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Research Paper

Biocompatible Synthesis of Porous Alumina Nanoparticles Using Thyme Extract and Evaluation of Their Antibacterial Activity Against Clinical Isolates of *Staphylococcus Aureus*

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Extended Abstract

Introduction The escalating threat of antibiotic resistance has emerged as a critical public health concern globally, with methicillin-resistant *Staphylococcus aureus* (MRSA) being one of the most dangerous pathogens in both hospital and community settings. MRSA is associated with a variety of severe infections, including sepsis, pneumonia, endocarditis, and complicated skin infections. Its resistance to a broad spectrum of β-lactam antibiotics and the reduced sensitivity to last-resort agents like vancomycin necessitate the exploration of alternative, sustainable, and biocompatible antimicrobial strategies. One promising avenue in this context is the application of green nanotechnology using medicinal plant extracts for the biosynthesis of nanomaterials. This approach leverages the reducing and stabilizing power of phytochemicals such as polyphenols, flavonoids, and terpenes to fabricate metal oxide nanoparticles with intrinsic antimicrobial properties. Among these, alumina (Al_2O_3) nanoparticles have garnered interest due to their notable biocompatibility, thermal stability, and surface functionality, making them suitable candidates for both therapeutic and industrial applications. This study aimed to develop porous alumina nanoparticles through a green synthesis method employing *Zataria multiflora* (thyme) extract, and to investigate their antibacterial efficacy against ten clinical isolates of Staphylococcus aureus, with an emphasis on MRSA strains producing extended-spectrum β-lactamase (ESBL).

Methods Clinical samples were randomly collected from the upper respiratory tracts (nose and throat) of hospital personnel using sterile swabs. These samples were promptly cultured on blood agar and incubated for 24 hours. Standard biochemical and phenotypic methods, including Gram staining, catalase and coagulase tests, and mannitol fermentation, were employed to confirm the presence of *Staphylococcus aureus*. The production of ESBL enzymes was assessed using a double-disk synergy test with cefotaxime and cefotaxime-clavulanic acid discs, following Clinical and Laboratory

Standards Institute (CLSI) guidelines. For the green synthesis of alumina nanoparticles, aluminum nitrate and sodium dodecyl sulfate (SDS) were used as precursors, with sodium hydroxide adjusting the pH to 8. The reaction mixture was heated and homogenized before being treated with 2 mL of thyme extract. Subsequent microwave-assisted heating cycles promoted nucleation and growth of the nanoparticles, which were then washed, centrifuged, and dried under vacuum. Characterization of morphology and size distribution was performed using Scanning Electron Microscopy (SEM), revealing mostly spherical particles with a mean size of 20–50 nm. The antibacterial properties were tested using the well diffusion method. Alumina nanoparticles were suspended at various concentrations (0.3–80 mg/mL) in a DMSO-methanol mixture and introduced into agar plates pre-inoculated with *Staphylococcus aureus* suspensions standardized to 0.5 McFarland turbidity. Zones of inhibition were measured after 24 hours of incubation. Additionally, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by serial dilution and culture regrowth assays.

Results and Discussion All ten isolates were confirmed as ESBL-producing *Staphylococcus aureus* based on their response to the combination disk test, fulfilling the ≥5 mm difference criterion between CTX and CTC inhibition zones. SEM images confirmed successful synthesis of alumina nanoparticles with uniform morphology and minimal surface irregularities. Over 80% of particles were below 50 nm, and the presence of porous structures was noted, indicating their potential for biomedical loading or adsorption applications. The antibacterial activity of the nanoparticles showed a clear dose-dependent response. At higher concentrations (40 and 80 mg/mL), the nanoparticles inhibited all S. aureus isolates effectively, with inhibition zones reaching up to 45 mm. MIC values ranged between 0.3 to 20 mg/mL across different strains. ANOVA and Tukey's post hoc analysis indicated a statistically significant difference ($p < 0.05$) among inhibition zones across the concentration gradient, confirming the efficacy of higher nanoparticle doses. The study also observed that the nanoparticles retained their bactericidal effect even at lower concentrations against several isolates, suggesting strong antimicrobial potency. The MBC values aligned closely with MICs, further validating their killing potential. The current findings are consistent with previous literature indicating that green-synthesized alumina nanoparticles exert antimicrobial effects primarily by disrupting bacterial cell walls and membranes. In this study, the integration of thyme extract enhanced the antibacterial potency, likely due to the synergistic effects of thymol, carvacrol, and other bioactive phytochemicals present in the extract. The results also demonstrate innovation in combining green synthesis techniques with nanomedicine. Previous studies utilizing silver or zinc oxide nanoparticles with plant extracts have shown similar efficacy, but alumina presents a lower toxicity profile and superior thermal stability, making it a more biocompatible alternative for medical applications. Given the multi-drug resistance profiles of MRSA and VRSA, novel agents that bypass traditional resistance mechanisms such as cell wall synthesis inhibition or efflux pump circumvention are essential. The thyme-mediated alumina nanoparticles offer such a strategy by leveraging multiple antibacterial mechanisms simultaneously.

Conclusion This research provides strong evidence supporting the development of biocompatible, porous alumina nanoparticles synthesized via green methods as potent antibacterial agents against multidrug-resistant Staphylococcus aureus. The use of *Zataria multiflora* extract not only facilitates a non-toxic synthesis process but also enhances the antimicrobial efficiency of the final nanomaterial. While the in vitro results are promising, further in vivo studies are essential to determine the cytotoxicity, pharmacokinetics, and therapeutic index of these nanoparticles. Additionally, evaluating their synergistic potential with existing antibiotics may lead to more effective combinatory therapies for resistant bacterial infections. The outcomes of this study mark an important step toward sustainable nanomedicine, merging traditional herbal knowledge with modern nanotechnology to address one of today's most pressing medical challenges.

Keywords: *Staphylococcus aureus*, Alumina nanoparticles, Thyme, Antibacterial activity.

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