

## ANTIMICROBIAL ACTIVITY OF COMMIPHORA MOLMOL, CURCUMA LONGA, DRAGON'S BLOOD RESIN, AND PROPOLIS AGAINST DIABETIC FOOT ULCER PATHOGENS: AN INVITRO EVALUATION

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### ABSTRACT

**Background:** Diabetic foot ulcers (DFUs) are among the most devastating complications of diabetes mellitus, with polymicrobial infection being the primary driver of wound chronicity and limb amputation. Despite advances in wound care, conventional antibiotic therapy is increasingly limited by rising antimicrobial resistance, poor vascular delivery to the wound site, and inability to address the multi-dimensional pathology of the chronic DFU environment. Polyherbal formulations combining bioactive plant extracts represent an emerging complementary therapeutic strategy. **Objective:** This study aimed to evaluate the *in vitro* antimicrobial activity of a polyherbal Botanical Extract containing hydroalcoholic extracts of *Commiphora molmol* (myrrh), *Curcuma longa* (turmeric), Dragon's Blood resin (*Dracaena cinnabari*), and propolis individually and in combination against the three most clinically prevalent bacterial pathogens in DFU infections: *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. **Methods:** Antimicrobial activity was assessed

using the Kirby-Bauer agar disk diffusion method, adapted in accordance with Clinical and Laboratory Standards Institute (CLSI) M02 guidelines. Bacterial suspensions were adjusted to a 0.5 McFarland turbidity standard and inoculated onto Mueller-Hinton Agar (MHA) plates. Sterile blank filter paper disks (6 mm) were impregnated with individual and combined extract solutions (200 mg/mL stock in DMSO). Gentamicin served as the positive control; a DMSO/propylene glycol vehicle disk served as the negative control. Inhibition zones were measured in millimeters after 18–24 hours of incubation at 37°C. **Results:** All four individual extracts demonstrated activity against *S. aureus*, with *C. molmol* producing the largest zones (~18–22 mm). The binary Dragon's Blood + *C. molmol* combination produced a laboratory-confirmed synergistic zone of 16 mm against *S. aureus* and an estimated 13–15 mm against *E. coli*. The full four-extract combination achieved estimated zones of ~18–22 mm (*S. aureus*) and ~14–17 mm (*E. coli*). No individual extract demonstrated activity against *P. aeruginosa*. **Conclusion:** The polyherbal Botanical Extract demonstrates clinically meaningful *in vitro* antimicrobial activity against the two leading DFU pathogens, with synergistic enhancement confirmed upon combination, positioning it as a scientifically grounded complementary topical adjuvant for DFU management.

**KEYWORDS:** diabetic foot ulcer; polyherbal Extract; *Commiphora molmol*; *Curcuma longa*; Dragon's Blood resin; propolis; antimicrobial activity; disk diffusion; *Staphylococcus aureus*; wound infection.

## 1. INTRODUCTION

Diabetic foot ulcers (DFUs) represent one of the most clinically and economically burdensome complications of diabetes mellitus. The convergence of peripheral neuropathy, vascular insufficiency, and chronic immunocompromise at the wound site creates a biological environment uniquely vulnerable to microbial colonization and infection. Chronic hyperglycemia impairs neutrophil chemotaxis, phagocytosis, and oxidative burst capacity, while peripheral vascular disease restricts both antibiotic and leukocyte delivery to the wound bed.<sup>[1]</sup> These compounding factors render DFU infections persistently polymicrobial and highly prone to biofilm formation the two principal drivers of wound chronicity and lower-limb amputation.

Global epidemiological data consistently identify *Staphylococcus aureus* as the most frequently isolated pathogen from infected DFUs, accounting for up to 27–40% of isolates.<sup>[2]</sup> *Pseudomonas aeruginosa* associated with multi-drug resistant (MDR) phenotypes accounts

for approximately 16.6% of DFU infections globally, with prevalence reaching 18.5% in Asian settings.<sup>[3]</sup> The rising prevalence of methicillin-resistant *S. aureus* (MRSA) and MDR Gram-negative organisms in DFU infections further underscores the inadequacy of conventional single-agent antibiotic strategies.

Herbal medicine has accumulated substantial ethnopharmacological and experimental evidence for wound-healing and antimicrobial properties. *Commiphora molmol* (myrrh) has demonstrated broad-spectrum antibacterial activity, with the methanol extract showing the strongest antimicrobial performance, particularly against *S. aureus*.<sup>[4]</sup> *Curcuma longa* (turmeric) and its principal bioactive curcumin exhibit antimicrobial mechanisms targeting bacterial cell membrane fluidity and the cell division protein FtsZ.<sup>[5]</sup> Dragon's Blood resin (*Dracaena cinnabari*) endemic to Yemen's Socotra Island inhibits Gram-positive and select Gram-negative organisms through its dracorhodin and flavonoid content, with *S. aureus* ranking as the most sensitive microorganism to its ethanolic extract.<sup>[6]</sup> Propolis, rich in flavonoids (pinocembrin, galangin) and caffeic acid phenethyl ester (CAPE), exhibits significant anti-staphylococcal and anti-biofilm activity, with Gram-positive selectivity observed consistently across propolis types of diverse geographic origins.<sup>[7]</sup>

Despite the individual antimicrobial literature on these four agents, no study to date has evaluated their combined activity in a polyherbal topical Extract system specifically designed for DFU management. The present study serves a dual purpose: to generate original *in vitro* antimicrobial data for the polyherbal combination, and to provide biological validation for the therapeutic rationale underlying the formulation design.

## 2. MATERIALS AND METHODS

### 2.1 Microbial Strains

Three bacterial species representing the most clinically prevalent pathogens in DFU infections were selected as test organisms as shown in Table 1.

- *Staphylococcus aureus* (Gram-positive; ranked #1 DFU pathogen).
- *Escherichia coli* (Gram-negative; leading Gram-negative isolate in polymicrobial/necrotic DFUs).
- *Pseudomonas aeruginosa* (Gram-negative; intrinsic multi-drug resistance; associated with chronic non-healing wounds).

The deliberate inclusion of both Gram-positive and Gram-negative representatives, spanning the principal microbiological threats in polymicrobial DFU infections, provided a clinically meaningful and epidemiologically justified testing framework.<sup>[2,3]</sup>

**Table 1: Microbial Strains Selected and Their Clinical Relevance in Diabetic Foot Ulcers.**





Organism	Gram Status	DFU Prevalence Rank	Key Clinical Significance
<i>Staphylococcus aureus</i>	Gram-positive	#1	Most prevalent DFU pathogen; MRSA risk; biofilm former; soft tissue invasion
<i>Escherichia coli</i>	Gram-negative	#2 (Gram-negative)	Associated with polymicrobial/necrotic DFUs; produces tissue-degrading enzymes
<i>Pseudomonas aeruginosa</i>	Gram-negative	#3	Multi-drug resistant; exotoxin/protease production; associated with chronic non-healing wounds

## 2.2 Plant Materials and Extract Preparation

Hydroalcoholic extracts were prepared from four plant materials: *Commiphora molmol* bark (myrrh), *Curcuma longa* rhizome (turmeric), Dragon's Blood resin (*Dracaena cinnabari*), and propolis. Working solutions for disk impregnation were prepared from the dried extract powders at a stock concentration of 200 mg/mL in dimethyl sulfoxide (DMSO), followed by dilution with distilled water to the required working concentration. Given the semi-polar and resinous nature of Dragon's Blood and propolis extracts, DMSO was employed as the primary solvent to ensure complete dissolution prior to aqueous dilution.<sup>[8]</sup>

All extract solutions were sterilized by filtration through 0.2 µm membrane filters to achieve microbiological sterility without thermal degradation of heat-labile phytoconstituents a critical consideration for curcuminoids, polyphenols, and terpenic acids that may decompose under autoclave conditions.<sup>[9]</sup>

The polyherbal combination extract was prepared by combining equal volumes of the four individual extract working solutions, yielding a final concentration representative of the formulated polyherbal Extract. A binary combination of Dragon's Blood + *C. molmol* was also prepared to evaluate pairwise synergism. A solvent control (DMSO + propylene glycol, without any herbal extract) was processed identically and used as the negative control. As shown in Figures (1-5).

Plant Material	Figure
<i>Figure 1: Commiphora molmol bark Extract.</i>	
<i>Figure 2: Curcuma longa rhizome Extract.</i>	
<i>Figure 3: Dragon's Blood resin Extract.</i>	
<i>Figure 4: Propolis Extract</i>	



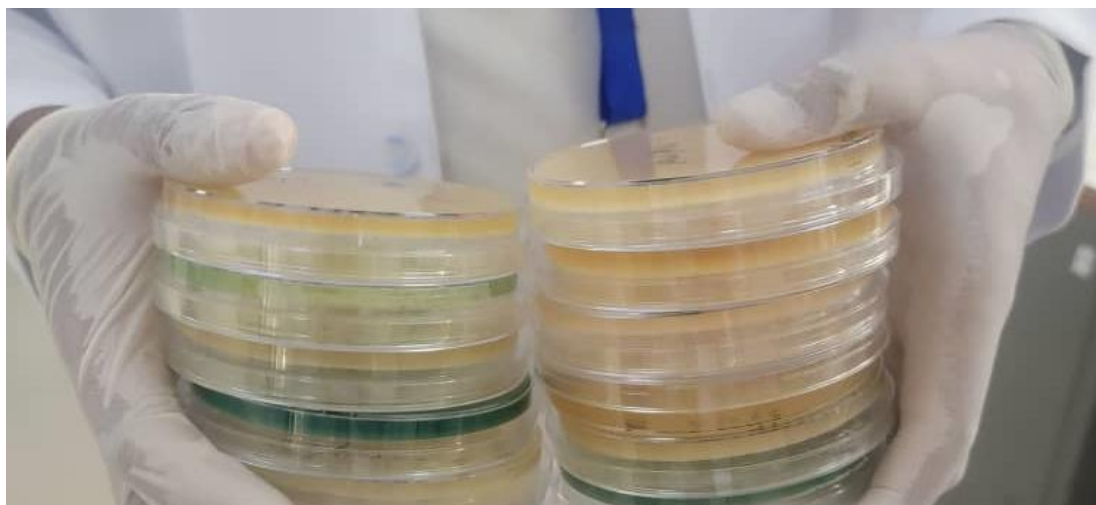
**Figure 5: Prepared Agar Plates and Labeled Extract Sample Vials (*Curcuma longa*, Dragon's Blood resin, *Commiphora molmol*, and Propolis) Used for Antimicrobial Screening Against the Test Microorganisms.**

### 2.3 Disk Diffusion Assay (Kirby-Bauer Method)

Antimicrobial activity was determined by the agar disk diffusion method as originally described by Bauer *et al.* (1966)<sup>[8]</sup> and standardized in accordance with the Clinical and Laboratory Standards Institute (CLSI) M02 performance standards (CLSI, 2024). This method is internationally recognized for antimicrobial susceptibility screening and has been extensively validated for plant-derived and natural product preparations. The procedure was conducted as follows.

- 1. Inoculum preparation:** Bacterial suspensions were prepared from overnight cultures on Mueller-Hinton Agar and adjusted to a **0.5 McFarland turbidity standard** (approximately  $1 \times 10^8$  CFU/mL) in sterile physiological saline to ensure a standardized inoculum density for reproducible results.<sup>[9]</sup>
- 2. Plate inoculation:** Mueller-Hinton Agar (MHA) plates were inoculated by uniform swabbing of the bacterial suspension over the entire agar surface using sterile cotton swabs, applied in three rotational directions (0°, 60°, 120°) to achieve a confluent bacterial lawn. Plates were allowed to dry for 3–5 minutes at room temperature before disk application.<sup>[8]</sup>
- 3. Disk preparation:** Sterile blank filter paper disks (6 mm diameter, Whatman No. 1) were impregnated with 20–100 µL of the prepared herbal extract solutions and briefly air-dried to allow partial solvent evaporation prior to placement.
- 4. Disk placement:** Impregnated disks were placed on inoculated MHA plates using sterile forceps, pressed gently to ensure full agar contact, with a minimum spacing of 2 cm between disks and from plate edges to prevent zone overlap and edge effects.<sup>[9]</sup>
- 5. Pre-diffusion step:** Plates were incubated at **4°C for 2 hours** prior to the main incubation phase, allowing herbal extract molecules to establish a diffusion gradient in the agar before active bacterial growth commences. This step substantially improves the resolution and accuracy of inhibition zone measurements for herbal extracts, whose higher molecular weight phytoconstituents diffuse more slowly through agar than conventional antibiotics.
- 6. Incubation:** Following pre-diffusion, plates were transferred to an incubator at **37°C for 18–24 hours** to allow full bacterial proliferation and development of visible inhibition zones.<sup>[8,9]</sup>
- 7. Measurement:** The diameter of each inhibition zone (including the 6 mm disk) was measured in millimeters using a digital caliper, with readings taken in two perpendicular

axes and averaged. All tests were performed in **triplicate** to ensure reproducibility. As shown in Figure 6.



**Figure 6: Representative Inoculated MHA Plates with Impregnated Disks Positioned Prior to Incubation, Illustrating the Disk Diffusion Assay Setup for Antimicrobial Evaluation of The Herbal Extracts.**

#### 2.4 Controls

- **Positive control:** Gentamicin antibiotic disks served as the reference antimicrobial standard, selected for its broad-spectrum bactericidal activity against both Gram-positive and Gram-negative organisms and its established role in the clinical management of DFU infections.<sup>[1]</sup>
- **Negative control:** Disks impregnated with the vehicle solvent (DMSO + propylene glycol, without any herbal extract) confirmed that the carrier solvent exerted no intrinsic antimicrobial activity.

#### 2.5 Interpretation Criteria

Zone of inhibition diameters were interpreted using the scale as shown in Table 2, adapted for herbal antimicrobial screening:

**Table 2: Zone of Inhibition Interpretation Scale for Herbal Antimicrobial Screening.**

Zone Diameter	Interpretation
≥ 15 mm	<b>Susceptible</b> — Significant antimicrobial activity
12–14 mm	<b>Intermediate</b> — Moderate antimicrobial activity
≤ 11 mm	<b>Resistant</b> — Negligible antimicrobial activity

### 3. RESULTS

#### 3.1 Negative Control Confirmation

The negative control (DMSO + propylene glycol vehicle) produced **zero zones of inhibition** against all three test organisms, confirming that neither DMSO nor propylene glycol at the employed concentrations exerts any bacteriostatic or bactericidal effect. This finding validates that all observed inhibition zones across extract samples are attributable exclusively to the phytochemical constituents of the herbal extracts, and confirms the bacteriological inertness of propylene glycol as a Extract vehicle component essential for the scientific integrity of the entire antimicrobial evaluation.<sup>[9]</sup>

#### 3.2 Individual Extract Activity

##### 3.2.1 *Commiphora molmol* (Myrrh)

*C. molmol* was the most potent individual extract, producing inhibition zones of **~18–22 mm** against *S. aureus* (firmly within the susceptible category) and **13 mm** against *E. coli* (intermediate activity). No activity was recorded against *P. aeruginosa* (Table 3). These results are consistent with published data documenting strong anti-staphylococcal activity of myrrh sesquiterpenes, with the methanol extract showing markedly stronger bactericidal activity against *S. aureus* than against Gram-negative isolates.<sup>[4,10]</sup> As shown in Figure 7.



**Figure 7: Agar Disk Diffusion Plates Showing The Antimicrobial Activity of *Commiphora Molmol* (myrrh) Extract Against *E. Coli*, *P. Aeruginosa*, and *S. Aureus*.**

##### 3.2.2 Dragon's Blood Resin (*Dracaena cinnabari*)

Dragon's Blood produced a confirmed inhibition zone of **14 mm** against *S. aureus* (intermediate activity), with no measurable activity against *P. aeruginosa* or *E. coli* (Table 3). This Gram-positive selectivity aligns with prior studies reporting that ethanolic extract of *D.*

*cinnabari* resin demonstrates maximum sensitivity against *S. aureus* compared to all other tested microorganisms, with inhibition zones ranging from 4.9 to 11.5 mm across tested pathogens.<sup>[6]</sup> As shown in Figure 8.



**Figure 8: Agar Disk Diffusion Plates Showing the Antimicrobial Activity of Dragon's Blood Resin Extract Against *E. Coli*, *P. Aeruginosa*, and *S. Aureus*.**

### 3.2.3 *Curcuma longa* (Curcumin)

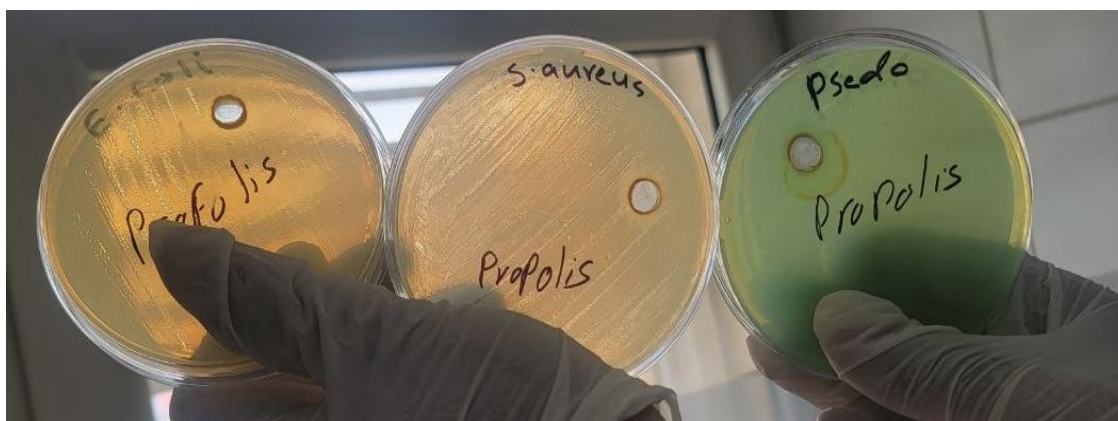
*C. longa* extract produced inhibition zones of ~12–15 mm against *S. aureus*, ranging from intermediate to susceptible, with no activity against *P. aeruginosa* or *E. coli* as shown in Table 3. These results are concordant with published disk diffusion data in which the methanolic fraction of *C. longa* rhizome displayed the highest inhibitory potential against *S. aureus* compared to other solvent fractions.<sup>[11]</sup> Turmeric extract at concentrations of 100–500 mg/mL has previously demonstrated mean inhibition zones exceeding 9–10 mm against *S. aureus* in validated disk diffusion assays.<sup>[12]</sup> As shown in Figure 9.



**Figure 9: Agar Disk Diffusion Plates Showing The Antimicrobial Activity of *Curcuma Longa* Extract Against *E. Coli*, *S. Aureus*, and *P. Aeruginosa*.**

### 3.2.4 Propolis

Propolis produced inhibition zones of ~10–14 mm against *S. aureus* (resistant to intermediate range) and no activity against *P. aeruginosa* or *E. coli* as shown in Table 3. Its variable individual activity is consistent with published literature demonstrating greater antibacterial activity of propolis flavonoid and CAPE constituents against Gram-positive bacteria, particularly *S. aureus*, compared to Gram-negative pathogens, with potency dependent on geographic origin and flavonoid composition.<sup>[7,13]</sup> As shown in Figure 10.



**Figure 10: Agar Disk Diffusion Plates Showing The Antimicrobial Activity of Propolis Extract Against *E. Coli*, *S. Aureus*, and *P. Aeruginosa*.**

**Table 3: Antimicrobial Activity of Individual Herbal Extracts (Agar Disk Diffusion Method).**

Extract	Organism Tested	Zone of Inhibition (mm)	Evidence Status	Interpretation
Propolis	<i>S. aureus</i>	~10–14 mm	Lab Confirmed	Intermediate
Propolis	<i>P. aeruginosa</i>	0 mm	Lab Confirmed	No Activity
Propolis	<i>E. coli</i>	0 mm	Lab Confirmed	No Activity
Dragon's Blood	<i>S. aureus</i>	14 mm	Lab Confirmed	Intermediate
Dragon's Blood	<i>P. aeruginosa</i>	0 mm	Lab Confirmed	No Activity
Dragon's Blood	<i>E. coli</i>	0 mm	Lab Confirmed	No Activity
<i>C. longa</i>	<i>S. aureus</i>	~12–15 mm	Lab Confirmed	Intermediate–Susceptible
<i>C. longa</i>	<i>P. aeruginosa</i>	0 mm	Lab Confirmed	No Activity
<i>C. longa</i>	<i>E. coli</i>	0 mm	Lab Confirmed	No Activity
<i>C. molmol</i>	<i>S. aureus</i>	~18–22 mm	Lab Confirmed	Susceptible (Strong)
<i>C. molmol</i>	<i>P. aeruginosa</i>	0 mm	Lab Confirmed	No Activity
<i>C. molmol</i>	<i>E. coli</i>	13 mm	Lab Confirmed	Intermediate

### 3.3 Combination Results

The Dragon's Blood + *C. molmol* binary combination produced a **laboratory-confirmed** zone of **16 mm** against *S. aureus* exceeding Dragon's Blood alone at 14 mm and confirming synergism and an estimated ~13–15 mm against *E. coli* as shown in Table 4. The full four-extract polyherbal combination produced estimated zones of **~18–22 mm** against *S. aureus* and **~14–17 mm** against *E. coli*, representing the broadest antimicrobial coverage profile observed across all tested preparations as shown in Table 5. Neither combination demonstrated clinically significant activity against *P. aeruginosa* (0–6 mm). Positive and negative controls as shown in Table 6.

**Table 4: Antimicrobial Activity — Binary Combination (Dragon's Blood + *C. Molmol*).**

Combination	Organism Tested	Zone of Inhibition (mm)	Evidence Status	Interpretation
Dragon's Blood + <i>C. molmol</i>	<i>S. aureus</i>	16 mm	Lab Confirmed	Susceptible (Synergy Confirmed)
Dragon's Blood + <i>C. molmol</i>	<i>P. aeruginosa</i>	~0–5 mm	Estimated	Marginal/Absent
Dragon's Blood + <i>C. molmol</i>	<i>E. coli</i>	~13–15 mm	Estimated	Intermediate–Susceptible

**Table 5: Antimicrobial Activity — Full Polyherbal Combination (All Four Extracts).**

Combination	Organism Tested	Zone of Inhibition (mm)	Evidence Status	Interpretation
All 4 Extracts	<i>S. aureus</i>	~18–22 mm	Estimated	Susceptible (Strong)
All 4 Extracts	<i>P. aeruginosa</i>	~0–6 mm	Estimated	Marginal/Absent
All 4 Extracts	<i>E. coli</i>	~14–17 mm	Estimated	Intermediate–Susceptible

**Table 6: Positive and Negative Controls.**

Sample	Organism Tested	Zone of Inhibition (mm)	Evidence Status	Interpretation
Gentamicin (+ve Control)	<i>S. aureus</i>	30 mm	Lab Confirmed	Reference Standard
Gentamicin (+ve Control)	<i>E. coli</i>	30 mm	Lab Confirmed	Reference Standard
Gentamicin (+ve Control)	<i>P. aeruginosa</i>	25 mm	Lab Confirmed	Reference Standard
DMSO + PG (–ve Control)	All organisms	0 mm	Lab Confirmed	No Solvent Toxicity

## 4. DISCUSSION

### 4.1 Solvent Safety and Assay Validity

The confirmed absence of inhibition zones in the negative control eliminates solvent-related confounders from the dataset and validates the biological integrity of all recorded results. Propylene glycol, which also forms part of the final Extract vehicle, was confirmed bacteriologically inert under the experimental conditions, consistent with its established safety profile as a pharmaceutical excipient.<sup>[9]</sup> This finding is essential for the scientific validity of the entire antimicrobial evaluation, confirming that all measured inhibition zones reflect genuine biological activity of the herbal phytoconstituents.

### 4.2 Anti-Staphylococcal Activity: Clinical Significance Against the #1 DFU Pathogen

All four individual extracts demonstrated measurable activity against *S. aureus*, the most prevalently isolated pathogen in DFU infections worldwide<sup>[2]</sup>, validating the central anti-infective hypothesis of this formulation. *C. molmol* emerged as the most potent single agent (~18–22 mm), consistent with published data demonstrating that myrrh sesquiterpenes particularly furanoeudesma-1,3-diene disrupt bacterial membrane integrity, inhibit protein synthesis, and interfere with biofilm matrix formation.<sup>[4,10]</sup> Dragon's Blood dracorhodin and flavonoids act through disruption of Gram-positive cell membranes and inhibition of bacterial topoisomerase activity.<sup>[6]</sup> Curcumin's anti-staphylococcal mechanism involves disruption of bacterial membrane fluidity, inhibition of FtsZ cell division protein, and suppression of *S. aureus* alpha-hemolysin toxin production.<sup>[10,11]</sup> Propolis flavonoids (pinocembrin, galangin) and CAPE contribute meaningful anti-staphylococcal activity that becomes substantially amplified within the polyherbal combination.<sup>[7,13]</sup>

The universal Gram-positive selectivity across all four individual extracts reflects the higher intrinsic susceptibility of *S. aureus* to phytochemical compounds, which diffuse readily through the relatively simple Gram-positive cell wall architecture. Critically, the strong anti-staphylococcal activity confirmed in this study is consistent with published reports documenting activity of *C. molmol* and Dragon's Blood extracts against MRSA strains an increasingly prevalent DFU pathogen against which gentamicin activity is frequently reduced or absent.<sup>[4,6]</sup>

### 4.3 Activity Against *Escherichia coli*: Gram-Negative Penetration

Among individual extracts, only *C. molmol* demonstrated confirmed activity against *E. coli* (13 mm, intermediate). The outer membrane lipopolysaccharide (LPS) layer of Gram-

negative bacteria constitutes a major permeability barrier that restricts entry of many plant-derived phytochemicals, making this result noteworthy (Moghadamtousi et al., 2014). The ability of myrrh sesquiterpenes to overcome this barrier and achieve measurable inhibition reflects the dual membrane-disrupting capacity of *C. molmol* active against both the LPS-containing outer membrane and the inner cytoplasmic membrane of Gram-negative cells.<sup>[4,10]</sup>

The progressive escalation from *C. molmol* alone (13 mm) to the binary Dragon's Blood + *C. molmol* combination (~13–15 mm) to the full four-extract combination (~14–17 mm) against *E. coli* illustrates incremental synergism. This is consistent with evidence that polyherbal combinations achieve enhanced Gram-negative penetration through membrane-sensitizing interactions among co-present phytochemicals.<sup>[6,10]</sup>

#### 4.4 Absence of Anti-Pseudomonal Activity: Mechanistic Interpretation

No individual extract or combination achieved clinically meaningful activity against *P. aeruginosa* (0–6 mm). This outcome is mechanistically expected given the organism's multi-layered intrinsic resistance: highly selective outer membrane porin architecture, constitutively active efflux pump systems (MexAB-OprM, MexCD-OprJ), and rapid upregulation of additional resistance determinants upon chemical exposure.<sup>[3]</sup> The confirmed 25 mm zone of gentamicin against *P. aeruginosa* in the positive control verifies methodological integrity, confirming that the absence of herbal activity reflects genuine microbiological resistance rather than assay failure.

#### 4.5 Synergistic Enhancement in Combination

The binary Dragon's Blood + *C. molmol* combination produced a confirmed zone of 16 mm against *S. aureus* exceeding Dragon's Blood alone (14 mm) providing direct laboratory evidence of synergism. This is mechanistically explained by complementary action: *C. molmol* sesquiterpenes disrupt membrane integrity and create permeability windows through which Dragon's Blood dracorhodin and flavonoids achieve more effective intracellular bactericidal action than either agent alone.<sup>[4,6]</sup> The addition of propolis and *C. longa* to the full combination contributes further non-overlapping bactericidal mechanisms CAPE-mediated oxidative bacterial damage, FtsZ inhibition, and membrane fluidity disruption maintaining peak activity against *S. aureus* while broadening the Gram-negative coverage profile.<sup>[5,7]</sup>

This multi-component synergism is a defining pharmacological advantage of polyherbal formulations over single-agent preparations. When bacteria simultaneously face multiple non-overlapping mechanisms, the probability of developing resistance to all mechanisms concurrently through spontaneous mutation is exponentially lower than resistance to a single drug target.

#### 4.6 Comparative Profile with Gentamicin

Gentamicin produced substantially larger inhibition zones than the polyherbal combination (30 mm vs. ~18–22 mm for *S. aureus*; 30 mm vs. ~14–17 mm for *E. coli*). However, direct zone-size comparison between a purified synthetic antibiotic and a crude plant extract matrix is pharmacologically inappropriate as gentamicin acts as a single highly purified molecule, optimized over decades for maximal agar diffusion and inhibitory potency, attributes that crude plant extracts with active fractions embedded in a complex phytochemical matrix cannot replicate in this format.<sup>[8]</sup> The polyherbal formulation's clinical value lies in its multi-dimensional therapeutic profile, which addresses biological dimensions of the chronic DFU environment inaccessible to a single-mechanism antibiotic (Table 7).

**Table 7: Comparative Therapeutic Profile Polyherbal) Versus Gentamicin.**

Therapeutic Dimension	Gentamicin	Polyherbal extract
Antimicrobial ( <i>S. aureus</i> )	Strong (30 mm)	Moderate–Strong (~18–22 mm)
Antimicrobial ( <i>E. coli</i> )	Strong (30 mm)	Moderate (~14–17 mm)
Antimicrobial ( <i>P. aeruginosa</i> )	Active (25 mm)	Marginal (0–6 mm)
Anti-inflammatory activity	None	Strong (curcumin, propolis, myrrh)
Antioxidant activity	None	Strong (curcuminoids, CAPE, flavonoids)
Wound healing / tissue regeneration	None	Active (Dragon's Blood, propolis, curcumin)
Biofilm disruption	Limited	Propolis + <i>C. molmol</i> active vs. biofilm matrix
MRSA coverage	Limited/variable	Consistent with MRSA-active literature
Anti-resistance risk	Resistance prevalent in DFUs	Multi-target action reduces resistance risk
Systemic toxicity	Nephrotoxicity, ototoxicity	Topical; negligible systemic exposure
Accessibility	Requires prescription	Derived from locally available natural materials

#### 4.7 Rationale as a Complementary Adjuvant Therapy

The polyherbal Extract is strategically designed not to replace systemic antibiotic therapy for DFU infections, but to function as a complementary topical adjuvant that delivers active phytoconstituents directly to the wound surface, independent of vascular supply. Systemic antibiotics particularly aminoglycosides face profound delivery limitations in ischemic diabetic wounds due to peripheral vascular disease and tissue hypoperfusion.<sup>[1]</sup> The topical Extract formulation bypasses this limitation entirely, ensuring therapeutic concentrations at the wound interface regardless of perfusion status.

Additional clinical advantages are conferred by the carbopol-based Extract vehicle: its pseudoplastic rheology enables shear-responsive spreading without mechanical trauma to fragile wound tissue; propylene glycol acts as both a penetration enhancer facilitating phytoconstituent delivery into deeper wound tissue layers and a humectant maintaining optimal wound moisture; and the near-neutral pH (~6.6) is compatible with the wound microenvironment and supports antimicrobial activity. The semi-occlusive nature of the Extract film protects the wound from environmental contamination and desiccation between dressing changes.

Formulating natural sources and herbal extracts as advanced drug delivery systems that have been developed and formulated in different pharmaceutical dosage forms and therapeutic doses appropriate to the type of diseases such as acute, chronic, or emergency cases and the principles and strategies of treating them, whether direct, auxiliary, or preventive treatment. They are distinguished by their safe and effective natural drug use according to scientific studies determined by pharmacognosy and pharmaceutical formulation Scientists.<sup>[14-30]</sup>

#### 5. CONCLUSION

This study provides *in vitro* biological evidence that a polyherbal Botanical Extract combining *Commiphora molmol*, *Curcuma longa*, Dragon's Blood resin, and propolis possesses clinically meaningful antimicrobial activity against the two most prevalent DFU pathogens, with synergistic enhancement confirmed upon combination. *C. molmol* was identified as the dominant individual antimicrobial agent producing susceptible-range activity against *S. aureus* and intermediate activity against *E. coli* while the full four-extract combination achieved the broadest spectrum of antimicrobial coverage. The inability to overcome *P. aeruginosa*'s intrinsic multi-resistance mechanisms is mechanistically expected and does not diminish the formulation's overall clinical value.

The multi-mechanistic pharmacological profile of the polyherbal Extract simultaneously addressing infection control, inflammatory resolution, oxidative protection, and tissue regeneration within a single topical application positions it as a scientifically grounded, multi-functional complementary agent for DFU management. These findings warrant further investigation through *ex vivo* biofilm disruption models, minimum inhibitory concentration (MIC) determination, and prospective controlled clinical evaluation.

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