Green Synthesis of Zinc Oxide Nanoparticles (ZnO NPs) from Filamentous Green Algae *Cladophora* sp. and their Antibacterial Activity

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Abstract

The use of metal and metal oxide nanoparticles in agricultural, medical, and biotechnological fields is attracting increasing attention. Zinc is considered safe for humans, and its use as a natural material in the treatment of various diseases is being extensively investigated. Physical and chemical methods used in nanoparticle synthesis are expensive and have negative environmental impacts. However, nanoparticle synthesis through the use of biological materials such as bacteria, fungi, and plant extracts is fast, environmentally friendly, and low-cost. Nanoparticles synthesized especially using algae are highly valuable due to advantages such as their stability in solution and their safe processing with various metabolites. *Cladophora* sp., a filamentous green alga, can be used in medical applications because of its abundance of bioactive components such as vitamins, amino acids, fatty acids, carbohydrates, and polyphenols. The aim of this study is to synthesize ZnO NPs using *Cladophora* sp. extract and to characterize the obtained nanoparticles in order to evaluate their antimicrobial activity against certain bacterial species. For this purpose, after preparing the aqueous extract of *Cladophora* sp., it was heated at 80 °C for 20 minutes using a magnetic stirrer, filtered with Whatman paper. The resulting extract was cooled and stored at +4 °C for the biosynthesis process. For the biosynthesis of ZnO NPs, $ZnSO_4$ was added to the extract as a zinc precursor, and the mixture was kept at room temperature on a magnetic stirrer for 24 hours. The completion of the synthesis was indicated by the reaction mixture turning white. The reaction product obtained was centrifuged at 4500 rpm for 15 minutes and then washed three times with distilled water to remove unreacted soluble substances and phytochemicals. In the final stage, the white precipitate formed was dried in a muffle furnace at 400 °C for 24 hours. The obtained ZnO NPs through green synthesis were examined in detail using multiple characterization techniques. In the optical analyses performed with UV–Vis spectroscopy, the sharp excitonic absorption peak observed at 365 nm confirmed the formation of ZnO. The zeta potential value was measured as −5.52 mV, and the results of X-ray diffractometry (XRD) and electron microscopy (SEM, TEM) indicated the presence of well-dispersed spherical particles with sizes ranging from 35 to 50 nm. Energy-dispersive X-ray spectroscopy (EDS) analyses confirmed the presence of zinc and oxygen elements and showed that the material contained no impurities. In the Fourier transform infrared spectroscopy (FTIR) analyses, characteristic Zn–O stretching vibrations were observed at approximately 450–500 cm^{-1}, along with functional groups attributed to biomolecules involved in nanoparticle stabilization. The broth microdilution assay revealed that the MIC values of the ZnO NPs against *Escherichia coli* and *Staphylococcus aureus* were 125 µg/mL and 250 µg/mL, respectively. Overall, the findings confirm the successful synthesis of pure, crystalline ZnO nanoparticles and highlight their potential applications in photocatalytic, antimicrobial, and biomedical fields.

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