (30µg); TE: Tetracycline (30µg); OF: Ofloxacin (5µg); GM: Gentamicin (10µg); AT: Azithromycin (1µg); LE: Levofloxacin (5µg).

Table 2 shows the percentage distribution of the susceptibility profiles of the isolated wound pathogens to antibiotics. All the organisms displayed varying susceptibilities to antibiotics used for the study. While all the *Enterobacter aerogenes* displayed absolute resistance to ceftriaxone (CR), absolute resistance was displayed by *E. coli* to tetracycline (TE). However, all the strains of *Klebsiella pneumoniae* isolated were resistant to cotrimoxazole (BA), cefotaxime (CF) and ceftriaxone (CR). All the strains of *Proteus mirabilis* isolated, on the other hand, were resistant to Ampicillin/Sulbactam (AS) and azithromycin (AT) while all the *Pseudomonas aeruginosa* strains isolated were resistant to cefotaxime.
Suffice it to say that some of the wound pathogens had multiple antibiotic resistance showing resistance to 3 or more different antibiotics used for the study.

Table 3: The minimum inhibitory concentrations (MICs) of the methanol extracts of *Nephrolepis biserrata, Platycerium stemaria* and *Platycerium angolense* against isolated wound pathogens

Codes	ISOLATES	Minimum Inhibitory Concentrations (MICs) (mg/mL) of		
		Nephrolepis biserrata	*Platycerium stemaria*	*Platycerium angolense*
1	*Bacillus spp*	12.5	-	12.5
2A	*Enterobacter aerogenes*	25	-	-
2B	*Staphylococcus aureus*	-	-	-
3B	*Klebsiella pneumoniae*	-	-	25
4A	*Proteus mirabilis*	25	-	-
5A	*Staphylococcus aureus*	12.5	12.5	12.5
6A	*Micrococcus luteus*	12.5	12.5	-

7A	*Staphylococcus aureus*	6.25	-	-
7B	*Micrococcus luteus*	12.5	12.5	12.5
8A	*Bacillus spp*	-	-	-
8B	*Pseudomonas aeruginosa*	6.25	3.125	-
10	*Pseudomonas aeruginosa*	-	-	6.25
12	*Escherichia coli*	-	12.5	12.5
13	*Micrococcus luteus*	12.5	-	12.5
14	*Enterobacter aerogenes*	3.125	-	6.25
15	*Pseudomonas aeruginosa*	3.125	-	3.125
17	*Staphylococcus aureus*	-	-	-
18	*Micrococcus luteus*	3.125	-	-
19	*Proteus mirabilis*	-	-	-
20	*Micrococcus luteus*	12.5	-	12.5

Table 3 shows the minimum inhibitory concentrations of the methanol extracts of *Nephrolepis biserrata, Platycerium stemaria* and *Platycerium angolense* against isolated wound pathogens. No MIC was recorded where the organism displayed equal susceptibility to the extract and the solvent control. *N. biserrata* was active against 2 of the 3 strains of *Ps. aeruginosa*, 1 of 2 *Proteus mirabilis*, 1 of 2 *Bacillus subtilis*, and 2 of 4 *S. aureus*. However, all the strains of *Enterobacter aerogenes* and *Micrococcus luteus* isolated in the study were susceptible with no activity recorded against *E. coli* and *K. pneumoniae*, respectively. *Platycerium stemaria* showed activity against *P. aeruginosa* (1 of 3), *E. coli*, *Micrococcus luteus* (2 of 5) and *S. aureus* (1 of 4). It has no activity against *P. mirabilis*, *K. pneumoniae*, *Enterobacter aerogenes* and *Bacillus subtilis*. While no activity was recorded for *Platycerium angolense* against *Proteus mirabilis* and *Bacillus subtilis*, it displayed activity against P. aeruginosa (2 of 3), *Enterobacter aerogenes* (1 of 2), *E. coli*, *K. pneumoniae*, *Micrococcus luteus* (3 of 5), and *S. aureus* (1 of 4).

The range of MIC of *Nephrolepis biserrata, Platycerium stemaria* and *Platycerium angolense* is also shown in Table 3. While *Nephrolepis biserrata* has activity against thirteen (13) of twenty (20) isolates with MIC between 3.125 and 25 mg/mL, *Platycerium stemaria* and *Platycerium angolense* have activity against five (5) and ten (10) isolates, respectively. However, the MIC of *P. stemaria* ranged between 3.125 and 12.5mg/mL while that of *P. angolense* ranged between 3. 125 and 25mg/mL.

Table 4: Qualitative phytochemical constituents of the plant extracts

S/N	Phytochemicals	*Nephrolepis Biserrata*	*Platycerium Stemaria*	*Platycerium angolense*
1	Alkaloids (Drangendorff and Mayer's)	-	-	-
2	Flavonoids	+	+	+
3	Glycosides	-	-	-
4	Tannins	+	-	+
5	Saponins	+	++	+
6	Phenol	+	+	+
7	Terpenoids	-	+	+

KEYS - ++: Present (Sustained); +: Present; -: Absent

Table 4 shows the distribution of phytoconstituents in the 3 selected lower plants. While none of the plants had alkaloids and glycosides as phytoconstituents, all the selected plants studied had flavonoids, saponins and phenol. However, while only the *Platycerium stemaria* did not contain tannins, only the *Nephrolepis biserrata* did not contain terpenoids.

Discussion:

Physical, chemical or mechanical agents can cause a loss of integrity and normal functions of the skin resulting in a wound[12-14]. A wound that has not yet progressed through the sequential stages of healing and takes between 7 and 10 days to heal is considered an acute wound while chronic wounds do not progress through the normal stages of healing and are not repaired in an orderly and timely manner[15]. One of the determinants of wound healing timeline is the wound infection. An infected wound affects the quality of life, and compromises the wound's healing rate[16]. Wound infections represent one third of nosocomial infections among surgical patients and are responsible for 70–80% of mortality[16-18]. Wound infections are associated with morbidity and mortality in patients especially in developing countries, regardless by the type of wound[16,17,19]. Several species of microorganisms have been reported to be associated with wound infections.

In this study, eight (8) species of bacteria were isolated. They include *E. coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter aerogenes, Proteus mirabilis, Micrococcus luteus, Bacillus subtilis* and *Staphylococcus aureus* (Table 1). Some of these bacterial isolates have been reported by several authors. For instance, Puca *et al*.[20] reported the association of *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Acinetobacter baumannii/haemolyticus* and *Staphylococcus aureus* with wounds in their study in Italy. However, while the percentage occurrences of Gram-negative and Gram-positive were 57.9 and 36.6, respectively in their study, the percentages were 45 and 55, respectively in this study. Moreover, fungi as *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, and *Candida stellatoidea* were also reported in their study. In a similar vein, Appapalam *et al*.[21] reported the presence of *Pseudomonas aeruginosa, Escherichia coli, Proteus* spp, *Acinetobacter* spp, *Enterobacter* spp, *Klebsiella pneumoniae, Citrobacter* spp, *K. oxytoca*, and

Stenotrophomonas spp as Gram-negative wound pathogens while Gram-positive bacteria such as *Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis, Corynebacterium* spp, and *Streptococcus dysgalactiae* were also identified in their study involving wound sites of diabetic foot ulcer patients. Dau *et al*.[22] reported the presence of *Acinetobacter* spp., coagulase-negative staphylococci, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* spp during their analysis of surgical wound infections of 1200 patients injured during the Libyan conflict in 2011. Odedina *et al*.[23] reported *Staphylococcus aureus, E. coli* and *P. aeruginosa* as bacterial isolates from wound infections at Federal medical Centre, Bida, Niger State, Nigeria. Gadzama *et al*.[24] identified *Staphylococcus aureus, E. coli, Klebsiella spp, Proteus spp* and *P. aeruginosa* as wound pathogens from their work at the University of Maiduguri Teaching Hospital, Maiduguri, Nigeria. In this study, *Micrococcus luteus* and *Staphylococcus aureus* were the predominant Gram-positive bacteria isolated with 25 and 20% occurrence respectively. These two organisms are structurally similar but phylogenetically different. While *M. luteus* is strictly an obligate aerobe capable of producing acid from glucose only aerobically, *S. aureus* is a facultative anaerobe which can produce acid from glucose both aerobically and anaerobically. Also, while *M. luteus* is oxidase positive, *S. aureus* is oxidase-negative. *M. luteus* is found in a range of habitats such as soil[25,26], sea water[27,28], fresh water[29,30] and surfaces such as clothing and human skin[31,32]. However, *S. aureus* is found on the skin surface, being its natural habitat. The strong association between the two species of bacteria and skin surfaces may be responsible for their being the predominant wound pathogens in this study. On the other hand, *Pseudomonas aeruginosa* was the predominant Gram-negative bacterial species isolated in this study. *P. aeruginosa* has been implicated in chronic wounds as its virulence factors have been reported to alter the ability of a wound to heal. The attributes of *P. aeruginosa* to delay wound healing include its capacity to (i) enhance the virulence of other pathogens with which it cohabitate the same wound site[33,34], (ii) alter the pharmacodynamics of antibacterials directed at other organisms[35,36], and (iii) influence the structure of polymicrobial biofilms[34,37].

The treatment of wounds is of great importance to human health as delay or lack of healing of wound may have social implications on the lives of the patients and their families, and results in the prolongation of treatment time, increased cost of treatment, and increased hospital visits[38,39]. The success of infected wound treatment using antibiotics lies on the infected organisms being susceptible to the antibiotic of choice.

In this study, susceptibility profiles of the isolated bacteria to conventional antibiotics were evaluated. The organisms displayed varying susceptibilities to the same and/or different antibiotics used in the study (Table 2). Mechanisms of action of these antibiotics involve inhibition of any of the processes of DNA replication, protein biosynthesis, cell wall biosynthesis and folic acid metabolism[40]. In this study, Piperacillin/Tazobactam and chloramphenicol are the drugs of choice as far as susceptibility profile study is concerned. Piperacillin inhibits cell wall synthesis by binding to bacterial cell membranes while Tazobactam inactivates bacterial beta-lactamase thereby protecting piperacillin from enzymatic degradation, extends its spectrum of activity, and prevents bacterial overgrowth. Resistance to this drug combination can be driven through hyperproduction of beta-lactamase enzyme.

Chloramphenicol, on the other hand, is a bacteriostatic antibiotic that binds to the 50S ribosomal subunit and inhibits the peptidyltransferase step in protein synthesis. Resistance to chloramphenicol is mostly due to inactivation of the antibiotic by a chloramphenicol acetyltransferase (CAT) enzyme that acetylates the antibiotic.

However, improper wound care using antibiotics can lead to more problems in patients. Example of such problems is the development of resistance by the infecting organisms to antibiotics. An increased resistance to antibiotics by wound pathogens has necessitated the search for more effective and efficacious antimicrobials from plant source. According to the World Health Organization (WHO), it is estimated that almost 80% of the world's population uses traditional practices as health treatment; of these, 85% use plant remedies[41].

While some medicinal plants have been evaluated for their wound healing property, lower plants as *Nephrolepis biserrata, Platycerium stemaria* and *Platycerium angolense* have not been evaluated although there are reports of their ethnobotanical uses for wound healing.

One of the properties often associated with agents used for wound healing is the antimicrobial activity, among others. Antimicrobial activity is often quantified using the minimum inhibitory concentration (MIC) which has been defined as the minimum concentration of an antimicrobial agent that will prevent the growth of a named organism under specified conditions. This implies that MIC varies with the concentration of the antimicrobial agent, number and nature of the organism and conditions of the test, among

other variables. The lower the MIC, the higher the potency of the agent.

In this study, antimicrobial activity of the methanol extracts of the 3 selected lower plants against the isolated wound pathogens was evaluated (Table 3). While *Nephrolepis biserrata* had activity against 65% of the isolates with MIC between 3.125 and 25 mg/mL, *Platycerium stemaria* and *Platycerium angolense* had activity against 25% and 50% of the isolates, respectively. However, the MIC of *P. stemaria* ranged between 3.125 and 12.5mg/mL while that of *P. angolense* ranged between 3.125 and 25mg/mL.

However, the study noted a correlation between azithromycin resistance and susceptibility to the activity of the 3 selected lower plants studied as all the isolated wound pathogens (2B, 8A, 17 & 19) that were resistant to azithromycin were also not susceptible to the methanol extracts of the 3 plants. One of the ways by which resistance to azithromycin can be developed is through decreased uptake of drugs via increased extrusion by efflux pumps[42]. The efflux pumps are the membrane proteins that export the antibiotics out of the cell and keep its intracellular concentrations at low levels. Efflux pumps affect all classes of antibiotics and vary in both their specificity and mechanism.

Antimicrobial activity of plant extracts has been attributed to the presence of effective antimicrobial phytochemicals at concentrations enough to prevent the growth of pathogens or to kill them.

In this study, phytochemical analysis of methanol extracts of the 3 selected lower plants was undertaken. The presence of flavonoids, Tannins, Saponins, phenol and terpenoids was established (Table 4). This finding is in agreement with the reports of Ibiye *et al*.[43] and Bode and Oyedapo[44]. However, the antimicrobial activity of all these phytochemicals had been reported[45-48].

Conclusion: The work concluded that methanol extracts of *N. biserrata, Platycerium stemaria and Platycerium angolense* leaves showed antimicrobial activity against isolated wound pathogens and could be useful in the management of wounds. The results from this study support the ethnobotanical uses of the plants for wound treatment.

References

1. Kujath P and Michelsen A (2008) Wounds–from physiology to wound dressing. *Deutsches Ärzteblatt International*, 105(13):239-248. https://doi.org/10.3238/arztebl.2008.0239
2. Basu S and Shukla V (2013) Complications of wound healing. In: Mani R, Romanelli M, Shukla V (eds). Measurements in Wound Healing: Science and Practice. 1st edn, Springer London, 109-144.
3. Falanga V, Isseroff RR, Soulika AM, Romanelli M, Margolis D, Kapp S, Granick M, Harding K (2022) Chronic wounds. *Nature Reviews Disease Primers*, 8(1):50 https://doi.org/10.1038/s41572-022-00377-3
4. Yazarlu O, Iranshahi M, Kashani HRK, Reshadat S, Habtemariam S, Iranshahy M, Hasanpour M (2021) Perspective on the application of medicinal plants and natural products in wound healing: A mechanistic review. *Pharmacological Research,* 174:105841. https://doi.org/10.1016/j.phrs.2021.105841
5. Puca V, Marulli RZ, Grande R, Vitale I, Niro A, Molinaro G, Prezioso S, Muraro R, Di Giovanni P (2021) Microbial species isolated from infected wounds and antimicrobial resistance analysis: Data emerging from a three-year retrospective study. *Antibiotics,* 10(10):1162. https://doi.org/10.3390/antibiotics10101162
6. Nishanthy M and Saikumar C (2017) Bacteriological Profile and Antibiotic Susceptibility Patterns of Bacteria Isolated From Wound Swab Samples from Patients Attending a Tertiary Care Hospital. *Scholars Journal of Applied Medical Sciences,* 5(11C):4512-4516. doi: 10.36347/sjams.2017.v05i11.040
7. Sorg H, Tilkorn DJ, Hager S, Hauser J, Mirastschijski U (2017) Skin wound healing: an update on the current knowledge and concepts. *European Surgical Research*, 58(1-2):81-94. https://doi.org/10.1159/000454919
8. Ahmad I, Malak HA and Abulreesh HH (2021) Environmental antimicrobial resistance and its drivers: a potential threat to public health. *Journal of Global Antimicrobial Resistance*, 27:101-111. https://doi.org/10.1016/j.jgar.2021.08.001
9. Cowan and Steel's Manual for the Identification of Medical Bacteria (1993) Cambridge University Press pp 175 – 178
10. Clinical and Laboratory Standard Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 30th Edition, M100, 2020
11. Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S (2023) Phytochemical Screening and

Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. *Journal of Experimental Pharmacology,* 15:51–62. https://doi.org/10.2147/JEP.S379805

12. Kumar B, Vijayakumar M, Govindarajan R, Pushpangadan P (2007) Ethnopharmacological approaches to wound healing–exploring medicinal plants of India. *Journal of Ethnopharmacology*, 114: 103–113. https://doi.org/10.1016/j.jep.2007.08.010
13. Enoch S and Leaper DJ (2008) Basic science of wound healing. *Surgery,* 26: 31–37. https://doi.org/10.1016/j.mpsur.2007.11.005
14. Imran H, Ahmad M, Rahman A, Yaqeen Z, Sohail T, Fatima N, Iqbal W, Yaqeen SS (2015) Evaluation of wound healing effects between *Salvadora persica* ointment and Solcoseryl jelly in animal model. *Pakistan Journal of Pharmaceutical Sciences,* 28: 1777–1780.
15. Li J, Chen J, and Kirsner R (2007) Pathophysiology of acute wound healing. *Clinics in Dermatology*, 25: 9–18. https://doi.org/10.1016/j.clindermatol.2006.09.007
16. Pallavali RR, Degati VL, Lomada D, Reddy MC, Durbaka VRP (2017) Isolation and in vitro evaluation of bacteriophages against MDR-bacterial isolates from septic wound infections. *PLoS ONE*, 12: e0179245. https://doi.org/10.1371/journal.pone.0179245
17. Anguzu JR and Olila D (2007) Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. *African Health Sciences*, 7: 148–154. https://doi.org/10.5555/afhs.2007.7.3.148
18. Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, Levin A (2007) Acute Kidney Injury Network. Acute Kidney Injury Network: Report of an initiative to improve outcomes in acute kidney injury. *Critical Care*, 11: R31. https://doi.org/10.1186/cc5713
19. Agnihotri N, Gupta V and Joshi RM (2004) Aerobic bacterial isolates from burn wound infections and their antibiograms–a five-year study. *Burns,* 30: 241–243. https://doi.org/10.1016/j.burns.2003.11.010
20. Puca V, Marulli RZ, Grande R, et al. (2021) Microbial Species Isolated from Infected Wounds and Antimicrobial Resistance Analysis: Data Emerging from a Three-Years Retrospective Study. *Antibiotics* (*Basel*), 10(10):1162. https://doi.org/10.3390/antibiotics10101162
21. Appapalam ST, Muniyan A, Vasanthi Mohan K, Panchamoorthy R (2021) A Study on Isolation, Characterization, and Exploration of Multiantibiotic-Resistant Bacteria in the Wound Site of Diabetic Foot Ulcer Patients. *International Journal of Lower Extremity Wounds*, 20(1):6-14. https://doi.org/10.1177/1534734619884430
22. Dau AA, Tloba S and Daw MA (2013) Characterization of wound infections among patients injured during the 2011 Libyan conflict. *Eastern Mediterranean Health Journal,* 19(4): 356-361
23. Odedina EA, Eletta EA, Balogun RA, Idowu O (2008) Isolates from wound infections at Federal Medical Centre, Bida. *African Journal of Clinical and Experimental Microbiology*, 9(2): 26-32
24. Gadzama GB, Zailani SB, Abubakar D, Bakari AA (2007) Bacterial pathogens associated with wound infections at the University of Maiduguri Teaching Hospital, Maiduguri, Nigeria. *Kanem Journal of Medical Sciences,* 1(1):26-28
25. Sims GK, Sommers LE, Konopka A (1986) Degradation of pyridine by *Micrococcus luteus* isolated from soil. *Applied and Environmental Microbiology,* 51:963–968. https://doi.org/10.1128/aem.51.5.963-968.1986
26. Ghosh A, Chaudhary SA, Apurva SR, Tiwari T, Gupta S, Singh AK, Katudia KH, Patel MP, Chikara SK (2013) Whole-genome sequencing of *Micrococcus luteus* strain Modasa, of Indian origin. *Genome Announcements*, 1:e0007613. https://doi.org/10.1128/genomeA.00076-13
27. Mohanrasu K, Premnath N, Siva Prakash G, Sudhakar M, Boobalan T, Arun A (2018) Exploring multi potential uses of marine bacteria: an integrated approach for PHB production, PAHs and polyethylene biodegradation. *Journal of Photochemistry and Photobiology B: Biology,* 185:55–65. https://doi.org/10.1016/j.jphotobiol.2018.05.014
28. Ferrari VB, Cesar A, Cayô R, Choueri RB, Okamoto DN, Freitas JG, Favero M, Gales AC, Niero CV, Saia FT, de Vasconcellos SP (2019) Hexadecane biodegradation of high efficiency by bacterial isolates from Santos Basin sediments. *Marine Pollution Bulletin*, 142:309–314.

https://doi.org/10.1016/j.marpolbul.2019.03.050
29. López L, Pozo C, Rodelas B, Calvo C, Juárez B, Martínez-Toledo MV, González-López J (2005) Identification of bacteria isolated from an oligotrophic lake with pesticide removal capacities. *Ecotoxicology,* 14:299–312. https://doi.org/10.1007/s10646-003-6367-y
30. Min KR, Zimmer MN, Rickard AH (2010) Physicochemical parameters influencing coaggregation between the freshwater bacteria *Sphingomonas natatoria* 2.1 and *Micrococcus luteus* 2.13. *Biofouling,* 26:931–940. https://doi.org/10.1080/08927014.2010.531128
31. Chiereghin A, Felici S, Gibertoni D, Foschi C, Turello G, Piccirilli G, Gabrielli L, Clerici P, Landini MP, Lazzarotto T (2020). Microbial contamination of medical staff clothing during patient care activities: performance of decontamination of domestic versus industrial laundering procedures. *Current Microbiology,* 77:1159–1166. https://doi.org/10.1007/s00284-020-01919-2
32. Khayyira AS, Rosdina AE, Irianti MI, Malik A (2020). Simultaneous profiling and cultivation of the skin microbiome of healthy young adult skin for the development of therapeutic agents. *Heliyon*, 6:e03700. https://doi.org/10.1016/j.heliyon.2020.e03700
33. Nguyen AT and Oglesby-Sherrouse AG (2016) Interactions between *Pseudomonas aeruginosa* and *Staphylococcus aureus* during co-cultivations and polymicrobial infections. Applied Microbiology and Biotechnology, 100: 6141–6148. https://doi.org/10.1007/s00253-016-7596-3
34. Cendra MDM and Torrents E (2021) *Pseudomonas aeruginosa* biofilms and their partners in crime. *Biotechnology Advances,* 49: 107734.https://doi.org/10.1016/j.biotechadv.2021.107734
35. Orazi G, Jean-Pierre F and O'Toole GA (2020) *Pseudomonas aeruginosa* PA14 Enhances the Efficacy of Norfloxacin against *Staphylococcus aureus* Newman Biofilms. *Journal of Bacteriology*, 202: e00159-20. https://doi.org/10.1128/JB.00159-20
36. Trizna EY, Yarullina MN, Baidamshina DR, Mironova AV, Akhatova FS, Rozhina EV, Fakhrullin RF, Khabibrakhmanova AM, Kurbangalieva AR, Bogachev MI, Kayumov AR (2020) Bidirectional alterations in antibiotics susceptibility in *Staphylococcus aureus-Pseudomonas aeruginosa* dual-species biofilm. *Scientific Reports*, 10: 14849. https://doi.org/10.1038/s41598-020-71834-w
37. Magalhães AP, Jorge P and Pereira MO (2019) *Pseudomonas aeruginosa* and *Staphylococcus aureus* communication in biofilm infections: Insights through network and database construction. *Critical Reviews in Microbiology*, 45: 712–728. https://doi.org/10.1080/1040841X.2019.1700209
38. Cacioppo JT and Hawkley LC (2003) Social isolation and health, with an emphasis on underlying mechanisms. *Perspectives in Biology and Medicine*, 46: S39–S52
39. Young A and McNaught C-E (2011) The physiology of wound healing. Surgery 29: 475–479. https://doi.org/10.1016/j.mpsur.2011.06.011
40. Halawa E M, Fadel M, Al-Rabia MW, Behairy A, Nouh NA, Abdo M, Olga R, Fericean L, Atwa AM, El-Nablaway M and Abdeen A (2024) Antibiotic action and resistance: updated review of mechanisms, spread, influencing factors, and alternative approaches for combating resistance. *Frontiers in Pharmacology,* 14:1305294. https://doi.org/10.3389/fphar.2023.1305294
41. Ayaz M, Subhan F, Ahmed J, Khan AU, Ullah F, Ullah I, Ali G, Syed NI, Hussain S (2015) Sertraline enhances the activity of antimicrobial agents against pathogens of clinical relevance. *Journal of Biological Research – Thessaloniki,* 22: 4. https://doi.org/10.1186/s40709-015-0028-1
42. Chiou C, Hong Y, Wang Y, Chen B, Teng R, Song H, Liao Y (2023) Antimicrobial Resistance and Mechanisms of Azithromycin Resistance in Nontyphoidal Salmonella Isolates in Taiwan, 2017 to 2018. *Microbiology Spectrum,* 11: e03364-22. https://doi.org/10.1128/spectrum.03364-22
43. Ibiye A, Green BO, Ajuru MG, Chikere LC (2024) Phytochemical and proximate compositions of frond extracts of *Nephrolepis biserrata*, *Phymatosorus scolopendria*, and *Microgramma mauritiana* in Rivers State University, Nigeria. *Asian Journal of Natural Product Biochemistry*, 22(1): 1-7.

https://doi.org/10.13057/biofar/f220101

44. Bode SO and Oyedapo OO (2010) Biological activities and phytoconstituents of the lower plant *Platycerium angolense*, Welwex Hook. *Journal of Medicinal Plants Research*, 5(8): 1321-1329

45. Yan Y, Li X, Zhang C, Lv L, Gao B, Li M (2021) Research Progress on Antibacterial Activities and Mechanisms of Natural Alkaloids: A Review. *Antibiotics (Basel*, Switzerland), 10(3): 318. https://doi.org/10.3390/antibiotics10030318

46. Shamsudin NF, Ahmed QU, Mahmood S, Ali Shah SA, Khatib A, Mukhtar S, Alsharif MA, Parveen H, Zakaria ZA (2022) Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative Interpretation. *Molecules (Basel, Switzerland),* 27(4): 1149. https://doi.org/10.3390/molecules27041149

47. Li J and Viviana M (2023) *In Vitro* and In Silico Studies of Antimicrobial Saponins: A Review. *Processes*, 11(10): 2856. https://doi.org/10.3390/pr11102856

48. Czerkas K, Olchowik-Grabarek E, Łomanowska M, Abdulladjanova N, Sękowski S (2024) Antibacterial Activity of Plant Polyphenols Belonging to the Tannins against *Streptococcus mutans*—Potential against Dental Caries. *Molecules*, 29: 879. https://doi.org/10.3390/molecules29040879