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Synthesis of Chitosan Functionalized Zinc Oxide Nanocomposite using *Moringa Stenopetala* Extract and Assessment of its Antibacterial and Cytotoxic Potentials

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Abstract

BACKGROUND/AIMS: Nanomaterials, especially nanocomposites, have garnered much attention due to their potential uses in antibacterial and cancer therapies. This research used *Moringa stenopetala* extract as a chelating agent to create a chitosan-functionalized zinc oxide nanocomposite (CZNM), and its cytotoxic and antibacterial properties were carefully assessed.

MATERIALS AND METHODS: The effective production of the nanocomposites was confirmed by their characterisation using fourier-transform infrared spectroscopy, energy-dispersive X-ray spectroscopy, X-ray diffraction, scanning electron microscopy, and UV-vis spectroscopy.

RESULTS: With an average size of 19 nm, the CZNMs had a spherical shape, and their stability was verified by zeta potential and UV-Vis studies. The produced CZNMs showed strong antibacterial action against *Escherichia coli* and *Salmonella typhi*. MDA-MB 231, and MCF-7, breast cancer cell lines were used in cytotoxicity tests, and the results showed concentration-dependent actions with IC50 values that were 63.4 µg/mL and 78.5 µg/mL, respectively.

CONCLUSION: Overall, CZNMs made from *Moringa stenopetala* extract have encouraging cytotoxic and antimicrobial properties, making them promising candidates for antimicrobial and cancer treatment.

Keywords: UV-vis spectroscopy, scanning electron microscopy, spherical morphology, *Salmonella typhi, Escherichia coli*

INTRODUCTION

Nanotechnology has emerged as a broad and advanced field focusing on developing eco-friendly methods to synthesize biosynthetic nanoparticles, such as zinc oxide (ZnO), silver, gold, and sodium nanoparticles. The distinct physicochemical features of these nanoparticles make them non-toxic, chemically stable, biocompatible, and useful as therapeutics for cancer,[1] as diabetes drugs,[2] as antibacterial agents,[3] in cell imaging devices,[4] as biological sensors,[5] as carriers for drugs,[6] and in aesthetics.[7]. Before the development of green synthesis techniques, metal oxide nanoparticles were usually made by chemical processes involving dangerous chemicals, which made the synthesis time-consuming, expensive, and toxic.[8] At the same time, to change and improve the materials' electrical work, optics, and mechanical

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characteristics, as well as their external chemical makeup, some nanoparticles, like metal oxide, are modified by completely replacing some metal ions with designated atoms.[9]

Therefore, this research study aims to provide an eco-friendly pathway that fits in the production of modified nanocomposites ZnO [chitosan-functionalized zinc oxide nanocomposites (CZNMs)], which involves the application of chitosans and of *Moringa stenopetala* extract, primarily as reducing and stabilising agents and zinc nitrate solution as a precursor, respectively. This approach has several advantages: simplicity, environmental coherence, affordability, high-purity precursors, and few by-products.[10] Furthermore, the synthesis of CZNMs does not include any toxic substances and occurs at an acceptable temperature.[11] This study fills a gap in the literature by synthesising a new, environmentally friendly, and sustainable CZNM for the multipurpose use in antibacterial therapies and cancer treatment using extract from *Moringa stenopetala*, an organic and naturally occurring resource. The individual uses of ZnO nanoparticles (ZnO NPs) and *Moringa* extract have been the subject of many investigations, but little work has integrated them to create an nanocomposite system that offers a thorough evaluation of both antimicrobial and cytotoxic properties.

MATERIALS AND METHODS

Synthesis of Chitosan-functionalized Zinc Oxide Nanocomposites

CZNMs using chitosan and *Moringa stenopetala* were attained using the method developed by Kavaz et al.[12] CZNMs were synthesized using 0.1 M zinc nitrate hexahydrate [$Zn(NO_3)_2{\cdot}6H_2O$] procured from Gibco by Life Technologies, United States of America. The synthesis was performed in a conical flask at a controlled temperature of 45 °C for 72 hours, with water serving as the solvent for the extraction process. Chitosan and *Moringa stenopetala* aqueous extract was introduced by drops to the zinc nitrate solution (1:1:9) while stirring continuously at 60 °C for 5 hours to enhance complex formation. Furthermore, the blend was vigorously calcined in a muffle furnace, as shown in Figure 1. The resulting residue comprised the synthesized CZNMs.

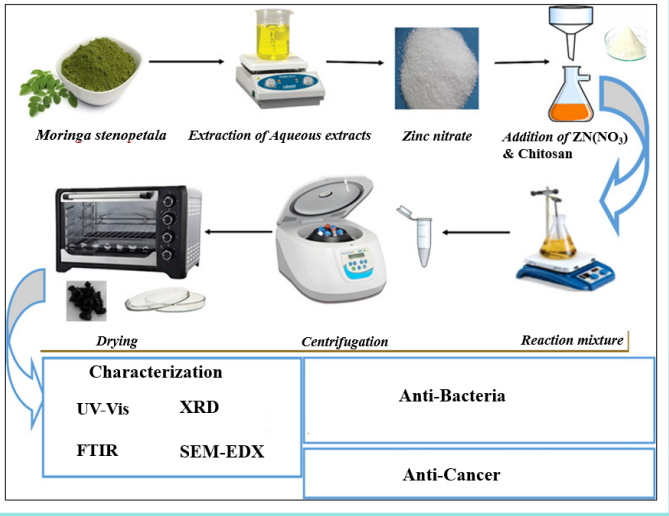

Figure 1. The representation of combined zinc oxide nanocomposites.

XRD: X-ray diffraction, FTIR: Fourier transform infrared, SEM: Scanning electron microscopy.

Characterization

Several analytical methods were used to characterize CZNMs. A Shimadzu UV-2450 UV-Vis spectrophotometer was used to capture the spectrum of UV-Vis absorption after the CZNMs were filtered via a 0.45 µm membrane, sonicated to achieve a constant dispersion, and then dissolved in deionized water. A 10 mm optical pathlength container was used to obtain ambient temperature spectra in the 300-800 nm wavelength range. Fourier transform infrared (FTIR) spectroscopy, used to find the functional categories in the CZNMs, was used, which has a frequency range of 500 to 4,000 cm^{-1} and a resolution of 4 cm^{-1}. The shape and structure of the nanocomposites were examined using scanning electron microscopy (SEM), and their crystallization was assessed with X-ray diffraction (XRD) employing a Rigaku ZSX Primus II.

Antimicrobial Activity

The antibacterial activity of synthesized CZNMs on *Salmonella typhi* (B-4420) and *Escherichia coli* (ATCC 8739) was assessed on Müller hinton's culture trays.[13] The sterilized empty disc was filled with an established amount of CZNMs, with ciprofloxacin (10 µg/disc) as a reference. Antibacterial efficacy was assessed with 30% DMSO.

Tryphan Blue Assay

The CZNMs' toxicity has been determined on MDA-MB 231 and MCF-7 cell lines using a slightly modified method of Umar.[14] The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum and L-glutamate (2%), and then grown to confluence in an incubator at 37 °C, 5% CO_2. The cells were subjected to nanoparticle concentrations (500, 250, 50, 10, 5, and 0 µg/mL) and then cultivated for 24 and 48 hours at 37 °C. After incubation, the treatment was removed, and the number of living and dead cells was counted independently. In the results section, the mean ± standard deviation was displayed.

Proliferation Assay

The 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to measure cell growth, following the methodology developed by Umar and Aliyu.[15] MDA-MB 231 cells (3×10,000) were incubated for 24 hours after exposure to CZNM doses of 0, 5, and 10 µg/mL. After incubation, the wells were filled with 600 µL of new growth media containing 150 µL of MTT solution (5 mg/mL) (Sigma-Aldrich Inc.). Using an ELX 800TM Absorption Microplate Reader, the absorption was measured at 570 nm. The experiment was carried out in triplicate to guarantee dependability, with each treatment repeated thrice.

Ethical Concern

The study involves using cell lines, not human subjects, and does not require ethical approval or informed consent.

Statistical Analysis

Data are shown as means ± SEM. The statistical analysis was performed using IBM SPSS Statistics version 21. The data were analysed using one-way ANOVA and the Student's t-test. All studies were done in triplicate ($n \geq 3$), and statistical significance was regarded as a p-value <0.05.

RESULTS

The biosynthesis of CZNMs using *Moringa stenopetala* and chitosan as reducing and stabilizing agents was confirmed by a visible color change and various characterization techniques. The UV-visible

spectrophotometer analysis showed a strong absorption peak at 380 nm (Figure 2a), indicating the formation of ZnO nanoparticles. The zeta sizer analysis determined the average particle size to be approximately 19 nm and confirmed the colloidal stability through zeta potential measurements.

FTIR spectroscopy revealed characteristic bands at 1446 cm^{-1} and 1033 cm^{-1} corresponding to C=C methyl groups and ZnO bonds, respectively (Figure 2b). The presence of alcohol groups suggests their role in stabilizing the nanoparticles. XRD analysis showed distinct diffraction peaks at 31.70°, 34.34°, 47.54°, 56.48°, 62.78°, 67.66°, 72.53°, and 76.58° (Figure 2c), consistent with the hexagonal wurtzite crystalline structure of ZnO nanoparticles. SEM imaging confirmed the spherical shape of the nanoparticles, with an average size of 19 nm (Figure 2d).

The antibacterial potential of the synthesized NPs was tested on *S. typhi* and *E. coli,* adopting the disc diffusion technique (Table 1). CZNMs had higher antibacterial activities than chitosan on both bacteria, with a significant difference compared to standard ($p<0.05$; $n\geq3$; Table 1).

The cytotoxic effect of CZNMs was evaluated on MDA-MB 231 and MCF-7 breast cancer cell lines using MTT and trypan blue assays. A concentration-dependent decrease in cell viability was observed, with the highest cytotoxic effect at 500 µg/mL after 24 hours of incubation (Figure 3a, b). No significant cytotoxicity was observed at lower concentrations (5 and 10 µg/mL), which were further tested for anti-proliferative potential (Figure 3c).

DISCUSSION

The biosynthesis of CZNMs using green methods was supported by the colour change and characteristic UV-Vis absorption peak at 380 nm, aligning with earlier findings by Kavaz et al.[16] The FTIR analysis confirmed the presence of functional groups from plant extract and chitosan that contribute to capping and stabilizing the nanoparticles, helping to prevent agglomeration.[17] Furthermore, the crystalline nature confirmed via XRD patterns is typical of ZnO nanoparticles and consistent with previous literature.[18] The SEM results further verified the uniform spherical morphology. Prior studies have emphasized that nanoparticle size and shape influence their interaction with cells and biological targets.[19] The nanoparticle size (19 nm) and high zeta potential support the synthesis of stable and monodispersed nanostructures. Smaller particle sizes often exhibit superior surface activity, improving biological interactions and stability.[20]

The significant antibacterial activity of CZNMs against *E. coli* and *S. typhi* demonstrates their potential as antimicrobial agents. Their enhanced efficacy over chitosan or zinc nitrate alone suggests synergistic effects when both components are combined. This is especially relevant for combating resistant bacterial strains.

In the cytotoxicity assays, the concentration-dependent effects are

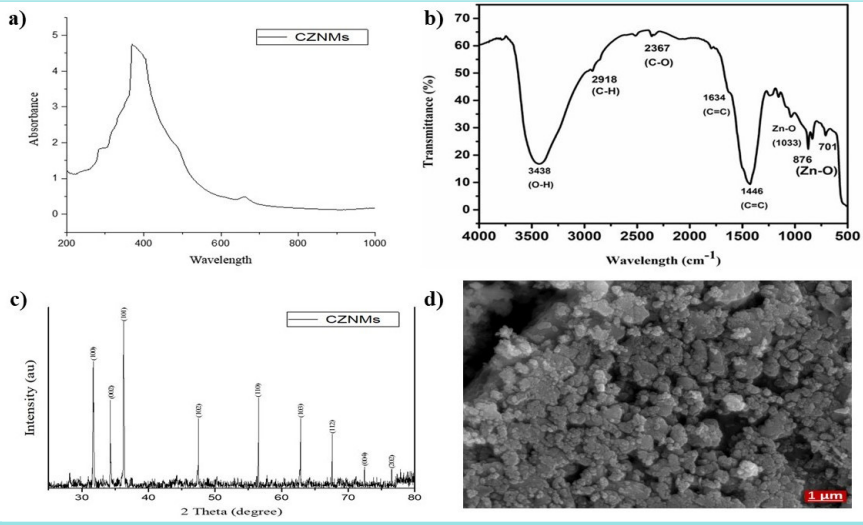

Figure 2. a) Absorption spectra of the CZNMs by UV-visible Spectrophotometer. b) FTIR of the CZNMs. c) XRD pattern of CZNMs. d) SEM image of CZNMs (scale bar 1µm).

CZNMs: Chitosan-functionalized zinc oxide nanocomposites, XRD: X-ray diffraction, FTIR: Fourier transform infrared, SEM: Scanning electron microscopy.

Table 1. Anti-microbial activity of chitosan-functionalized zinc oxide nanocomposites (CZNMs)

Zone of Inhibition (mm)		
Bacterial strains	***Escherichia coli* (mean ± SEM)**	***Salmonella typhi* (mean ± SEM)**
Standard ciprofloxacin (10 µg/disc)	14.0±0.20	12.53±0.50
CZNMs	11.6±0.47**	10.80±0.20**
Chitosan	5.8±0.15**	6.20±0.10**
Zinc nitrate solution (0.1M)	1.20±0.10**	1.22±0.20**
DMSO (30%)	-	-

CZNMs: Chitosan-functionalized zinc oxide nanocomposites, DMSO: Dimethyl sulfoxide, SEM: Scanning electron microscopy, ** $p<0.01$ vs. control (standard group).

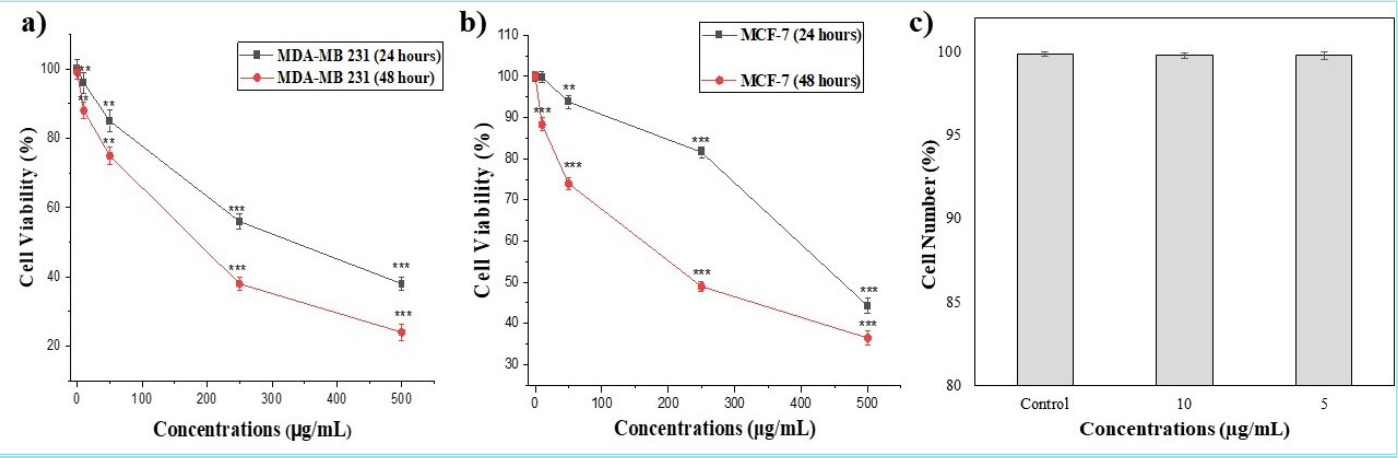

Figure 3. Effect of CZNMs on the viability of a) MDA-MB 231, and b) MCF-7 cells. c) Effect of CZNMs on the viability of a) MDA-MB 231 cells.
CZNMs: Chitosan-functionalized zinc oxide nanocomposites.

consistent with existing literature on green-synthesized ZnO NPs.[21] The more pronounced cytotoxicity observed in MDA-MB 231 cells compared to MCF-7 suggests possible differential sensitivity between triple-negative and hormone receptor-positive breast cancer cells. Furthermore, lower concentrations did not significantly affect viability, indicating that the CZNMs might be safe at low doses-an essential consideration for therapeutic applications. Our findings are in agreement with prior studies, such as those by Taher et al.[22] who showed that chitosan-based nanoparticles have dose-dependent anti-proliferative effects. Similarly, thymoquinone-loaded nanoparticles also demonstrated comparable apoptotic activity, reinforcing the potential of natural product-based nanocarriers in cancer therapy.[23]

Study Limitations

The absence of other analyses, such as lateral mobility, migration, and invasion assays, is a study limitation. These tests are required to verify that concentrations determined to be non-toxic to cells have no discernible effects on cellular behaviours, including proliferation.

CONCLUSION

In conclusion, there is considerable antibacterial and cytotoxic potential in the manufacture of CZNMs utilizing extract from *Moringa stenopetala*. In addition to their dose- and time-dependent cytotoxic effects on MDA-MB 231 and MCF-7 breast cancer cells, the nanocomposites demonstrated potent antibacterial activity against *S. typhi* and *E. coli*. These results imply that the CZNMs have potential as therapeutic candidates, especially in cancer treatment, due to their efficacy and biocompatibility. More research into their mode of action and *in vivo* efficacy is necessary to explore their clinical potential.

MAIN POINTS

- The chitosan-functionalized zinc oxide nanocomposites (CZNMs) were successfully synthesized using *Moringa stenopetala* extract, and characterized using various spectroscopic and microscopic techniques in which spherical morphology and an average size of 19 nm was observed.
- The synthesized CZNMs revealed potent antibacterial effects against *Escherichia coli* and *Salmonella typhi*. Compared to chitosan and zinc nitrate solution alone, suggesting their effectiveness as an antibacterial agent.
- Dose-dependent cytotoxicity on MCF-7 and MDA-MB 231 breast cancer cell lines was observed when treated with CZNMs.

ETHICS

Ethics Committee Approval: Not available.

Informed Consent: Not available.

Footnotes

Authorship Contributions

Concept: H.U., N.U., B.B.D., D.U.O., M.R.A., Design: H.U., N.U., D.U.O., M.R.A., Data Collection and/or Processing: H.U., B.B.D., D.U.O., M.R.A., Analysis and/or Interpretation: H.U., N.U., D.U.O., Literature Search: H.U., B.B.D., D.U.O., M.R.A., Writing: H.U., N.U., B.B.D., D.U.O., M.R.A.,

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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