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RESEARCH ARTICLE

Antibacterial Properties of Zinc Oxide Nanoparticles Synthesized Using Green Chillie as Reducing Agent

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ABSTRACT

The study is on the green synthesis of zinc oxide nanoparticles and to determine its antibacterial properties as zinc oxide has characteristic physical and chemical properties. The reducing agent green chillie is used to prepare zinc oxide nanoparticles by eco-friendly methods at room temperature. Characterization studies indicated the ZnO nanoparticles were in the range of 67 to 92nm. SEM analysis indicated spherical and cylindrical shaped nanoparticles. Reducing and stabilization characteristics were identified by the presence of functional groups through Fourier Transform Infrared Spectroscopy (FT-IR) analysis. Color change established the synthesis of nanoparticles using the reducing agent of green chili extract. Antibacterial characteristics of the prepared nanoparticles were determined by measuring the zone of inhibition, and good results were obtained.

Keywords: Green chillie, zinc oxide nanoparticles, antibacterial properties

1 Introduction

Nanoparticles are defined as particles with sizes ranging from 1 to 100 nm. They can be categorized into various types based on their properties, structure, size, or shape [1]. These tiny particles exhibit unique physical and chemical characteristics due to their small size and large surface area. As a result, nanoparticles play a crucial role in numerous fields, including biomedical applications such as drug delivery systems and imaging contrast agents, as well as in agriculture, catalysis, construction, cosmetics, energy, electronics, environmental science, mechanical applications, and sensor technology [2]. They have transformed multiple sectors, particularly in drug delivery, agriculture, and the food industry. Nanoparticles can be synthesized using various methods, including physical, chemical, and biological approaches, with the chemical method being the most commonly favored [3]. Techniques for synthesizing nanoparticles include emulsion solvent extraction, double emulsion, salting out, solvent displacement/precipitation, evaporation, and emulsion diffusion methods [4]. Metal-based nanoparticles serve as effective antimicrobial agents against common pathogenic microorganisms; for instance, zinc oxide nanoparticles exhibit both antibacterial and antifungal properties. The eco-friendly synthesis of nanoparticles is particularly significant, as it is more energy-efficient and cost-effective compared to other methods [5]. Zinc oxide nanoparticles can be synthesized using various sources, including plants and microorganisms such as bacteria, fungi, viruses, and algae [6]. Among these, plant-based methods are particularly advantageous, as they simplify the process by eliminating the need to maintain complex microbial cultures and media. Additionally, plant extracts are increasingly favored due to their simplicity and cost-effectiveness, making them the most widely studied biological source for nanoparticle synthesis [7]. This study is motivated by the limited literature available on the synthesis of zinc oxide nanoparticles using plant-based methods.

In this research, an in vitro approach is employed, utilizing plant extracts to reduce zinc sulfate salt. Plants contain a variety of primary and secondary metabolites, including phenols, flavonoids, tannins, polypeptides, terpenoids, starches, and saponins, which serve as both reducing and capping agents in the synthesis process [8]. Plant metabolites are extracted using solvents like water, dilute ethyl alcohol, or methyl alcohol, which interact with zinc solutions under various conditions to achieve optimal yields [9]. This study focuses on the green synthesis of zinc oxide nanoparticles utilizing a reducing agent, such as green chilies. These nanoparticles possess numerous medical applications due to their enhanced properties compared to traditional zinc oxide. ZnO nanoparticles exhibit semiconducting characteristics, making them suitable for use in sensors designed to detect environmental pollutants and monitor epidemic disease outbreaks [10]. Additionally, these nanoparticles have potential applications in water purification, fertilizers, and plant protection products. Chilli is recognized for its antioxidant properties and as a source of natural dye extract [11]. The goals of this study are to synthesize zinc oxide using chili extract, characterize the green-synthesized ZnO nanoparticles through Fourier Transform Infrared (FTIR) spectroscopy and Scanning Electron Microscopy (SEM), and evaluate the antibacterial activity of these green-synthesized ZnO nanoparticles using the Agar well diffusion method.

2 Materials and Methods

2.1 Green synthesis of ZnO nanoparticles

Zinc sulfate with a purity of 90% was sourced from Hi-Media, and distilled water was utilized throughout the experiments for synthesizing ZnO nanoparticles. Fresh green chilies were purchased from the market, ensuring they were free from infection, thoroughly washed, and rinsed with sterile distilled water. 0.2 mM solution of zinc sulfate was prepared by mixing it with distilled water. To create the chilli extract, 20 grams of fresh chilies were ground with 100 ml of distilled water. The mixture was combined in a 1:4 ratio and heated in a water bath at 60°C for 30 minutes, until a color change was observed. The mixture was then filtered using Whatman No. 1 filter paper. Subsequently, it underwent centrifugation at 6000 rpm for 10 minutes and was washed with water for 2-3 minutes [12]. The resulting nanoparticles were dried in a hot air oven at 60°C and stored for future use. The formation of nanoparticles was observed using UV-Vis spectroscopy within the wavelength range of 250 to 500 nm [13]. In this study, a peak at 370 nm was detected, indicating the presence of zinc nanoparticles. The mixture was then centrifuged at 10,000 rpm for 15 minutes at 4°C. The resulting solid product, which exhibited a light orange color, was dried overnight by incubating at 60°C, resulting in the production of small-sized zinc oxide nanoparticles.

2.2 Characterization of ZnO nanoparticles

The UV-Vis spectra of the sample were obtained by measuring absorbance with a UV-Vis spectrophotometer. The FTIR spectra of ZnO nanoparticles synthesized with green chili were examined using a spectrometer and the KBr pellet method, covering a wavenumber range of 4000 to 400 cm^{-1}. The morphology of the ZnO nanoparticles was assessed through SEM analysis.

2.3 Antibacterial activity of ZnO nanoparticles by agar well diffusion method

Escherichia coli (E. coli) was isolated from tap water through a serial dilution (10^{-6}) using distilled water. The bacteria were cultured on agar media and incubated at 37°C for 24 hours. Isolated colonies were then transferred to agar growth medium and inoculated onto disk diffusion plates. The antibacterial activity of zinc oxide nanoparticles was evaluated against selected gram-negative pathogens using the Kirby-Bauer disk diffusion susceptibility test [14]. Aseptic transfer of the agar medium was performed onto petri plates, allowing it to solidify. Test bacterial strains were then swabbed onto the surface of the agar using a sterile L-rod. Sterile Whatman filter paper disks were employed to assess antibacterial activity. Each disk was loaded with 10 µL of diluted zinc nanoparticles and placed on the agar plate, which was incubated for 24 hours at 37°C. After the incubation period, a zone of inhibition was observed. Antibacterial activity was noted by measuring the diameter of the zone of inhibition formed around the disks [15].

3 Results and discussion

3.1 Green synthesis of ZnO nanoparticle using green chillie extract

The synthesis of ZnO nanoparticles using green chili extract solutions is illustrated in Figure 1. The extract contains various phytochemicals, including polyphenols, flavonoids, glycosides, and tannins, which serve as both reducing and stabilizing agents in the nanoparticle synthesis process. This formation occurs through interactions between the phytochemicals and metal ions. The appearance of a light brown color indicates the successful formation of zinc oxide nanoparticles, signifying the reduction of zinc ions [16]. This reduction is attributed to the presence of free electrons, which contribute to the development of a surface plasmon resonance absorption band.

Figure 1: *ZnO nanoparticles from green chillie extract*

3.2 Characterization of ZnO nanoparticles

A preliminary analysis of the nanoparticles was performed using UV-spectroscopy, which showed absorbance values within a wavelength range of 250 to 500 nm (Figure 2). In addition, ZnO nanoparticles were examined using FT-IR spectroscopy and SEM techniques.

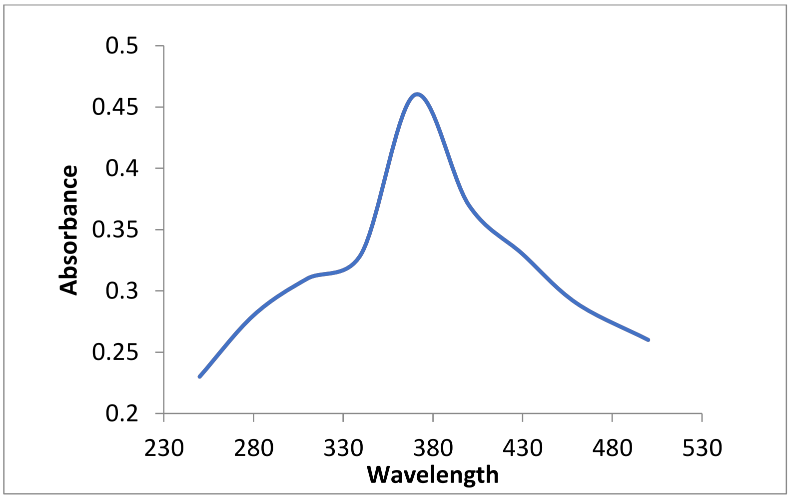

Figure 2: *UV spectra for Zinc oxide nanoparticles*

Figure 3 illustrates the FT-IR absorption spectra of ZnO nanoparticles synthesized through a green method. The various peaks identified in the FTIR analysis correspond to different functional groups, such as N-H groups in amines, C=O stretching in polyphenols and carboxyl groups, C=C stretching in aromatic rings, C–O stretching in amino acids, and C–H bonds in the aliphatic region [17]. Moreover, the peaks in the IR spectrum suggest the presence of biomolecules like polyphenols, alkaloids, and flavonoids within the ZnO nanoparticles, which are crucial for their stabilization [18]. The SEM analysis of ZnO nanoparticles revealed the formation of spherical and cylindrical nanocrystals. The nanoparticles synthesized using green chili extract exhibited diameters ranging from 67 to 92 nm. The size, shape, and distribution of the zinc oxide nanoparticles were assessed through SEM analysis.

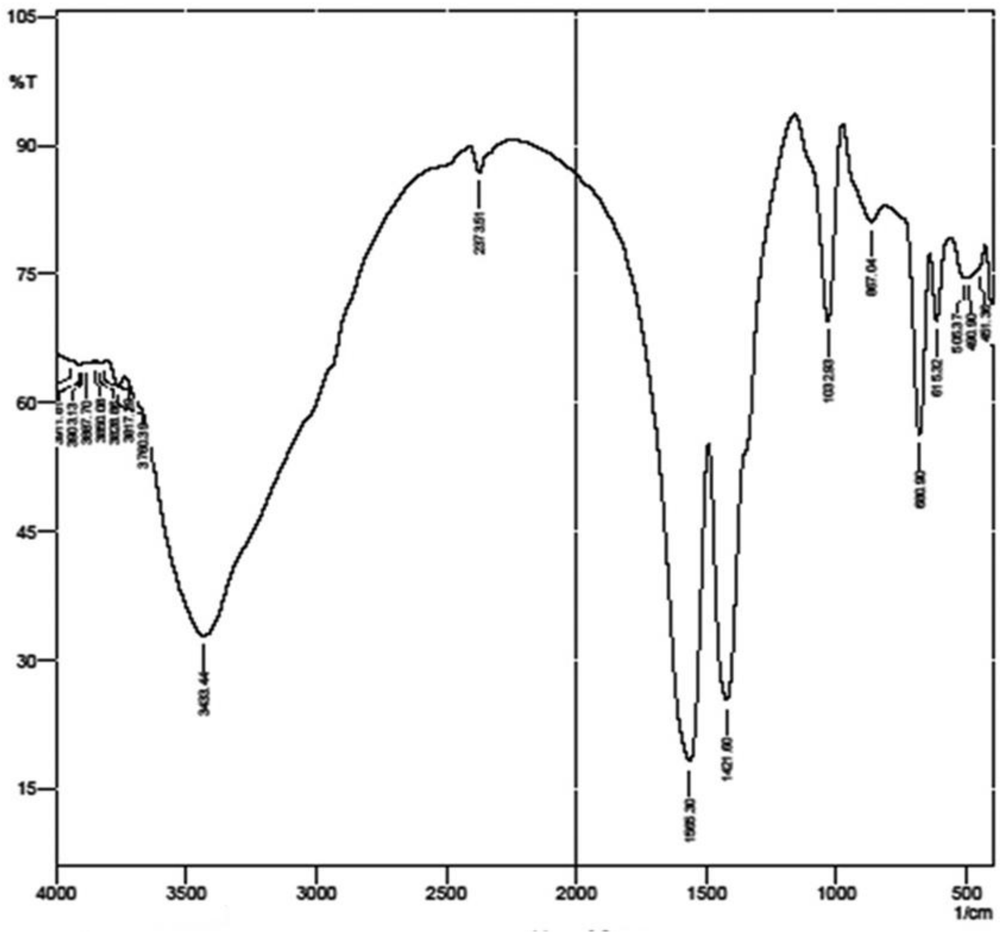

Figure 3: *FT-IR spectrum for zinc oxide nanoparticles*

3.3 Antibacterial activity

The antibacterial properties of ZnO nanoparticles synthesized using green chili as a reducing agent were assessed against the Gram-negative waterborne pathogen *Escherichia coli* through the disc diffusion method. The bactericidal effectiveness of the zinc oxide nanoparticles was evaluated by measuring the zone of inhibition [19]. Discs containing diluted ZnO nanoparticles exhibited a distinct clear zone, indicating antimicrobial activity that inhibits the growth of *Escherichia coli*. The measured zone of inhibition was 7 mm in diameter, likely attributed to the presence of functional groups such as proteins and amides in the biosynthesized ZnO nanoparticles. Electrostatic interactions between the bacterial cell surface and nanoparticles may contribute to the inhibition of bacterial growth and enhance the production of hydrogen peroxide from ZnO nanoparticles. These interactions can aid in the penetration of nanoparticles into the bacterial cell membrane, leading to cellular damage and ultimately resulting in bacterial death [20]. As a result, green extracts with antimicrobial properties are essential for effective treatment. Employing biological methods for nanoparticle synthesis, especially through plant and microbial sources, presents a practical approach, as the reduction of metal ions can occur efficiently under ambient temperature and pressure conditions. Thus, this study aimed to synthesize ZnO nanoparticles using green chili extract, characterize them, and assess their antibacterial properties.

The studies were conducted using FTIR spectroscopy and SEM analysis. FTIR analysis reveals the presence of biomolecules in the green extract, including phenolic groups, flavonoids, and alkaloids [21]. These biomolecules play a crucial role in the reduction and formation of amino acids and amide linkages in proteins, and they are also integral to the stabilization process of the nanoparticles. The IR spectrum of the ZnO nanoparticles distinctly shows the presence of these biomolecules, which are involved in the reduction and stabilization/capping processes [22]. The size and shape of the nanoparticles were determined through SEM analysis, which revealed the presence of both spherical and cylindrical forms [23]. The diameter of the ZnO nanoparticles varied between 67 and 92 nm, while their lengths ranged from 1 to 3μm (Figure 4).

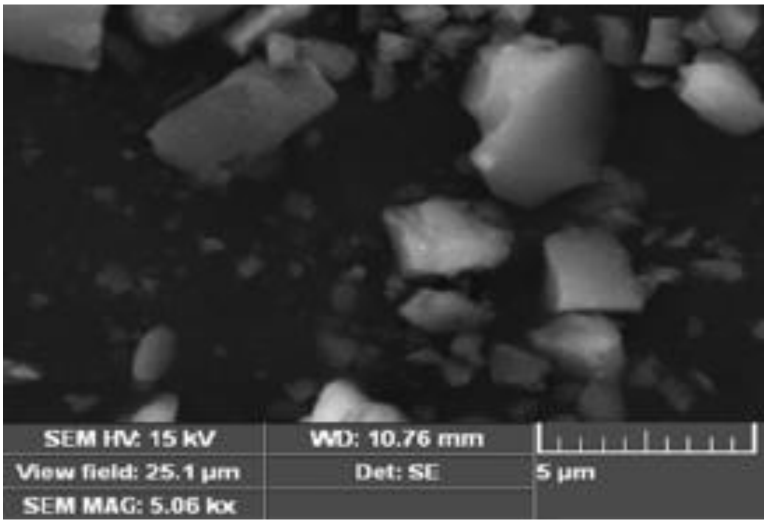

Figure 4: *SEM analysis of green synthesized zinc oxide nanoparticles*

The antibacterial properties of green synthesized nanoparticles demonstrated effectiveness in suppressing the growth of bacterial isolates (Figure 5). This efficacy can be attributed to the presence of various functional groups, including proteins and amides. These groups likely facilitate electrostatic interactions between the bacterial cell surface and the nanoparticles, resulting in growth inhibition and the production of hydrogen peroxide from ZnO nanoparticles [24].

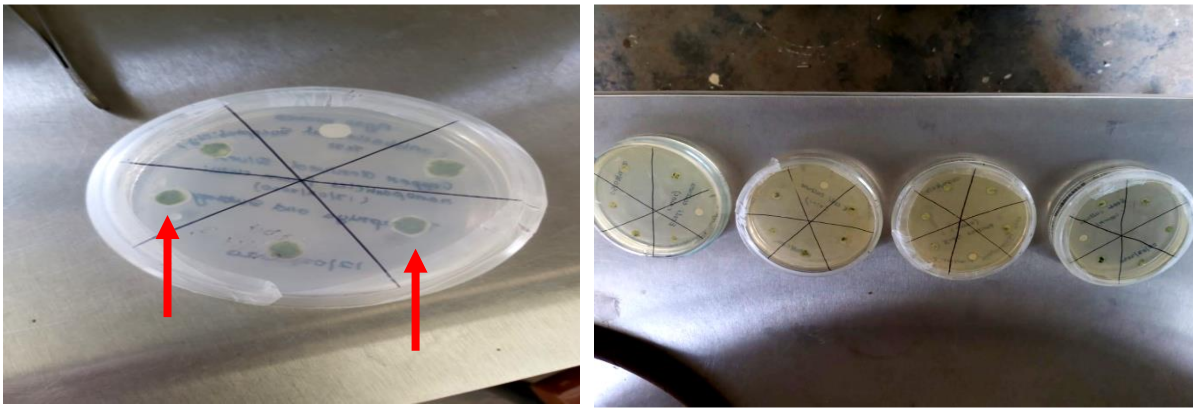

Figure 5: *Antibacterial susceptibility of the zinc oxide nanoparticles tested with Disk diffusion method. The arrow mark indicates the clear zone of Inhibition*

4 Conclusion

The synthesis of ZnO nanoparticles using green chili is a straightforward and efficient process that requires minimal time and is cost-effective. This method is stable, non-toxic, and environmentally friendly, making it suitable for large-scale production. FT-IR analysis confirmed the presence of biomolecules that serve as reducing and capping agents. SEM analysis revealed the formation of spherical and cylindrical nanoparticles, with diameters ranging from 67 to 92 nm and lengths between 1 to 3 µm. Consequently, the green synthesis route for nanoparticles has garnered significant interest. These nanoparticles have demonstrated potential as effective catalysts for organic reactions, highlighting the versatility of those produced through eco-friendly methods for various applications.

5 Publisher's Note

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