

Biological and apoptotic potential of titanium dioxide nanoparticles synthesized from *Stereospermum colais*: Antioxidant and quantitative polymerase chain reaction-based Anti-Proliferative Study

Running title: Green TiO₂ NPs: Antioxidant & Anticancer Potential

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Abstract

This research investigates the green synthesis of titanium dioxide nanoparticles from *Stereospermum colais* (ScTiO₂ NPs), a medicinal plant that is rich in bioactive molecules. The TiO₂ NPs are characterized using XRD, FTIR, Raman, SEM, DLS, and Zeta potential studies and then evaluated for antioxidant, antimicrobial, antiproliferative, and apoptotic activities. A dose-dependent response is observed by the DPPH assay for antioxidant activity, with an IC₅₀ of 132 µg/mL. Antimicrobial activity against *Staphylococcus aureus*, a gram-positive bacteria, and *Pseudomonas aeruginosa*, a gram-negative bacteria, is observed at MIC values of 80 µg/mL and 160 µg/mL, respectively. Antiproliferative activity of the green synthesized TiO₂ nanoparticles is also investigated in the MCF-7 breast cancer cells and exhibits dose-dependent inhibition of cell viability with an IC₅₀ value of 155.4 µg/mL. The apoptotic activity is validated by qPCR analysis, and it exhibits upregulation of pro-apoptotic genes, thus establishing that TiO₂ nanoparticles induce apoptosis in cancer cells. Thus, the results highlight the significance of green synthesis in developing eco-friendly nanomaterials with versatile biomedical applications.

Keywords: MIC, MCF-7, radical scavenging, *Stereospermum colais*, ScTiO₂NPs

1. Introduction

Titanium dioxide nanoparticles (TiO₂ NPs) are highly renowned for their biocompatibility, chemical stability, and photocatalytic behavior. They have been employed in various applications, from cancer therapy to antimicrobial coatings.^{1,2} Traditionally, TiO₂ nanoparticles (NPs) are synthesized using various physicochemical methods, including sol-gel, hydrothermal, chemical precipitation, and microemulsion.¹ Nevertheless, applications of physicochemical methods are restricted owing to the reason that they entail pressure, high temperature, and harmful chemicals. To mitigate these challenges, eco-friendly green synthesis methods have gained tremendous attention these days.²

Plant extract-mediated green synthesis of nanoparticles has been identified as an environmentally friendly substitute for conventional techniques.^{3,4} This strategy utilizes plant-based phytochemicals such as polyphenols, terpenoids, and proteins as natural reducing and stabilizing agents that eliminate the need for hazardous chemicals and enhance biocompatibility.^{5,6} Plant-mediated

nanoparticle synthesis has been enhanced due to its benefits, such as cost savings, reduced environmental impact, and increased stability and homogeneity of nanoparticles.⁷ Titanium dioxide nanoparticles derived from plant extracts have shown promising applications in photocatalytic reactions, antibacterial therapy, and environmental decontamination processes.⁸ Nevertheless, few studies are reported on synthesizing TiO₂ NPs by medicinal plants of known therapeutic qualities. *Stereospermum colais* is a Bignoniaceae medicinal plant with versatile uses in Indian traditional medicine. Known by the common name the "Yellow Snake Tree" in English and "Padre" in Hindi, where different parts of these plants are utilized to treat asthma, fever, wounds, and dyspepsia.⁹ Additionally, *S. colais* phytochemicals promote neuroprotection and suppress oxidative stress and, hence, are a promising candidate for synthesizing TiO₂ NPs.

In addition, TiO₂ NPs have also shown potential against antimicrobial resistance (AMR) and oxidative stress. The photocatalytic activity and strong oxidizing power of TiO₂ NPs impart in them excellent antibacterial activity against Gram-positive and Gram-negative bacterial strains.¹⁰ When combined with antibiotics, TiO₂ NPs can enhance antibacterial and antibiofilm activity and, hence, potentially restrict the spread of AMR.¹¹ The reactive oxygen species (ROS) generation potential of TiO₂ NPs under UV irradiation has been employed in photodynamic therapy to treat different diseases, such as neoplasms and antibiotic-resistant bacterial infections.¹² TiO₂ NPs have also been shown to be cytotoxic against breast cancer cell lines.¹³ TiO₂ NPs prepared from *Terenna asiatica* fruit extract showed high antioxidant, anti-inflammatory, and anticancer activities against the MCF-7 breast cancer cell line.¹⁴ TiO₂ NPs prepared from *Syringodium isoetifolium* also showed high antioxidant, antibacterial, and anticancer activities against breast cancer cells, along with their photocatalytic activity against the degradation of dyes.¹⁵ Although TiO₂ NPs have demonstrated cytotoxic effects on breast cancer cells, most studies focus solely on cell viability assays, providing limited insight into the molecular mechanisms underlying their anticancer effects. Further, the apoptotic pathways activated by plant-derived TiO₂ NPs remain largely unexplored, particularly regarding the regulation of key genes.

Herein, we aim to synthesize TiO₂ NPs using *S. colais* extract and evaluate their biological potential. After thorough characterization, they are screened for antioxidant, antimicrobial, antiproliferative, and apoptotic effects. Their antioxidant activity is assessed via the DPPH radical scavenging assay,

while their antimicrobial potential is evaluated against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*) bacteria. Further, the antiproliferative and apoptotic effects of TiO₂ NPs on MCF-7 breast cancer cells are explored using MTT assays and qPCR analysis of apoptotic gene expression. Moreover, this study contributes to the green synthesis of TiO₂ NPs with applications in antioxidant therapies, antimicrobial treatments, and cancer therapeutics.

2. Materials and methods

2.1. Materials

A dry, coarsely powdered sample of *Stereospermum colais* was used for nanoparticle synthesis. TiO₂ salt was obtained from Sigma-Aldrich and prepared at concentrations of 1 mM, 2 mM, and 5 mM. All chemicals used in the study were of analytical grade quality and did not require further purification prior to use.

2.2. Instrumentation

The morphological features of the TiO₂ nanoparticles were evaluated through a Hitachi S3400N scanning electron microscope (Hitachi, Japan). To improve contrast and prevent aggregation, the nanoparticle suspension was diluted in distilled water (1:10) prior to imaging. The composition of phases, crystallinity, and TiO₂ NPs were assessed on a Bruker D8 X-ray diffractometer. Diffraction patterns were collected between 10° to 90° to determine peaks and the respective crystalline phases. The functional groups and intermolecular interactions of the TiO₂ NPs were examined using Fourier transformed infrared (FTIR) spectroscopy. The powdered sample was mixed with KBr to form a pellet and arrange for the FTIR spectra to be achieved in the range of 4000–400 cm⁻¹. The hydrodynamic size distributions, polydispersity index (PDI) and surface charge (zeta potential) of the TiO₂ nanoparticles was measured using a Microtrac dynamic light scattering (DLS) analyzer (Microtrac, USA). Raman spectroscopy was conducted in order to investigate the chemical structure, phase polymorphism, crystallinity, and the molecular interactions of the synthesized TiO₂ NPs. Measurements were achieved using a high-resolution Raman spectrometer (Witech, Germany) at an excitation wavelength of 532 nm so that we could precisely characterize the vibrational modes.

2.3. Phyto synthesis of TiO₂NPs

To prepare the leaf extract of *Stereospermum colais*, 10g of the leaves were boiled in 100mL distilled water for approximately 15 minutes, after which the boiled extract was cooled and then filtered with Whatman filter paper. 20mL of the fresh leaf extract was then mixed with 80mL of a 1mM solutions of TiO₂ salt in a glass container. The container was then incubated on a shaker set at 200 rpm for 24 hours at room temperature. The obtained pellets were centrifuged at 10,000 rpm for 10 minutes. The developed pellet was washed and dried at 70°C. The dried product was calcinated at 400°C to obtain S. colais-derived TiO₂ NPs (ScTiO₂ NPs).

2.4. Antioxidant Activity: DPPH Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed to evaluate the antioxidant capacity of ScTiO₂ NPs. The reduction of DPPH radicals by antioxidants leads to a decrease in absorbance, which is quantitatively measured. ScTiO₂ NPs were prepared at 10 to 320 µg/mL concentrations. A DPPH solution (1 mg/mL in methanol) was mixed with varying concentrations of the test sample. After 15 minutes of incubation at room temperature, absorbance was measured at 517 nm using a UV-vis spectrophotometer. The percentage of radical scavenging activity was calculated using the following formula:

$$\text{Radical scavenging activity (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

2.5. Antimicrobial Activity: MIC Determination

The minimum inhibitory concentration (MIC) was determined to assess the antimicrobial potential of ScTiO₂ NPs against *Staphylococcus aureus* and *Pseudomonas aeruginosa* using the resazurin-based colorimetric assay. ScTiO₂ NPs were tested at concentrations ranging from 10 to 320 µg/mL. *Pseudomonas aeruginosa* (gram-negative) and *Streptococcus aureus* (gram-positive) bacterial inoculum were prepared following CLSI guidelines and inoculated in 96-well plates with the ScTiO₂ NPs CLSI.¹⁶ After 24 hours of incubation at 37°C, resazurin (0.015%) was added and further incubated at 37°C for 2-4 hours to observe the metabolism of Resazurin by active bacterial cells leads to the reduction of Resazurin (Purple-blue) to resorufin (pink-colorless) Pink color. The OD was taken at 600nm. The wells showing no color change (blue

resazurin color) were considered above the MIC value.

2.6. MTT Assay for Cytotoxicity

The MCF-7 cell line was obtained from ATCC and cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were maintained at 37°C in a humidified incubator with 5% CO₂.

A stock solution of 32 mg/mL TiO₂ NPs was prepared in DMSO, and the working concentration was adjusted to 10 µg/mL in DM EM media through serial dilutions. 50,000 cells/well were seeded into 96-well plates and incubated for 24 hours. After incubation, the cells were treated with different concentrations of TiO₂ NPs for 24 hours. The MTT reagent (5 mg/mL) was then added to each well and incubated for 4 hours. Formazan crystals formed by viable cells were dissolved in DMSO, and absorbance was measured at 590 nm using a microplate reader. The IC₅₀ values were calculated using nonlinear regression analysis via GraphPad Prism software. All experiments were performed in triplicates, and the data were expressed as mean ± standard deviation.

2.7. Expression of apoptotic genes

2.7.1. Cell Culture and Treatment

MCF-7 cells (ATCC CCL-247) were cultured in plain RPMI (Rosewell Park Memorial Institute medium) growth medium without fetal bovine serum (FBS). Based on the available literature, the selection of 40 µg/mL and 80 µg/mL concentrations for ScTiO₂ NPs in cytotoxicity studies is consistent with previous research that has explored a range of concentrations to assess biological effects.¹⁷ Hence, Cells were treated with the concentrations of ScTiO₂ NPs—40 µg/mL and 80 µg/mL—and Cisplatin (40 µM) as a positive control for 24 hours.

2.7.2. RNA Isolation and cDNA Synthesis

RNA was isolated from treated cells using TRIzol reagent and purified with chloroform and isopropanol. cDNA was synthesized from 500 ng of RNA using the PrimeScript RT Reagent Kit (TAKARA) following the manufacturer's instructions.

2.7.3. Quantitative PCR (qPCR) Analysis

Gene expression of Caspase-3, BAX, and BCL-2 was analyzed using qPCR with SYBR Green dye (Table

1). GAPDH was used as a housekeeping gene to normalize expression levels. The relative expression of the target genes was determined using the 2^{-ΔΔC_q} method. An unpaired t-test was performed to determine the significance value between each experimental group.

3. Results and Discussions

3.1. XRD Analysis

The X-ray diffraction analysis confirms that the synthesized TiO₂ nanoparticles, using the green synthetic route with *Stereospermum colais* extract, were highly crystalline and existing mostly in the anatase phase. The diffraction pattern shows some well-defined peaks (Figure 1), with the most intense peak occurring at 25–27°, corresponding to the (101) plane, along with peaks located at about 37°, 48°, 55°, 63°, and 75° corresponding to the (004), (200), (211), (204), and (215) planes respectively, in agreement with the standard patterns for anatase TiO₂ (PDF 01-075-2545), as well as other recent research studies,¹⁸ which confirm that the measured phase was indeed anatase. The peaks exhibited sharpness and definition, indicating high crystallinity and phase purity of the nanoparticles, as no evidence of rutile or brookite phases were observed.

Furthermore, crystallite size estimation using the Debye–Scherrer equation yielded sizes in the 10–50 nm range, in agreement with reports on green-synthesized TiO₂ nanoparticles.¹⁹⁻²¹ The bioactive molecules present in the *Stereospermum colais* extract are likely to play a critical role as both reducing and stabilizing agents, thereby promoting controlled nucleation and growth. This results in nanoparticles with narrow size distribution and enhanced surface area features crucial for improving photocatalytic performance and broadening their applicability in biomedical and environmental remediation applications.²²

3.2. FTIR Analysis of ScTiO₂ NPs

Fourier-transform infrared (FTIR) spectroscopy was used to determine the functional groups of the likely biomolecules involved in capping and stabilizing TiO₂ NPs (Figure 2). The broad peak at 3200-3500 cm⁻¹ reveals O-H stretching, thus confirming the presence of phenolic compounds in *S. coalis* leaf extracts.²³ Peaks at 2917 cm⁻¹ and 2849 cm⁻¹ are characteristic of asymmetric and symmetric C-H stretching vibrations, respectively.²⁴ A broad peak at 1615-1629 cm⁻¹ confirms the presence of C=O groups, which are mainly linked with amides of

protein residues on the surface of TiO₂ NPs.²⁵ The intense peak at 1021 cm⁻¹ arises from the C-O bond of ether groups.²⁶ Peaks at 400–700 cm⁻¹ are attributed to the Ti–O stretching and bridging stretching mode of Ti–O–Ti.²⁷

3.2. Raman Analysis of TiO₂ NPs

The Raman spectrum of the synthesized TiO₂ nanoparticles exhibits prominent peaks around 188, 393, 514, and 637 cm⁻¹, which are characteristic of the anatase phase of TiO₂ (Figure 3). The observed strong band at 142.56 cm⁻¹ corresponds to the Eg mode and is sensitive to particle size and stoichiometry.²⁸ Other strong peaks are the B1g mode at 393.82 cm⁻¹, the A1g/B1g mode at 514.03 cm⁻¹, and another Eg mode at 637.34 cm⁻¹, all of the anatase phase of TiO₂ nanoparticles.^{29,30} Furthermore, a peak at 3675.06 cm⁻¹ shows the presence of hydroxyl groups on the surface or adsorbed water molecules.³¹

3.3. DLS and Zeta Potential Study

DLS and Zeta potential study reveal the hydrodynamic diameter and surface charge of the nanoparticles, respectively. The red bars (histogram) in Figure 4a indicate the distribution of particle sizes, and the green line represents the cumulative percentage of particles below a given size. The smallest particles detected are around 18.06 nm with a %Pass value of 3.92, indicating about 3.92% of the particles are smaller than 18.06 nm. The %Pass value of 0.00 at this size indicates that no particles are below this size. The smallest particles detected are around 18.06 nm with a %Pass value of 3.92, indicating about 3.92% of the particles are smaller than 18.06 nm. The zeta potential findings (Figures 4b, c) reveal a very low surface charge (-0.1 mV) that shows negligible electrostatic repulsion of the TiO₂ NPs from one another. This can imply that the sample will readily aggregate or flocculate because there is insufficient repulsion or repulsive force between the particles. While there is a negative charge to be measured, it is very little and this too implies that aggregation can be more easily observed because repulsion is not enough.³¹ This tendency to aggregation can be significant with respect to the performance and use of nanoparticles particularly within biological systems and catalytic reactions where dispersion stability is of utmost significance.

3.4. SEM Analysis

SEM images indicate that ScTiO₂ NPs exhibit a degree of agglomeration, as demonstrated by the irregular morphology observed in Figures 5a-5c. The observed particle size range for the ScTiO₂ NPs is from 9 nm to 231 nm with a mean value of 93.673 nm. The spatial organization of the particles suggests they are close packed, with some regions observed to be porous or containing gaps. In addition, the surface texture was heterogeneous, consisting of coarse and fine particles. The disparity in particle size can influence physical and chemical properties of the ScTiO₂, such as reactivity and surface area, both of which are of importance to biological or catalytic applications.

3.5. Antioxidant Activity

ScTiO₂ NPs exhibited considerable antioxidant activity in the DPPH assay, demonstrated as a significant and dose dependent activity (Figure 5-6). The IC₅₀ value for TiO₂ NPs was 158.3 µg/mL, indicating that it has moderate radical scavenging ability in comparison to ascorbic acid, which had an IC₅₀ value of 8.124 µg/mL (Table 1). As such, TiO₂ NPs have the potential for scavenging free radicals, and might in fact mitigate tissue oxidative stress in biological systems (Figure 6a-6c). Doxorubicin is a potent therapeutic agent, meaning it maintains efficacy at low concentrations. In contrast, some natural or newly synthesized pharmaceuticals, including nanomaterials, may have a lower overall potency and therefore must be utilized at a higher concentration to achieve the same biological activity.

ScTiO₂ NPs may have a different mechanism of action or bioefficacy, compounding to greater concentration required to reach a similar biological response to doxorubicin. The requirement for potential therapeutic agents to be used at higher doses in initial testing is often standard, especially in testing prior to the optimization of a therapeutic agent's method of action. As for the substances tested in the DPPH assay, the moderate level of scavenging activity demonstrates a capacity for TiO₂ NPs to mitigate oxidative stress during reaction by potentially scavenging free radicals.

3.6. Antimicrobial Activity

TiO₂ NPs displayed antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The MIC for *S. aureus* was found to be 80 µg/mL, while the MIC for *P. aeruginosa* was 160 µg/mL.

The inhibition of bacterial growth increased in a concentration-dependent manner, with the highest inhibition observed at 320 µg/mL. The calculated MIC values suggest that TiO₂ NPs are more effective against Gram-positive bacteria. Significant ($p < 0.05$) for both bacterial strains, confirming the relationship between concentration and antimicrobial effect. The results of this study demonstrate that TiO₂ NPs possess both antioxidant and antimicrobial activities. The antimicrobial studies showed that TiO₂ NPs are effective against both Gram-positive and Gram-negative bacteria, with *S. aureus* being more susceptible than *P. aeruginosa* (Figure 7a, b).

The antimicrobial action of TiO₂ NPs is attributed to their ability to generate reactive oxygen species (ROS) under light, damaging bacterial cell walls and membranes. These properties make TiO₂ NPs attractive candidates for developing antimicrobial coatings and treatments, particularly in healthcare settings where antibiotic resistance is a concern. In terms of antimicrobial activity, TiO₂ is more effective against *S. aureus* than *P. aeruginosa*, which is reflected in the lower mean inhibition and higher variability against *P. aeruginosa*. These findings suggest that TiO₂ nanoparticles may have more consistent performance in antioxidant assays compared to their antimicrobial effectiveness, which varies more across different bacterial strains.

3.6.1 Cytotoxicity of TiO₂ NPs

TiO₂ NPs demonstrated a dose-dependent inhibition of MCF-7 cell proliferation. The cytotoxic effects increased with the concentration of TiO₂ NPs, as seen in Figure 7c. The test drug has shown marked cytotoxicity in MCF 7 cells. The ScTiO₂NPs showed IC₅₀ values of 132 µg/mL in MCF-7 cells, whereas Doxorubicin, a standard chemotherapeutic agent, was used as a positive control. The standard drug Doxorubicin has shown an IC₅₀ value of 21.4 µM against MCF-7 cell lines. The results of this study indicate that TiO₂ NPs exhibit markable cytotoxicity against MCF-7 breast cancer cells.

The dose-dependent inhibition of cell proliferation suggests that TiO₂ NPs can effectively reduce the viability of cancer cells at higher concentrations. However, compared to doxorubicin, a well-established chemotherapeutic agent, TiO₂ NPs have a significantly higher IC₅₀, implying that they may be less potent as direct anticancer agents (Figure 7c-7j).

3.7. Gene Expression Results

RNA yield varied across treatment groups, with the control group showing 260.96 ng/µL, while treated groups yielded for Cisplatin (40 µM): 181.6 ng/µL for ScTiO₂ Sample at 40 and 80 µg/mL was obtained respectively as 196.64 ng/µL and 314.32 ng/µL.

Caspase-3: Upregulated in cells treated with titanium nanoparticles. At 40 µg/mL, Caspase-3 showed a 1.6-fold increase, while at 80 µg/mL, the increase was 2.3-fold, compared to control. Cisplatin treatment resulted in a 2.8-fold increase. **BAX:** Pro-apoptotic gene BAX was similarly upregulated. The highest expression occurred at 80 µg/mL, with a 2.3-fold increase, and Cisplatin induced a 1.8-fold increase.

BCL-2: Anti-apoptotic gene BCL-2 was downregulated. The highest downregulation was observed at 80 µg/mL, with a fold change of 0.6 compared to the control. Cisplatin treatment showed a fold change of 0.3 (Figure 7k-7m, Figure S1).

The results indicate that the titanium nanoparticles synthesized from *Stereospermum colais* significantly upregulate pro-apoptotic markers Caspase-3 and BAX while downregulating the anti-apoptotic gene BCL-2 in MCF-7 breast cancer cells. The upregulation of Caspase-3 suggests enhanced apoptotic activity, while increased BAX expression and reduced BCL-2 expression confirm the promotion of cell death via the intrinsic apoptotic pathway. This data aligns with previous findings on nanoparticle-induced apoptosis in cancer cells. Furthermore, the green synthesis of nanoparticles adds a layer of biocompatibility and eco-friendliness, making this approach viable for future cancer therapies.

4. Conclusions

In conclusion, Green synthesis of TiO₂ NPs was achieved using *Stereospermum colais*. Structural analysis, such as XRD and Raman spectroscopy, confirmed the presence of highly crystalline anatase-phase TiO₂ NPs, and FTIR analysis confirmed the presence of biocompatible functional groups. DLS analysis indicated the average size within the nanoscale range, while zeta potential measurements indicated a negative surface charge, reflecting low electrostatic repulsion. SEM analysis indicated a non-uniform particle size distribution with high surface roughness and variation in particle size.

The antioxidant property of TiO₂ NPs displays a strong dose-response relationship with high correlation and statistically significant regression and reveals that higher concentrations of TiO₂ NPs increase the activity of radical scavenging capacity.

In addition, the antimicrobial activity of TiO₂ NPs also displays a moderate to strong positive correlation with the concentration, which is more efficient against Gram-positive bacteria (*Staphylococcus aureus*) compared to Gram-negative bacteria (*Pseudomonas aeruginosa*). Linear regression analysis reveals that antimicrobial activity is increased proportionally with the concentration of TiO₂ NPs. Cytotoxicity was tested in the MCF-7 breast cancer cells, which showed an inhibitory effect of IC₅₀ value equal to 132 µg/mL. Apoptotic gene expression experiments confirmed the induction of Caspase-3 and BAX and the repression of BCL-2, thus substantiating their participation in apoptosis-induced inhibition of cancer cells.

These findings emphasize the utility of green synthesis of ScTiO₂ NPs for biomedical applications, specifically antimicrobial and anticancer therapy. Further studies, especially in vivo, are recommended to validate these findings and explore additional mechanisms of action.

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Figure

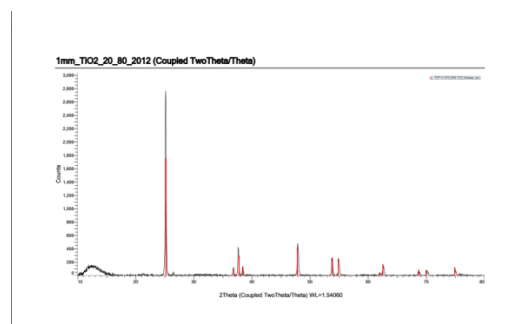
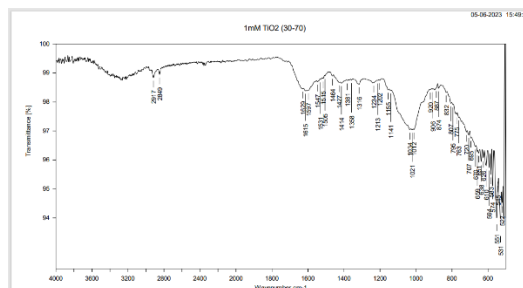
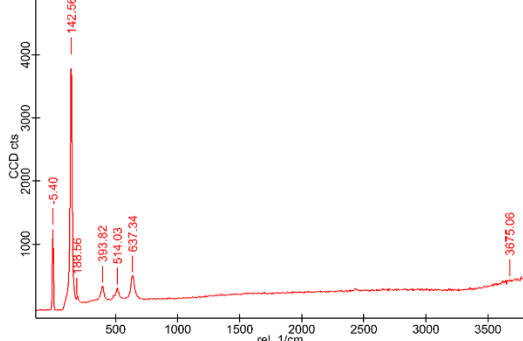
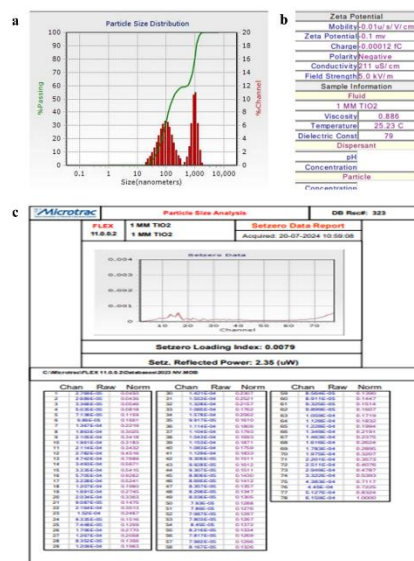
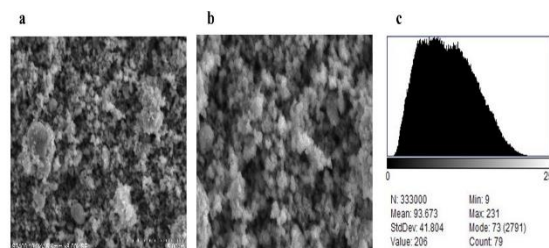
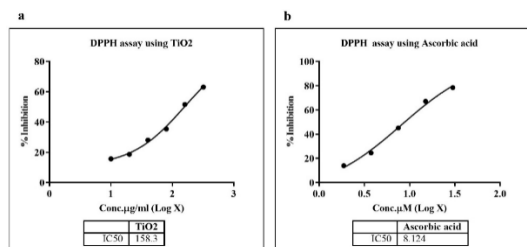
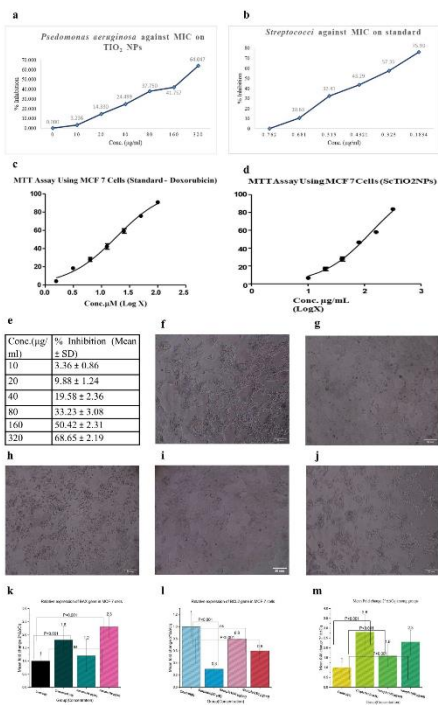
Fig 1 XRD patterns of ScTiO₂ NPs.Fig 2 FTIR spectra of ScTiO₂NPs.Fig 3 Raman Spectra ScTiO₂NPs.Fig 4 (a) DLS and (b, c) Zeta potential of ScTiO₂NPs.

Fig 5 SEM images and Histogram of ScTiO₂ NPs.**Fig 6** Antioxidant activity of (a) Ascorbic acid and (b) ScTiO₂ NPs.**Fig 7** Biological evaluation TiO₂: (a) antimicrobial activity against *Staphylococcus aureus*; (b) antimicrobial activity against *Pseudomonas aeruginosa*; (c) Tabular data showing inhibition of MCF-7 cells by TiO₂ NPs; (d) Cytotoxicity of doxorubicin; (e) Cytotoxicity of ScTiO₂; (f) MCF-7 Control (g) MCF-7 treated with Doxorubicin: low dose; (h) MCF-7 treated with Doxorubicin: high dose; (i), MCF-7 treated with ScTiO₂ NPs (40 $\mu\text{g/mL}$); (j) MCF-7 treated with ScTiO₂ NPs (80 $\mu\text{g/mL}$); Normalized expression of (k) Casp 3, (l) BAX and (m) BCL2 gene in MCF-7 Cells.**Fig S1** qPCR details of GAPDH, Caspase3, BAX, BCL2

Table**Table 1.** Details of qPCR primers for the target genes

Sl. no.	Primer	Sequence	Base pairs	Annealing temperature
1	GAPDH	GTCTCCTCTGACTTCAACAGCG	4	60
		ACCACCCTGTTGCTGTAGCCAA	4	
2	Caspase 3	CAACGTCCCCTCTGAAAAA	4	45
		TGGAATTGATGCGTGATGTT	0	
3	BAX	TCAGGATGCGTCCACCAAGAAG	4	60
		TGTGTCCACGGCGGCAATCATC	4	
4	Bcl-2	ATCGCCCTGTGGATGACTGAGT	4	57
		GCCAGGAGAAATCAAACAGAGGC	4	