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IN VITRO EVALUATION OF ANTIBACTERIAL ACTIVITY OF BERBERINE NANO GEL ON STANDARD BACTERIAL STRAINS ASSOCIATED WITH BURN WOUND INFECTIONS

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ABSTRACT

Objective: *To evaluate the In vitro antibacterial activity of berberine nanogel against reference bacterial strains and compare it with a reference control.*

Subjects and methods: *In vitro experimental design. Antibacterial activity was quantified using the agar disk diffusion method (zone of inhibition, mm). Time-kill assays were conducted using twofold serial dilutions (from 1/2 to 1/128) with with bacterial growth assessed at 2, 6, and 24 hours for Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa.*

Results: *The berberine nanogel produced zones of inhibition against both Gram-positive and some Gram-negative bacteria. Specifically: E. coli 17.11 ± 1.44 mm vs. the reference control 23.49 ± 2.16 mm ($p < 0.001$); P. aeruginosa 14.14 ± 1.61 mm vs. 14.25 ± 1.88 mm ($p > 0.05$); Acinetobacter sp. 19.79 ± 1.37 mm vs. 10.59 ± 1.19 mm ($p < 0.001$); Enterobacter sp. 15.26 ± 1.58 mm vs. 24.26 ± 0.97 mm ($p < 0.001$). For Gram-positive bacteria, S. aureus achieved 16.47 ± 1.54 mm vs. 11.20 ± 1.37 mm ($p < 0.001$). In time - kill testing, no bacterial growth was observed after 24 hours at dilutions ≥1/16 for E. coli and P. aeruginosa, and at ≥ 1/32 for S. aureus. At higher concentrations (≥1/4), early inhibition/bactericidal activity was evident as early as 2 hours.*

Conclusion: *Berberine nanogel demonstrated significant In vitro antibacterial activity, with pronounced effects against S. aureus and Acinetobacter sp., and efficacy comparable to the reference control against P. aeruginosa.*

Keywords: *Berberine nanogel; In vitro antibacterial activity; antimicrobial susceptibility testing; burn wound infection.*

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1. INTRODUCTION

Burn wound infection is a leading cause of delayed wound healing, sepsis, and mortality in burn patients. Common pathogens include *S. aureus, P. aeruginosa, Acinetobacter spp*. and *Enterobacter spp*.; many of which can form biofilms, complicating treatment and increasing antibiotic resistance [1] .

Berberine, a natural alkaloid, has been shown to possess a broad antibacterial spectrum against both Gram-positive and Gram-negative bacteria. Its mechanisms of action include membrane disruption, interaction with DNA, inhibition of drug efflux pumps, reduction of biofilm formation, and interference with quorum sensing [2] . Beyond its standalone activity, berberine exhibits synergistic effects when combined with certain antibiotics. Recently, berberine-loaded nano and gel/ hydrogel systems have shown potential to improve solubility, permeability, sustain release, and enhance antibacterial activity, while also supporting wound healing in skin infection models [3] . These findings suggest the feasibility of berberine nanogel formulations for topical application in infected wounds.

However, direct In vitro comparative data against typical burn wound pathogens remain limited and require quantitative characterization using standardized assays. To address this evidence gap, the study was designed to evaluate the In vitro antibacterial activity of berberine nanogel against *S. aureus, E. coli, P. aeruginosa,* and *Acinetobacter sp.* using agar diffusion and time-kill assays.

2. METHODS

2.1. Research design

An In vitro, parallel-controlled study was conducted to evaluate the antibacterial activity of berberine nanogel against common reference bacterial strains found in burn wounds, comparing it with a positive control (standard antibiotics) and a standard burn treatment control used at the facility. Two main assays were performed: agar diffusion (measuring the zone of inhibition in mm) and time-kill assays at serial dilutions, with readings at 2, 6, and 24 hours.

2.2. Research site and time

- Department of Paraclinical Medicine - Le Huu Trac National Burn Hospital/Military Medical Academy; conducted from June to December 2021.

2.3. Materials and reagents

- Nano berberine gel: Prepared by the Department of Pharmacy - Le Huu Trac National Burn Hospital.

- Positive control (reference standard): Streptomycin sulfate 16 IU/mL (for Gram-negative bacteria) and Benzathine penicillin 20 IU/mL (for Gram-positive bacteria).

- Culture media and materials: Nutrient broth and agar medium; sterile Petri dishes; 6 mm filter paper discs; calipers (accuracy 0.02 mm); 37^0C incubator.

- Bacterial test: Bacterial strains provided by the Department of Paraclinical Medicine:

S. aureus ATCC 29213; E. coli ATCC 25922

P.aeruginosa ATCC 27853; Acinetobacter sp.; Enterobacter sp.

2.4. Procedures

- Bacterial suspension standardization: Test bacteria were cultured in broth for 18 - 24 hours at 37^0C to a density of 10^7 cells/mL (confirmed by dilution and comparison with a standard series) for use in subsequent assays.

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- Agar disk diffusion test (adapted using gel-impregnated paper discs)

+ Preparation of seeded agar: Sterile agar was cooled to 45-50°C, inoculated with the test bacteria at a ratio of 2.5 mL per 100 mL, mixed thoroughly, and poured into Petri dishes (20 mL/dish) to solidify.

+ Disc impregnation: Sterile filter paper discs were impregnated three times with the test sample solution, dried at < 60^0C after each impregnation. The same procedure was applied for the positive control, SSD cream, and negative control.

+ Disc placement and incubation: The impregnated discs were placed on the surface of the inoculated agar and incubated at 37^0C for 18 - 24 hours.

+ Measurement of inhibition zones: The diameter (mm) was measured using calipers (accuracy 0.02 mm). The mean ($\overline{X}$) and standard deviation (SD) were calculated from repeated measurements (n).

+ Replication: Each condition was tested with ≥3 discs per experiment and independently repeated ≥ 3 times.

- Time-kill test and determination of operational MIC/MBC

+ Setup: Bacterial suspensions were prepared at the standard density. Berberine nanogel was added in twofold serial dilutions: 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128 (relative to the original preparation). Positive controls (streptomycin/benzathine penicillin according to Gram stain), SSD cream, and a negative control were set up in parallel.

+ Sampling and incubation: Samples were taken at 2, 6, and 24 hours; bacterial colonies were cultured and quantified after incubation at 37^0C according to the unit's standard procedure.

+ Reading and definitions: Bacterial density (CFU/mL) was recorded for each time point and concentration. The operational Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration allowing growth of 1-3 colonies. The Minimum Bactericidal Concentration (MBC) was defined as the lowest concentration yielding no growth.

+ Data presentation: The "concentration × time" matrix for *E. coli, S. aureus,* and *P. aeruginosa* is presented in the Results section.

- Outcome variables and measures

+ Primary outcome: Zone of inhibition diameter (mm) of berberine nanogel compared to the positive control and SSD cream for each bacterial species.

+ Secondary outcomes: (i) Bacterial density (CFU/mL) over time in the time-kill assay; (ii) The lowest concentration resulting in no growth after 24 hours (MBC) and the concentration allowing 1-3 colonies (MIC).

- Sample size and repetition

Each condition was tested with at least 3 discs per time point, independently repeated ≥ 3 times (total observations ≥ 9 per comparison), ensuring reliability for estimating the $\overline{X}$ and SD, and for evaluating differences.

2.5. Data analysis

Data were analyzed using SPSS 26.0 software. Means ± SD were calculated (or medians if data were non-normal). Comparisons between berberine nanogel and the positive control/ SSD cream for each species were performed using the Shapiro-Wilk test for normality and Levene's test for homogeneity of variances. If assumptions were met, a two-sample t-test was used; otherwise, the Mann-Whitney U test was applied. Two-tailed p-values were reported, with a significance level of $p < 0.05$.

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2.6. Research ethics

In vitro study on bacterial strains, no human/animal intervention, therefore no IRB/IACUC approval required; compliance with appropriate biosafety and institutional waste disposal procedures.

3. RESULTS

3.1. Agar disk diffusion method

Table 1. Antibacterial activity of Berberine nanogel against Gram-negative bacteria

(Unit: mm)

Microorganism	Research Sample		Standard sample		p
	Zone diameter	SD	Zone diameter	SD	
E. coli	17.11	1.44	23.49	2.16	< 0.001
P. aeruginosa	14.14	1.61	14.25	1.88	> 0.05
Acinetobacter sp.	19.79	1.37	10.59	1.19	< 0.001
Enterobacter sp.	15.26	1.58	24.26	0.97	< 0.001

Berberine nanogel exhibited significant antibacterial activity. Compared to the standard sample, it was significantly more effective against *Acinetobacter* sp. (nearly twice the zone size, $p < 0.001$). The gel was less effective than the standard against *E. coli* ($p < 0.001$) and *Enterobacter* sp. ($p < 0.001$). Against *P. aeruginosa*, the activity of Berberine nanogel was equivalent to the standard ($p > 0.05$).

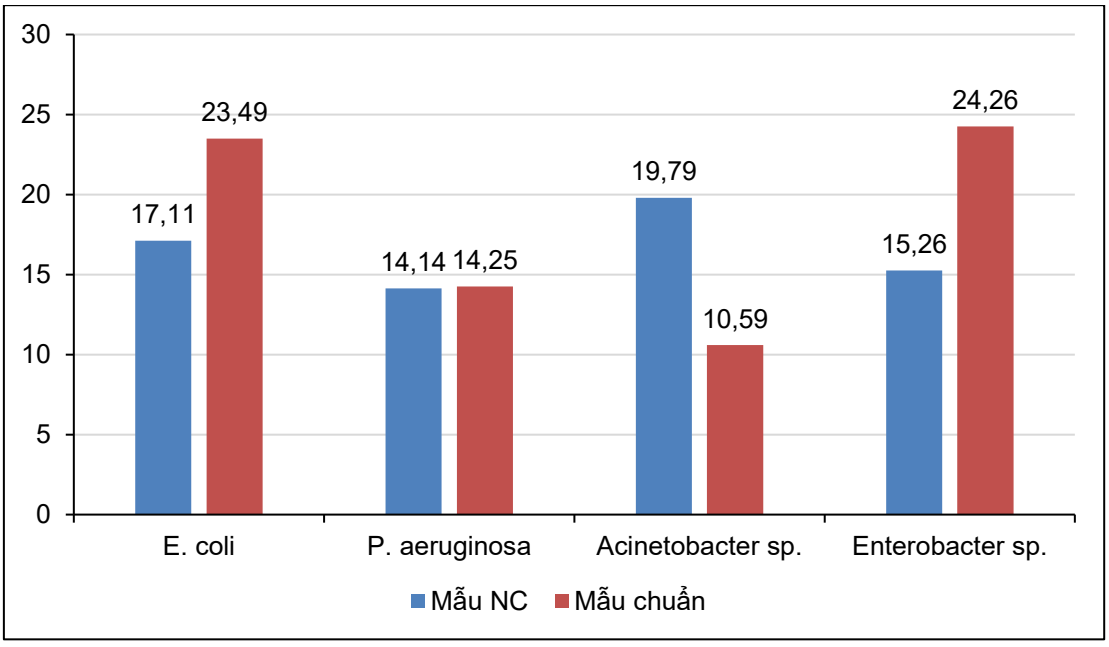

Figure 1 Diameter of the inhibition zone of the drug against Gram-negative bacteria (mm)

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Table 2. Antibacterial activity of Berberine nanogel against Gram-positive bacteria (Unit: mm)

Microorganism	Research sample		Standard sample		p
	Zone diameter	SD	Zone diameter	SD	
S. aureus	16.47	1.54	11.20	1.37	< 0.001

Berberine nanogel was effective against the Gram-positive bacterium *S. aureus* ($p < 0.001$), showing significantly stronger activity than the standard antibiotic. *S. aureus* is a common bacterium in burn wounds, known for its high environmental resistance and strong drug resistance.

3.2. Time-kill assay results

Table 3. Bacterial count (CFU/mL) in relation to drug concentration and exposure time

Concentration		*E. coli*			*S. aureus*			*P. aeruginosa*		
		2h	6h	24h	2h	6h	24h	2h	6h	24h
Berberine nanogel	1/2	0	0	0	0	0	0	0	0	0
	1/4	0	0	0	0	0	0	0	0	0
	1/8	10^3	0	0	0	0	0	10^3	0	0
	1/16	10^4	10^2	0	0	0	0	10^3	0	0
	1/32	10^5	10^3	0	10^4	0	0	10^4	0	0
	1/64	10^6	10^4	0	10^6	10^4	0	10^5	10^3	0
	1/128	10^7	10^5	10^4	10^7	10^6	10^4	10^6	10^4	10^2

Berberine nanogel at dilutions up to 1/16 reduced bacterial counts after 2 hours of exposure. At a dilution of 1/64, the formulation achieved complete bactericidal effect after 24 hours. At a dilution of 1/128, it only reduced bacterial counts even after 24 hours. After 2 hours of exposure, Berberine nanogel at a dilution of 1/4 exhibited complete bactericidal activity against *E. coli, S. aureus,* and *P. aeruginosa.*

4. DISCUSSION

This study demonstrated that berberine nanogel possesses in vitro antibacterial activity against both Gram-positive and some Gram-negative bacteria relevant to burn wound infections. In the agar diffusion assay, berberine nanogel produced larger zones of inhibition than the control against *S. aureus* (16.47 ± 1.54 mm vs. 11.20 ± 1.37 mm; $p < 0.001$) and *Acinetobacter sp.* (19.79 ± 1.37 mm vs. 10.59 ± 1.19 mm; $p < 0.001$), was equivalent to the control against *P. aeruginosa* ($p > 0.05$), but was less effective against *E. coli* and *Enterobacter sp.* Time-kill assays demonstrated early inhibition/bactericidal activity at high concentrations (≥1/4 after 2 hours) and no growth after 24 hours at dilutions ≥1/16 for *E. coli* and *P. aeruginosa*, and ≥1/32 for *S. aureus.* These

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data suggest notable efficacy of berberine nanogel against *S. aureus* and *Acinetobacter*, while maintaining significant activity against *P. aeruginosa*, a major pathogen in burn wounds.

The above results are consistent with the epidemiology of burn wounds: the early stage is often dominated by Gram-positive bacteria (especially *S. aureus*), then shifts to multidrug-resistant Gram-negative bacteria, in which *P. aeruginosa* and *Acinetobacter* spp. account for a large proportion and easily form biofilm, increasing treatment failure and mortality [1], [4], [5]. Recent reviews also emphasize the burden of MDR in burn patients and the importance of infection control strategies and choosing topical therapies with more durable anti-biofilm/anti-microbial activity [1], [4] .

Mechanistically, berberine is an alkaloid that disrupts cell membranes, binds nucleic acids, inhibits drug efflux pumps (e.g., MexAB-OprM *in P. aeruginosa*; AdeABC in *A. baumannii)*, reduces biofilm formation, and enhances antibiotic efficacy when combined [2], [3], [6]. This may explain the superior sterile zone on *S. aureus* and *Acinetobacter* in the study. In addition, hydrogel/nano-berberine systems have demonstrated improved solubility, sustained release, antibacterial-anti-inflammatory-antioxidant properties, and accelerated wound healing in a diabetic mouse model, suggesting the benefits of berberine-loaded gels for infected wounds [3] .

A notable point is the equivalent efficacy against *P. aeruginosa* - the most common pathogen in late-burn wounds. Recently, In vitro studies have suggested synergy between berberine and fluoroquinolones against *P. aeruginosa,* including reduced MIC, biofilm inhibition, and limited emergence of drug-resistant mutations; this may be a direction for future optimization of formulations or combination regimens [3], [6], [7].

However, this study still has some methodological limitations such as (i) The diffusion method using gel-impregnated paper discs is a non-standard variation; the diffusion capacity depends on viscosity and active ingredient loading. It is recommended that diffusion tests should follow CLSI-M02 or EUCAST guidelines (media standardization, seed size, ring reading) and report the amount of active ingredient/disk for comparison between studies [8], [9]. (ii) MIC/MBC determination should be performed by broth microdilution according to CLSI M07 Ed12 - the reference standard for interpolating breakpoints and comparing with published data [10]. (iii) The new study used standard strains; it needs to be extended to multidrug-resistant clinical strains, biofilm testing, and exudate wound models to better reflect the burn environment. Finally, although “SSD cream” is listed as the treatment control, a direct quantitative comparison with SSD (loop, MIC/MBC, time-kill) is needed to conclude the position of berberine nano gel relative to the practice standard.

With its strong potency against *S. aureus/ Acinetobacter* and modest potency against *P. aeruginosa*, berberine nanogel is a potential candidate for local care of infected burns. Next steps should include anti-biofilm testing and ex vivo/in vivo burn wound models; (evaluating local safety (irritation, tissue toxicity) and local pharmacokinetics); head-to-head testing with SSD and/or other antibacterial dressings, before proceeding to early-phase clinical studies [2].

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The current results support the feasibility of berberine nanogel as a targeted topical antibacterial agent for burns. Standardizing methods according to CLSI/EUCAST, extending testing to multidrug-resistant clinical strains, and utilizing near-clinical biological models will be key to translating these in vitro findings into compelling clinical evidence.

5. CONCLUSION

The in vitro evaluation demonstrated that berberine nanogel has significant antibacterial activity against common burn wound pathogens. Superior activity was observed against *S. aureus* (16.47 ± 1.54 mm vs. 11.20 ± 1.37 mm; $p < 0.001$) and *Acinetobacter sp.* (19.79 ± 1.37 mm vs. 10.59 ± 1.19 mm; $p < 0.001$); its efficacy was comparable to the reference standard against *P. aeruginosa* (14.14 ± 1.61 mm vs. 14.25 ± 1.88 mm; $p > 0.05$). In the time-kill assays, no bacterial growth was observed after 24 hours at concentrations up to 1/64 for *E. coli, S. aureus*, and *P. aeruginosa*; at concentrations ≥ 1/4, early inhibition/bactericidal effects were observed within 2 hours. These results support the potential application of berberine nanogel as a topical agent for infected burns and provide a basis for further preclinical and clinical studies.

REFERENCES

1. Roy S., Mukherjee P., Kundu S., et al. (2024). Microbial infections in burn patients. Acute and Critical Care, 39(2): 214.
2. Zhou H., Wang W., Cai L., et al. (2023). Potentiation and mechanism of berberine as an antibiotic adjuvant against multidrug-resistant bacteria. Infection and Drug Resistance : 7313-7326.
3. Hu Z., Zhao K., Chen X., et al. (2023). A berberine-loaded Bletilla striata polysaccharide hydrogel as a new medical dressing for diabetic wound healing. International journal of molecular sciences, 24(22): 16286.
4. Elsheikh R., Makram A.M. (2024). Multidrug-resistant organisms: the silent plight of burn patients. Journal of Burn Care & Research, 45(4): 877-886.
5. Youssouf A.I., Benaissa E., Belouad E.M., et al. (2024). Epidemiological Profile of Bacterial Infections in Burn Patients Over a Five-Year Period. Cureus, 16(11).
6. Li X., Song Y., Wang L., et al. (2021). A potential combination therapy of berberine hydrochloride with antibiotics against multidrug-resistant Acinetobacter baumannii. Frontiers in Cellular and Infection Microbiology, 11: 660431.
7. Zeraatpisheh A., Moradi M., Arshadi M., et al. (2025). The In vitro evaluation of synergistic effects of ciprofloxacin and berberine hydrochloride against Pseudomonas aeruginosa. BMC microbiology, 25(1): 487.
8. Clinical and Laboratory Standards Institute (CLSI) (2024), M02 - Performance Standards for Antimicrobial Disk Susceptibility Tests. 14th ed.
9. European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2024), EUCAST Disk Diffusion Test Methodology; version 12.0.
10. Clinical and Laboratory Standards Institute (CLSI) (2024), M07 - Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 12th ed.