



Green synthesis of Titanium Dioxide nanoparticles from *Capsicum annum* extract and its Characterization and Evaluation of antioxidant activity

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Abstract

The green synthesis approach for producing nanoparticles has significantly increased due to their adaptable and flexible nature. Titanium dioxide nanoparticles (TiO₂ NPs) were synthesised using *Capsicum annum* extract through an eco-friendly green synthesis approach. Plant-derived phytochemicals acted as natural reducing and stabilising agents, enhancing nanoparticle bioactivity. The antioxidant potential of the synthesized TiO₂ nanoparticles was evaluated using DPPH, Ferrozine, and FRAP assays. The nanoparticles exhibited significant free-radical scavenging activity with a clear dose-dependent response, strong ferric-reducing capacity, and surface accessible ferrous ion reactivity, indicating functional antioxidant behaviour. Structural and chemical characterisation using UV-Visible spectroscopy and FTIR spectroscopy confirmed formation of nanoparticles and phytochemical functionalization. The results demonstrate that *Capsicum annum*-mediated TiO₂ nanoparticles are structurally stable, functionally active, and biologically relevant, highlighting their potential for biomedical and pharmaceutical applications involving oxidative stress modulation.

Keywords: Green synthesis; Titanium dioxide Nanoparticles; *Capsicum annum*; Antioxidant Activity.

Introduction

Nanotechnology has gained considerable attention in recent years due to the unique physicochemical properties of nanoparticles, particularly their nanoscale dimensions and high surface area-to-volume ratio¹. Nanoparticles smaller than 100 nm exhibit tunable surface characteristics that enable a wide range of biomedical, environmental, and industrial applications². Based on structural geometry, nanomaterials are classified as zero, one, two, and three-dimensional systems, each displaying distinct functional properties³. Nanoparticles can be synthesised using top-down or bottom-up approaches. Top-down methods involve the physical breakdown of bulk materials and often result in structural defects, whereas bottom-up approaches enable controlled assembly at the atomic or molecular level with improved efficiency and reduced material waste⁴. Conventional chemical and physical synthesis routes frequently require high energy input and hazardous reagents. In contrast, green synthesis has emerged as a sustainable alternative due to its eco-friendly nature, cost-effectiveness, and scalability⁵. Green synthesis utilises biological resources, particularly plant extracts, as natural reducing and stabilising agents, enabling nanoparticle formation under mild conditions with minimal environmental impact⁶. Phytochemicals such as flavonoids, phenolics, and terpenoids facilitate nanoparticle reduction and stabilisation while enhancing biological activity⁷. Among metal oxide nanoparticles, titanium dioxide nanoparticles (TiO₂-NPs) have attracted significant interest due

to their non-toxicity, chemical stability, photocatalytic efficiency, and antimicrobial properties. TiO₂-NPs are widely applied in environmental remediation, agriculture, cosmetics, pharmaceuticals, and biomedical fields, including antioxidant and drug delivery applications⁸. Medicinal plants are increasingly employed in green nanoparticle synthesis, as their bioactive compounds enhance nanoparticle functionality while minimising chemical toxicity⁹. *Capsicum annum*, a member of the *Solanaceae* family, is rich in antioxidants, flavonoids, and essential nutrients, contributing to its medicinal and economic importance^{10,11}. In the present study, TiO₂ nanoparticles were synthesised using *Capsicum annum* extract via a green synthesis approach. The antioxidant activity of the synthesised nanoparticles was evaluated using DPPH, ferrozine, and FRAP assays. Structural and chemical characterisation was carried out using UV-Visible and FTIR spectroscopy. The study highlights the effectiveness of phytochemical-mediated green synthesis and demonstrates the enhanced antioxidant potential of *Capsicum annum*-functionalised TiO₂ nanoparticles.

Materials and Methods

Sample Drying: The sample selected for the study was *Capsicum annum* (green capsicum). Two medium-sized capsicums were washed, finely chopped, and sundried for one day. The dried sample was then ground into fine powder and used for extraction.

Metabolite Extraction: The dried plant material (7.37g) was soaked in approximately 150 mL of distilled water and subjected to repeated boiling in a water bath with intermittent stirring for 6–7 cycles. The extract was filtered sequentially through single- and double-layered cheesecloth to remove coarse and fine debris. From the filtrate, 40 mL was collected, with a portion retained as stock. Of this, 20 mL was set aside as the aqueous control, while the remaining 20 mL was mixed with an equal volume of 70% methanol and incubated in a water bath at room temperature for approximately 1.5 h to facilitate extraction.

Preparation of TiO₂: Titanium dioxide was chosen as a metal salt to form NPs using plant extracts. 0.4g of TiO₂ was added in 50mL of distilled water to obtain a 0.1M concentration. About 1.5mL of 0.1M TiO₂ was diluted to 50mL with distilled water to get 3mM concentration.

Mixing of extracted metabolites with titanium salt solution: To the 50mL of 3mM solvents in culture jars, 45mL of Capsicum extract was added.

Formation of Nanoparticles: After mixing the extract with the solvent, the culture jars were placed on a magnetic stirrer at 200 rpm and maintained at pH 6.8–7 for 36–40 hours to facilitate nanoparticle formation. The observed colour change confirmed successful synthesis of Capsicum–Ti (C–Ti) nanoparticles.

Colour indication and centrifugation of formed nanoparticles: A visible colour change in the C–Ti reaction mixture confirmed nanoparticle formation. The initially white, turbid solution gradually turned light blue after stirring on a magnetic stirrer. For spectroscopic analysis, 2mL of the colour-changed suspension was transferred to a test tube, and absorbance was recorded across different wavelengths. The remaining volume was centrifuged at 4000 rpm for 8min to collect the nanoparticle pellet, which was washed with deionised water and recentrifuged under the same conditions. The final pellet was resuspended in a small volume of deionised water, transferred onto a watch glass, and dried in a hot air oven at 35°C. Slow drying was preferred to avoid thermal degradation of polyphenols and phytochemicals, thereby preserving nanoparticle stability and bioactivity.

Collection of dried nanoparticles: The dried C–Ti contents in the watch glass were scraped using a spatula and divided into 2 aliquots in a microcentrifuge tube. To one of the tubes, 2mL of deionised water was added, and the NPs were dissolved by vortexing, which is utilised for antioxidant application¹². The second aliquot was used for characterisation.

Characterisation of the Synthesised TiO₂ Nanoparticles: Characterisation of Titanium Dioxide nanoparticles are as fellow.

Zeta potential: The synthesised NPs were suspended in deionised water and treated with ultrasonication to ensure proper homogenisation for evaluating the particle size distribution. The mixture was filtered and centrifuged at 5000 rpm for 10 mins at 25°C. The supernatant was diluted 4 to 5 times and analysed using a computer-controlled particle size analyser¹³.

XRD: XRD analysis of the synthesised TiO₂ nanoparticles confirms that the TiO₂ NPs crystallized in the tetragonal anatase phase and is crystalline in structure.

UV Vis spectra: The standard titanium dioxide nanoparticles and green synthesised nanoparticles were subjected to estimate the spectra at 200 to 800nm using a UV Vis spectrophotometer.

FTIR analysis of NPs: To validate the conjugation of extract to NPs on the surface modification and the functional groups and chemical bonds present, FTIR was performed in the wavelength ranging from 400 to 4000cm⁻¹ using the KBr pellet method¹⁴.

Antioxidant Tests: Antioxidant Tests such as DPPH Test, Ferrozine Assay and FRAP Assay are as fellow.

DPPH Test: To evaluate the antioxidant activity, 0.5 mL of distilled water was taken in a test tube as a blank. A set of test tubes was prepared by adding varying volumes of standard ascorbic acid (0, 2, 4, 6, 8 and 10µg/mL), and the total volume in each tube was adjusted to 0.5 mL with distilled water. To each tube, including the blank, 2 mL of DPPH solution was added. About 0.1mL of titanium dioxide nanoparticles (TiNPs) were used as control. The reaction mixtures were allowed to stand at room temperature for 30 minutes in the dark. After incubation, the absorbance (OD) of each tube was measured at 520 nm using a colorimeter¹⁵.

Ferrozine Assay: In a test tube 0.5mL of distilled water was taken as a blank. Different volumes of citric acid (0, 100, 200, 300, 400 and 500µg/mL) were pipetted into separate test tubes, and the total volume in each tube was adjusted to 0.5mL with distilled water. In a separate tube, 0.1 mL of titanium dioxide nanoparticles (TiO₂NPs) was added. Subsequently, 0.5mL of FeCl₂ solution was added to all test tubes, followed by 0.2 mL of Ferrozine reagent. The reaction mixtures were incubated at room temperature for 10 minutes, after which the absorbance (OD) of each tube was measured at 562 nm using a colorimeter¹⁶.

FRAP Assay: Different concentrations of FeSO₄ (0, 30, 60, 90, 120 and 150µg/mL) were pipetted into separate test tubes to prepare standard solutions, and the total volume in each tube was adjusted to 0.5 mL with distilled water. For the blank, 0.5 mL of distilled water was used without FeSO₄. To each tube, 3 mL of FRAP reagent was added. In a separate tube, 0.2 mL of titanium dioxide nanoparticles (TiO₂NPs) was mixed with 3 mL of FRAP reagent. All reaction mixtures were incubated at room

temperature for 30 minutes, after which the absorbance (OD) of each tube was measured at 420 nm using a colorimeter¹⁷.

Statistical analysis: All the experiments were done in triplicates and represented as value $\pm$ SD. ANOVA was used for analysis and the P value was remembered at 0.05.

Results and Discussion

Characterisation of synthesised TiO₂ nanoparticles: Particle size Zeta Potential TiO₂ nanoparticles and Spectral analysis are given as follow.

Particle size: Particle size analysis revealed that 56% of the synthesized nanoparticles possessed an average diameter of 100 nm, indicating a predominant size distribution within this range (Figure-1).

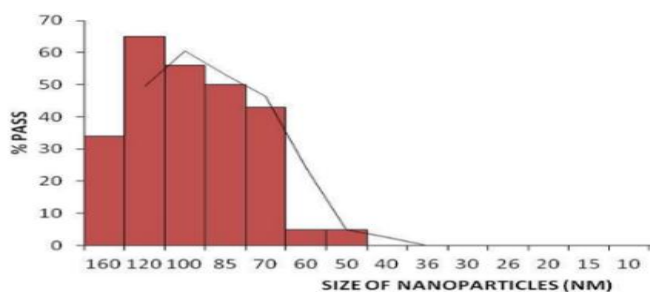


Figure-1: Graph represents the size of synthesised nanoparticle.

Zeta Potential: The zeta potential measures -3.11 mV. Characteristic diffraction peaks were observed at 102(26.4°), 200 (48.5°), 105 (54.3°), 204(64.2°), 115 (69.2°), 218 (75.4°), 226 (83.2°) as shown (Figure-2).

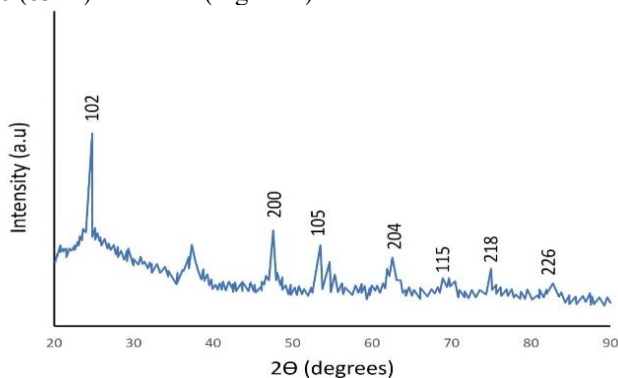


Figure-2: Characteristic diffraction peaks.

UV Vis spectra: The samples synthesized were subjected to estimate the spectra at 200 to 800nm using a UV spectrophotometer (Shimadzu, 1800) (Figure-3).

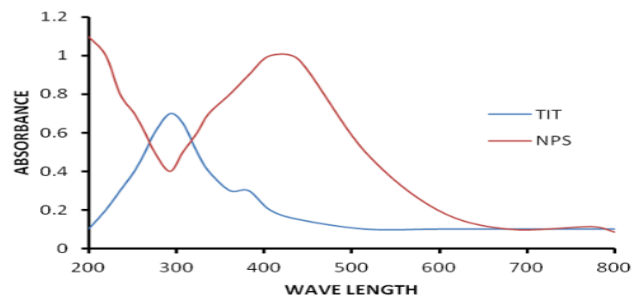


Figure-3: The titanium dioxide NPs showed absorbance at 290nm, which was clearly seen shifted to 415nm with the formation of NPs with capsicum extract.

FTIR analysis of NPs: FTIR spectra of standard titanium dioxide nanoparticles and green synthesized titanium dioxide nanoparticles are as follows.

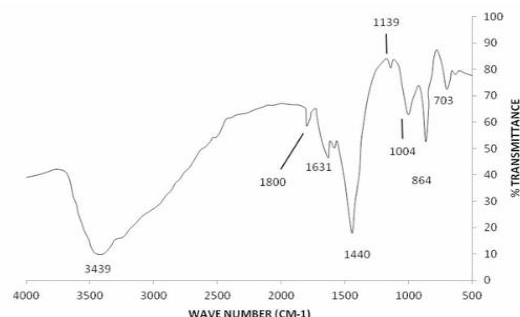


Figure-4: The FTIR spectra of standard titanium dioxide nanoparticles.

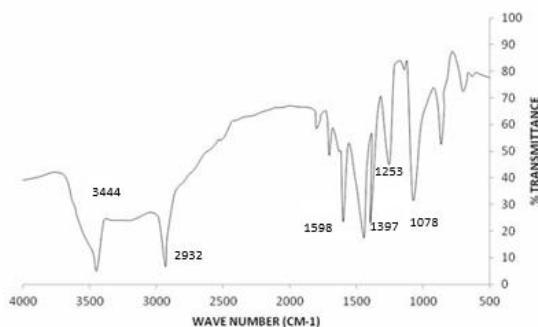


Figure-5: The FTIR spectra of green synthesized titanium dioxide nanoparticles.

FTIR spectra of standard titanium dioxide nanoparticles: 703cm⁻¹: Vibration of the TiO₂ bond, 1800cm⁻¹: asymmetrical C-O vibration of the anhydride group, 3441cm⁻¹: OH group, 2924cm⁻¹: C-H stretching and bending, 1631cm⁻¹: C=O stretching, 1440cm⁻¹: C-O vibration, 1139 – 1004cm⁻¹: corresponds to C-N stretch.

FTIR spectra of green synthesised titanium dioxide nanoparticles: FTIR verified that the conjugation of extract with TiO₂NPs was stable. Figure-4 displays the FTIR spectra of green synthesised titanium dioxide NPs from capsicum. The sharp bands mentioned below indicate the conjugation of

capsicum-extracted compounds at specific wavelengths. 3444cm^{-1} : NH stretching, $2932\text{-}2923\text{cm}^{-1}$: CH groups, 1598cm^{-1} : Amides I, carboxyl and C=C bonds, 1397cm^{-1} : C-O bonds, 1253cm^{-1} : Methyl groups, 1078cm^{-1} : Bands of aromatic rings and C-O aliphatic groups¹⁸.

DPPH: The DPPH assay revealed that, vitamin C standards (2,4,6,8 and 10 $\mu\text{g/mL}$) exhibited a concentration-dependent decrease in absorbance at 520 nm, from 0.70 to 0.34, confirming assay validity. The TiNPs test sample recorded an absorbance of 0.63 at 520 nm, corresponding to antioxidant activity comparable to that of 4 $\mu\text{g/mL}$ vitamin C, thereby indicating significant free radical scavenging potential of the synthesised nanoparticles.

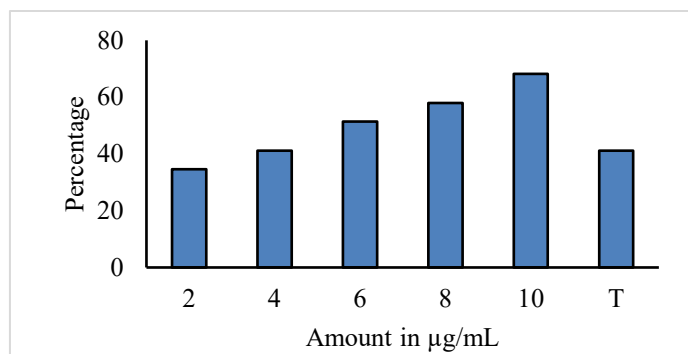


Figure-6: From the graph, it is shown that the synthesised nanoparticles exhibited 41% free radical scavenging activity at a concentration of 3.74 $\mu\text{g/mL}$ in the DPPH assay, with an IC_{50} value of 5.84545 $\mu\text{g/mL}$.

Ferrozine Assay: In the ferrozine assay, citric acid standards showed measurable Fe^{2+} formation, confirming effective ferric ion reduction. The TiNP test sample exhibited a higher absorbance of 0.61 at 562 nm, indicating stronger ferrous ion-chelating and reducing ability. This enhanced response suggests that the synthesised titanium nanoparticles possess notable iron-reducing capacity, supporting their antioxidant potential.

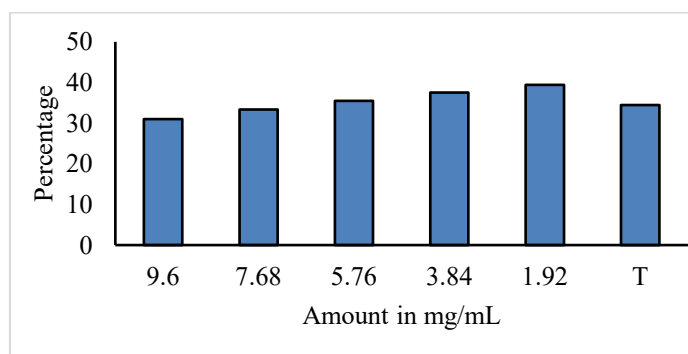


Figure-7: Illustrates the ferrozine assay results, showing the ferrous ion-chelating and reducing ability of the synthesised nanoparticles, which exhibited 34.43% scavenging activity at a concentration of 6.60 $\mu\text{g/mL}$ and an IC_{50} value of 7.70863 $\mu\text{g/mL}$.

FRAP ASSAY: In the FRAP assay, FeSO_4 standards exhibited a concentration dependent increase in absorbance at 420 nm, rising from 1.10 to 1.67. The TiNP test sample showed an absorbance of 1.23 at 420 nm, compared to the standard, indicating strong ferric reducing antioxidant power of the synthesised titanium nanoparticles.

The results of the DPPH, FRAP, and Ferrozine assays collectively confirm the strong antioxidant potential and favourable surface bioactivity of the *Capsicum annuum*-mediated titanium nanoparticles. The DPPH assay demonstrated a clear dose-dependent free-radical scavenging activity, with the unadjusted test sample achieving approximately 41% scavenging activity at 3.74 $\mu\text{g/mL}$, indicating intrinsic antioxidant functionality. This activity is attributed to phytochemical-derived surface functionalisation, which enables effective electron donation and oxidative radical neutralisation. The Ferrozine assay revealed ferrous ion-chelating and reducing ability by the TiNPs. The FRAP assay further supported these findings, as the nanoparticles exhibited a high ferric-reducing capacity, with an IC_{50} value of 44.75 $\mu\text{g/mL}$. This confirms their strong reducing power and effectiveness in oxidative environments and suggests efficient green synthesis mediated by *Capsicum annuum*, resulting in favourable surface chemistry and potential redox activity.

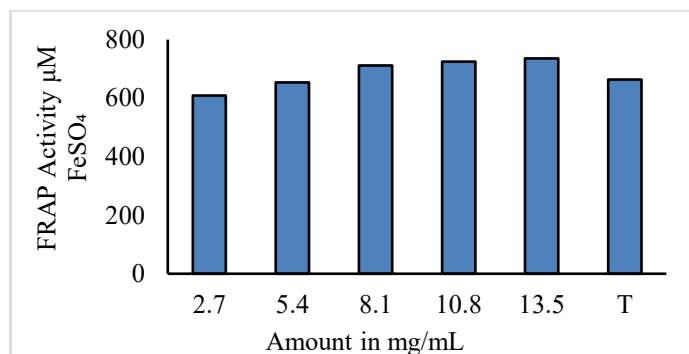


Figure-8: From the graph, it is evident that the synthesised nanoparticles demonstrated ferric reducing antioxidant activity, exhibiting 664.0625 μM , with an IC_{50} value of 44.75 mg/mL .

Discussion: Green synthesis using *Capsicum annuum* extract successfully produced TiO_2 nanoparticles with enhanced antioxidant functionality, attributed to phytochemical-mediated surface modification. Rahman A. et al.¹⁹ did an investigation on optical properties of lemon juice and noticed an absorbance peak at 315nm and Narh, D. et al.²⁰ synthesised Silver-Ti NPs using citrus sinensis peel extract and observed a strong absorbance at 442nm.

In our study the synthesis of C-TiNPs were confirmed by the increase in the wavelength of UV-Vis spectra of green synthesised TiO_2 NPs (415nm) compared to that of TiO_2 NPs (290nm) clearly suggests the formation of NPs with capsicum extract.

FTIR Spectroscopy was performed in the range of 4000 – 500 cm^{-1} to identify functional groups associated with nanoparticle synthesis and stabilization. The spectrum exhibited several characteristics absorption bands. A medium band at 3444 cm^{-1} corresponds to N-H stretching vibrations, indicating the presence of primary amine groups. The band observed at 2932 cm^{-1} was attributed to C-H stretching of alkane groups. An absorption band at 1598 cm^{-1} was assigned to C=C stretching vibrations, suggesting cyclic alkene structures. Additionally, a strong band at 1253 cm^{-1} was associated with C-O stretching, indicating alkyl aryl ether groups. The presence of these functional groups implies that phytochemicals present in the plant extract may play a significant role in the reduction and stabilization of the synthesized Capsicum-TiNPs.

The DPPH assay demonstrated a clear dose-dependent free-radical scavenging response. Joseph et al.²¹ reported 64% scavenging activity for ZnO–TiO₂ nanoparticles at a concentration of 10 $\mu\text{g/mL}$. In contrast, the Capsicum-derived TiNPs in the present study exhibited an IC₅₀ value of 5.84 $\mu\text{g/mL}$, thereby confirming their intrinsic antioxidant potential.

FRAP analysis demonstrated significant reducing capacity, with an IC₅₀ of 44.75 $\mu\text{g/mL}$. This value reflects the concentration at which maximal reducing activity was achieved, highlighting the nanoparticles ability to donate electrons and maintain redox balance under oxidative stress.

Ferrozine assay results revealed stronger ferrous ion–chelating and reducing ability of 34.43% scavenging activity at a concentration of 6.60 $\mu\text{g/mL}$, which is a favourable when optimised under controlled conditions. The assays confirm that the synthesized TiO₂ nanoparticles are structurally stable and functionally active. Phytochemical enrichment enhances antioxidant performance, making these nanoparticles suitable for biomedical applications such as oxidative stress mitigation, wound healing, and drug delivery systems.

The current study didn't fall within the standard range, that is $>+30$ mV or < -30 mV generally associated with stable nanoparticle. The zeta potential value we obtained in this experiment is -3.11, indicating the nanoparticles might have minimal repulsive forces, which may increase the likelihood of particle aggregation and reduced suspension stability overtime. Despite this, the nanoparticle may exhibit initial structural and functional stability, as demonstrated by the antioxidant activity observed in this study.

The presence of bioactive compounds from the plant extract used in the synthesis could contribute to partial stabilization and biological activity of the nanoparticles. Low zeta potential probably achieved due to many factors such fluctuation in pH or when colloidal suspension reaches its isoelectric point, the particles will aggregate. Further studies need to be done to enhance the stability of NPs by introducing steric stabilizing

agents, adjusting pH, controlled particle size distribution, ionic strength adjustment, and surface modification.

Conclusion

The present study demonstrates an efficient green synthesis of titanium dioxide nanoparticles using *Capsicum annuum* extract, resulting in phytochemically functionalised and biologically active nanoparticles. Antioxidant evaluation through DPPH, FRAP, and Ferrozine assays confirmed strong free-radical scavenging ability, and reducing power. These findings validate the functional antioxidant nature of the synthesised TiO₂ nanoparticles and highlight the potential of *Capsicum annuum* derived TiO₂ in biomedical and pharmaceutical applications, particularly in oxidative stress-related therapies.

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