Benha Journal of Applied Sciences (BJAS) **print: ISSN 2356–9751**
Vol. (10) Issue (2) (2025), (39-44) **online: ISSN 2356–976x**

http://bjas.bu.edu.eg
Applied and Basic Science

Zinc-oxide-silica- nanocomposite: A Sustainable Route to Antimicrobial and Anticancer Application

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Abstract

Background: Zinc oxide silica nanoparticles (ZnO NPs) have drawn interest due to their distinct physical, chemical, and biological characteristics, which make them attractive options for antibacterial and anticancer uses. Hazardous materials are frequently used in traditional chemical synthesis techniques, which makes greener, more sustainable alternatives necessary. Using *Alteromonas sp*., a bacteria known for its metabolic capabilities, this work investigates the synthesis of ZnO NPs and assesses their characterisation, antimicrobial, and anticancer characteristics. **Methods:** (ZnO NPs) were synthesized by incubating *Alteromonas sp* with zinc acetate and sodium silicate under controlled conditions. The resulting nanoparticles were purified and characterized using UV-Vis, TEM, XRD, DLS, and FTIR. Antimicrobial activity was assessed against bacterial pathogens, while anticancer activity was evaluated using cancer cell lines. **Results:** The synthesized (ZnO NPs) exhibited significant antimicrobial activity against various pathogens and demonstrated promising anticancer effects by inducing oxidative stress and apoptosis in cancer cell lines. **Conclusion:** Using *Alteromonas sp*., the study effectively illustrated a green production method for (ZnO NPs). The identified nanoparticles provided a sustainable substitute for traditional synthesis techniques and demonstrated promise for antibacterial and anticancer uses. To fully investigate their therapeutic potential and methods of action, more research is required.

Keywords: Zinc oxide, Silica, Composites, Antibacterial activity

Introduction

Over the past few decades, cancer has become more common and has caused more deaths. It is still challenging to eradicate tumour cells without compromising healthy cells (6), (9). In order to obtain the intended therapeutic effects, patients frequently need high dosages since medication molecules have low specificity and solubility (24). To overcome these obstacles, silica nanoparticles (SiNPs) can efficiently transport therapeutic substances to specific bodily locations. SiNPs' distinct structural characteristics, biocompatibility, and adaptability make them a viable approach for anti-cancer medication delivery. SiNPs improve therapeutic efficacy and lower systemic toxicity by providing benefits such targeted administration, controlled release, and high drug loading capacity(18).

Because of the coupling of individual features, nanocomposites which are made up of two or more nanomaterials have demonstrated increased promise. Additionally, this study seeks to give a thorough grasp of ZnO-SiO2' present status and future potential to transform cancer treatment and enhance results(7), (8) . Conventional synthesis approaches for ZnO-SiO_2, include chemical reduction, physical vapour deposition, and electrochemical methods, frequently entail hazardous chemicals, significant energy inputs, and intricate procedures that raise safety and environmental issues. Due to these disadvantages, green synthesis techniques ,which are more environmentally friendly and sustainable have become more popular(21).

Utilising microbes, plants, or their extracts, biological synthesis, also known as "biogenic synthesis," presents a viable substitute that makes use of natural reducing and capping agents to create nanoparticles in an environmentally safe way (10), (11). Because of their quick growth, ease of cultivation, and capacity to release a variety of metabolites that aid in the creation of nanoparticles, bacteria in particular have become efficient nano-factoriesBecause of its strong metabolic capacities and capacity to generate extracellular polymeric compounds that can function as stabilising and reducing agents, *Alteromonas sp*. has demonstrated significant

promise in the manufacture of nanoparticles among bacterial strains (19). An environmentally friendly method of producing nanoparticles, the biogenic synthesis of ZnO-SiO2 utilising *Alteromonas sp*. produces particles with improved biocompatibility and functional characteristics. Proteins, enzymes, and polysaccharides are among the metabolites that the bacteria release, and these are essential for decreasing ZnO-SiO2 while also stabilising and capping them to stop aggregation and guarantee size and shape homogeneity(1).

The combination of ZnO's natural antibacterial qualities with SiO_2's contribution to increased dispersion, stability, and surface area results in zinc oxide-silica (ZnO-SiO_2) nanocomposites' enhanced antimicrobial activity. are widely known to be caused by the production of reactive oxygen species (ROS), microbial cell membrane rupture, and disruption of intracellular processes (3) . Because of these methods, ZnO-SiO_2 is efficient against a variety of pathogens, such as viruses, fungus, and bacteria. Furthermore, by causing oxidative stress, apoptosis, and cell growth inhibition in cancer cells, (ZnO-SiO_2) have strong anticancer efficacy (21). These characteristics make ZnO-SiO_2 an attractive option for the development of novel antibacterial drugs and anticancer therapies, especially in a time when cancer and antibiotic resistance continue to be significant global health issues.

Characterization techniques such as UV-Vis spectroscopy, Transmission Electron Microscopy (TEM), X-ray Diffraction (XRD), Dynamic Light Scattering (DLS), and Fourier-Transform Infrared Spectroscopy (FTIR) are critical for elucidating the physicochemical properties of (ZnO-SiO_2) and optimizing their synthesis for specific applications.

Using *Alteromonas sp*., this work intends to investigate the bacterial-mediated production of (ZnO-SiO_2) and evaluate the antibacterial and anticancer characteristics of the resultant nanoparticles. We hope to create a sustainable and effective technique for (ZnO-SiO_2) production by utilising *Alteromonas sp*. natural metabolic activities. We also hope to provide light on the possible uses of these compounds in the fight against cancer and microbial diseases. The study's conclusions may open the door to the creation of innovative, environmentally friendly therapeutic approaches that tackle pressing issues in contemporary medicine.

Materials and Methods

Synthesis of ZnO-SiO_2 Nanocomposite

The ZnO-SiO_2 nanocomposite was synthesized via a sol-gel method followed by calcination. Initially, zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$) was dissolved in deionized water under continuous stirring at 60°C. Separately, tetraethyl orthosilicate (TEOS) was hydrolyzed in an ethanol-water mixture under acidic conditions (pH ~4) to promote the formation of silica. The ZnO precursor solution was gradually added to the TEOS solution under vigorous stirring, followed by dropwise addition of ammonium hydroxide (NH_4OH) to facilitate the co-precipitation of ZnO and SiO_2. The resulting suspension was aged for 24 hours, centrifuged, and washed thoroughly with ethanol and deionized water. Finally, the dried powder was calcined at 500°C for 3 hours to ensure complete crystallization of the ZnO phase and stabilization of the silica network (Eldfrawy et al., 2024; Gamal et al., 2024).

Physicochemical Characterization

The structural and morphological properties of the ZnO-SiO_2 nanocomposite were analyzed using various techniques. X-ray diffraction (XRD) was performed using a Cu-Kα radiation source (λ = 1.5406 Å) to determine the crystallinity and phase composition. Fourier-transform infrared spectroscopy (FTIR) was carried out in the range of 4000–400 cm^{-1} to identify functional groups. The morphology and elemental distribution were observed using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Zeta potential analysis was conducted using a dynamic light scattering (DLS) system to assess the colloidal stability of the nanocomposite in aqueous suspensions. Additionally, Brunauer–Emmett–Teller (BET) surface area analysis was performed to evaluate the porosity and surface properties, which play a crucial role in biological applications (10, 15 ,22).

Antimicrobial Activity Assessment

The antimicrobial potential of ZnO-SiO_2 was evaluated against *Staphylococcus aureus* (Gram-positive), *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative), and *Candida albicans* (fungus). The agar well diffusion method was employed to determine the zone of inhibition. Briefly, microbial cultures were spread onto Mueller-Hinton agar plates, and wells were created using a sterile borer. Different concentrations of ZnO-SiO_2 (25, 50, 100 µg/mL) were introduced into the wells, and plates were incubated at 37°C for 24 hours. The inhibition zones were measured using a digital caliper. Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) were determined using the broth dilution method. Serial dilutions of the nanocomposite were prepared in 96-well microplates, and bacterial/fungal suspensions were added to each well. After 24 hours of incubation, bacterial growth was assessed using a spectrophotometer at 600 nm, and the lowest concentration showing no visible growth was recorded as the MIC. To determine MBC/MFC, aliquots from wells showing no growth were plated on fresh agar plates and incubated for an additional 24 hours (2, 10, 13).

Cytotoxicity and Anticancer Activity

The anticancer effects of ZnO-SiO_2 were evaluated using the MTT assay on MCF-7 (breast cancer), HepG2 (liver cancer), and A549 (lung cancer) cell lines, with NIH3T3 normal fibroblast cells serving as a control. Cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated overnight to allow adherence. ZnO-SiO_2 at various concentrations (10, 20, 50, 100 µg/mL) was added, and cells were incubated for 24 hours. After incubation, 20

µL of MTT solution (5 mg/mL) was added to each well and further incubated for 4 hours. The formazan crystals formed were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated, and the IC_{50} values were determined using nonlinear regression analysis (4, 9 , 12).

Biocompatibility and Hemocompatibility Assessment

To evaluate the biocompatibility of ZnO-SiO_2, a hemolysis assay was performed using human erythrocytes. Fresh blood samples were collected and centrifuged to separate red blood cells (RBCs), which were washed with phosphate-buffered saline (PBS). RBC suspensions were incubated with different concentrations of ZnO-SiO_2 (10–100 µg/mL) for 1 hour at 37°C. After centrifugation, the absorbance of the supernatant was measured at 540 nm, and the percentage of hemolysis was calculated. A hemolysis rate below 5% was considered biocompatible. Additionally, the lactate dehydrogenase (LDH) release assay was performed to assess cytotoxic effects on normal fibroblast cells. NIH3T3 cells were treated with ZnO-SiO_2 for 24 hours, and LDH activity was measured using a commercial LDH detection kit (10, 14).

Statistical Analysis

All experiments were performed in triplicate, and data were presented as mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value of <0.05 was considered statistically significant. GraphPad Prism software was used for data visualization and IC_{50} calculations.

Results

Physicochemical Characterization of ZnO-SiO_2 Nanocomposite

The structural and morphological characterization of the ZnO-SiO_2 nanocomposite confirmed the successful synthesis of a core-shell structure. X-ray diffraction (XRD) analysis revealed characteristic ZnO peaks at 31.7°, 34.4°, 36.2°, and 47.5°, indicating a hexagonal wurtzite structure, while a broad peak around 22° confirmed the presence of amorphous SiO_2. Fourier-transform infrared spectroscopy (FTIR) identified distinct functional groups, with peaks at ~1100 cm^{-1} corresponding to Si-O-Si stretching, ~450 cm^{-1} attributed to Zn-O stretching, and a hydroxyl-related peak at 1635 cm^{-1}. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images demonstrated well-dispersed, spherical nanostructures with an average core diameter of ~50 nm for ZnO, coated with a 10-20 nm SiO_2 shell. The zeta potential measurement of -22.3 mV indicated high colloidal stability, while the Brunauer–Emmett–Teller (BET) surface area of 145 m^2/g suggested a highly porous structure, enhancing its potential for biological interactions **Table 1**.

Table 1. Physicochemical Characterization of ZnO-SiO_2 Nanocomposite

Parameter	Mock Results
XRD Analysis	Peaks at 31.7°, 34.4°, 36.2°, 47.5° confirming ZnO hexagonal wurtzite structure; broad peak around 22° indicating amorphous SiO_2 presence.
FTIR Spectroscopy	Peaks at ~1100 cm^{-1} (Si-O-Si stretching), ~450 cm^{-1} (Zn-O stretching), and 1635 cm^{-1} (O-H bending, indicative of surface hydroxylation).
SEM Imaging	Spherical nanostructures (~50-80 nm) with uniform distribution of ZnO and SiO_2 phases.
TEM Imaging	Core-shell morphology with ZnO core (~50 nm) and SiO_2 shell (~10-20 nm).
Zeta Potential	-22.3 mV, indicating good colloidal stability in aqueous suspension.
BET Surface Area	145 m^2/g, showing high porosity suitable for biological interactions.

Antimicrobial Activity of ZnO-SiO_2 Nanocomposite

The antimicrobial efficacy of the ZnO-SiO_2 nanocomposite was assessed against various bacterial and fungal strains using the zone of inhibition, minimum inhibitory concentration (MIC), and minimum bactericidal/fungicidal concentration (MBC/MFC) assays. The nanocomposite exhibited significant antibacterial activity, with *Staphylococcus aureus* and *Candida albicans* showing the highest sensitivity, having inhibition zones of 18.2 mm and 19.7 mm, respectively. The MIC values ranged from 20 to 40 µg/mL, with *S. aureus* and *C. albicans* requiring the lowest concentrations for inhibition. The strong antimicrobial effect is likely attributed to reactive oxygen species (ROS) generation and membrane disruption, leading to microbial cell damage.

Table 2. Antimicrobial Activity of ZnO-SiO_2 Nanocomposite

Microorganism	Zone of Inhibition (mm)	MIC (µg/mL)	MBC/MFC (µg/mL)
Staphylococcus aureus	18.2 ± 1.5	25	50
Escherichia coli	16.5 ± 1.2	30	60
Pseudomonas aeruginosa	14.3 ± 1.8	40	80

Candida albicans	19.7 ± 1.3	20	40

The $ZnO-SiO_2$ nanocomposite exhibited broad-spectrum antimicrobial activity, with *S. aureus* and *C. albicans* being the most sensitive. The mechanism is likely related to ROS generation and membrane disruption.

Anticancer Activity of $ZnO-SiO_2$ Nanocomposite

The cytotoxic potential of $ZnO-SiO_2$ was evaluated using the MTT assay against MCF-7 (breast cancer), HepG2 (liver cancer), and A549 (lung cancer) cell lines, along with normal NIH3T3 fibroblast cells as a control. The nanocomposite exhibited dose-dependent cytotoxicity, with IC_{50} values of 21.4 µg/mL for MCF-7, 27.8 µg/mL for HepG2, and 33.5 µg/mL for A549, while showing minimal toxicity toward normal fibroblast cells **Table 3**.

Table 3. Cytotoxicity (MTT Assay) on Cancer Cell Lines

Cell Line	IC_{50} (µg/mL)	Viability Reduction (%) at 100 µg/mL
MCF-7 (*Breast cancer*)	21.4 ± 2.1	82.3%
HepG2 (*Liver cancer*)	27.8 ± 1.8	75.6%
A549 (*Lung cancer*)	33.5 ± 2.0	68.4%
NIH3T3 (*Normal fibroblasts*)	>100	15.2%

The nanocomposite demonstrated selective cytotoxicity towards cancer cells while being less toxic to normal cells.

Biocompatibility and Hemocompatibility

To assess the potential biomedical application of $ZnO-SiO_2$, hemolysis and lactate dehydrogenase (LDH) release assays were conducted. The nanocomposite induced only 3.8% hemolysis at 100 µg/mL, remaining within the biocompatible threshold of 5%. Furthermore, LDH release analysis confirmed that concentrations below 50 µg/mL did not induce significant cytotoxicity in normal cells. These findings suggest that $ZnO-SiO_2$ is a promising candidate for further therapeutic exploration due to its high biocompatibility and selective cytotoxicity toward cancer cells **Table 4**.

Table 4. Biocompatibility and Hemocompatibility

Test	Results
Hemolysis Assay	3.8% hemolysis at 100 µg/mL (biocompatible threshold <5%).
Lactate Dehydrogenase (LDH) Release	No significant toxicity at concentrations <50 µg/mL.

Discussion

Alteromonas sp. is used in this study to synthesise $ZnO-SiO_2$ in a novel way, demonstrating the potential of the green synthesis process to produce nanoparticles with potent antibacterial and anticancer effects. Following the successful completion of the synthesis, the resulting $ZnO-SiO_2$ was characterised using a variety of techniques to assess its physical, chemical, and biological properties.The cytotoxic nature of zinc oxide nanoparticles (ZnO NPs) and their potential as drug delivery vehicles have attracted a lot of interest in cancer research(5).

The anticancer effectiveness of $ZnO-SiO_2$ composites against different cancer cell lines has been investigated in recent studies.The antibacterial and anticancer activities of ZnO and SiO_2-coated ZnO nanoparticles were assessed in a study by Bhadra et al. (2019). When compared to uncoated ZnO nanoparticles, the study showed that SiO_2-coated ZnO nanoparticles had stronger lethal effects on cancer cells(16). This improvement is ascribed to the SiO_2 coating's increased stability and dispersibility, which promote better cellular uptake and interaction with cancer cells(24). Additionally, there have been encouraging outcomes when ZnO NPs are used with traditional chemotherapy drugs. The combination therapy disrupted microbial cell membranes, interfered with intracellular processes, and increased the generation of reactive oxygen species (ROS), which in turn enhanced apoptosis in cancer (ZnO NPs)(5).

These results are corroborated by the antimicrobial activity found in this investigation, which indicates that the ZnO NPs made with *Alteromonas sp*. have strong antibacterial qualities. *Alteromonas sp*. green synthesis of ZnO NPs has a number of benefits over conventional chemical synthesis techniques, such as improved biocompatibility, lower cost,

and less environmental effect. This study's method produces nanoparticles with similar size, shape, and stability to previous bacterial-mediated synthesis procedures, like those employing Bacillus subtilis or E. coli (17). *Alteromonas sp*. is used in the biogenic approach, which reduces the use of hazardous ingredients and utilises natural metabolic processes, as opposed to chemical synthesis approaches, which frequently call for toxic reducing agents and stabilisers. Through the use of biological capping agents, the procedure becomes more ecologically friendly and may enhance the functional characteristics of the nanoparticles (20).

Conclusion:

The study effectively illustrates a sustainable Alteromonas sp. synthesis strategy for ZnO NPs. According to the characterisation results, stable, well-defined nanoparticles with potential uses in antibacterial and anticancer treatments have formed. These results, when compared to previous research, clearly show that bacterial-mediated synthesis is a good substitute for conventional techniques, offering a productive and environmentally benign way to produce nanoparticles. The precise mechanisms of action and therapeutic efficacy of these ZnO NPs could be investigated in more detail.

Conflict of interest: NA

Reference

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