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RESEARCH ARTICLE

Synthesis of Titanium Dioxide Nanoparticles Using *Zingiber officinale* Extracts: Their Role in Anticancer Potential

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Abstract

Objective: This study aimed to develop an eco-friendly nanotherapeutic strategy by synthesizing titanium dioxide nanoparticles (TiO_2 NPs) using *Zingiber officinale* (ginger) extract and to evaluate their apoptosis-mediated anticancer activity against A549 human lung carcinoma cells. The study emphasizes the distinctive role of ginger phytochemicals in enhancing nanoparticle bioactivity compared to conventional plant mediated syntheses. **Methods:** TiO_2 nanoparticles were biosynthesized using *Zingiber officinale* extract as a natural reducing and stabilizing agent, eliminating the need for toxic chemicals. The synthesized nanoparticles were characterized using transmission electron microscopy (TEM) to determine morphology and size, and Fourier-transform infrared (FTIR) spectroscopy to identify phytochemical functional groups involved in nanoparticle capping and stabilization. The anticancer efficacy of TiO_2 NPs was assessed against A549 lung cancer cells and normal lung fibroblasts using the MTT assay. Apoptosis associated morphological changes were examined microscopically, and apoptotic cell death was further confirmed by acridine orange/ethidium bromide (AO/EtBr) dual staining. **Results:** TEM analysis confirmed the formation of uniformly distributed nanosized TiO_2 particles, while FTIR spectra revealed the presence of amine, ketone, and alkene groups, indicating effective surface functionalization by ginger-derived phytochemicals. The biosynthesized TiO_2 NPs exhibited significant dose-dependent cytotoxicity against A549 cells and normal lung fibroblasts, with a low IC_{50} value of 12 ± 0.5 µg/mL. Treated cells showed characteristic apoptotic features, including cell shrinkage, membrane blebbing, chromatin condensation, and orange-red fluorescence in AO/EtBr staining. **Conclusion:** The findings indicate that ginger-mediated TiO_2 nanoparticles effectively induce apoptosis in A549 lung cancer cells and exhibit strong anticancer activity. These biosynthesized TiO_2 NPs represent a promising eco-friendly and biocompatible nanotherapeutic candidate for lung cancer treatment.

Keywords: Green synthesis- *Zingiber officinale*- Titanium dioxide nanoparticles- A549 lung cancer cells- Cytotoxicity

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Introduction

The production of plant-mediated nanoparticles with powerful therapeutic uses is one area of biomedical research that has benefited greatly from the recent breakthroughs in nanotechnology [1, 2]. Among these, Titanium dioxide nanoparticles (TiO_2 NPs) have attracted considerable attention due to their unique physicochemical properties, including high surface area, photocatalytic activity, and biocompatibility [3]. Traditional chemical and physical synthesis methods, however, often involve toxic reagents and high energy input, prompting the search for more sustainable and eco-friendly alternatives. Green synthesis, using plant extracts, has emerged as a viable solution, offering a cost-effective, safe, and scalable approach to nanoparticle production [4].

Zingiber officinale (commonly known as ginger) is a medicinal plant widely recognized for its rich phytochemical profile, including gingerols, shogaols, and zingerone compounds known for their strong antioxidant, anti-inflammatory, and anticancer properties [5, 6]. Leveraging these bioactive constituents, ginger extract

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can act as both a reducing and stabilizing agent in the synthesis of TiO_2 NPs, eliminating the need for hazardous chemicals. Not only does this green approach support sustainable nanoparticle synthesis, but it also imparts therapeutic functionality to the nanoparticles themselves [7].

Recent studies have highlighted the anticancer potential of plant-mediated TiO_2 nanoparticles, particularly through mechanisms involving reactive oxygen species (ROS) generation, mitochondrial dysfunction, and the induction of apoptosis in cancer cells [8]. The phytochemical-functionalized surface of ginger-mediated TiO_2 NPs may enhance cellular uptake and bioavailability, potentially leading to improved efficacy against various cancer types while minimizing side effects on normal cells [9]. Mathesh et al., study explores the green synthesis of TiO_2 nanoparticles using Spirulina as a natural reducing and stabilizing agent. This eco-friendly approach enhances biocompatibility and minimizes toxic byproducts. The study emphasizes their structural characterization and antibacterial potential. Special focus is placed on combating multidrug-resistant pathogens [10]. The biosynthesized nanoparticles were thoroughly characterized to confirm their structure, size, and stability. Their ability to disrupt biofilm formation, reduce exopolysaccharide production, and induce protein leakage was assessed. The findings highlight their promising role as nano-antibiotics for dental implant coatings [11]. Among plant-mediated strategies for the green synthesis of titanium dioxide nanoparticles, *Zingiber officinale* is a particularly relevant choice due to its rich phytochemical profile and established biomedical activity. Ginger rhizomes contain phenolic compounds such as gingerols, shogaols, and zingerone, which possess well documented antioxidant, anti-inflammatory, and anticancer properties [7]. During nanoparticle synthesis, these bioactive molecules act as natural reducing and stabilizing agents and remain associated with the nanoparticle surface, as evidenced by FTIR analysis. This phytochemical functionalization is likely to enhance nanoparticle cell interactions and modulate apoptotic responses in lung cancer cells, positioning ginger-mediated TiO_2 nanoparticles as a biologically informed and functionally enhanced green nanotherapeutic system rather than a purely eco-friendly alternative [12]. This study aims to synthesize and characterize *Zingiber officinale* mediated TiO_2 nanoparticles and evaluate their anticancer activity, providing insights into their mechanism of action and potential application in nanomedicine. By combining the therapeutic benefits of ginger with the advanced functionality of TiO_2 NPs, this work represents a promising step toward the development of biocompatible nanotherapeutics for future cancer treatment strategies.

Materials and Methods

Materials

Titanium (IV) isopropoxide (TTIP; CAS 546-68-9), used as the titanium precursor, was procured from Sigma-Aldrich. Glutaraldehyde (CAS 111-30-8), sodium hydroxide pellets (CAS 1310-73-2), ethanol (CAS 64-17-5), and acetic acid (CAS 64-19-7) were purchased from SRL and HiMedia. A549 human lung carcinoma cells were sourced from NCCS, Pune. Cell culture plasticware was obtained from TPP (SRL distributor), and phosphate-buffered saline (PBS; HiMedia) was used for washing procedures. All laboratory glassware and plasticware (Borosil/ HiMedia) were properly cleaned, dried, and sterilized prior to use. Fresh *Zingiber officinale* (ginger) rhizomes were collected locally for extract preparation.

Preparation of Zingiber officinale Rhizome Extract

Fresh ginger rhizomes were thoroughly washed under running tap water, followed by two rinses with distilled water to remove surface contaminants. The rhizomes were then peeled and finely chopped. An aqueous extract was prepared at a 1:10 (w/v) ratio by mixing 10 g of the chopped material with 100 mL of distilled water in a 250 mL beaker. The mixture was heated to a gentle boil and maintained for approximately 15 minutes, during which a light yellowish-brown color developed, indicating the extraction of bioactive compounds. After cooling to room temperature, the extract was filtered through Whatman No. 1 filter paper, and the resulting clear filtrate was used immediately for the synthesis of TiO_2 nanoparticles.

Green Synthesis of Zingiber officinale-Mediated Titanium Dioxide Nanoparticles

Preparation of Titanium Precursor Solution

A titanium precursor solution was prepared by dissolving titanium (IV) isopropoxide (TTIP) in ethanol under continuous stirring to obtain a uniform solution of desired molarity (typically 0.1-0.2 M).

Interaction with Zingiber officinale Extract

The freshly prepared ginger extract was introduced slowly into the titanium precursor solution under constant magnetic stirring at approximately 40 °C. The reaction mixture was maintained under continuous agitation, allowing phytochemicals present in the extract to act as reducing and stabilizing agents. A gradual change in the turbidity of the solution signified the initiation of nanoparticle formation.

Alkaline pH Adjustment and Nanoparticle Formation

To facilitate the formation of titanium dioxide nanoparticles, the pH of the reaction mixture was carefully adjusted to around 10 using sodium hydroxide solution added dropwise under constant stirring. As the pH increased, a whitish precipitate developed, confirming TiO_2 nanoparticle formation. The mixture was allowed to age for complete particle maturation. The resulting nanoparticles were collected by centrifugation, followed by repeated washing with distilled water and ethanol to remove unreacted residues and phytochemical impurities. The purified TiO_2 nanoparticles were finally dried and stored for subsequent anticancer evaluation and hydrogel loading.

Transmission Electron Microscopy

Transmission Electron Microscopy (TEM) was used to analyze the size and morphology of titanium dioxide nanoparticles synthesized using *Zingiber officinale* extract. A drop of the nanoparticle suspension was placed on a carbon-coated copper grid, air-dried, and examined under a TEM. The images revealed spherical and uniformly distributed nanoparticles, and particle size was measured using ImageJ software. Selected Area Electron Diffraction (SAED) patterns confirmed the crystalline nature of the nanoparticles [13].

FTIR Characterization

Fourier transform infrared (FTIR) spectroscopy was utilized to analyze the functional groups of TiO_2 nanoparticles using the KBr pellet method. The spectra were recorded using a Nicolet 5700 spectrometer (Thermo Scientific, USA) in the range of 500-4000 cm^{-1}. The analysis was conducted to identify characteristic peaks corresponding to functional groups associated with TiO_2 NPsnanoparticles and phytochemical constituents from *Z. officinale* [14, 15].

Cell Culture Maintenance

The human lung cancer (A549) cells were supplied by the National Centre for Cell Sciences (NCCS), which is situated in Pune, India. 10% fetal bovine serum (FBS) (Gibco, USA), 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 1.5 g/L glucose, 2 mM L-glutamine, and 10 mM HEPES buffer were added to Dulbecco's Modified Eagle's Medium (DMEM), which was used to develop the cells. To prevent bacterial contamination, 100 IU/mL penicillin and 100 µg/mL streptomycin were added to the medium. Cells were maintained at 37°C in a humidified incubator with 5% CO2 [16].

Morphological Analysis

A549 cells were seeded onto sterile cover slips at a density of 1×10^5 cells per cover slip and left to adhere for the entire night in order to assess morphological changes. The cells were fixed with a 3:1 (v/v) ethanol and acetic acid solution after being treated with TiO_2 nanoparticles. This fixing method preserved cellular structure by stabilizing membranes and preventing further biological activity. The ethanol-acetic acid combination effectively crosslinked proteins and cellular components, ensuring their integrity for microscopic analysis. The fixed cells were placed on glass slides and observed at 10× magnification using a bright-field inverted light microscope (Nikon, Japan). Images of three randomly selected fields per experimental group were captured to record morphological changes in the cells [17].

Cytotoxicity Evaluation

The MTT assay was used to evaluate the cytotoxic effect of TiO_2 nanoparticles on A549 cells. The cells were cultivated till 80% confluency after being seeded at a density of 1×10^5 cells per well in 96-well plates. The cells were treated with samples of serially diluted TiO_2 nanoparticles and then incubated for a further 48 hours. After the incubation period, 100 µL of MTT solution (0.5 mg/mL in PBS) was applied to each well after the medium was withdrawn. To enable the development of formazan crystals, the plates were incubated for four hours at 37°C. Following the removal of the supernatant, 50 µL of DMSO was added to dissolve the formazan, and an ELISA microplate reader (ThermoMultiskan EX, USA) was used to detect absorbance at 620 nm [18]. The percentage of viable cells was calculated using the formula:

Cellviability(%)=(ODoftreatedsample/ODofcontrol) x 100

Fluorescence Microscopic Analysis of Apoptotic Cell Death

Using a dual staining method with acridine orange (AO) and ethidium bromide (EtBr), apoptotic cell death was examined. Phosphate buffered saline (PBS, pH 7.2) was used to suspend A549 cells (1×10^5 cells/mL), and 1 µL of a dye combination containing 100 µg/mL AO and 100 µg/mL EtBr was added. The treated cells were incubated with dye for two minutes and then washed twice with PBS. The stained cells were then viewed at 10× magnification using a fluorescent microscope (Nikon Eclipse, Japan) with an excitation filter set at 580 nm. Because of EtBr absorption, apoptotic cells fluoresced orange-red, whereas live cells fluoresced green [19].

Results

Transmission Electron Microscopy

The TEM micrograph showed polydispersed, predominantly spherical TiO_2 nanoparticles with an average size below 100 nm (Figure 1). Moderate aggregation was observed, and the particles appeared embedded in an amorphous matrix, suggesting capping by phytochemicals from the ginger extract.

FTIR Analysis

To determine which functional groups are responsible for the stability and synthesis of TiO_2 nanoparticles mediated by *Z. officinale*, Fourier transform infrared (FTIR) spectroscopy was employed. Many unique peaks were visible in the spectrum, which may be shown in Figure 2. A significant signal at 1509 cm^{-1} was found to correspond with the N-H bending of amine groups.

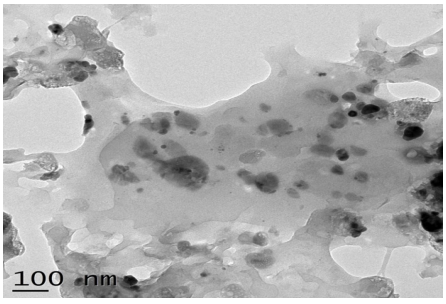

Figure 1. TEM Image of TiO_2 Nanoparticles Synthesized Using *Zingiber Officinale* Extract. Nanoparticles are mostly spherical, polydispersed, and below 100 nm in size with slight agglomeration.

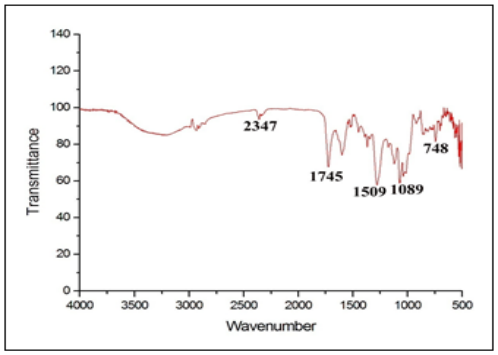

Figure 2. FTIR Spectra of TiO2 Nanoparticles Showing Key Functional Groups from *Zingiber officinale*

These amine groups are responsible for stability of the nanoparticles. The minuscule peaks that were observed at 821 cm^{-1} were attributed to stretching vibrations of the C=C bond, which indicated the presence of alkene groups. It was determined that the CO-O-CO stretching (anhydride) was responsible for the large peak at 1089 cm^{-1}. This could be because of the presence of bioactive compounds from *Z. officinale*, which is known for its potential to inhibit cancer growth. Furthermore, a prominent signal at 1745 cm^{-1} suggested the existence of C=O stretching vibrations, which are frequently connected to aliphatic ketone groups. The results presented here reveal the successful incorporation of phytochemicals derived from *Z. officinale* onto TiO_2 nanoparticles, which has the potential to enhance the biological activity of these nanoparticles, particularly in terms of inhibiting the growth of A549 lung cells.

Cytotoxicity Assay

The MTT assay was carried out to understand how ginger mediated TiO_2 nanoparticles affect cancer and normal cells. The results show that cell response changes clearly with concentration. In Figure 3 at lower doses the effect on A549 lung cancer cells was mild. Cell viability stayed relatively high up to 10 µg/mL which suggests limited toxicity at the initial exposure level. As the concentration increased a steady drop in A549 viability was observed. At 25 and 50 µg/mL the reduction became more noticeable and at 100 µg/mL the viability fell to nearly 20%. This strong decrease indicates that the nanoparticles interfere with metabolic activity in cancer cells and reduce their survival in a dose dependent manner. In contrast normal lung fibroblasts behaved very differently. Across all tested concentrations these cells maintained much higher viability. Even at the maximum dose of 100 µg/mL more than 70% of the fibroblasts remained viable. This pattern suggests that the nanoparticles are less harmful to normal cells when compared with cancer cells. The selective response may be linked to the ginger derived compounds present on the nanoparticle surface. These bioactive molecules are known to enhance oxidative stress in cancer cells which can disrupt mitochondrial function and reduce MTT reduction. Normal cells appear more resistant under the same conditions overall the results indicate that ginger mediated TiO_2 nanoparticles show promising anticancer activity while preserving good biocompatibility. This selective cytotoxic behavior supports their possible use in future biomedical applications though further studies are still needed.

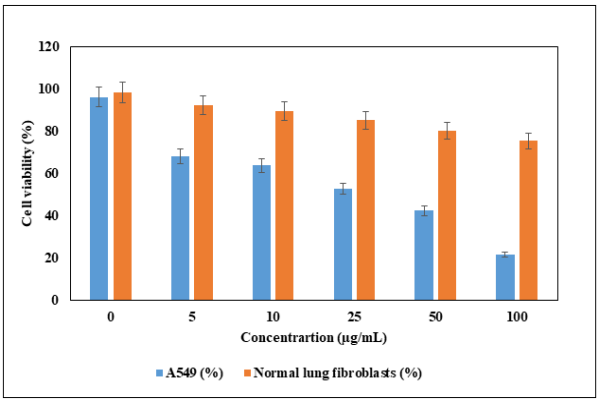

Figure 3. Cell Viability (%) of A549 Lung Cancer Cells and Normal Lung Fibroblasts after Treatment with Ginger-mediated TiO_2 Nanoparticles at Different Concentrations

Cell Morphology Analysis

The morphological changes in A549 cancer cells upon exposure to TiO_2 nanoparticles at varying concentrations were examined (Figure 4a). Control cells exhibited normal morphology without significant changes. However, cells treated at IC_{50} (12µg/mL) concentrations displayed notable alterations, including shrinkage, membrane blebbing, and detachment, forming floating cells (Figure 4b). These cytological changes support the antiproliferative effects of TiO_2 nanoparticles, which disrupt membrane integrity and cytoskeletal stability, ultimately leading to cell death.

Cell Death Analysis Using Fluorescent Microscopy

Fluorescence microscopy was utilized to investigate apoptotic cell death using ethidium bromide (EtBr) and acridine orange (AO) stains (Figure 5a). In untreated control cells, uniform green fluorescence was observed, indicating viable cells. In contrast, TiO_2-treated cells at IC_{50} (12µg/mL) showed a shift from green to orange/red fluorescence (Figure 5b), signifying apoptosis induction and nuclear condensation. This colour transition confirms the apoptogenic effect of TiO_2 nanoparticles, demonstrating their ability to trigger programmed cell death in A549 cancer cells.

Discussion

The current study effectively illustrates the environmentally friendly manufacture of titanium dioxide (TiO_2) nanoparticles utilizing extract from *Z. officinale* and assesses the anticancer potential of these nanoparticles against A549 human lung carcinoma cells. By adding phytochemicals to the nanoparticles, the biosynthetic method not only does away with the need for hazardous chemicals but also increases the biological activity of the particles [8, 20]. FTIR analysis confirmed the involvement of phytochemical constituents in nanoparticle formation

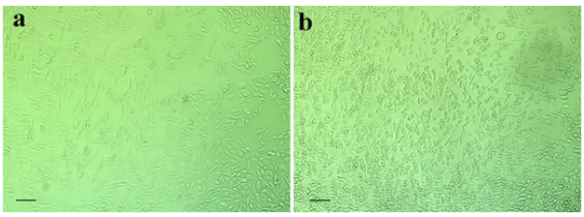

Figure 4. Morphological Changes in A549 Cells after TiO2 Nanoparticle Treatment: (A) Control cells showing normal morphology. (B) IC_{50}-treated cells (12µg/mL) displaying shrinkage, membrane blebbing, and detachment.

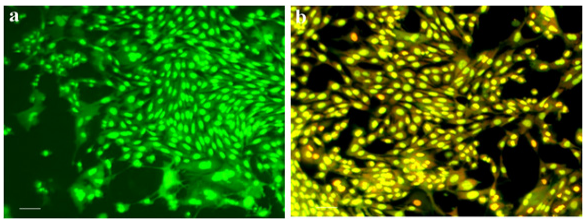

Figure 5. Fluorescence Microscopy of A549 Cells Stained with AO/EtBr: (A) Green fluorescence, viable control cells. Treatment of cells with TiO2 nanoparticles at IC_{50} (12µg/mL).

and stabilization. The strong absorption band at 1509 cm^{-1} associated with N-H bending suggests the presence of amine groups that stabilize the nanoparticles [21]. The presence of C=C and C=O functional groups indicates the contribution of bioactive ginger compounds, such as gingerols and shogaols, known for their anticancer potential. These functional groups not only act as capping agents but may also facilitate cellular uptake and interaction with cancer cell membranes, enhancing cytotoxicity [22]. The MTT assay results revealed a potent cytotoxic effect of ginger-mediated TiO_2 NPs. This significant inhibition of cancer cell viability supports the therapeutic potential of TiO_2 NPs synthesized through this green route. Comparatively, chemically synthesized TiO_2 NPs often require higher concentrations to achieve similar effects, indicating the synergistic impact of incorporated phytochemicals [23]. Morphological analysis further validated the cytotoxic potential. Cells treated at IC_{50} concentration exhibited classical apoptotic features such as cell shrinkage, membrane blebbing, and detachment, contrasting the intact morphology of control cells. These changes are indicative of programmed cell death, aligning with prior findings on ROS-induced mitochondrial dysfunction by TiO_2 NPs [24]. Fluorescence microscopy using AO/EtBr staining confirmed apoptosis as the primary mode of cell death. Treated A549 cells displayed orange-red fluorescence, signifying membrane permeability changes and nuclear condensation, hallmarks of late apoptosis [25]. This suggests that the synthesized TiO_2 NPs initiate apoptosis via intrinsic pathways, likely through ROS generation and mitochondrial damage mechanisms that spare normal cells and are favorable in cancer therapy. Comparative literature shows that plant-mediated TiO_2 NPs using neem, tulsi, and aloe vera have exhibited similar anticancer effects. However, ginger-mediated NPs may offer an edge due to the rich profile of potent anti-inflammatory and antioxidant compounds. The biocompatibility and dual therapeutic-synthetic role of ginger make it a superior candidate for green nanomedicine development. Furthermore, the small size and high surface area of the synthesized TiO_2 NPs are crucial for efficient internalization into cancer cells and targeted drug delivery [26]. This nanoplatform could also serve as a carrier for chemotherapeutic drugs, improving efficacy while reducing systemic toxicity [27, 28]. While apoptotic cell death was clearly observed, the underlying molecular pathways were not directly examined in this study. The involvement of reactive oxygen species, mitochondrial impairment, or ginger derived phytochemicals is therefore suggested based on existing reports rather than confirmed experimentally. Future work including ROS quantification and caspase activity assays would help clarify these mechanisms. In addition, a more critical comparison with related green synthesized TiO_2 nanoparticles such as those derived from Spirulina or neem would further position the present findings within the broader literature [29].

In conclusion, this study underscores the potential of Zingiber officinale-mediated TiO_2 nanoparticles as a novel, biocompatible anticancer agent. The green synthesis approach not only supports sustainable nanotechnology but also enriches the therapeutic profile of the nanoparticles through the incorporation of phytochemicals. With an IC_{50} of 12 µg/mL, evident apoptotic induction, and significant morphological alterations in A549 cells, the biosynthesized TiO_2 NPs exhibit strong anticancer properties. These findings lay the groundwork for further in vivo investigations, optimization for drug delivery applications, and clinical translation in the treatment of lung and possibly other epithelial cancers. The integration of nanotechnology with traditional medicinal plants offers a promising path forward in precision oncology.

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CRediT authorship contribution statement

Akshaya Viswanathan - Original draft, Conceptualization, Formal analysis and Review editing.

Deepika A - Conceptualization, Formal analysis and Review editing.

Vimal S - Writing draft, Review editing, Formal analysis, Conceptualization and Supervision.

Data availability

The data supporting the findings of this study are available from the all authors request. All relevant data are included within the article and its supplementary materials.

Declarations

Ethical approval - Not required.

Competing interests

The authors declare no competing interests.

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