

Research Article

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Synthesis and characterization of Pluronic F-127-coated titanium dioxide nanoparticles synthesized from extracts of *Atractylodes macrocephala* leaf for antioxidant, antimicrobial, and anticancer properties

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Abstract: Globally, nanotechnology is generating significant interest because of its promise in a wide range of industries. The most commonly used nanoparticles are titanium dioxide nanoparticles (PF-127 coated TiO₂ NPs), which can be formulated with physical, chemical, and environmental factors. The establishment of an economical and environmentally beneficial method for its fabrication is due to increasing concerns about human health impacts. In this exploration, green Pluronic F-127 (PF-127) coated TiO₂ NPs using leaf extracts of *Atractylodes macrocephala* have been formulated and studied through various methods. PF-127 coated TiO₂ NPs were 60 nm large and a polygonal rutile-type crystalline structure was observed. Moreover, the NPs' antimicrobial capacity against several pathogens was investigated. The cytotoxicity of the NPs against HEP-2, KB, and Vero cell lines was assessed using the MTT test. Increased antimicrobial potential of PF-127 coated TiO₂ NPs against several pathogens was noted. Furthermore, NPs displayed remarkable antioxidant activity, which increased with concentration. The NPs exhibited significant cytotoxic effects against HEP-2 and KB cell lines but failed to demonstrate toxicity against Vero cells. This is indicative of their cytotoxic potential against cancer cell lines and non-toxic nature towards healthy cells. This indicates that PF-127 coated TiO₂ NPs possess beneficial antimicrobial and antitumor properties.

Keywords: Pluronic F-127, titanium dioxide nanoparticles, *Atractylodes macrocephala* leaf extract, HEP-2 cancer cells, KB cell line, Vero cell line

1 Introduction

Nanotechnology is a fascinating technological advancement focused on developing materials with superior properties and innovative capabilities. The utilization, as well as applicability of nanoparticles (NPs), are particularly prevalent in the fields of chemicals, medicine, healthcare, automobiles, cosmetics, and energy [1]. In comparison to the starting materials, the produced NPs typically have a high surface-to-volume ratio [2].

Noble metal NPs have attracted the most popularity among inorganic NPs owing to their unique properties. These properties enable major implementation in several disciplines like photonics, microelectronics, medicine, and catalysis [3]. Titanium nanoparticles (Pluronic F-127 coated TiO₂ NPs), particularly, are regarded as desirable nanomaterials due to their simplicity of management, low cost, excellent resistance to chemical erosion, nontoxicity, excellent stability, and antimicrobial and photocatalytic activities [4–6]. It is an N-type semiconductor and is utilized in a plethora of applications like wastewater treatment, dye-sensitized solar cells, photoionized circuits, and lithium-ion batteries [7].

Pluronic F-127 (PF-127) coated TiO₂ NPs are typically formulated by sol-gel, chemical vapor deposition, hydrothermal, and chemical precipitation processes. However, these processes have drawbacks because of high energy, pressure, temperature, and harmful chemicals [8,9]. Hence, environmentally friendly and inexpensive synthesis approaches for PF-127 coated TiO₂ NPs are required

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to make them appropriate for large-scale production. The utilization of naturally derived products as environmentally friendly reducing agents to produce metal oxide NPs is a result of the rising focus on green chemistry [10]. In order to synthesize stable, appropriate-sized, and soluble NPs with minimal energy usage, green synthesis strategies are required [11].

Green synthesis techniques utilizing biological preparations as reducing agents allow us to synthesize PF-127 coated TiO₂ NPs at a low cost and with no environmental impact. In the past, a variety of fungi, bacteria, microorganisms, and plant extracts were utilized to fabricate NPs [12]. Plants offer excellent availability, biocompatibility, and biodegradability among these life forms [13]. Numerous phytochemicals from plants, including alkaloids, polyphenols, tannins, terpenoids, and alcoholic compounds, are easily obtainable in herbal extracts. They can stabilize and reduce the environmental impact of NP synthesis. Additionally, the metabolites present in plant extracts are essential NP stabilizing or capping agents. Further, some research has indicated that plant extracts can yield relatively more stable metal NPs than those produced by microorganisms and can reduce metal ions more efficiently than bacteria [14].

In the literature, it has been observed that PF-127 coated TiO₂ NPs produced from plant extracts exhibit inhibitory action against a plethora of microbial pathogens [15]. Plant extracts of *Aloe barbadensis* [16], *Trigonella foenum-graecum* [11], *Citrus limon* [17], *Cochlospermum gossypium* [18], *Artemisia haussknechtii* [19], *Eichhornia crassipes* [20], *Echinacea purpurea* [21], *Syzygium cumini* [22], *Carica papaya* [23], and *Citrus sinensis* [24] are now being extensively employed in synthesis techniques to produce green PF-127 coated TiO₂ NPs [19]. However, the leaves from the plant *Atractylodes macrocephala* have not been employed for green PF-127 coated TiO₂ NP synthesis.

The formation of stable structures for effective drug delivery is essential when designing nanodrugs for therapeutic delivery. Therefore, delivery methods have shifted to more regulated and stable systems, including polymeric NPs [25]. Particularly, as these polymers can be combined with both hydrophilic and hydrophobic compounds, attention has recently increased on polymeric amphiphiles as core components. Pluronic, an amphiphilic block copolymer, has drawn considerable attention from the drug delivery sector [26].

PF-127 is an amphiphilic copolymer, comprising a central hydrophilic poly-ethylene oxide chain [27]. PF-127 is recognized as an excellent medium for effective drug administration by a variety of parenteral and non-

parenteral routes due to its strong solubilizing properties, low toxicity, and biocompatibility [28]. Over the last decades, extensive research into the functional characteristics of these polymers has led to the creation of several systems widely used as drug delivery mechanisms [29]. An earlier study presented a TiO₂@MWCNTs nanocomposite photoanode for photoelectrochemical water splitting, improving the absorption of visible light and electron transfer. The nanocomposite has higher current density under solar irradiation and improved solar-to-hydrogen conversion efficiency [30]. Researchers developed a low-cost method to reduce pristine TiO₂ in 30 min using minimal sodium borohydride, producing colored samples. UV-DRS, X-ray diffraction (XRD), ESR, XPS, field emission scanning electron microscopy (FESEM), and TEM are used as characterization techniques [31].

Previous research explored the green synthesis of copper oxide NPs (CuO NPs) using plant extracts. The review also discusses the cost-effective and environmentally friendly process, revealing aspects of plant physiology and their relationship to NP synthesis, and the multifunctional applications of CuO NPs synthesized with plant extracts in environmental remediation, sensing, catalytic reduction, photocatalysis, biological activities, energy storage, and organic transformations. The previous study also aimed to serve as a guide for readers interested in plant extract-mediated biosynthesis of CuO NPs and their potential applications [32]. Chromic oxide NPs (Cr₂O₃ NPs) are a significant inorganic NP with numerous applications in various fields. Despite their ecological and economic challenges, biological methods using plant materials have shown potential for their synthesis. These NPs have numerous health, environmental, economic, and medicinal benefits, including antibacterial, antifungal, antioxidant, anticancer, antileishmanial, antiviral, and anti-diabetic properties. However, challenges in clarifying formation reactions remain unresolved. Modern advancements in the synthesis, characterization, and applications of photosynthesized Cr₂O₃ NPs are discussed [33].

In traditional Chinese medicine, *A. macrocephala* Koidz has gained attention because it is an herb from the Compositae family. In China, it has been used for millennia as food and medicine. A variety of compounds have been identified in *A. macrocephala*, such as essential oils, amino acids, sesquiterpenoids, resins, polysaccharides, and other substances [34]. Pharmacological studies and clinical experience indicate that the plant extracts have a wide range of bioactive properties against diarrhea, stomach discomfort, intestines, liver, and kidney disorders [35]. The digestive tract is frequently treated with it, and the gastrointestinal tract is also regulated by it [36]. Lactones

from the plant also possess anticancer properties [37]. The ethanolic extract of *A. macrocephala* fresh leaf acts as a reducing, stabilizing, and capping agent in the green synthesis of TiO₂ NPs. The bioactive compounds present in the extract not only contribute to the NP formation process but also impart unique properties that make the synthesized NPs suitable for various applications such as antioxidant, antimicrobial, and anticancer activities, including their potential as biocompatible and bioactive agents.

We aim to synthesize and characterize TiO₂ NPs coated with PF-127 using *A. macrocephala* leaf extracts for antioxidant, antimicrobial, and anticancer properties. As an eco-friendly and sustainable alternative to chemical reagents, natural extracts may enhance the properties of NPs. The NPs coated with PF-127 provide improved biocompatibility, making them versatile for antimicrobial and anticancer, and displayed remarkable antioxidant activity, which increased with concentration. Synergistic effects may result from combining the NPs with the bioactive compounds contained in *A. macrocephala* leaf extract. The NPs exhibited significant cytotoxic effects against HEP-2 and KB cell lines but failed to demonstrate toxicity against Vero cells. This indicates their cytotoxic potential against cancer cell lines and non-toxic nature towards healthy cells. Various applications are possible for the NPs due to their comprehensive evaluation, which covers antioxidant, antimicrobial, and anticancer properties. The research aligns with green nanotechnology principles, reducing reliance on traditional chemical methods. Understanding the mechanisms behind NPs' properties could lead to more targeted therapies. Optimizing the coating consistency, NP size, or extract concentration could be included in future optimizations. The interdisciplinary approach encourages collaboration and knowledge exchange among experts in different fields, fostering innovation.

In the present investigation, the biosynthesis of PF-127 coated TiO₂ NPs with the leaf extract of *A. macrocephala* and their biomedical applications such as antioxidant, antimicrobial, and anticancer activities were assessed.

2 Materials and methodology

2.1 Materials

Chemicals such as Titanium isopropoxide (TTIP) and PF-127 were procured from Sigma Aldrich, USA. All the corresponding assay kits to estimate biochemical markers were purchased from Abcam, USA.

2.2 Preparation of *A. macrocephala* leaf extract

10 g *A. macrocephala* fresh leaf extract was mixed with 100 ml ethanol and heated for 20 min at 80°C. The suspension was filtered using filter paper, and the resultant extract was stored at 4°C.

2.3 Preparation of PF-127 coated TiO₂ NPs

To prepare PF-127 coated TiO₂ NPs, 0.1 M of TTIP and 0.5 g of PF-127 were added with 100 ml of *A. macrocephala* extract (pH of solution 8). The green-yellow suspension was stirred continually for 5 h at 80°C. The white precipitate was dried at 120°C for 1 h. The formed PF-127-coated TiO₂ NPs were calcined for 5 h at 800°C.

2.4 Characterization of PF-127 coated TiO₂ NPs

The PF-127-coated TiO₂ NPs were studied with an XRD (Bruker-AXS D5005). The test was conducted under Cu-K α radiation at $\lambda = 0.1541$ nm and scanned at 2θ angle between 20 and 90°. A DLS study was performed to examine the average particle size. The test was conducted utilizing NPs at a 90° scattering angle and 25°C. The appearance of PF-127-coated PF-127 coated TiO₂ NPs was observed with FESEM (Hitachi s-4800II) and EDAX was employed to determine the elemental composition. To investigate the NP size, transmission electron microscopy (TEM; Jeol Jem-2010F) was employed. The NPs were spread on a copper grid, and exposed to electronic radiation under vacuum. Additionally, an electron beam was utilized to take images after transmitting them via material. Based on fourier-transform infrared spectroscopy (FTIR) spectroscopy (NicoletIS50), functional groups present in PF-127 coated TiO₂ NPs were analyzed. The infrared (IR) spectrum was taken using the reflectance method. The developed NPs were ground with KBr at a 1:100 ratio. The blend was flattened into a disc to make potassium bromide (KBr) discs and scanned at 400–4,000 cm⁻¹ wavelength. The formed NPs were examined using UV-Visible spectroscopy (Shimadzu UV-2550, made in the USA) to validate the development and observe the surface plasmon resonance. The NPs were examined at 1,200–200 nm and the study was conducted three times. Photoluminescence (PL) study

scrutinized the optical properties (Roithner Lasertechnik). The spectra were investigated at 350–550 nm at $\lambda_{\text{exc}} = 470$ nm. Triplicate tests were conducted, and the energy band gap was investigated by plotting the Tauc curve with the equation given below

$$(h\nu)^{1/n} = A(h\nu - E_g) \quad (1)$$

where h is the Planck's constant; A is a constant proportion; α is the absorption coefficient; ν is the vibration frequency; E_g is the band gap; and n is the sample transition nature.

2.5 Antimicrobial activity

The antimicrobial potentials of the PF-127 coated TiO₂ NPs against *S. aureus*, *B. megaterium*, *B. subtilis*, *S. dysenteriae*, *E. coli*, *P. aeruginosa*, and *C. albicans* strain was evaluated by disc diffusion technique. For this investigation, molten nutrient agar medium was utilized. Micropipettes were used to transfer samples at 1, 1.5, and 2 mg·ml⁻¹ dosages along with the positive control onto the bacteria-seeded plates after inoculation. After that, the plates were left for incubation for 24 h. The inhibition zones were noted following the incubation. The positive control against all the tested microorganisms employed was amoxicillin (Hi-Media) [38,39].

2.6 Evaluation of antioxidant activity

DPPH was utilized to evaluate the capacity of PF-127 coated TiO₂ NPs to quench free radicals with a few minor adjustments. In brief, 500 ml of 0.2 mM DPPH solution (in 50% ethanol) were combined with NPs at varied concentrations (1.25, 2.5, 5, 10, 20, and 40 µg·ml⁻¹). The reaction solution was shaken for 30 min, and absorbance at 517 nm was calculated and compared to a control. Increased antioxidant action is shown by a decrease in DPPH absorption. The equation to estimate the radical scavenging potential is given below

$$\begin{aligned} \text{Scavenging effect (\%)} \\ = (1 - A_{\text{samples, 517 nm}}/A_{\text{control, 517 nm}}) \times 100 \end{aligned} \quad (2)$$

2.7 In vitro anticancer activity of PF-127 coated TiO₂ NPs

2.7.1 Culture and maintenance of cell lines

HEp-2 (human laryngeal cancer cells), KB (mouth), and Vero cells (normal) were acquired from ATCC, USA, and grown in DMEM medium for 24 h. The cells were collected

after reaching 80% confluency and employed in further investigations.

2.7.2 Cytotoxicity assay

The cytotoxicity of PF-127 coated TiO₂ NPs was assessed using MTT assay. Using 24-wellplate, the cells were cultivated for 24 h and following the incubation time, the cells were exposed to the NPs at 2, 4, 8, 16, 32, and 64 µg·ml⁻¹ for 24, 48, and 72 h. Afterwards, the cells were added to MTT (5 mg·ml⁻¹) for 3 h. After that, DMSO was mixed to liquefy the formazan sediments before the absorbance was taken at 540 nm. Using the program OriginPro8, the IC₅₀ concentration of the NPs were measured.

2.8 Statistical analysis

All studies were executed in triplicate, and then one-way ANOVA and a post hoc Tukey test were applied to statistically assess the data. Data are given as mean value ± SD with $p < 0.05$ as significant.

3 Results

3.1 Spectral characterization of PF-127 coated TiO₂ NPs

UV-Vis spectroscopy is the procedure that is employed most frequently to characterize the structural properties of NPs.

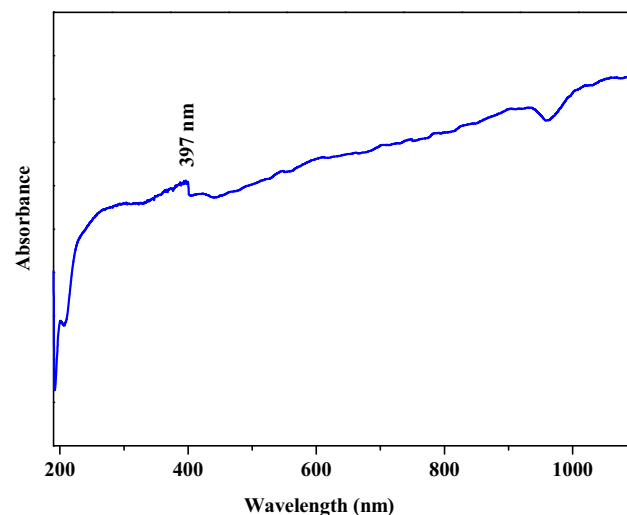


Figure 1: UV-Vis spectrophotometer analysis of synthesized of PF-127 coated TiO₂ NPs.

The absorption spectra of green PF-127 coated TiO₂ NPs annealed at 600°C is depicted in Figure 1. The NP absorption was estimated in the range between 200 and 1,100 nm and the PF-127 coated TiO₂ NP in its rutile phase form exhibits an optical absorption range of around 397 nm.

The FTIR spectra of green synthesized PF-127 coated TiO₂ NPs is illustrated in Figure 2. Peaks corresponding to O–H stretching and bending was noticed at 3,427 and 1,639 cm⁻¹, respectively. The typical peaks were noticed at 2,924 cm⁻¹ for asymmetric stretching, 2,854 cm⁻¹ for symmetric stretching, and 1,384 cm⁻¹ for an O–H bending [40]. The strong band below 1,050 cm⁻¹ corresponds to Ti–O stretching vibrations. The O–Ti–O stretching band was located at 779, 548, and 478 cm⁻¹ [41].

Figure 3 demonstrates the PL spectrum of PF-127 coated TiO₂ NPs with an excitation wavelength of 325 nm. For PF-127 coated TiO₂ NPs, PL emission was noted at 430, 438, 484, 513, and 527 nm. The blue emission bands at 430, 438, and 484 nm are attributed to singly ionized tin vacancies. The green emission indicates that the oxygen vacancy (Ov) band is located between 513 and 527 nm [42].

3.2 Morphology and chemical elements of green synthesized PF-127 coated TiO₂ NPs

Surface morphological characteristics of green PF-127 coated TiO₂ NPs were observed using FESEM/TEM/SAED patterns, as revealed in Figures 4 and 5. The polygonal structure of PF-127 coated TiO₂ NPs is noticeable in FESEM

(Figure 5a) and TEM (Figure 4a–c) images. The size of 54 nm, as exhibited by XRD, is consistent with the findings.

The SAED pattern authorizes the PF-127 coated TiO₂ rutile crystalline phase generation (Figure 4d). EDAX analysis, with elemental mapping and spectrum analysis, can verify NP composition and distribution patterns. Each element has a peak in an X-ray area that corresponds to its energy level [43]. The chemical constitution of the green PF-127 coated TiO₂ NPs was determined by an EDAX spectrum, as depicted in Figure 5b. In the PF-127 coated TiO₂ NPs, copper (Cu), titanium (Ti), and iron (Fe) were the predominant atomic percentages. During the TEM analysis, copper elements might be present due to the copper grid.

3.3 XRD study

An XRD study was conducted to determine the crystallinity of the PF-127 coated TiO₂ NPs, as depicted in Figure 6a. The crystal structure of the produced PF-127 coated TiO₂ NPs was discovered to be in the rutile phase based on the XRD pattern (JCPDS Card No. 21-1272) [44]. The diffraction peaks of PF-127 coated TiO₂ NPs in the rutile phase of 2θ angle at 27.18°, 35.81°, 38.92°, 40.99°, 43.81°, 54.07°, 56.41°, 62.51°, 63.80°, 68.73°, and 69.53°, with diffraction plans (110), (101), (200), (111), (210), (211), (220), (002), (310), (301), and (112), respectively. The crystallinity of the NPs is determined by Debye–Scherrer's equation [45].

$$\text{Crystallite size } (D) = \frac{0.9\lambda}{\beta \cos \theta} \quad (3)$$

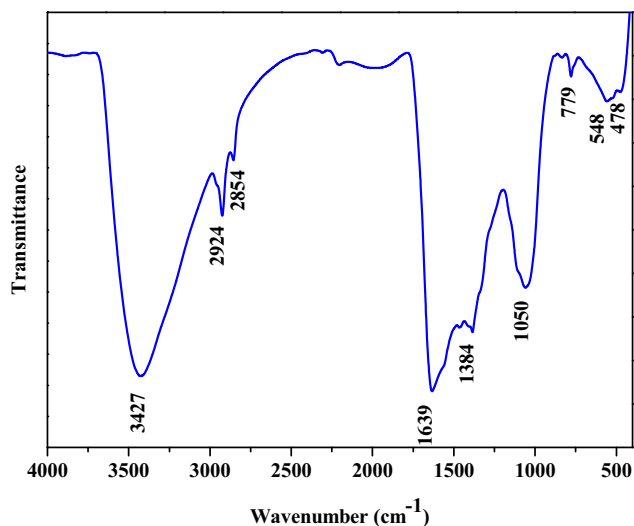


Figure 2: FTIR transmittance vs wavenumber chart of PF-127 coated TiO₂ NPs derived from IR analysis.

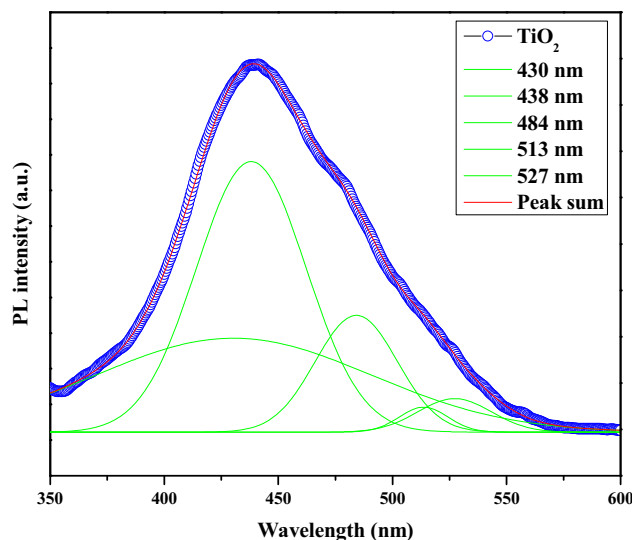


Figure 3: PL spectrum of PF-127 coated TiO₂ NPs.

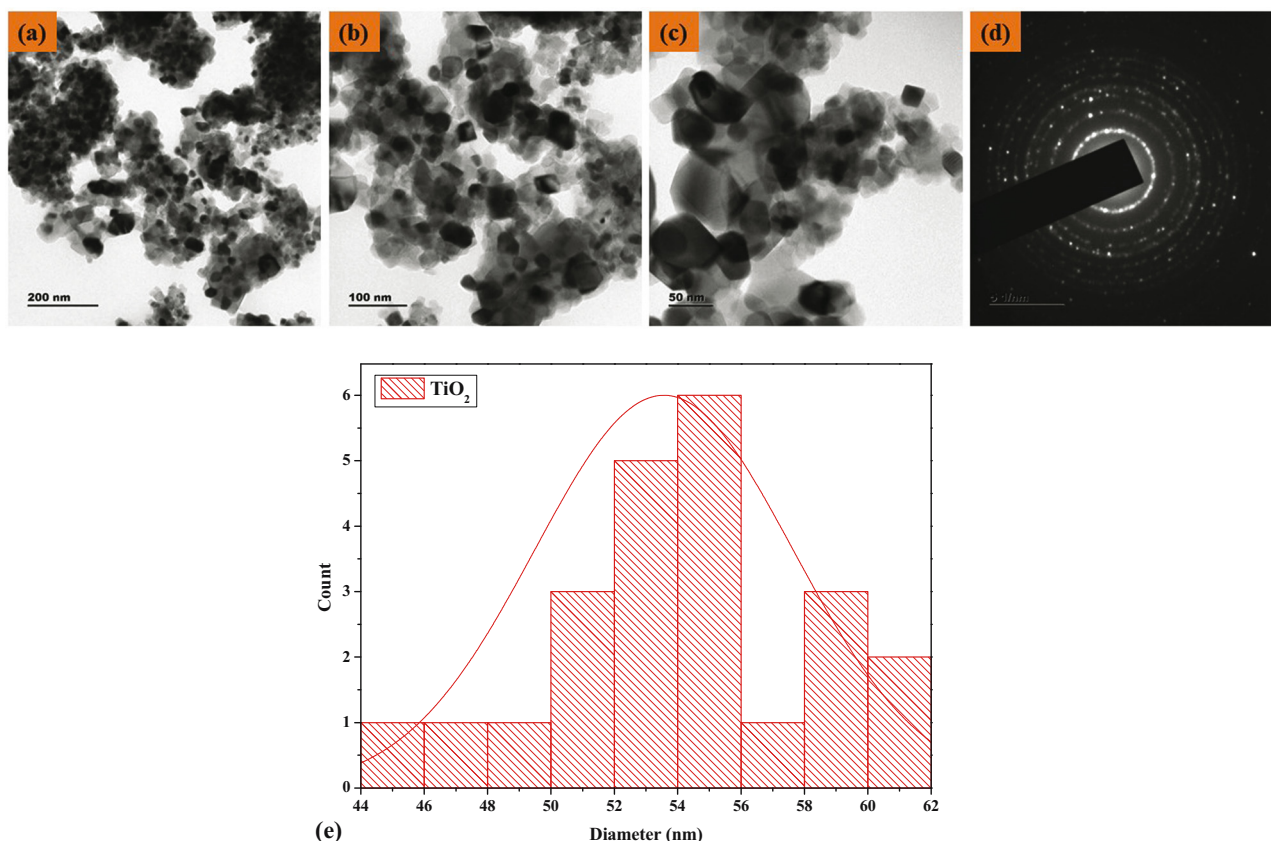


Figure 4: (a–c) TEM images and (d) SEAD pattern of green synthesized PF-127 coated TiO₂ NPs: Lower and Higher magnification TEM image. (e) Histogram of PF-127 coated TiO₂ NPs.

where λ is the X-ray wavelength (1.54060 Å), θ is the Bragg's diffraction angle, and β is the angular peak width at half maximum (radians). After successful synthesis, the particle size of the PF-127 coated TiO₂ NPs has been primarily scrutinized using Debye–Scherrer's equation in addition to analyzing the crystallinity of the material. The Scherrer's equation, which combines the 2θ and full width at half maximum level from XRD finding, is regarded to be the most essential and often employed equation to measure particle size [46].

The size of the PF-127 coated TiO₂ NPs is 60 nm. The hydrodynamic diameter of PF-127 coated TiO₂ NPs was assessed using DLS to obtain particle size information (Figure 6b). The PF-127 coated TiO₂ NPs size was observed at 164 nm because an aqueous medium surrounded the PF-127 coated TiO₂ NPs. This is known as hydrodynamic size. The stability of the NPs was assessed based on their zeta potential, as illustrated in Figure 3b. The results revealed a significant negative zeta potential value of -16.7 mV, indicating a robust colloidal nature of the particles. This observation suggests that the particles exhibited good stability and maintained their colloidal state effectively.

3.4 Green PF-127 coated TiO₂ NPs induce antimicrobial activity

The antimicrobial property of the green synthesized PF-127 coated TiO₂ NPs was assessed against *S. aureus*, *B. megaterium*, *B. subtilis*, *E. coli*, *S. dysenteriae*, *P. aeruginosa*, and *C. albicans* pathogens at various sample concentrations (1, 1.5, and 2 mg·ml⁻¹). The inhibition zones of PF-127 coated TiO₂ NPs and conventional antibiotics like amoxicillin against bacterial and fungal strains are depicted in Figure 7a and b. The results revealed that for all of the pathogens examined, increase in the PF-127 coated TiO₂ NPs concentration enhanced the diameters of the inhibitory zones.

This study demonstrates that the green-synthesized PF-127 coated TiO₂ NPs have potential as antimicrobial agents against a range of bacterial and fungal pathogens. The results also suggest that increasing the concentration of these NPs can enhance their antimicrobial efficacy. The study's findings may contribute to the development of new antimicrobial materials or treatments. For most pathogens, as the concentration of PF-127 coated TiO₂ NPs increases,

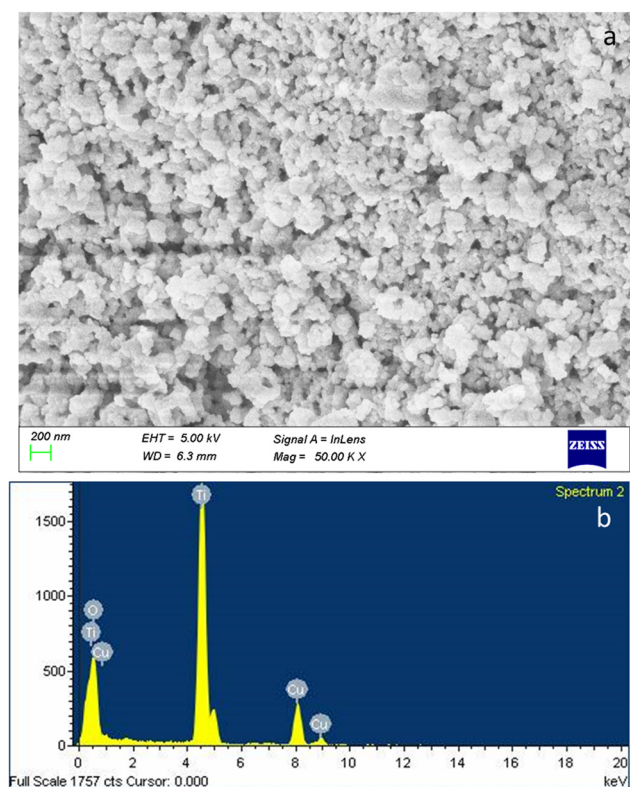


Figure 5: FESEM image of PF-127 coated TiO_2 NPs (a). Elements, weight%, and atomic% of the composition obtained by EDX (b).

the inhibition zone diameter tends to increase. This suggests that higher NP concentrations have a stronger inhibitory effect against these pathogens. In some cases, the inhibition zone diameter for the highest concentration of PF-127 coated TiO_2 NPs ($2 \text{ mg} \cdot \text{mL}^{-1}$) is greater than that of

amoxicillin, indicating that the NPs might be more effective against those particular pathogens. The effectiveness of the NPs can vary depending on the pathogen. Some pathogens show higher susceptibility to the NPs at lower concentrations, while others might require higher concentrations for effective inhibition. The presented data suggest that the green-synthesized PF-127 coated TiO_2 NPs have potential as antimicrobial agents, and their effectiveness varies based on the concentration and the target pathogen (Figure 7a and b).

3.5 Green PF-127 coated TiO_2 NPs exhibits antioxidant activity

The antioxidant potential of the PF-127 coated TiO_2 NPs was evaluated by examining the potential of the NPs to quench the stable DPPH radical into nonradical state. A high free radical scavenging potential was observed upon treatment with the biosynthesized PF-127 coated TiO_2 NPs. Moreover, the activity was found to increase the antioxidant activity in a concentration-dependent fashion, with the highest concentration sample exhibiting an inhibition percentage of 75% (Figure 8).

3.6 PF-127 coated TiO_2 NPs induce cytotoxicity against HEP-2 and KB cell lines

The biosynthesized PF-127 coated TiO_2 NPs was scrutinized for their cytotoxicity against HEP-2 (human laryngeal

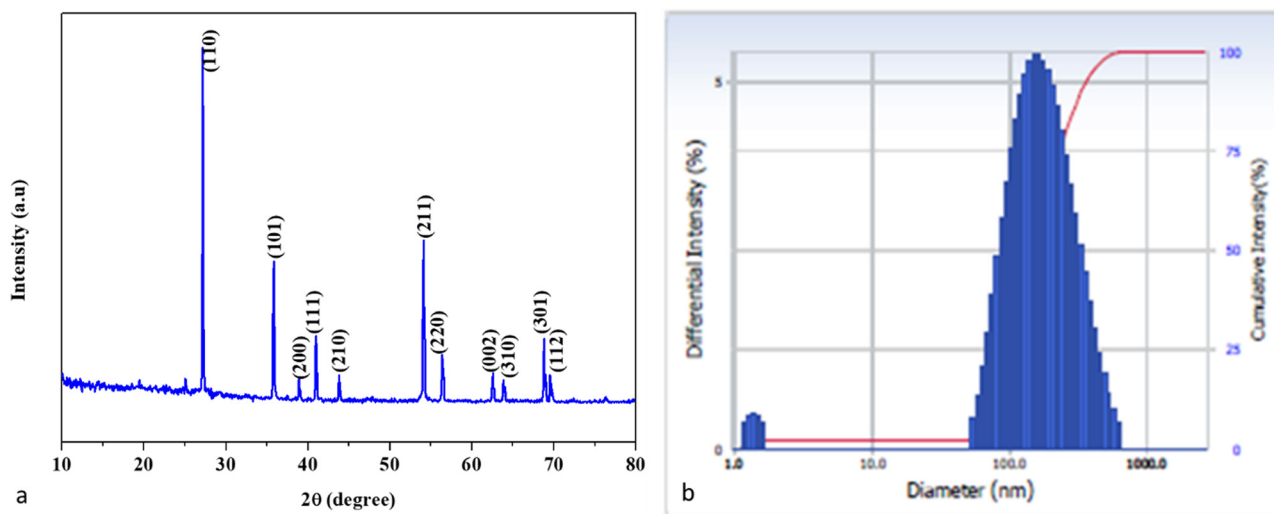


Figure 6: An XRD and DLS pattern of TiO_2 NP coated with PF-127 is shown in (a) and (b).

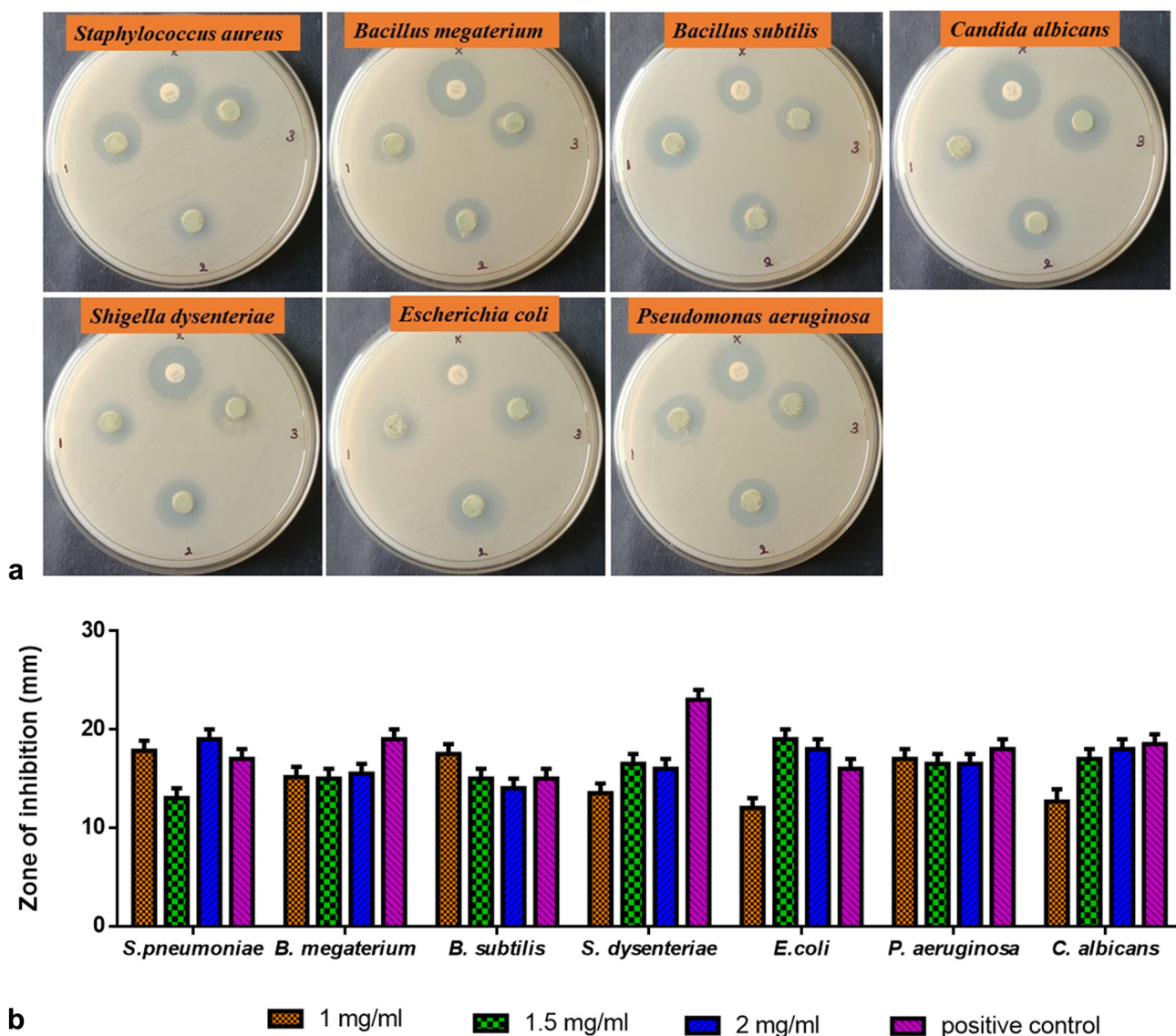


Figure 7: Antibacterial activity of PF-127 coated TiO_2 NPs. NPs inhibit the growth of bacteria (a). Antibacterial activity was determined for PF-127 coated TiO_2 NPs by measuring zone of inhibition (b).

cancer cell line), KB (human epithelial carcinoma cells), and Vero cell lines (Monkey kidney epithelial cell lines) at diverse dosages (2, 4, 8, 16, 32, 64 $\mu\text{g}\cdot\text{ml}^{-1}$) at 24, 48, and 72 h. The NPs exhibited significant cytotoxic effects against HEP-2 and KB cells with an IC_{50} level of 37.9 and 42.2 $\mu\text{g}\cdot\text{ml}^{-1}$ at 24 h; 30.09 and 18.64 $\mu\text{g}\cdot\text{ml}^{-1}$ at 48 h; and 16.72 and 11.79 $\mu\text{g}\cdot\text{ml}^{-1}$ at 72 h, respectively (Figure 9).

4 Discussion

As a metal oxide semiconductor, PF-127 coated TiO_2 NP in its rutile phase form exhibits an optical absorption range

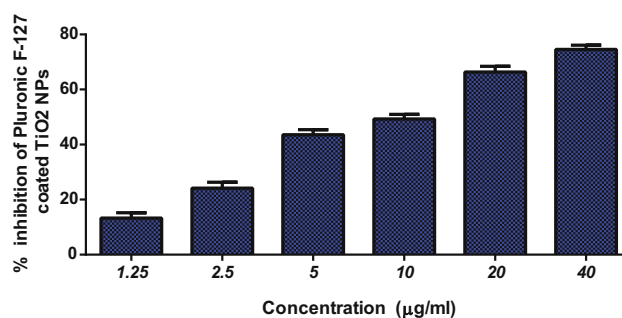


Figure 8: Antioxidant activity and percentage of inhibition of DPPH free radical by synthesized PF-127 coated TiO_2 NPs. Values are presented as mean value $\pm$ SD ($n = 3$). Statistical analysis was performed by Student's t -test. $**p < 0.01$; $***p < 0.001$.

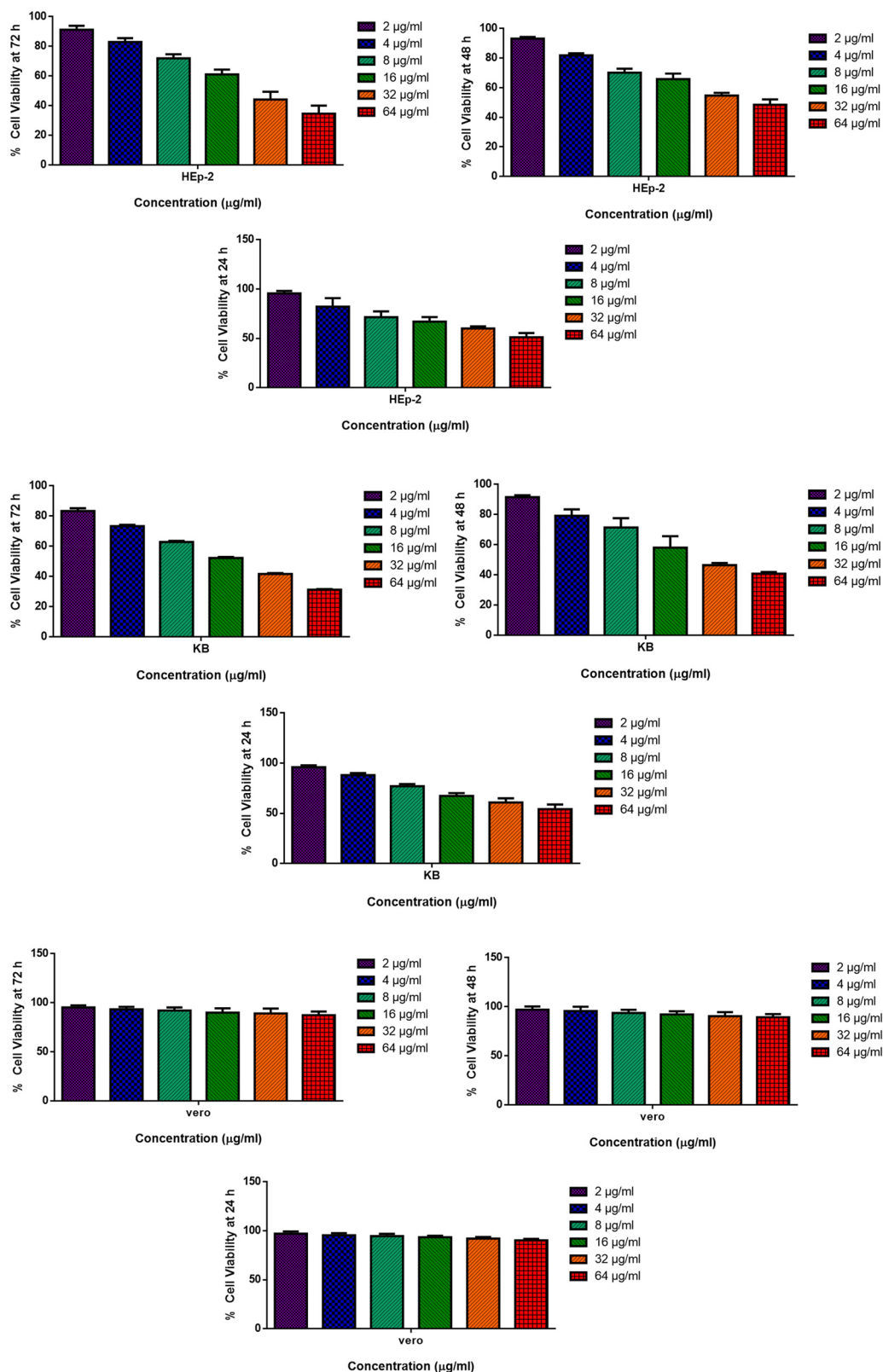


Figure 9: PF-127 coated TiO₂ NPs cause cytotoxicity in HEP-2 (laryngeal cancer cells), KB (mouth), and vero cells (normal). HEP-2, KB, and Vero cells were treated with different concentrations (2–64 µg·ml⁻¹) of PF-127 coated TiO₂ NPs for 24, 48, and 72 h. The cells were subjected to MTT assay and the values were depicted as mean value ± SD of three individual experiments.

of around 397 nm [37,47]. The outcomes of the current investigation are in concordance with those of other investigations that reported the synthesis of PF-127 coated TiO₂ NPs using *Trigonella foenum* and *Vitex negundo* extracts, where the presence of TiO₂ bands were observed at 400 and 380–400 nm, respectively [11,48]. By analyzing the chemical composition, FTIR could be utilized to assess a variety of materials in bulk and nano forms. The FTIR study revealed information on the biological molecules on the NP surface [3]. It is evident from the FTIR spectroscopic study that *A. macrocephala* plant extract contains the phytochemicals necessary to reduce the Ti ions in the precursor that is a necessary step in the PF-127 coated TiO₂ NPs. The results are consistent with those obtained from PF-127 coated TiO₂ NP production using extracts from *T. portulacastrum* and *C. quinoa* [7]. PL spectroscopic study revealed that the PF-127 coated TiO₂ NPs were related to band defects or transitions or to exciton recombination [49].

Techniques including SEM and TEM were extensively utilized to investigate the morphological characteristics of the NPs. These techniques can also be used to establish the size of NPs that have been obtained [50]. The form and surface of the PF-127 coated TiO₂ NPs produced by *A. macrocephala* were different. This might be because *A. macrocephala* leaf extracts contain phytochemicals including terpenoids, polyphenols, steroids, flavonoids, antioxidants, alkaloids, and tannins, whereas chemically produced NPs are typically spherical shaped, arranged in clusters with a relatively larger diameter, and porous in nature [7].

NP build-up on the microbial surface also serves a function in this mechanism by causing the destruction of the cell envelope and the release of intracellular components. The area of interaction between NPs and pathogenic bacteria increases as a result of their enhanced surface area, making them appropriate as antimicrobial agents [51]. The generation of Ti²⁺ ions and their electrostatic interaction with the bacterial cell wall might contribute to the antibacterial action of the NPs. Reactive oxygen species (ROS) are generated due to this interaction that can damage proteins and DNA. This leads to the death of microbes [52,53]. Since Ti NPs contain hydroxyl groups, they can dissolve bacterial outer membranes, causing the bacterium to die [54].

PF-127 coated TiO₂ NPs have displayed remarkable antibacterial activity against several pathogens [55]. Additionally, PF-127 coated TiO₂ NPs produced with fenugreek displayed effective antimicrobial potential and green PF-127 coated TiO₂ NPs synthesized with lemon also exhibited antibacterial activity against *Dickeya dadantii* [11,14]. These outcomes aligned with the prior study since PF-127 coated

TiO₂ NPs synthesized from *Kniphofia foliosa* root extract exhibited antibacterial effects against *E. coli*, *S. pyogenes*, *K. pneumonia*, and *S. aureus* [56].

Free radicals are generated by external substances or by regular metabolic processes in the body. They can immediately begin the peroxidation of membrane lipids, which results in the excessive accumulation of lipid peroxide [57]. The electron density transfer from the one positioned at O₂ to the electron present at nitrogen in DPPH, leading to a reduced intensity of $n \rightarrow \pi^*$ transitions, majorly contributes to the antioxidant effectiveness of the SnO₂ NPs [58]. A similar significant dose-dependent antioxidant activity by green PF-127 coated TiO₂ NPs synthesized with the leaves extract of *Malva parviflora* and *Artemisia haussknechtii* have been documented in various investigations [3,59].

Cancer causes high fatalities in developing countries. It was demonstrated that oxidative stress serves immensely in the initiation and progression of numerous illnesses that affect vital organs, particularly cancer [60]. The therapeutic index of current chemotherapeutic agents is extremely confined, they have poor solubility, and they are toxic to normal tissues [61]. Based on this theory, polymer-based drug delivery devices can ensure highly localized chemotherapeutic drug level in tumor site with minimal negative effects on normal cells [62].

Furthermore, the cell growth was noticed to reduce with higher NP dose. Nonetheless, the NPs did not reveal cytotoxicity to the Vero cells, which demonstrate their non-toxicity nature to non-malignant cells. Higher ROS levels cause oxidative stress inside the cells, which ultimately causes programmed cell death. Additionally, elevated ROS levels have been linked to cell death via damaging mitochondrial membranes [63]. As per the earlier investigations, green-synthesized SnO₂ NPs have revealed considerable antitumor properties [64–66]. To improve the stability of NPs in an aqueous condition, PF127 were employed for encapsulation [67]. It was highlighted that PF-127 can sensitize tumor cells to several drugs [10].

The orange peel extract-derived and *Coleus aromaticus* leaf-derived PF-127 coated TiO₂ NPs inhibited 41% of the A549 cell line [55]. The ROS that accumulated on the surface of the cells may have been induced by the occurrence of capped components of the leaf extract on the PF-127 coated TiO₂ NPs and by the excess electrons that the leaf extract may have provided to the NPs. It elevated oxidative stress, damaged cell membranes, accelerated lipid peroxidation, decreased glutathione levels, and eventually caused cell death [68] (Table 1).

Green Synthesis Approach: The use of *A. macrocephala* leaf extracts for the synthesis of TiO₂ NPs is a green and

sustainable method. Unlike conventional chemical methods that may involve toxic chemicals and high energy consumption, this approach is environmentally friendly and reduces potential negative impacts on human health and the environment. *A. macrocephala* is known for its potential bioactive compounds, and these compounds may contribute to the unique properties of the synthesized TiO₂ NPs. The coating of TiO₂ NPs with Pluronic F-127 is an additional step that enhances their stability, dispersibility, and potential for biological applications. This innovative modification can contribute to improved properties and interactions with cells. The thorough characterization techniques applied in this study provide a detailed insight into the physical and chemical properties of the synthesized NPs. This ensures the accurate assessment of their structure, size, and composition, providing a solid foundation for evaluating their properties. The study extensively evaluates the synthesized TiO₂ NPs for their antioxidant, antimicrobial, and anticancer activities. This comprehensive assessment allows for a holistic understanding of the NPs' potential applications and benefits. The evaluation of cytotoxicity against multiple cell lines, including HEp-2, KB, and Vero cells, highlights the selectivity of the synthesized NPs. This information is crucial for determining their potential as anticancer agents while maintaining non-toxicity to normal cells. The study suggests that the PF-127-coated TiO₂ NPs could serve as a platform for nanodrug delivery. This aspect could have far-reaching implications for targeted therapy and controlled drug release. The innovative and environmentally friendly approach, along with the multifunctional assessment, positions these TiO₂ NPs as promising candidates for various biomedical applications.

HEp-2 and KB cells show similar trends in response to PF-127 coated TiO₂ NPs, with decreasing viability as

concentrations increase. KB cells tend to have slightly lower cell viability than HEp-2 cells. HEp-2 cells tend to have the highest initial viability but decrease more steeply with the increase in the PF-127 coated TiO₂ NPs concentrations compared to the other cell lines. Vero cells (normal cells) exhibit consistent viability, showing a relatively negligible amount of cytotoxicity across concentrations and at each time point compared with both HEp-2 and KB cells. A comparison of the IC₅₀ values of KB cells and HEp-2 cells shows that KB cells consistently exhibit the lowest values, indicating a higher sensitivity to the inhibitory effects of PF-127 coated TiO₂ NPs. There is generally a difference in IC₅₀ values depending on the cell line and the time point. KB cells consistently show the lowest IC₅₀ values, suggesting that they are generally more sensitive to PF-127 coated TiO₂ NPs' inhibitory effects in terms of cell viability reduction. As a result of these IC₅₀ values, we are able to determine the potency of PF-127 coated TiO₂ NPs when it comes to inhibiting cell viability for different exposure durations. The decreasing trend in IC₅₀ values over time suggests that the cytotoxicity of PF-127 coated TiO₂ NPs becomes more pronounced with longer exposure periods. It should be noted that KB cells consistently showed lower IC₅₀ values than HEp-2 cells across all time points, which indicates that KB cells were more sensitive to the effects of PF-127 coated TiO₂ NPs.

Various techniques were used to synthesize and characterize green PF-127 coated TiO₂ NPs with *A. macrocephala* leaf extracts. Their antimicrobial, antioxidant, and anticancer properties have also been investigated. The crystallite size of PF-127 coated TiO₂ NPs were determined to be 60 nm, and the NPs had a polygonal rutile-type crystalline structure. Green PF-127 coated TiO₂ NPs demonstrated high antimicrobial efficacy against a variety of pathogens. Furthermore, the NPs demonstrated

Table 1: An overview of the efficiency of TiO₂ NPs compared with previous reports

Plant source	Green synthesis method	Antioxidant activity	Antimicrobial activity	Anticancer activity
<i>Aloe vera</i> and <i>Aloe barbadensis</i> [16]	Plant extract	ROS scavenging	Inhibition of pathogens	Induction of apoptosis
Green tea [69]	Leaf extract	Free radical scavenging	Antibacterial effects	Inhibition of cancer cells
Turmeric [70]	Rhizome extract	Antioxidant properties	Antimicrobial potential	Growth inhibition
Neem [71]	Leaf extract	Scavenging of ROS	Broad-spectrum activity	Apoptosis induction
Mexican mint [72]	Leaf extract	ROS neutralization	Antimicrobial effects	Cytotoxicity
Basil [73]	Leaf extract	Antioxidant potential	Inhibition of microorganisms	Cell cycle arrest
Common guava [74]	Leaf extract	ROS quenching	Antibacterial activity	Anti-proliferative effect
Lemon [17,24]	Fruit extract	Free radical scavenging	Antimicrobial properties	Apoptosis induction
Pomegranate [75]	Peel extract	Antioxidant effects	Inhibition of pathogens	Suppression of growth
Ashwagandha [76]	Leaf extract	ROS scavenging	Antimicrobial activity	Antiproliferative action

remarkable antioxidant activity, which increased concentration-dependently. The NPs demonstrated significant cytotoxicity against HEP-2 and KB cell lines, but not against Vero cell lines, indicating their cytotoxic potential against cancer cells. Therefore, PF-127 coated TiO₂ NPs demonstrate remarkable salutary properties to be utilized as a potential antibacterial and anticancer candidate. As a result, further research will focus on utilizing the newly synthesized PF-127 coated TiO₂ NPs for effective design of nanodrug delivery platforms.

5 Conclusion

In conclusion, the PF-127 coated TiO₂ NPs derived from *A. macrocephala* leaf extracts possess a diverse array of salutary properties. Their antimicrobial, antioxidant, and selective anticancer effects make them promising candidates for applications in antibacterial and anticancer strategies. The synthesized NPs exhibited an average crystallite size of 60 nm and a distinct polygonal rutile-type crystalline structure, confirming their successful formation. Notably, the PF-127 coated TiO₂ NPs displayed heightened antimicrobial efficacy against a diverse range of pathogens, suggesting their potential as potent antibacterial agents. The NPs also demonstrated impressive antioxidant activity, with their efficacy increasing proportionally with concentration. This attribute highlights their potential application as effective antioxidants, holding promise for various health-related applications. A significant finding was the NPs' remarkable cytotoxic effect on cancer cell lines HEP-2 and KB. This selective cytotoxicity underscores their potential as candidates for anticancer therapies, as they effectively target cancer cells while sparing healthy cells from harm. Importantly, the NPs exhibited a non-toxic nature towards Vero cells, indicating their safety profile in relation to healthy cells. This selectivity enhances their attractiveness as potential candidates for cancer treatments with reduced side effects. The next step in the research will involve harnessing the unique properties of these NPs for the development of innovative nanodrug delivery platforms, thereby potentially revolutionizing targeted therapeutic interventions in the field of medicine.

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