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RESEARCH ARTICLE

Calophyllum inophyllum -Derived ZnO Nanoparticles: A Promising Strategy against Breast Cancer Cell Lines

Balaji MB[1], Thomas N[2], Anugraha Mani[1] and Vimal S[1]*
[1]Department of Biochemistry, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.
[2]Department of General Medicine, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.

*Corresponding Author
Vimal S

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Abstract: The biomedical sciences have revolutionized because of nanotechnology, which has produced innovative methods for treating cancer. There has been a lot of interest in plant-based technology for fabricating zinc oxide nanoparticles (ZnO NPs) since they are more cost-effective, ecologically friendly, and biocompatible. In this work, the production of ZnO NPs utilizing extract from calophyllum inophyllum is investigated and they undergo investigation for their tumoricidal potential. FTIR analysis confirmed the presence of functional groups, including hydroxyl (-OH), carboxylate (-COO), and carbonyl (C=O), which play a role in nanoparticle stabilization. SEM imaging showed pentagonal-shaped nanoparticles with a tendency to aggregate, suggesting that plant-based compounds influence their formation. Cytotoxicity was assessed using an MTT assay on MCF-7 breast cancer cells, revealing a concentration-dependent decrease in cell viability with an IC50 value of 22 µg/mL. AO/EtBr staining has taken which validated nuclear condensation and programmed cell death, further corroborated the apoptotic alterations seen by microscopic examination, including membrane streaking and cell shrinkage. The findings revealed that ZnO nanoparticles synthesized using C. inophyllum exhibit significant anticancer activity, indicating their potential as a Prospective cure targeting tumors

Keywords: Nanotechnology, Zinc Oxide Nanoparticles (ZnO NPs), Calophyllum inophyllum, Breast Cancer, MCF-7 Cells, Cytotoxicity.

INTRODUCTION

Cancer associated with breasts continues to be a major cause of death for women and a major worldwide epidemic. While advancements in treatment options such as chemotherapy, radiation, and targeted therapies have improved patient outcomes, there remains a need for alternative therapeutic approaches that are both effective and less toxic . In this regard, nanotechnology has emerged as a promising field, offering innovative solutions for cancer treatment by facilitating targeted drug delivery with minimal side effects[1]. Among various nanomaterials, zinc oxide nanoparticles (ZnO NPs) have gained attention due to their selective cytotoxicity against cancer cells, making them potential candidates for anticancer therapy. ZnO NPs possess distinct physicochemical properties such as high surface area, enhanced reactivity, and the ability to generate reactive oxygen species (ROS), which can lead to apoptosis in cancer cells[2]. Despite their potential, traditional ZnO NP synthesis methods often rely on hazardous chemicals, raising environmental and biocompatibility concerns. To mitigate these issues, green synthesis using plant extracts has emerged as an eco-friendly alternative. This method not only eliminates toxic reagents but also enhances the biological efficacy of the synthesized nanoparticles [3][4]. Plant-derived substances act as natural catalysts and protectants, aiding in the generation of nanoparticles with enhanced medical effectiveness. Commonly referred to as tamanu, Calophyllum inophyllum is a medicinal plant having an array of pharmacological effects, which comprises as tumor-suppressive qualities, pathogen suppression, immunological modulation, and free radical scavenging. Its bioactive components, which include alkaloids, saponins, and phenolic compounds, greatly aid in the creation of nanoparticles, enhancing their durability and therapeutic effectiveness. These phytochemicals have been reported to inhibit tumor growth by influencing cellular pathways associated with apoptosis, oxidative stress, and immune responses. Studies suggest that plant-derived ZnO NPs exhibit greater cytotoxic effects on cancer cells compared to their chemically synthesized counterparts, making them highly advantageous for biomedical applications[5]. Furthermore, plant-mediated ZnO NPs demonstrate enhanced stability, controlled drug release, and improved solubility, making them ideal for sustained drug delivery in cancer therapy. They induce cell death in tumors by disrupting mitochondrial function, DNA fragmentation, and ROS generation has been extensively researched. Additionally, the biocompatibility of C. inophyllum-derived ZnO NPs ensures reduced adverse effects, offering a safer alternative to conventional chemotherapeutic agents[6][7]. This research investigated the production of ZnO nanoparticles (NPs) utilizing C. inophyllum leaf extract and examined their cancer-fighting properties against MCF-7 breast cancer cells. The produced nanoparticles underwent characterization through FTIR and SEM techniques to determine their structural and morphological features. The potential toxicity to cancer cells was evaluated using MTT assays, while apoptotic cell death was observed through fluorescence

microscopy. This work contributes to the expanding realm of plant-derived nanomedicine, highlighting the promise of eco-friendly nanotechnology in improving breast cancer therapies.

METHODOLOGY

2.1 Fabrication of Zinc Oxide Nanostructures Mediated by Calophyllum inophyllum

To produce zinc oxide nanoparticles through a green route, Calophyllum inophyllum leaves were first collected, rinsed thoroughly, and left to dry at room temperature. The dried material was then heated in deionized water to extract bioactive compounds, and the resulting solution was filtered to obtain a clear plant extract. Separately, a zinc acetate solution (0.01 M) was prepared and mixed with the extract in equal volumes under gentle stirring at elevated temperatures (60–70°C) for about two hours. A change in the appearance of the solution suggested nanoparticle generation. The mixture was then cooled and subjected to high-speed centrifugation to isolate solid particles. The collected material was washed repeatedly with water and ethanol to remove residual organic matter and then dried in an oven. To improve structural properties, the dried powder underwent thermal treatment at 400°C. The resulting ZnO nanopowder was stored in a sealed container for subsequent use.

2.2 Infrared Spectral Analysis (FT-IR)

Infrared spectroscopy was employed to detect functional moieties responsible for reducing and stabilizing ZnO nanoparticles. The examination utilized a Nicolet 5700 FT-IR spectrometer (Thermo Fisher Scientific, USA), implementing the KBr pellet technique. Measurements of the spectra were taken across a wavenumber range spanning 4000–500 cm^{-1}, aiming to detect characteristic functional groups from C. inophyllum phytochemicals that interact with ZnO NPs [8].

2.3 Surface Morphology Analysis (SEM)

A scanning electron microscope (JSM5600LV, JEOL, Japan) was employed to examine the morphological characteristics of the synthesized ZnO NPs. The specimens were dried in air on a polycarbonate substrate and subsequently coated with a thin gold layer using a sputter coater. To evaluate the surface morphology of the nanoparticles, SEM analysis was performed at 20 kV, yielding high-resolution images[1][9].

2.4 Cell Culture

MCF-7 human breast carcinoma cells were obtained from the National Center for Cell Sciences (NCCS), Pune, India, for experimental use. The cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM), enriched with several additives to support growth. The medium was supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 1 mM sodium pyruvate, and 10 mM HEPES. To prevent microbial contamination, antibiotics-penicillin and streptomycin-were incorporated at a concentration of 100 IU/mL each. The cultures were maintained at 37°C in a humidified incubator under a 5% carbon dioxide atmosphere. [8].

2.5 Cytotoxicity Evaluation (MTT Assay)

The effect of ZnO nanoparticles on MCF-7 cell viability was examined through the MTT assay. A total of 1×10^4 cells per well were plated into 96-well microplates and incubated for 48 hours to allow proper attachment before treatment. Following exposure to different ZnO NP concentrations, Following incubation, 100 µL of MTT reagent (5 mg/mL) was introduced into each well and left for 4 hours at 37°C to enable formazan crystal formation. Subsequently, 50 µL of dimethyl sulfoxide (DMSO) was added to dissolve the crystals. Absorbance was then recorded at 620 nm using a microplate spectrophotometer. Cell viability was calculated using the following formula:

$$Cell\ viability(\%) = \left(\frac{OD\ of\ treated\ sample}{OD\ of\ control}\right) x\ 100$$

2.6 Fluorescence Microscopy for Apoptotic Cell Death

The apoptosis-inducing effect of ZnO NPs was examined using acridine orange (AO) and ethidium bromide (EtBr) staining. A mixture of AO (100 µg/mL) and EtBr (100 µg/mL) was applied to the cell suspension and incubated for 2 minutes. Fluorescence microscopy (Nikon Eclipse) was used at 400× magnification with an excitation filter at 580 nm to differentiate apoptotic and necrotic cell populations [11].

RESULTS

3.1 FTIR Spectral Analysis and Functional Group Identification

Fourier Transform Infrared Spectroscopy was employed to examine the chemical functional groups present in the produced ZnO nanoparticles.The spectra (Figure 1) exhibited characteristic absorption bands indicative of biomolecules involved in nanoparticle stabilization and reduction. A broad peak at 2996 cm^{-1} corresponded to –OH stretching vibrations from hydroxyl groups, commonly found in plant-derived metabolites[8], [12]. Peaks at 2345 cm^{-1} were attributed to -CH stretching vibrations, while a prominent peak at 1879 cm^{-1} indicated carboxylate group stretching, contributing to nanoparticle stabilization. The absorption peak at 1098 cm^{-1} suggested C–O stretching vibrations associated with carbonyl groups such as ketones, aldehydes, and carboxylic acids, reinforcing the role of secondary metabolites in ZnONP formation.

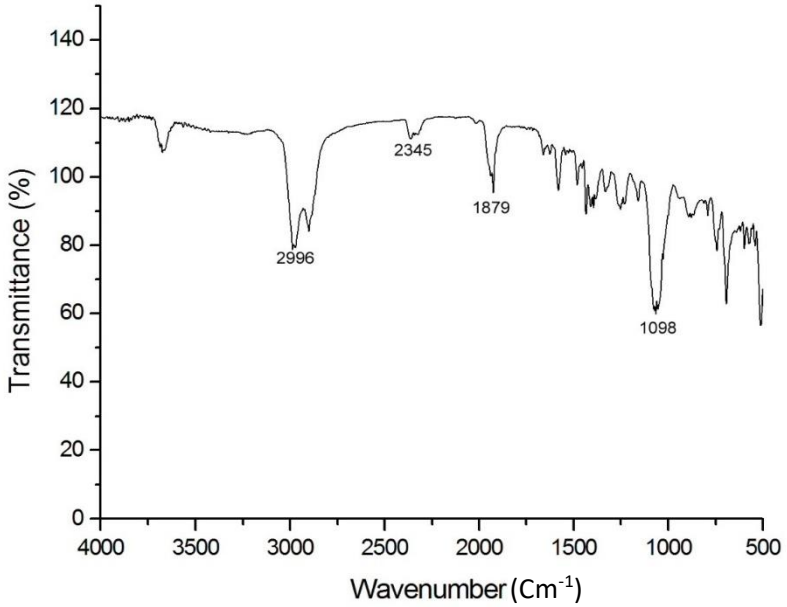

Figure 1: FT-IR spectra of the ZnO nanoparticles (ZnONPs).

3.2 Scanning Electron Microscopy Analysis

SEM analysis was performed to examine the size and morphology of the ZnONPs. The images (Figure 2) revealed nanoparticles with a pentagonal shape and a tendency to aggregate. This aggregation suggests that plant-derived metabolites influence the nucleation and stabilization of ZnONPs. The observations indicate that metal ions undergo limited nucleation, leading to the formation of larger nanoparticle clusters. These observations validate the successful synthesis of ZnONPs and underscore the influence of plant-derived compounds on the nanoparticles structural characteristics [13].

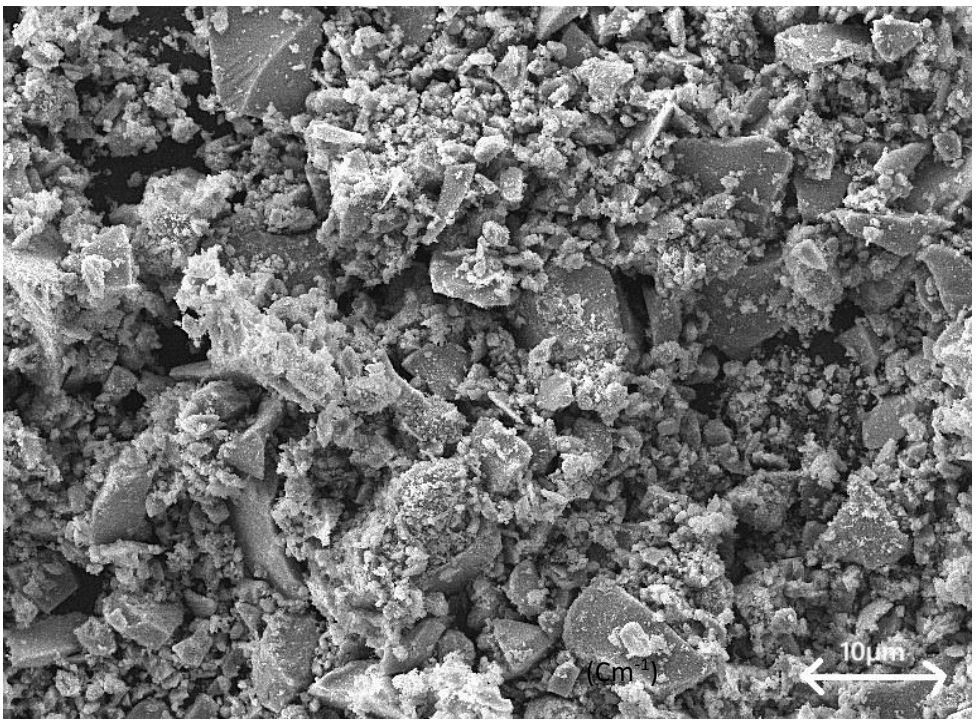

Figure 2: Scanning Electron Microscopy (SEM) analysis showing the morphology and aggregation of ZnONPs.

3.3 Cytotoxic Assay

ZnONP cytotoxicity was evaluated using the MTT assay against MCF-7 breast cancer cells across various concentrations (up to 100 µg/mL). The results (Figure 3) revealed a dose-dependent inhibition of cell proliferation. The IC50 value was determined to be 22 µg/mL, indicating notable cytotoxic activity. A low IC50 value suggests that ZnONPs effectively suppress cancer cell growth, reinforcing their potential as anticancer agents. These results align with previous studies highlighting ZnONPs cytotoxic effects on different cancer cell lines [14].

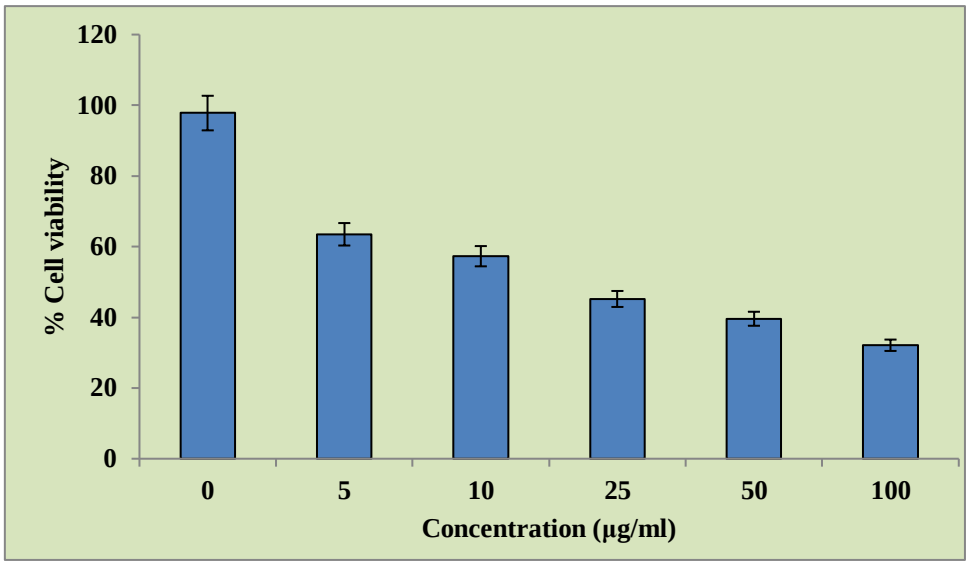

Figure 3: Cytotoxic effects of ZnONPs on MCF-7 breast cancer cells, showing dose-dependent inhibition of cell proliferation with an IC50 value of 22 µg/mL.

3.4 Cell Morphology Analysis

Microscopic examination of MCF-7 cells treated with ZnONPs demonstrated significant apoptotic morphological changes (Figure 4a and 4b). Untreated control cells retained their normal structure, whereas ZnONP-exposed cells exhibited membrane blebbing, shrinkage, and apoptotic body formation [15]. These structural changes suggest that ZnONPs interfere with cancer cell cytoskeletal integrity, prompting apoptosis. Such findings are in accordance with existing reports on the cytotoxic mechanisms of metallic nanoparticles [16].

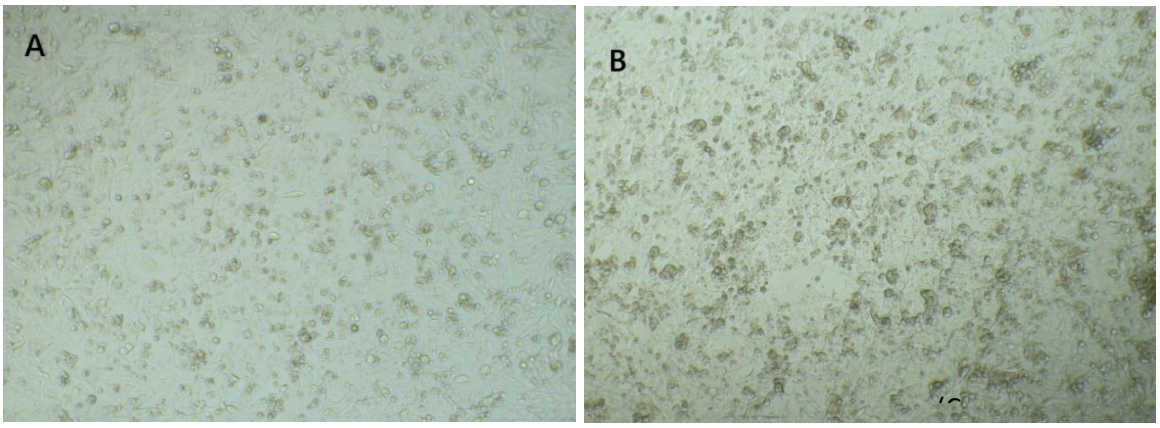

Figure 4: Morphological changes in MCF-7 cells after ZnONP treatment, with (a) Control and (b) IC50-treated cells showing cell shrinkage, membrane blebbing, and floating cells.

3.5 Acridine Orange/Ethidium Bromide (AO/EtBr) Staining

AO/EtBr staining and fluorescence microscopy (Figure 5a and 5b) were used to further confirm ZnONP-induced apoptosis in MCF-7 cells [17]. Control cells displayed uniform green fluorescence, indicating viable cells, whereas ZnONP-treated cells showed orange-red fluorescence, indicative of apoptosis and nuclear condensation. These findings substantiate ZnONPs role in promoting cell death, supporting their potential as anticancer agents [18, 19].

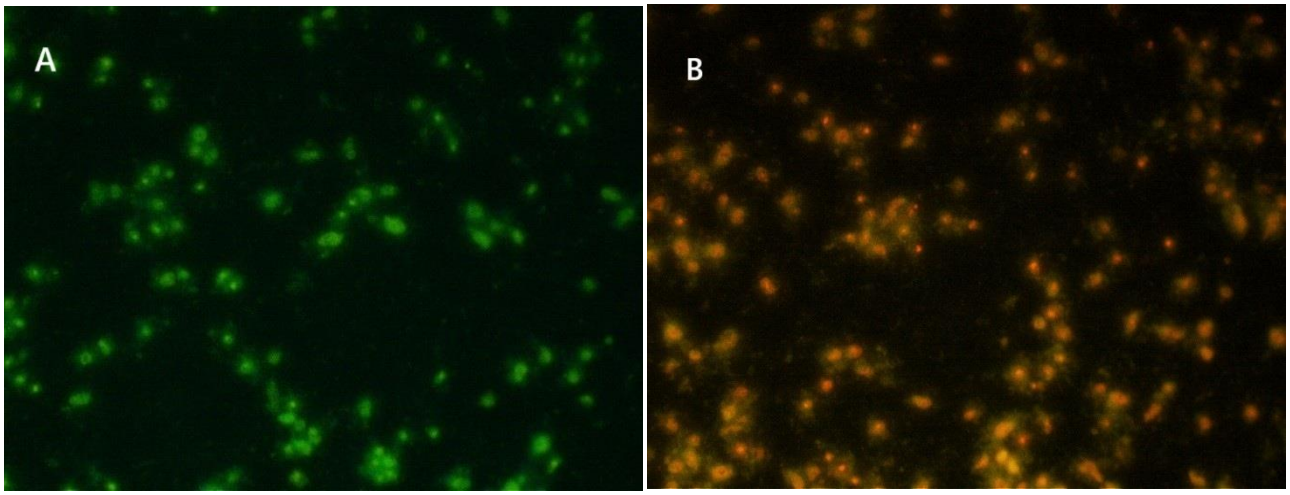

Figure 5: Fluorescence microscopy images of MCF-7 cells showing apoptosis induction and nuclear condensation after ZnONP treatment, with (a) Control and (b) IC50-treated cells

DISCUSSION

This study successfully demonstrated the biosynthesis of ZnONPs using plant extracts and evaluated their structural and functional properties. FTIR analysis confirmed the presence of biomolecules aiding in nanoparticle formation and stability [20]. SEM imaging revealed distinct nanoparticle morphology with a tendency for aggregation due to plant-derived secondary metabolites. Furthermore, cytotoxicity assays provided substantial evidence of ZnONPs anticancer potential against MCF-7 cells, as reflected in the low IC50 value and observed apoptotic effects [21]. Prior research has indicated that ZnONPs induce cytotoxicity through oxidative stress, mitochondrial dysfunction, and apoptosis activation [22]. The contribution of Free radicals of oxygen in ZnONP-mediated cytotoxicity has been widely reported, contributing to cellular damage and programmed cell death. The morphological changes observed in this study further corroborate these mechanisms, reinforcing ZnONPs potential as effective anticancer agents [23]. Additionally, green synthesis presents an eco-friendly alternative to conventional synthesis methods, reducing harmful by products while maintaining biocompatibility. The significant cytotoxic effects observed in this study highlight ZnONPs applicability in biomedical research, particularly in targeted cancer therapy [24].

CONCLUSION

This study highlights the successful biosynthesis of zinc oxide nanoparticles (ZnONPs) using plant-mediated techniques, a green and eco-friendly approach that leverages natural phytochemicals as reducing and stabilizing agents. The plant-derived biomolecules not only assist in the formation of nanoparticles but also contribute to their functional properties, such as biocompatibility and potential therapeutic effects. Overall, the study suggests that plant-mediated ZnONPs are promising candidates for anticancer therapy. Their ability to selectively induce apoptosis in cancer cells, combined with their environmentally friendly synthesis, makes them highly attractive for future biomedical applications. These findings warrant further mechanistic studies, including molecular signaling pathways involved in apoptosis, and preclinical in vivo evaluations to assess therapeutic efficacy, biodistribution, and safety.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval: Not required.

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