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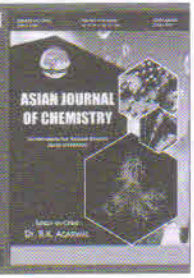

Effect of Green Silver Nanoparticle Synthesised by *W. coagulans* on Carbapenamse Resistant Gene Down Regulation Antibacterial Efficacy

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The present study investigates the phytochemical profile, phylogenetic relationships and antibacterial activity of *Withania coagulans*, along with the green synthesis of silver nanoparticles (AgNPs) and their effects on cytotoxicity and the downregulation of pathogenicity-related genes. Phytochemicals were extracted using Soxhlet extraction and AgNPs were synthesized *via* a one-step green synthesis approach. The synthesized nanoparticles were characterized using UV-Visible, FTIR and TEM techniques. The antibacterial efficacy of AgNPs against imipenem-resistant pathogens was evaluated, followed by cytotoxicity assessment. The results revealed that methanolic and chloroform extracts were rich in bioactive phytochemicals, which likely contributed to the enhanced antibacterial activity and biocompatibility of the synthesized AgNPs. These extracts also showed notable antibacterial activity at 100 µg/mL, producing inhibition zones of 13-22 mm especially against *Klebsiella pneumoniae* and *Streptococcus pneumoniae*. UV-Visible spectrophotometry confirmed characteristic peak at 210-230 nm attributed by phytochemical reducing agents. Size of AgNP were 20 to 95 nm and spherical in nature. The biosynthesised AgNPs demonstrated strong antibacterial effects, with inhibition zones of 17-21 mm across all tested clinical isolates. The qPCR analysis further showed substantial down-regulation (70-95%) of resistance associated genes, with fold-change values ranging from 0.045 to 0.51, indicating potent transcriptional suppression. Cytotoxicity assessment on A549 cells showed mild toxicity, maintaining high viability (91.8-95%) up to 100 µg/mL with preserved cell morphology confirming the biocompatibility of the nanoparticles. The findings establish *W. coagulans* as a genetically authenticated and phytochemically rich source for biosynthesised AgNPs with significant antibacterial and gene inhibitory activities and limited cytotoxicity, supporting its potential for biomedical applications.

Keywords: *W. coagulans*, Phenol, Nanoparticle, RNA expression, Antibacterial activity, Carbapenem.