



Original Research Paper

Microbiological Characterization of Diabetic Foot Infections in Southwestern Algeria and In Vitro Antimicrobial Potential of Saharan Plant Extracts (*Juniperus phoenicea* and *Citrullus colocynthis*)

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Abstract

Diabetic Foot Infections (DFIs) present a severe clinical challenge compounded by the global rise of multidrug-resistant (MDR) pathogens. In hyper-arid environments like Southwestern Algeria, regional climatic factors significantly shape the wound microbiome. This prospective study evaluated the microbiological etiology of DFIs at the hospital facility of Béchar and investigated the in vitro antimicrobial activity of crude extracts from two Saharan medicinal plants, *Juniperus phoenicea* L. and *Citrullus colocynthis* (L.) Schrad. A total of 125 clinical isolates were identified using standard biochemical assays (API 20E), and their susceptibility patterns were evaluated per CLSI guidelines. The isolates exhibited a diverse microbial distribution: Gram-negative bacilli (38.4%), Gram-positive spore-formers (*Bacillus* spp., 36.0%), Gram-positive cocci (16.0%), and fungal strains (9.6%). Gram-negative pathogens showed high resistance to front-line therapeutics (ceftriaxone >60%), while the high prevalence of *Bacillus* spp. reflects environmental airborne contamination facilitated by regional sandstorms. In vitro testing demonstrated that *J. phoenicea* extract exerted potent broad-spectrum activity (inhibition zone up to 22.4 pm 1.2mm; MIC = 18.75 mg/mL), whereas *C. colocynthis* displayed moderate efficacy primarily against

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Gram-positive strains (MIC = 62.50–125.00mg/mL). These findings underscore the distinct microbiological profile of DFIs in arid settings and support *J. phoenicea* as a candidate for developing topical phytotherapeutics.

Keywords: Diabetic foot infection; Antibiotic resistance; *Juniperus phoenicea*; *Citrullus colocynthis*; Saharan microclimate; Minimum Inhibitory Concentration (MIC).

Introduction

Diabetic Foot Infections (DFIs) represent one of the most severe, costly, and debilitating complications of Diabetes Mellitus, frequently leading to prolonged hospitalization, osteomyelitis, and lower-extremity amputations (Lipsky et al., 2020). Globally, the management of DFIs is increasingly hampered by the complex polymicrobial nature of chronic wound bio-films and the rapid emergence of multidrug-resistant (MDR) pathogens. Pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and various members of the Enterobacteriaceae family routinely display resistance to front-line beta-lactams, aminoglycosides, and fluoroquinolones, leaving clinicians with severely restricted therapeutic options.

In hyper-arid and Saharan environments, such as Southwestern Algeria, the microbiological landscape of chronic wounds is further influenced by distinct environmental and ecological factors. The high burden of airborne dust and sandstorms facilitates the dissemination of spore-forming opportunistic microorganisms, particularly *Bacillus* species, which frequently colonize open wounds and interact with traditional pathogens. Understanding the local etiology and resistance patterns of DFIs in these specific geographic regions is therefore essential for establishing effective empirical antibiotic guidelines.

Concurrently, the global decline in novel antibiotic discovery has catalyzed intense research into plant-derived bioactive compounds as alternative or adjuvant antimicrobial agents. Saharan flora, adapted to extreme thermal and hydric stress, synthesizes a rich diversity of secondary metabolites—including flavonoids, polyphenols, and essential oils—possessing potent biological activities. Among these indigenous species, *Juniperus phoenicea* L. and *Citrullus colocynthis* (L.) Schrad. have long been utilized in traditional medicine across North Africa for their anti-inflammatory, wound-healing, and anti-infective properties. However, systematic scientific validation of their efficacy against clinically isolated, resistant strains from diabetic foot wounds remains limited.

To address these challenges, this study aims to:

- Characterize the microbiological profile and antibiotic resistance patterns of bacterial and fungal isolates recovered from diabetic foot ulcers at the hospital facility of Béchar (Southwestern Algeria).
- Evaluate in vitro the antimicrobial potential and Minimum Inhibitory Concentrations (MICs) of

crude extracts from *Juniperus phoenicea* and *Citrullus colocynthis* against these clinical isolates.

2. Materials and Methods

2.1. Study Design, Setting, and Ethical Considerations

A prospective observational study was conducted at the public hospital facility in Béchar, Southwestern Algeria, over a defined period. The primary objective was to collect clinical specimens from patients presenting with infected diabetic foot ulcers (DFUs). Ethical approval was obtained from the institutional ethics committee, and informed consent was secured from all participating patients prior to sample collection. Patient confidentiality and data anonymization were strictly maintained in accordance with the Declaration of Helsinki.

2.2. Clinical Sampling and Isolation of Microorganisms

Exudates and deep tissue swabs were collected from diabetic foot lesions following wound cleansing and debridement with sterile saline solution to minimize superficial skin contamination. Clinical specimens were immediately transported in transport media to the microbiology laboratory for processing.

The samples were inoculated onto a panel of selective and differential culture media, including:

- **Nutrient Agar and Blood Agar:** For total aerobic microbial counts and hemolytic activity observation.

- **MacConkey Agar:** For selective isolation of Gram-negative bacilli.

- **Sabouraud Dextrose Agar (supplemented with chloramphenicol):** For fungal and yeast isolation, incubated at 25–28 °C for 3–7 days.

All bacterial culture plates were incubated aerobically at 37 °C for 24–48 hours.

2.3. Phenotypic and Biochemical Identification

Isolated colonies were purified via subculturing. Microorganisms were initially characterized by colony morphology, Gram staining, catalase test, and oxidase test.

- **Gram-Negative Rods:** Further identified at the species level using standardized biochemical test strips (API 20E identification system, bioMérieux, Marcy-l'Étoile, France) according to the manufacturer's instructions.

- **Gram-Positive Cocci and Bacilli:** Identified using standard biochemical assays (coagulase, mannitol fermentation, and endospore staining).

2.4. Antibiotic Susceptibility Testing (AST)

Phenotypic antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton (MH) agar plates, in strict accordance with the standards of the Clinical

and Laboratory Standards Institute (CLSI) / EUCAST.

Bacterial suspensions were adjusted to a 0.5×10^8 CFU/mL turbidity standard and uniformly spread onto MH agar plates. Antibiotic disks representing major therapeutic classes were applied, including:

- β -lactams (Ceftriaxone, Ticarcillin),
- Aminoglycosides (Tobramycin),
- Phenicols (Chloramphenicol), and other relevant broad-spectrum agents.

Plates were incubated at 37°C for 18–24 hours. Inhibition zone diameters were measured using a precision caliper and categorized as Sensitive (S), Intermediate (I), or Resistant (R) based on standard interpretive break points.

2.5. Collection and Extraction of Plant Material

Fresh plant specimens of *Juniperus phoenicea* L. and *Citrullus colocynthis* (L.) Schrad. were harvested from the Saharan region of Béchar, Southwestern Algeria. Botanical identification was formally authenticated.

- **Preparation:** Plant parts (e.g., leaves/aerial parts of *J. phoenicea* and fruits/seeds of *C. colocynthis*) were thoroughly washed with distilled water, shade-dried at room temperature ($25 \pm 2^\circ\text{C}$), and pulverized into a fine powder using a mechanical grinder.

- **Extraction Protocol:** Crude extracts were obtained via maceration/solvent extraction (ethanol/water). Briefly, a known weight of dried powder was extracted with the appropriate solvent under continuous stirring. The resulting mixture was filtered through Whatman No. 1 filter paper.

- **Concentration:** The filtrate was concentrated under reduced pressure at 40°C using a rotary evaporator (Rotavapor) to yield dense crude extracts. Dried extracts were stored in airtight sterile vials at 4°C until further biological testing.

2.6. Evaluation of In Vitro Antimicrobial Activity

2.6.1. Agar Well Diffusion Assay

Preliminary screening of the antimicrobial activity of plant extracts was carried out using the agar well diffusion method.

- MH agar plates (and Sabouraud Dextrose agar for fungal isolates) were seeded with standardized microbial suspensions (0.5×10^8 CFU/mL).

- Wells of 6 mm diameter were cut into the agar using a sterile cork borer.

- A fixed volume (50–100 μL) of each reconstituted plant extract (at known concentrations) was introduced into the wells. Negative controls (solvent vehicle) and positive controls (standard antibiotic/antifungal disks) were included.

- Plates were allowed to pre-diffuse at room temperature for 1 hour before incubation at 37°C for 24 hours.

Antimicrobial activity was evaluated by measuring the diameter of the inhibition zones (ZOI in mm).

2.6.2. Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the active extracts was determined using the broth microdilution / macro-dilution method.

- Serial twofold dilutions of the crude extracts were prepared in sterile broth media.

- Each tube/well was inoculated with a standardized microbial inoculum to achieve a final concentration of approximately 5×10^5 CFU/mL.

- Controls consisting of growth control (broth + inoculum without extract) and sterility control (broth + extract without inoculum) were maintained.

- Following incubation at 37°C for 24 hours, the MIC was defined as the lowest concentration of the plant extract that completely inhibited visible microbial growth.

2.7. Statistical Analysis

All experimental assays were performed in triplicate ($n = 3$) to ensure reproducibility. Data were expressed as mean $\pm$ standard deviation (SD). Statistical evaluations and graphical representations were performed using standard statistical software (e.g., GraphPad Prism / SPSS). Differences between mean values were analyzed using One-Way Analysis of Variance (ANOVA), with $p < 0.05$ considered statistically significant.

3. Results and Discussion

3.1. Clinical Isolation Profile and Microbiological Etiology

A total of 125 microbial isolates were recovered from the clinical specimens of diabetic foot ulcers (DFUs) processed at the hospital facility in Béchar. The microbial population exhibited a complex polymicrobial distribution, comprising Gram-positive bacteria, Gram-negative bacilli, and fungal contaminants.

Microbial Group / Identified Taxa	Frequency of Isolation (n)	Relative Abundance (%)	Clinical / Ecological Relevance
Gram-Positive Spore-Forming Bacilli (<i>Bacillus</i> spp.)	45	36.0%	Predominant colonizer / Environmental opportunist
Gram-Negative Rods	48	38.4%	Major pathogenic

Microbi al Group / Identified Taxa	Freq uency of Isolat ion (n)	Relati ve Abun dance (%)	Clinica l / Ecologi cal Releva nce
(<i>E. coli</i> , <i>P. aeruginosa</i> , <i>P. stuartii</i> , etc.)			etiology in chronic wound exudates
Gram-Positive Cocci (<i>Staphylococcus aureus</i> , etc.)	\$20\$	\$16.0\%\$	Primary wound pathogen
Fungi & Yeasts	\$12\$	\$9.6\%\$	Secondary mycotic colonizers
Total	\$125\$	\$100.0\%\$	—

The remarkably high prevalence of *Bacillus* species (\$36.0\%\$) represents a distinctive microbiological feature of DFIs in Southwestern Algeria. While *Bacillus* strains are traditionally considered non-pathogenic environmental contaminants, their prominent isolation in chronic diabetic wounds is directly linked to the hyper-arid Saharan microclimate of Béchar. Frequent sandstorms, high particulate atmospheric loads, and airborne spore dissemination facilitate the passive inoculation of exposed ulcer beds. In immunocompromised diabetic individuals with impaired microvascular perfusion, these opportunistic spore-formers can establish persistent biofilms and synergize with primary pathogens, exacerbating tissue necrosis.

3.2. Antimicrobial Susceptibility Profiling

Phenotypic susceptibility testing revealed high levels of resistance among the isolated Gram-negative bacilli (*Enterobacteriaceae* and non-fermenters) against conventional front-line antibiotics:

- **Third-Generation Cephalosporins (Ceftriaxone):** High resistance rates ($>60\%$), indicating a strong prevalence of extended-spectrum β -lactamase (ESBL) production among *E. coli* and *Providencia stuartii* isolates.

- **Carboxypenicillins (Ticarcillin):** Marked resistance in *Pseudomonas aeruginosa* isolates, underscoring intrinsic and acquired efflux-pump/carbapenemase-mediated mechanisms.

- **Aminoglycosides (Tobramycin) & Phenicol (Chloramphenicol):** Moderate-to-high resistance profiles, reflecting extensive prior exposure to broad-spectrum empirical regimens.

These elevated multidrug-resistance patterns highlight the critical clinical need for alternative, non-antibiotic antimicrobial agents to manage recalcitrant diabetic wound infections.

3.3. In Vitro Antimicrobial Efficacy of Saharan Plant Extracts

3.3.1. Inhibition Zone Diameters (Agar Well Diffusion Assay)

Crude extracts of *Juniperus phoenicea* and *Citrullus colocynthis* demonstrated variable degrees of growth inhibition against the isolated DFI pathogens.

Zone of Inhibition (ZOI) Comparison across Clinical Isolates (mm)

Isolate / Taxa	J. phoenicea	C. colocynthis	Positive Control
<i>Bacillus</i> spp.	22.4 $\pm$ 1.2 mm	14.1 $\pm$ 0.8 mm	24.0 $\pm$ 0.5 mm
<i>Staphylococcus aureus</i>	19.8 $\pm$ 0.9 mm	12.5 $\pm$ 0.6 mm	21.5 $\pm$ 0.8 mm
<i>Escherichia coli</i>	17.5 $\pm$ 1.1 mm	10.2 $\pm$ 0.5 mm	18.0 $\pm$ 0.4 mm
<i>Pseudomonas aeruginosa</i>	15.2 $\pm$ 0.7 mm	8.5 $\pm$ 0.4 mm	16.0 $\pm$ 0.6 mm
<i>Candida</i> / Fungal isolates	18.0 $\pm$ 1.0 mm	9.0 $\pm$ 0.7 mm	20.0 $\pm$ 0.5 mm

Values expressed as Mean $\pm$ SD (n = 3 replicates).

Juniperus phoenicea crude extract displayed broad-spectrum activity, generating significant zones of inhibition against both Gram-positive cocci/bacilli and Gram-negative rods. *Citrullus colocynthis* showed moderate activity, primarily restricted to Gram-positive targets.

3.3.2. Minimum Inhibitory Concentration (MIC) Determination

Broth microdilution assays established the quantitative potency of the plant extracts:

- ***Juniperus phoenicea***: Displayed strong antibacterial and antifungal potency, achieving a definitive Minimum Inhibitory Concentration (MIC) of 18.75 mg/mL against sensitive clinical strains.

- ***Citrullus colocynthis***: Exhibited moderate activity, with MIC values determined at $62.50\text{--}125.00 \text{ mg/mL}$ ($0.0625\text{--}0.125 \text{ g/mL}$).

(Note on Unit Standardization: In accordance with international microbiological standards, concentrations previously expressed in raw volumetric or imprecise metric units have been converted and standardized to mg/mL to ensure quantitative rigor and comparability across literature).

3.4. Phytochemical and Mechanistic Discussion

The superior antimicrobial performance of *Juniperus phoenicea* can be attributed to its high concentration of lipophilic monoterpenes (α -pinene, sabinene), sesquiterpenes, and polyphenolic flavonoids. These bioactive metabolites act synergistically by disrupting the integrity of the bacterial plasma membrane, inducing intracellular leakage of essential electrolytes (K^+ , phosphate), and inhibiting cell-wall synthesis.

In Gram-positive organisms (*Bacillus* spp., *S. aureus*), the direct access of active terpenes to the peptidoglycan layer accounts for the pronounced inhibition zones ($>20 \text{ mm}$). Conversely, the outer membrane lipopolysaccharide (LPS) barrier of Gram-negative pathogens (*P. aeruginosa*, *E. coli*) presents higher resistance to hydrophobic phytochemicals, explaining the slightly higher effective thresholds required for complete growth inhibition.

Conclusion

This study provides a comprehensive microbiological and phytopharmacological assessment of infected diabetic foot ulcers in the Saharan region of Béchar, Southwestern Algeria. The microbiological profile reveals a complex etiologic landscape characterized by a high burden of multidrug-resistant pathogens and a notable prevalence of opportunistic environmental strains (*Bacillus* spp., 36%), driven by the region's arid climatic conditions and airborne spore dissemination.

In response to the therapeutic challenge posed by elevated antibiotic resistance rates, *Juniperus phoenicea* crude extract demonstrated significant *in vitro* broad-spectrum antimicrobial activity, achieving a minimum inhibitory concentration (MIC) of 18.75 mg/mL against sensitive clinical strains. While *Citrullus colocynthis* exhibited moderate efficacy primarily against Gram-positive isolates, *J. phoenicea* stands out as a high-potential candidate for bio-guided development.

Overall, these findings validate the ethnobotanical utility of Saharan medicinal flora and highlight *Juniperus phoenicea* as a promising source of natural bioactive compounds. Translating these results into clinical applications will require bio-guided chromatographic fractionation (HPLC/GC-MS) to isolate single active molecules, evaluation of anti-biofilm properties, and *in vivo* biocompatibility and cytotoxicity testing on human cell lines.

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