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Biosynthesis of silver nanoparticles using *Camonea bifida* leaf extract and investigation of antimicrobial properties

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Silver nanoparticles (AgNPs) have been used in nanomedicine as an alternative antimicrobial agent and disinfectant. The biological synthesis of silver nanoparticles has gained much attention due to its eco-friendly and less toxic nature. The present study was focused on the biosynthesis of AgNPs using *Camonea bifida* leaf extract and the investigation of their antimicrobial and antibiofilm properties. Aqueous leaf extract (5 g/100 mL) of *Camonea bifida* was subjected to heat treatment at 60°C. For the optimization of synthesis, parameters including $AgNO_3$ concentration, $AgNO_3$: plant extract ratio, and reaction temperature were studied. The formation of AgNPs was confirmed by the UV-Visible absorption spectrum. Particle characterization was performed using Transmission electron microscopy (TEM), Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), and stability study. Antimicrobial activity was studied using well diffusion method against *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 1706), *Candida glabrata* (ATCC 90030), *Candida albicans* (ATCC 10231), and *Candida tropicalis* (ATCC 13803). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were performed for *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Gentamicin (600 µg/mL) and Fluconazole (500 µg/mL) were used as positive controls. The antibiofilm activity of AgNPs against *Pseudomonas aeruginosa* and *Candida glabrata* was performed using crystal violet assay. Color change and UV-Visible peak around 450 nm confirmed the formation of AgNPs. FT-IR spectrum indicated the presence of different functional groups of biomolecules such as O-H, C-H, C=O, C-N on the surface of AgNPs. The phytochemical analysis confirmed the presence of flavonoids, phenols, tannins, terpenoids, and chalcones as reducing and capping agents. TEM and SEM results indicated spherical-shaped AgNPs with an average size of 34 nm. AgNPs were stable for more than 4 weeks under room conditions. *Pseudomonas aeruginosa* (15.0±0.0 mm) and *Staphylococcus aureus* (16.0±0.6 mm) showed the highest sensitivity towards AgNPs while *Escherichia coli* showed moderate results (12.7±0.9 mm). *Klebsiella pneumoniae* and other *Candida* species did not respond except *Candida tropicalis* (10.3±0.3 mm). MIC and MBC of AgNPs against *Staphylococcus aureus* were 20.6 µg/mL and 41.3 µg/mL. MIC and MBC of AgNPs against *Pseudomonas aeruginosa* were 5.2 µg/mL and 10.3 µg/mL respectively. The percentage inhibition of biofilm formation of *Pseudomonas aeruginosa* and *Candida glabrata* was 69% and 61% respectively for 650 µg/mL AgNPs concentration. The overall results indicated the potential of using *Camonea bifida* mediated AgNPs as a promising antimicrobial and antibiofilm agent.

Keywords: Antibiofilm activity, Antimicrobial activity, *Camonea bifida*, Nanomedicine, Silver nanoparticles.