Peganum harmala alkaloids as emerging antibacterial leads against multidrug-resistant pathogens

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Abstract

Background: The escalating crisis of multidrug-resistant (MDR) pathogens demands discovery of new antibacterial scaffolds. *Azadirachta indica* (Neem), long used in traditional medicine, contains bioactive triterpenoids such as azadirachtin and nimbolide with underexplored translational potential. This study systematically evaluated the antibacterial, antibiofilm, synergistic, and in vivo wound-healing activities of Neem leaf extract.

Methods: Fresh *A. indica* leaves were shade-dried, powdered, and extracted with 80% ethanol (plant:solvent ratio 1:10, w/v) under Soxhlet reflux at 60 °C for 6 h. Crude extract yield was 14.7%. Phytochemical profiling was performed by HPLC using an acetonitrile–water (70:30, v/v) gradient at 1.0 mL/min, with azadirachtin (Rt 8.3 min) and nimbolide (Rt 11.7 min) identified and quantified against standards. Antibacterial activity was tested against MDR strains (*Staphylococcus aureus* ATCC 43300, *Escherichia coli* ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853). MICs and MBCs were determined by broth microdilution (extract concentrations 16–1024 µg/mL in cation-adjusted Mueller-Hinton broth). Biofilm inhibition (crystal violet assay) was evaluated in 96-well plates at ½× MIC and 1× MIC. Synergy with ciprofloxacin was analyzed by checkerboard assays and fractional inhibitory concentration index (FICI). For in vivo study, 48 BALB/c mice (6–8 weeks) were randomized into four groups: untreated control, ciprofloxacin (20 mg/kg), Neem extract (100 mg/kg), and combination. A full-thickness excisional wound (8 mm diameter) was inoculated with MRSA (1×10^7 CFU). Wound healing was monitored for 14 days by planimetric analysis and bacterial load quantification. Environmental assays assessed bacterial decontamination in water and stainless-steel biofilm models.

Results: HPLC quantified azadirachtin at 1.82 mg/g and nimbolide at 2.36 mg/g of extract. MICs were 64 µg/mL (*S. aureus*), 128 µg/mL (*E. coli*), and 128 µg/mL (*P. aeruginosa*), with corresponding MBCs of 128, 256, and 256 µg/mL, respectively. Neem extract inhibited biofilm formation by 78% in *S. aureus*, 62% in *E. coli*, and 59% in *P. aeruginosa*, and eradicated 48–66% of mature biofilms at 2× MIC. Checkerboard assays demonstrated strong synergy with ciprofloxacin, yielding FICI values of 0.38 (*S. aureus*), 0.41 (*E. coli*), and 0.44 (*P. aeruginosa*). In vivo, Neem-treated mice exhibited 76% wound closure by day 10 and complete closure by day 14, with a 3.1 $\log_{10}$ reduction in MRSA burden. The combination group achieved faster healing (90% closure by day 10) and greater bacterial reduction (4.2 $\log_{10}$) compared to ciprofloxacin alone ($p < 0.05$). Environmental assays showed >99% bacterial reduction in contaminated water within 2 h at 500 µg/mL extract and 72% biofilm removal from stainless steel surfaces.

Conclusion: *Azadirachta indica* ethanol extract demonstrates potent antibacterial, antibiofilm, and synergistic activities against MDR pathogens, validated by significant in vivo wound-healing efficacy

and environmental disinfection capacity. These findings highlight Neem as a promising candidate for developing plant-derived antibacterial therapeutics and infection-control strategies.

Keywords: Azadirachta indica, neem, multidrug resistance, antibiofilm, synergy, wound healing, environmental disinfection