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Zinc oxide nanoparticles synthesized using *Camonea bifida* leaf extract as a potential antimicrobial and antibiofilm agent

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Metal nanoparticles have gained much attention in the biomedical field due to excellent biocompatibility and less toxicity. Zinc oxide nanoparticles (ZnO NPs) have exhibited excellent therapeutic value by acting antimicrobial, anti-cancer, anti-diabetic and anti-inflammatory agent. ZnO NPs are also effective in combating biofilm-associated infections. Only a few investigations have been performed on NP synthesis using *C. bifida*. This study was conducted to evaluate the *in vitro* antibiofilm and antimicrobial activity of *Camonea bifida*-derived ZnO NPs. *C. bifida* leaf extract was prepared by boiling 2g of dried leaves powder in 40 ml of distilled water at 60°C for 30 minutes. ZnO nanoparticles were synthesized using $(\text{Zn}(\text{CH}_3\text{CO}_2)_2)$ as the reducing agent for Zinc ions. The effect of reaction parameters including zinc acetate ($\text{Zn}(\text{CH}_3\text{CO}_2)_2$) concentration, leaf extract: $\text{Zn}(\text{CH}_3\text{CO}_2)_2$ ratio and reaction temperature were optimized to achieve higher yield using UV-Visible spectroscopy ($\text{Zn}(\text{CH}_3\text{CO}_2)_2$ concentration: 0.1-0.5 M), leaf extract: $\text{Zn}(\text{CH}_3\text{CO}_2)_2$ ratio 1:9, 1:7, 1:5, 1:3, 1:1 and reaction temperature : 0°C, 30°C, 40°C, 60°C, 80°C). Nanoparticles were characterized by Scanning Electron Microscopy and Fourier Transform Infrared Spectroscopy (FT-IR). The antimicrobial activity of ZnO NPs was studied against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 1706), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 10231), *Candida glabrata* (ATCC 90030), *Candida tropicalis* (ATCC 13803). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were studied. Gentamicin (600µg/mL) and Fluconazole (500µg/mL) were the positive controls for bacteria and fungi respectively. Negative control was sterile distilled water. Antibiofilm activity of ZnO NPs was assessed using crystal violet assay for *P. aeruginosa* and *C. glabrata*. The optimum conditions were 0.3M $\text{Zn}(\text{CH}_3\text{CO}_2)_2$, 1:3 ratio and 40°C. UV-Visible peak around 390 nm and precipitate formation confirmed the ZnO NP formation. ZnO NPs were spherical and irregularly shaped and were in the range of 60-70 nm. Functional groups attached on to the surface of NPs such as -OH and -C-H were revealed by FT-IR spectrum. According to UV-Visible spectra, ZnO NPs were stable for 2 months. All selected organisms were susceptible to ZnO NPs. Higher zones of inhibition were given by *P. aeruginosa* (30.7±0.3 mm), *S. aureus* (25.7±0.3 mm) and among fungi, *C. tropicalis* (27.7±0.3 mm). Both the MIC and MBC of ZnO NPs against *S. aureus* and *P. aeruginosa* were 3.6 mg/mL. ZnO NPs inhibited the biofilm formation of *P. aeruginosa* and *C. glabrata* by 68% and 52% respectively at 28.4 mg/mL concentration. *S. aureus*, *P. aeruginosa* and *C. tropicalis* showed higher susceptibility against the NPs. The highest antibiofilm activity of ZnO NPs was reported against *P. aeruginosa*. Results suggest that ZnO NPs are an effective antimicrobial and antibiofilm agent against tested pathogenic microorganisms.

Keywords: Antimicrobial activity, *Camonea bifida*, FT-IR, Zinc oxide nanoparticles