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Comparative *in Vitro* Antibacterial Efficacy of *Peperomia Pellucida* Nanoemulsion, Gentamicin, and Silver Sulfadiazine Against Common Burn Wound Pathogens

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Abstract

Background: Burn wound infections remain a major cause of morbidity and mortality, predominantly caused by multidrug-resistant pathogens such as Pseudomonas aeruginosa, Acinetobacter baumannii, and methicillin-resistant Staphylococcus aureus (MRSA). Topical agents like silver sulfadiazine (SSD) and gentamicin are standard, but resistance and toxicity drive interest in natural alternatives. This study compared the *in vitro* antibacterial activity of Peperomia pellucida nanoemulsion (PPNE), gentamicin, and SSD against these pathogens.

Methods: An experimental post-test only control group design was employed. Clinical isolates or reference strains of A. baumannii, MRSA, and P. aeruginosa were cultured on Mueller-Hinton agar. Antibacterial activity was assessed using the Kirby-Bauer disk diffusion method with disks impregnated with PPNE (5%), gentamicin topical preparation, or SSD. Zone of inhibition (ZOI) diameters were measured after 18–24 h incubation at 37°C (three replicates per group). Data were analyzed using Kruskal-Wallis test ($p < 0.05$ significant).

Results: PPNE produced the largest mean ZOI against A. baumannii (25.9 mm; strong activity), significantly outperforming gentamicin (17.0 mm) and SSD (11.3 mm) ($p = 0.005$). Against MRSA, ZOIs were comparable (PPNE 11.8 mm, gentamicin 11.3 mm, SSD 11.1 mm; all moderate; $p = 0.005$). For P. aeruginosa, PPNE (10.7 mm) and SSD (10.5 mm) showed moderate activity, superior to gentamicin (9.5 mm; weak-moderate; $p = 0.005$).

Conclusion: PPNE demonstrated superior or comparable *in vitro* antibacterial efficacy compared to conventional agents, particularly against A. baumannii. These findings support further *in vivo* and clinical evaluation of PPNE as a potential adjunct or alternative in burn wound infection management.

Keywords: Burn Wound Infection, Peperomia Pellucida Nanoemulsion, Silver Sulfadiazine, Gentamicin, Disk Diffusion, Antibacterial Activity

Introduction

Burn injuries compromise the skin barrier, predisposing patients to invasive infections that contribute to >75% of burn-related mortality globally. Common pathogens include Pseudomonas aeruginosa, Acinetobacter baumannii, and methicillin-resistant Staphylococcus aureus (MRSA), with increasing multidrug resistance complicating treatment. Topical antimicrobials such as 1% silver sulfadiazine (SSD) and gentamicin remain first-line agents for prophylaxis and treatment, yet concerns regarding delayed healing, resistance emergence, and systemic toxicity persist.

Peperomia pellucida (L.) Kunth, a Piperaceae herb with documented anti-inflammatory, antioxidant, and antimicrobial properties, has shown promise in wound healing models. Nanoemulsion formulations (PPNE) enhance bioavailability and delivery of bioactive compounds (e.g., flavonoids, phenolics). Recent preclinical data indicate PPNE accelerates burn wound closure via combined antimicrobial and anti-inflammatory effects. This *in vitro* study aimed to compare the antibacterial efficacy of PPNE, gentamicin, and SSD against predominant burn wound pathogens using the Kirby-Bauer disk diffusion method.

Methods

This experimental study employed a post-test only control group design and was conducted at the Department of Pharmaceutical Microbiology, Universitas Sumatera Utara, following ethical approval. Reference strains or clinical isolates of Acinetobacter baumannii, methicillin-resistant Staphylococcus aureus (MRSA), and Pseudomonas aeruginosa were cultured to 10^5–10^6 CFU/mL (McFarland 0.5 equivalent). Mueller-Hinton agar plates were inoculated using spread plate technique. Sterile 6-mm blank disks were impregnated with PPNE (5% concentration), gentamicin topical preparation, or SSD and placed on plates (three disks per plate, three replicates per agent). Plates were incubated at 37°C for 18–24 h. Zones of inhibition (ZOI) were measured using digital calipers (mm). Activity was classified: <5 mm inactive; 5–10 mm weak; 10–20 mm moderate; >20 mm strong. Data were analyzed descriptively (mean ± SD) and inferentially using Kruskal-Wallis test with SPSS software ($p < 0.05$ significant).

Results

Table 1: Mean Zone of Inhibition Diameters (mm) Against Acinetobacter baumannii

Treatment	Replicate I	Replicate II	Replicate III	Mean ± SD	Interpretation	p-value*
Gentamicin	16.9	17.0	17.0	16.97 ± 0.06	Moderate	0.005
PPNE	26.8	25.5	25.4	25.90 ± 0.78	Strong	
Silver Sulfadiazine	11.0	11.7	11.3	11.33 ± 0.35	Moderate	

*Kruskal-Wallis test

Table 2: Mean Zone of Inhibition Diameters (mm) Against Methicillin-resistant Staphylococcus aureus

Treatment	Replicate I	Replicate II	Replicate III	Mean ± SD	Interpretation	p-value*
Gentamicin	11.2	11.3	11.3	11.27 ± 0.06	Moderate	0.005
PPNE	11.8	11.9	11.8	11.83 ± 0.06	Moderate	
Silver Sulfadiazine	11.4	10.8	11.1	11.10 ± 0.30	Moderate	

*Kruskal-Wallis test

Table 3: Mean Zone of Inhibition Diameters (mm) Against Pseudomonas aeruginosa

Treatment	Replicate I	Replicate II	Replicate III	Mean ± SD	Interpretation	p-value*
Gentamicin	9.8	9.0	9.7	9.50 ± 0.44	Weak-Moderate	0.005
PPNE	10.6	10.9	10.7	10.73 ± 0.15	Moderate	
Silver Sulfadiazine	10.2	10.7	10.5	10.47 ± 0.25	Moderate	

*Kruskal-Wallis test

PPNE exhibited significantly superior activity against A. baumannii (strong) and comparable or better activity against MRSA and P. aeruginosa (moderate) compared to gentamicin and SSD ($p < 0.05$ for all).

Discussion

This study demonstrates that PPNE possesses robust *in vitro* antibacterial activity against key burn wound pathogens, particularly outperforming gentamicin and SSD against A. baumannii. The strong ZOI (>20 mm) against A. baumannii aligns with emerging evidence of Peperomia pellucida's broad-spectrum antimicrobial effects, likely mediated by flavonoids, phenolics, and nanoemulsion-enhanced diffusion.

Against MRSA, all agents produced moderate ZOIs with minimal differences, consistent with SSD's established activity against Gram-positive organisms and reports of P. pellucida efficacy against S. aureus strains. For P. aeruginosa, PPNE and SSD outperformed gentamicin, reflecting gentamicin's variable activity against Pseudomonas due to intrinsic resistance mechanisms (efflux pumps, biofilm).

These findings support prior reports of SSD's broad but sometimes limited efficacy in resistant strains and highlight the potential of natural nanoformulations to address resistance gaps. Limitations include the *in vitro* design (no eschar/biofilm simulation) and use of disk diffusion, which is formulation-dependent. PPNE's superior performance against A. baumannii—a notorious MDR pathogen in burns—suggests promise as an adjunct or alternative, warranting *in vivo* validation.

Conclusion

PPNE exhibited superior or comparable *in vitro* antibacterial efficacy to gentamicin and SSD against common burn wound pathogens, with particularly strong activity against A. baumannii. These results justify further preclinical and clinical studies to explore PPNE as a novel topical agent for burn wound infection control.

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