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ZnO Nanoparticles to Penetrate Bacterial Cell Membranes

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Abstract

This study demonstrates the synthesis of ZnO nanoparticles from the leaf extract of Mentha as a natural reducing and stabilizing agent. Phytochemical analysis of the methanolic extract identified active components. Nanotechnology has emerged as a revolutionary area in modern science that involves the design and manipulation of materials at the nanoscale in the range of 1-100 nm. These nano-scale materials exhibit unique physicochemical properties enabling novel applications across medicine, environmental science, and industry areas.

Keywords- Leaf, ZnO, Nanoparticles, Green synthesis, Mentha, Antimicrobial etc.

Introduction-

Among the diverse nanomaterials, nanoparticles—including carbon-based, metallic, polymeric, and semiconductor types- have garnered significant interest because of their high surface-area-to-volume ratio, tunable characteristics, and potential for multifunctionality. Metallic nanoparticles have shown immense promise for various applications, with ZnO nanoparticles standing out due to their versatility in antimicrobial treatments, catalysis, energy storage, and sustainable environments. Zn has a critical role in enzymatic functions, antioxidant defences, and the elimination of pathogens in living organisms.

The nanoscale form of ZnO oxide combines these biological benefits with enhanced reactivity and adsorption capacity, enabling unique properties such as antibacterial, antioxidant, and anticancer activities. These attributes make ZnO nanoparticles highly attractive for use in healthcare, agriculture, and beyond on the environment. Green synthesis methods have emerged as a sustainable and practical alternative to produce nanoparticles with minimal environmental impact and enhanced functional properties. Plant-based green synthesis consists of an abundance of phytochemicals such as phenols, flavonoids, and terpenoids that help in reducing and stabilizing nanoparticles during synthesis. The genus Mentha (mint) has long been valued in traditional medicine and industry for its rich bioactive composition, offering antimicrobial, antioxidant, and anti-inflammatory benefits. Recent research has shown that mint extracts, rich in phytochemicals such as polyphenols and terpenoids effectively aid in the green synthesis of nanoparticles like ZnO nanoparticles from the green method hold great promise in addressing the growing challenge of antibiotic resistance.

They show activity against both Gram-positive and Gram-negative bacteria by disrupting cell membranes, leading to leakage of intracellular contents and cell death. This mechanism makes them a potential solution against multidrug resistance against microbes. This study explores the synthesis of ZnO nanoparticles using mint leaf extracts followed by analysis through analytical methods and evaluation of antimicrobial properties. By employing plant-based green synthesis, this research adheres to the principles of green chemistry, emphasizing environmental sustainability and biocompatibility.

Results and Discussion –

Additionally, the study contributes to the main functions of phytochemicals in nanoparticle formation and their uses in biomedicine and environmental protection. **Material and Method-** The collected fresh leaves were carefully cleaned and dried in a shaded area or under mild sunlight for 5 days. The dried leaves were pulverized and kept in sealed containers to protect them from contamination. For extraction, the powdered plant material was immersed in distilled water. The mixture was stirred while maintaining a temperature of 60°C for one hour, followed by a standing period of 24 hours at room temperature. This extract was used for both nanoparticle synthesis and phytochemical screening [1]. **Synthesis of ZnO Nanoparticles** Approximately 0.1 M solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was prepared by dissolving 25 g of the compound in water and diluting it to 1 L in a volumetric flask.

The mixture was stirred continuously for one hour using a magnetic stirrer, with 1 M NaOH being introduced midway through the stirring process to adjust the pH to 10. Initially, the solution exhibited a dark brownish colour. **Preparation of Mint Extract for Phytochemical Screening** The mixture was allowed to macerate for 96 hours at room temperature, with intermittent shaking to enhance the extraction. After the maceration period, the mixture was passed through filter paper using a funnel to separate the solid residues from the liquid extract. The collected filtration was then concentrated by evaporating the methanol in a water bath set at 45 °C. To identify its phytochemical profile, a range of standardized methodologies were employed to detect specific bioactive constituents.

The presence of alkaloids was confirmed through Mayer's test μL of the respective sample solution, a negative control, DMSO (C-) and a standard kanamycin (Sigma Aldrich-USA, Purity: 95%) solution (5 mg/mL, 10 μL) for evaluate the optical properties, A UV-Vis spectrum of the sample, prepared as a dilute suspension in distilled water, was recorded by scanning the wavelength range from 200 to 600 nm. Functional groups related to the sample, acting as stabilizing and reducing agents, were identified through Fourier- transform infrared spectroscopy (FTIR) between 4000–400 cm^{-1} wavenumber and 4 cm^{-1} scan resolution μL of the respective sample solution, a negative control, DMSO (C-) and a standard kanamycin (Sigma Aldrich-USA, Purity: 95%) solution (5 mg/mL, 10 μL) for evaluate the optical properties, a SPECORD 200 Plus UV-vis spectrophotometer (Germany) was employed. A UV-Vis spectrum of the sample, prepared as a dilute suspension in distilled water, was recorded by scanning the wavelength range from 200 to 600 nm. Functional groups related

to the sample, acting as stabilizing and reducing agents, were identified through Fourier- transform infrared spectroscopy (FTIR) between 4000–400 cm^{-1} wavenumber and 4 cm^{-1} scan resolution.

As mentioned in published literature, this research also proves the leaf extract does not contain alkaloids, quinones, in plant metabolites like flavonoids, phenols, alkaloids, proteins, and quinones This interaction results in the reduction of copper ions from Zn(II) which then undergoes oxidation to form ZnO seed particles. Over time, these seed particles aggregate and nucleate, eventually forming stable znO nanoparticles **Antimicrobial Study.** tannins but possesses positive tests for flavonoids, saponins, and terpenoids Studies suggest that phytochemicals such as tannins and flavonoids exhibit antibacterial properties and play a significant role in scavenging free radicals .

However, the process of synthesizing nanoparticles based on natural resources is complex, as the precise mechanisms are not yet fully understood. Studies have emphasized that functional groups such as hydroxyl, carbonyl, amine, and methoxide are critical for the reduction and stabilization of nanoparticles. These groups are commonly found

Conclusion-

The higher activity of ZnO-NPs towards Gram- negative bacteria might be attributed to their thin cell membrane, which allows ZnO nanoparticles to penetrate bacterial cell membranes more effectively. Interestingly, the highest zone of inhibition (1.5 cm) was seen against *Candida albicans*. This study underscores the potential of plant-based methods for synthesizing antimicrobial nanoparticles, offering promising prospects for applications in medicine, agriculture, and sustainable development.

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