

ABSTRACT

The mounting increase in multidrug-resistant bacterial pathogens has become a major global health concern, necessitating the investigation of eco-friendly and novel antimicrobial agents. The research aimed to investigate the antibacterial efficiency and phytochemical profile of *C. sinensis*, *C. limetta* and *C. limon* coat and seed extracts against two clinically significant Gram-negative pathogens, *Pseudomonas aeruginosa* and *Escherichia coli*. Isolation of respective bacterial strains were done from sputum samples of patients with lung illness, and diarrheal stool, and their identity were affirmed by morphological and genetic characterization.

Fresh coat and seed of three Citrus species were subjected to extraction by using aqueous solvent followed by green-synthesis of AgNO₃ nanoparticles. Phytochemical screening of both aqueous extracts discovered multiple active compounds that play significant role in their antimicrobial efficiency such as flavonoids, tannins, saponins, phenolics, terpenoids and alkaloids. UV-Visible spectroscopy and scanning electron microscopy confirmed their formation while Fourier-transform infrared spectroscopy identified multiple functional groups, scanning electron microscopy revealed morphology and distribution of nanoparticles, and GC-MS analysis validated the presence of volatile compounds like limonene,

phenols and phenolic acids of both extracts and AgNPs.

Antibacterial efficiency was evaluated using agar-well diffusion method in order to determine zones of inhibition (mm) values. Results demonstrated that both extracts and green-synthesized AgNPs showed antibacterial activity against tested pathogens. Among samples, nanoparticles showed greater antibacterial effects because of their enhanced surface interaction with bacterial cells and nano-scale size followed by coat extracts. Least inhibition zones were observed by seed extracts. Among tested species, *C. sinensis* exhibited greater antibacterial activity against both bacterial strains followed by the *C. limetta* and *C. limon*. In the statistical analysis, one-way ANOVA accompanied by the Tukey's post hoc test validated that the observed differences among control vs extracts, antibiotics and citrus-synthesized AgNPs were statistically significant ($p < 0.05$).

The study's overall findings highlights the comparative antibacterial capability of *C. sinensis*, *C. limetta* and *C. limon* and identifies all three species mediated nanoparticles as promising, sustainable and biocompatible substitute for

traditional antibiotics. The development of natural plant-derived nanotherapeutics for managing multi-drug resistant pathogenic strains is sufficiently aided by such researches.

Keywords: *C. sinensis*, *C. limetta*, *C. limon*, green-synthesized AgNO₃ nanoparticles, *P. aeruginosa*, *E. coli*, phytochemicals, characterizations, one-way ANOVA multidrug-resistance, antibacterial activity