

Bio - Green Synthesis of Ag-Doped TiO₂ Nanoparticles Using *Beta vulgaris* Leaf Extract: Photocatalytic Degradation of Diclofenac Sodium and Biomedical Applications

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Abstract

Ag/TiO₂ nanoparticles were produced using extract from beta Vulgaris leaves. The characterization of these nanoparticles was performed through various methods, including scanning electron microscopy combined with energy dispersive X-ray and elemental mapping, transmission electron microscopy, as well as Fourier-transform infrared and UV-Vis spectroscopy analyses. The obtained elemental mapping indicated the presence of Ti, Ag, K, Cl, S, P, Si, and O elements distributed throughout the nano hybrid. XRD analysis verified the crystalline characteristics of Ag/TiO₂ nanoparticles, revealing an average particle size ranging from 8 to 60 nm, while higher sizes beyond 100 nm occurred due to the aggregation of nanoparticles. The catalytic performance of Ag/TiO₂ was assessed by examining its ability to degrade diclofenac sodium in the presence of UV-Vis light. Approximately 90% degradation was accomplished after 40 minutes of irradiation. The study also explored the biomedical potential of Ag/ TiO₂, specifically its anticancer properties using the MCF-7 cell line. The kinetics of degradation were modelled according to the pseudo-first-order model. Antioxidant capabilities were evaluated through the DPPH assay, and the antimicrobial effects of Ag/TiO₂ nanoparticles were investigated. The research included four varieties of bacterial strains and two types of fungal strains, yielding positive results in antimicrobial activity.

Keywords: Green synthesis, Ag/ TiO₂, Beta Vulgaris, drug degradation and Biomedical Application

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Introduction

The presence of pharmaceutical residues in aquatic environments has become a growing global concern in recent years. Pharmaceuticals and personal care products (PPCPs) are recognized as emerging contaminants that encompass a wide range of medicines, cosmetic products, and other synthetic organic compounds associated with human activities [1]. The continuous release of these substances into the environment may adversely affect ecosystems and human health [2]. Among the various PPCPs, diclofenac sodium is one of the most frequently detected contaminants. It is a widely prescribed non-steroidal anti-inflammatory drug (NSAID) used worldwide for the treatment of pain and inflammation [3]. Studies have reported its occurrence in both the influent and effluent streams of wastewater treatment facilities, as well as in surface waters and drinking water sources. Conventional wastewater treatment methods, including filtration and activated sludge processes, are often ineffective in completely removing diclofenac sodium because of its high stability, resistance to degradation [4], and low biodegradability. As a result, these methods mainly transfer the contaminant between environmental compartments rather than achieving

its complete elimination.

To overcome these limitations, advanced oxidation processes (AOPs) have emerged as promising technologies for the degradation of persistent organic pollutants. The Fenton process is one of the most widely investigated AOPs due to its ability to generate highly reactive hydroxyl radicals ($\bullet\text{OH}$), which can rapidly oxidize a broad spectrum of organic contaminants [5,6]. Despite its effectiveness, the conventional Fenton process faces several challenges related to the use of hydrogen peroxide (H₂O₂). Hydrogen peroxide is chemically unstable and can undergo self-decomposition, reducing its efficiency and causing operational difficulties due to the heat generated during the reaction. Therefore, the development of alternative Fenton reagents and optimized reaction conditions has become an important area of research.

Diclofenac sodium is extensively used as an analgesic, anti-inflammatory, anti-rheumatic, and anti-arthritis medication. Approximately 15% of the administered dose is excreted from the human body in its unchanged form, resulting in its continuous introduction into aquatic environments and contributing to its widespread environmental

occurrence [7].

Although diclofenac is known to undergo rapid degradation through direct photolysis under typical environmental conditions [8], it remains one of the most commonly detected pharmaceutical contaminants in aquatic ecosystems. Concentrations of diclofenac have been reported in sewage treatment plant (STP) effluents and surface waters ranging from 0.14 to 1.48 µg/L, with levels reaching up to 0.59 µg/L in groundwater [9], 15 µg/L in surface waters, and 28.4 µg/L in various aquatic environments [10]. Its widespread occurrence highlights its continuous release and persistence in the environment.

The ecological risks associated with diclofenac contamination have been extensively documented. In particular, the dramatic decline of *Gyps* vulture populations in India and Pakistan has been linked to the veterinary use of diclofenac as an analgesic and anti-inflammatory drug. Residues of the drug accumulated in vultures through the consumption of carcasses of treated livestock, leading to renal failure and causing population declines exceeding 95% in certain species since the early 1990s [11].

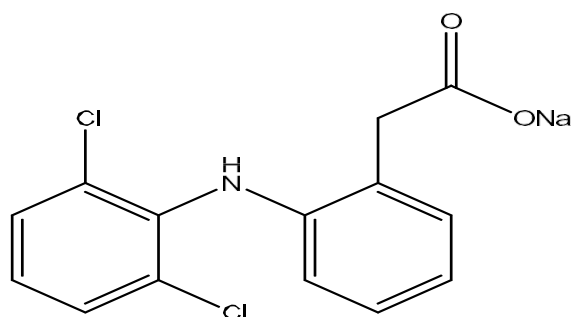


Fig 1 Chemical structure of

Diclofenac sodium

Furthermore, laboratory studies have demonstrated that environmentally relevant concentrations of diclofenac can adversely affect aquatic organisms. For example, exposure to concentrations as low as 1 µg/L induced vitellogenin production in male Japanese medaka (*Oryzias latipes*), indicating endocrine-disrupting effects [12]. Similarly, cytopathological alterations in the liver, kidneys, and gills of rainbow trout have been observed at the same concentration level [13,14].

Given these adverse environmental impacts, the effective removal of diclofenac from contaminated water bodies is of considerable importance. Among the various treatment technologies developed over recent decades, advanced oxidation processes (AOPs) have attracted significant attention due to their high efficiency in degrading pharmaceutical contaminants in aqueous systems. These processes rely on the generation of highly reactive hydroxyl radicals (•OH), which possess stronger oxidation potential than most conventional chemical oxidants

and are capable of achieving complete mineralization of organic pollutants [15]. Among the different AOPs, the Fenton oxidation process has emerged as one of the most promising techniques because of its relatively low cost, operational simplicity, and effectiveness in degrading persistent and non-biodegradable organic compounds [16].

In recent years, several advanced treatment technologies have been investigated for the degradation of diclofenac (DCF) in aqueous environments. Effective removal of DCF has been reported using various processes, including ozonation (O₃) [17], UV/H₂O₂ oxidation [18], electron beam irradiation [19], UV-activated peroxymonosulfate (PMS) [20], UV/TiO₂ photocatalysis [21], three-dimensional electrochemical reactors [22], visible-light-driven photocatalytic systems [23], UV/chlorine treatment [24], and LED-assisted irradiation techniques [25]. Despite the extensive research on these advanced oxidation technologies, studies focusing on the conventional Fenton oxidation process for DCF degradation remain limited. Therefore, the present work aims to evaluate the effectiveness of the Fenton process in the degradation and mineralization of diclofenac in aqueous solutions through kinetic investigations. Particular emphasis is placed on assessing the influence of the initial drug concentration and determining the corresponding pseudo-second-order rate constants.

In addition, Ag/TiO₂ nanoparticles were synthesized through a green synthesis approach using *Beta vulgaris* leaf extract, with the objective of developing an environmentally friendly and simple method for modifying photocatalytic materials while minimizing the use of hazardous chemicals and reagents. The synthesized nanoparticles were comprehensively characterized to examine their structural, morphological, elemental, spectral, and optical properties. Characterization techniques included Scanning Electron Microscopy (SEM), Energy-Dispersive X-ray Spectroscopy (EDX) coupled with elemental mapping, Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FT-IR), and UV-Visible spectroscopy.

Although the photocatalytic performance of Ag/TiO₂ nanoparticles has been widely reported in the literature, the present study investigates their catalytic efficiency under combined UV-Visible light irradiation. The obtained results are compared with previously reported photocatalytic performances to demonstrate the enhanced degradation efficiency of the UV-Visible catalytic system. Such an approach offers a promising strategy for the effective treatment of pharmaceutical contaminants and their safe removal

prior to discharge into aquatic environments. Fig1 have showed chemical structure of diclofenac sodium

2. Materials and Methods

2.1 Synthesis of Ag/TiO₂

Fresh leaves of *Beta vulgaris* were procured from a local vegetable market in Chidambaram, Cuddalore District, Tamil Nadu, India. The collected leaves were thoroughly washed three times with tap water, followed by rinsing with deionized water to remove adhering dust and impurities. Subsequently, the leaves were shade-dried for seven days to eliminate residual moisture and then finely ground into powder [26].



Fig 2 Scheme of green synthesis of Ag/ TiO₂ Nanoparticles

2.2. Characterization, drug degradation and biomedical applications

2.2.1 Scanning electron microscopy

The morphology and particle size of the synthesized Ag/TiO₂ nanoparticles were examined using Scanning Electron Microscopy (SEM) (Nikon, Japan). For sample preparation, the nanoparticles were dispersed in deionized water at a concentration of 1 mg/mL and sonicated in an ultrasonic bath to obtain a uniform and stable suspension [27]. The resulting stock suspension was subsequently diluted twenty-fold for particle size analysis. A drop of the diluted suspension was deposited onto a clean glass substrate and allowed to dry completely under ambient conditions. The dried sample was then coated with a thin layer of gold to enhance conductivity prior to SEM analysis. SEM imaging was employed to investigate the surface morphology, particle size distribution, and structural characteristics of the synthesized Ag/TiO₂ nanoparticles.

2.2.2 EDS

Energy-Dispersive X-ray Spectroscopy (EDS) was employed to determine the elemental composition of the synthesized nanoparticles. This technique was used to verify the presence of silver (Ag), titanium (Ti), and oxygen (O) within the Ag/TiO₂ nanostructures, as well as to identify any additional elements present in the sample. In addition to qualitative elemental identification, EDS enables the semi-quantitative estimation of elemental concentrations [27]. For EDS analysis, the nanoparticle suspension was diluted 100-fold with deionized water. Subsequently, 10 µL of the diluted sample was deposited onto a carbon-coated stub and

allowed to air dry. The EDS spectra were recorded using an accelerating voltage of 20 kV with a data acquisition time of 19 s. Elemental mapping was performed using pseudo-color imaging to visualize the two-dimensional spatial distribution of the detected elements within the sample. All analyses were carried out using a JEOL JSM-6360 scanning electron microscope equipped with an EDS detector.

2.2.3 Transmission electron microscopy

The particle size and morphological characteristics of the synthesized Ag/TiO₂ nanoparticles were investigated using High-Resolution Transmission Electron Microscopy (HR-TEM) (JEOL 3010, Japan) operated at an accelerating voltage of 200 kV, following a previously reported method with minor modifications [28]. For sample preparation, the nanoparticles were dispersed in deionized water at a concentration of 1 mg/mL and sonicated in an ultrasonic bath until a homogeneous suspension was obtained. To facilitate particle size analysis, the sonicated stock suspension was diluted twenty-fold to achieve a final concentration of 0.5 mg/mL. A small drop of the diluted Ag/TiO₂ nanoparticle suspension was then deposited onto a carbon-coated copper grid and allowed to air dry at room temperature. The prepared samples were subsequently examined by HR-TEM to evaluate the particle size, morphology, and structural features of the synthesized Ag/TiO₂ nanoparticles.

2.2.4 X-ray Diffraction study

The crystalline structure and phase composition of the synthesized Ag/TiO₂ nanoparticles were analyzed using X-ray Powder Diffraction (XRD). Diffraction patterns were recorded with an X'Pert PRO diffractometer (PANalytical Ltd., The Netherlands) equipped with a goniometer radius of 240 mm. The measurements were performed using nickel-filtered Cu K α radiation with a wavelength of 1.54060 Å [29]. A divergence slit of 1° and a receiving slit of 0.1 mm were employed during data acquisition. The XRD analysis was conducted under operating conditions of 40 kV tube voltage and 30 mA tube current. Diffraction data were collected over a 2 θ scanning range of 10°–90° to identify the crystalline phases and evaluate the structural properties of the synthesized Ag/TiO₂ nanomaterials.

2.2.5 Dynamic Light Scattering (DLS) analysis

Dynamic Light Scattering (DLS) analysis was performed using a Micrometrics Nano Plus instrument following a standard protocol with minor modifications. The system is capable of determining particle size distributions of colloidal suspensions ranging from 0.1 nm to 12.3 µm, covering sample concentrations between 0.00001% and 40%, and is sensitive to molecular weights as low as 250 Da. For analysis, Ag/TiO₂ nanoparticle suspensions were prepared at a concentration of 100 µg/mL and sonicated for 2 min to ensure proper dispersion.

Subsequently, two drops of the nanoparticle suspension were diluted in 10 mL of Milli-Q water. After complete dispersion of the nanoparticles in the medium, the samples were subjected to DLS measurement. Each experiment was conducted multiple times to ensure reproducibility, and the average particle size was reported.

2.2.6 FT-IR spectroscopy

The functional groups and chemical bonding characteristics of the synthesized Ag/TiO₂ nanoparticles were analyzed using Fourier Transform Infrared (FT-IR) spectroscopy. The measurements were performed using a PerkinElmer Spectrum Two FT-IR spectrometer [31], operating in the spectral range of 400–4000 cm⁻¹ with a resolution of 4 cm⁻¹, which allows detailed identification of vibrational modes associated with various chemical bonds. For sample preparation, the conventional potassium bromide (KBr) pellet technique was employed. In this method, a small quantity of the dried nanoparticle sample was thoroughly mixed with spectroscopic-grade KBr powder and compressed under high pressure to form a transparent pellet. The prepared pellet was then placed in the sample holder for spectral acquisition. The resulting FT-IR spectra were used to identify the presence of functional groups and to confirm the successful synthesis and surface modification of the Ag/TiO₂ nanomaterials.

2.2.7. UV–vis spectroscopy

The absorption maximum of the biosynthesized Ag/TiO₂ nanoparticles was determined using UV–visible spectroscopy. The samples were analyzed at room temperature [32] with a PerkinElmer Lambda 35 UV–Vis spectrophotometer, operating at a spectral resolution of 1 nm over a wavelength range of 190–1100 nm, following the method reported Dash et al. 2014

2.2.8 Drug degradation

The concentration of diclofenac sodium (DCF) was determined using a SPECORD 210 PLUS UV–Visible spectrophotometer at a wavelength of 276 nm. To develop a calibration curve relating the percentage degradation of DCF to degradation time, standard solutions were prepared at three different pH values (10.5, 7.0, and 2.5) and analysed according to previously reported methods [33–39]. A linear correlation between degradation percentage and degradation time was observed across the studied pH range. Water samples collected from the reactor were analysed immediately after sampling, and the percentage degradation of DCF was calculated using the established calibration curve. The pH of the samples was monitored using a Lovibond pH 100 digital pH meter. Furthermore, the extent of mineralization was evaluated to assess the complete breakdown of DCF into inorganic end products.

2.2.9. Cytotoxicity by MTT assay

The cytotoxicity of the synthesized nanoparticles (NPs) was determined using the MTT assay. Briefly, Vero cells were seeded in a 96-well plate and allowed to adhere under standard culture conditions as previously described. After incubation, 10 mL of MTT solution was added to each well, and the plate was incubated for 4 h in a CO₂ incubator. Subsequently, the supernatant was discarded, and 200 mL of DMSO was added to each well to dissolve the formed formazan crystals. The absorbance was measured at 550 nm using a microplate reader.

2.2.10. Anticancer activity by MTT assay

Human breast cancer cell line (MCF-7) was obtained from the National Centre for Cell Science (NCCS), Pune, India, and cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% fetal bovine serum (FBS). The cells were maintained in a humidified atmosphere containing 5% CO₂ at 37 °C and allowed to grow for 24 h. Following incubation, the cells were treated with varying concentrations of Ag/TiO₂ nanoparticles (6.25, 12.5, 25, 50, and 100 µg/mL) and further incubated for 48 h under the same conditions. Cells cultured in medium without nanoparticles served as the untreated control for cell viability assessment. All experiments were performed in triplicate. After treatment with Ag/TiO₂ nanoparticles, the cells were washed with 600 µL of phosphate-buffered saline (PBS, pH 7.0) and centrifuged at 3000 rpm for 10 min to remove cellular debris and obtain a clear supernatant. After treatment with Ag/TiO₂ nanoparticles, the cells were washed with 600 µL of phosphate-buffered saline (PBS, pH 7.0) and centrifuged at 3000 rpm for 10 min to remove cellular debris and obtain a clear supernatant.

The percentage of cell viability was calculated using the following equation:

$$\% \text{ Cell Viability} = (\text{Absorbance of Test Sample} / \text{Absorbance of Control}) \times 100$$

2.2.11 Anti-oxidant studies by DPPH method

The antioxidant activity of Ag/TiO₂ nanoparticles (NPs) was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay according to the method described by Vishaka et al., with slight modifications. The assay was performed in a 96-well microplate. Briefly, 20 µL of the test sample was added to the respective wells, followed by 180 µL of DPPH solution to obtain a final reaction volume of 200 µL. Ascorbic acid was used as the reference standard. The reaction mixtures were incubated at room temperature for 30 min in the dark. The reduction of DPPH radicals was indicated by a color change from violet to yellow, reflecting the antioxidant potential of the nanoparticles. The absorbance of the reaction mixture was measured at 517 nm using a microplate reader. The percentage of DPPH

radical scavenging activity was calculated using the following equation:

$$\% \text{ DPPH Scavenging Activity} = [(A_0 - A_1) / A_0] \times 100$$

where A₀ is the absorbance of the control and A₁ is the absorbance of the test sample.

2.2.12. Antibacterial assay

The antibacterial activity of Ag/TiO₂ nanoparticles (NPs) was evaluated using the disc diffusion method as described by Vishaka et al.. Four pathogenic bacterial strains were used in this study: two Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus cereus*, and two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*. The bacterial cultures were refreshed in nutrient broth and incubated at 37 °C for 24 h prior to the assay. Nutrient agar medium was used for the cultivation of the bacterial strains. Sterile filter paper discs (6 mm diameter) were prepared from Whatman No. 1 filter paper. The culture media, filter paper discs, and all other materials used in the experiment were sterilized by autoclaving. All procedures were carried out under aseptic conditions in a microbiological safety cabinet. The bacterial cultures were uniformly spread onto labeled nutrient agar plates. Ampicillin was used as the positive control. The synthesized Ag/TiO₂ NPs were loaded onto the sterile discs at a concentration of 20 mg per disc, and the discs were carefully placed on the inoculated agar plates. The plates were then incubated at 37 °C for 24 h. Antibacterial activity was assessed by measuring the diameter of the zones of inhibition surrounding each disc after incubation.

2.2.13 Antifungal assay

The antifungal activity of Ag/TiO₂ nanoparticles (NPs) synthesized using Beta vulgaris leaf extract was evaluated against two fungal strains, *Candida albicans* and *Trichoderma viride*. The fungal cultures were refreshed in Sabouraud Dextrose Broth (SDB) and maintained on Sabouraud Dextrose Agar (SDA) plates. The antifungal activity was assessed using a slightly modified disc diffusion method described previously. All media, filter paper discs, and laboratory apparatus were sterilized by autoclaving prior to use. The experiments were performed in triplicate under aseptic conditions in a microbiological safety cabinet. Sterile filter paper discs impregnated with the nanoparticle suspension were placed on SDA plates previously inoculated with the test fungal strains. Amphotericin B was used as the positive control, while Ag/TiO₂ NPs were applied at a concentration of 20 mg per disc. The plates were incubated at 28 °C for 24 h, after which the antifungal activity was determined by measuring the diameter of the zones of inhibition surrounding the discs.

3. Result and discussion

3.1. Confirmation of Morphology

3.1.1. SEM and EDS analysis

The SEM micrographs of the synthesized

Ag/TiO₂ nanoparticles (Figure 3a–c) revealed a mixed morphology consisting of spherical, rod-shaped, and cauliflower-like structures. The EDS spectra (Figure 3d–e) confirmed the presence of titanium (Ti), silver (Ag), and oxygen (O) in the nanocomposite. Quantitative EDS analysis indicated Ti contents of 40.02 wt% and 45.28 at%, Ag contents of 54.61 wt% and 46.50 at%, and O contents of 39.77 wt% and 54.57 at%. The elemental mapping analysis (Figure 3d) demonstrated a homogeneous distribution of Ti and Ag throughout the nanohybrid, with oxygen atoms uniformly associated with the metal oxide matrix. SEM observations (Figure 3e) further revealed spherical TiO₂ particles surrounded by flaky rod-like structures, indicating the successful formation of the Ag/TiO₂ nanocomposite. The EDS results confirmed the presence of Ag and TiO₂ in the nanohybrid, with Ag and TiO₂ loadings of approximately 61 wt% and 39 wt%, respectively. Furthermore, elemental mapping demonstrated the uniform distribution of Ag and TiO₂ nanoparticles across the surface, while the spherical and rod-like morphologies were observed to be integrated within the metal oxide framework. The EDS and elemental mapping analyses confirmed the stoichiometric composition and successful formation of the Ag/TiO₂ nanohybrid.

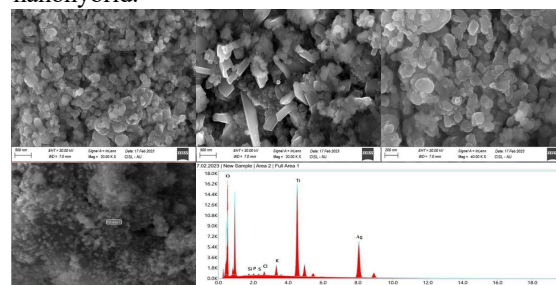


Fig 3 SEM and EDS Analysis of Ag/ TiO₂ Nanoparticles from Beta vulgaris leave extract

3.1.2 TEM analysis

The results of TEM analysis showed in figure 4 (a–f), the formation of distorted spherical particles ranging between 10 and 15 nm, Figure 4a and 4b were revealed spherical and clusters of rod like aggregates forming a dense bundle of structure., From figure 4c, showed a mixed morphology of spherical, Figure 4d and 4e, showed mixed types of crystalline structures, Figure 4f, showed SAED pattern to showed definite polycrystalline structure. The average particle size was observed to be ranging between 23 and 35 nm. The appearance of mixed morphology in the case of Ag/ TiO₂ confirmed the formation of the nanoparticles.

Fig 4 TEM Analysis of Ag/ TiO₂ Nanoparticles from Beta vulgaris leaves extract

3.1.3 XRD Analysis

The XRD pattern of Ag/TiO₂ synthesized using Beta vulgaris leaf extract (Figure 5a) exhibited intense diffraction peaks at 2θ = 23.8°, 26.7°, 39.1°, 47.2°, 49.3°, 65.3°, 69.4°, and 73.6°,

which correspond to the (101), (100), (004), (200), (105), (204), (220), and (215) crystallographic planes, respectively, indicating a face-centered cubic structure of Ag-doped TiO₂. These peaks were found to be consistent with the standard JCPDS card No. 21-1272. Additional peaks associated with Ag–O–Ti–O bonding were observed at 2θ values of 23.8° (101), 47.2° (200), and 65.9° (204), further confirming the formation of the Ag/TiO₂ nanocomposite. The average crystallite size calculated using the Debye–Scherrer equation was approximately 36 nm. Furthermore, the XRD pattern showed weak reflections at $2\theta = 16.5^\circ, 17.6^\circ, 19.5^\circ, 26.4^\circ, 28.3^\circ, 34.9^\circ, 39.4^\circ, 54.9^\circ,$ and 67.4° , which are attributed to the phytochemical constituents of the *Beta vulgaris* leaf extract. The relatively low intensity and partial absence of some of these peaks suggest their encapsulation or masking by the Ag/TiO₂ nanoparticles, confirming successful nanoparticle formation and stabilization by the plant extract.

3.1.4 DLS Analysis

Dynamic light scattering (DLS) is widely used to determine the particle size distribution of Ag/TiO₂ nanoparticles in their dispersed state. The DLS results (Figure 5b) indicated that the particle size ranged from approximately 8 to 80 nm, with a predominant peak centered at 23.8 nm. The relatively symmetric size distribution suggests a reasonably uniform dispersion and homogeneity of the synthesized Ag/TiO₂ nanoparticles. However, a broader size distribution was also observed, which may be attributed to the aggregation of nanoparticles during the DLS measurement process. Such agglomeration can lead to an apparent increase in particle size compared to other techniques.

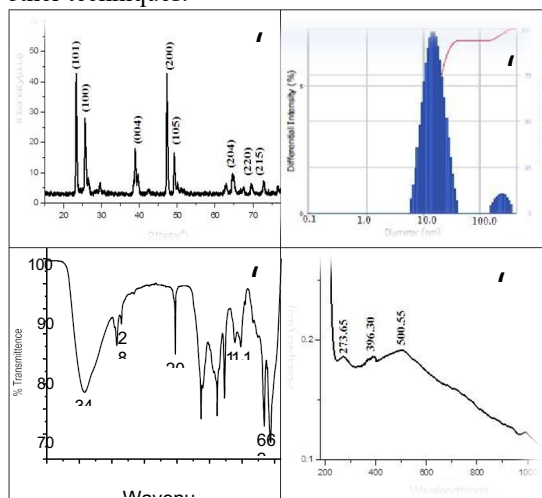


Fig 5 XRD pattern, DLS, FTIR and UV spectrum images of Ag/ TiO₂ Nanoparticles using *Beta vulgaris* leaf extract

3.2 Spectral Studies

3.2.1 FTIR Analysis

The FTIR spectra of Ag/TiO₂ nanoparticles are presented in Figure 5c. A broad absorption band

observed at 3418 cm⁻¹ is attributed to the O–H stretching vibration of adsorbed water molecules on the nanoparticle surface. The peak at 2923 cm⁻¹ corresponds to C–H stretching vibrations. The band at 1632 cm⁻¹ is assigned to carbonyl (C=O) stretching vibrations, while the peak at 1384 cm⁻¹ is associated with O–H bending vibrations. The absorption bands observed at 1271 cm⁻¹, 1112 cm⁻¹, and 1022 cm⁻¹ are attributed to aliphatic amine groups and overlapping C–H and =CH₂ out-of-plane bending vibrations. The peaks at 662 cm⁻¹ and 566 cm⁻¹ are characteristic of Ag–Ti–O–Ag–O asymmetric and symmetric stretching vibrations, confirming the formation of Ag–TiO₂ nanoparticles.

3.2.2 UV Spectroscopy

The UV–Vis absorption spectrum of Ag/TiO₂ nanoparticles is shown in Figure 5d. The optical properties of the silver-doped TiO₂ nanoparticles were analysed in the wavelength range of 200–600 nm using a UV–Vis spectrophotometer. The Ag-doped TiO₂ exhibited enhanced absorption in the ultraviolet region with absorption peaks at 273.65 nm, 396.30 nm, and 500.55 nm. A noticeable shift in the absorption band toward lower wavelengths (blue shift) was observed, which is attributed to the surface Plasmon resonance effect of silver nanoparticles incorporated into the TiO₂ matrix. The overall increase in absorbance indicates improved light-harvesting ability due to Ag doping. In the Ag/TiO₂ nanocomposite system, the reduction in peak intensity at 273.65 nm suggests electron transfer from the TiO₂ conduction band to Ag nanoparticles, which helps reduce electron–hole recombination and enhances optical performance [14].

3.2.3 UV and Visible Light Assisted Degradation of diclofenac sodium drug

The degradation of diclofenac sodium (DCF) in aqueous solution using the Fe²⁺/H₂O₂ system is illustrated in Figure 6. The results revealed that approximately 98% degradation of DCF was achieved within 40 min. This high efficiency is attributed to the generation of highly reactive species such as sulfate radical anions (•SO₄⁻), hydroxyl radicals (•OH), or a combination of both, produced through the Fe²⁺/H₂O₂ reaction system. These radicals are well known for their strong oxidative capability in degrading pharmaceutical pollutants. In comparison, nearly 80% degradation was achieved using the Ag/TiO₂/*Beta vulgaris* leaf extract nanocomposite. The plots of percentage degradation versus time under both UV and visible light irradiation for Ag/TiO₂, *Beta vulgaris* leaf extract, and Ag/TiO₂/*Beta vulgaris* systems demonstrated pseudo-first-order kinetics in all cases. Fig 6 Drug degradation mechanisms of diclofenac sodium drug with Ag/ TiO₂ Nanoparticles using *Beta vulgaris* leaf extract

The photocatalytic degradation of DCF

using Ag/TiO₂/Beta vulgaris leaf extract was significantly influenced by pH. The degradation efficiency was evaluated over a pH range of 2.5 to 10.5 at a fixed catalyst dose (50 mg/L) under UV irradiation for different time intervals (0–40 min), as shown in Figure 7. The highest degradation efficiency was observed under acidic conditions, with optimum performance at pH 2.5. In mildly acidic media, pharmaceutical molecules exist predominantly in their neutral form, while the catalyst surface becomes positively charged due to protonation, enhancing electrostatic attraction and adsorption of the pollutant. This facilitates the generation of hydroxyl radicals through interaction of hydroxide ions with photogenerated holes, thereby improving degradation efficiency. However, under strongly acidic conditions, excessive H⁺ concentration may suppress •OH formation, leading to a decrease in degradation efficiency. In neutral and alkaline conditions, both the catalyst surface and drug molecules may carry negative charges, resulting in electrostatic repulsion and reduced adsorption, which further decreases photocatalytic activity. Figure 7 illustrates the proposed mechanism for the degradation of diclofenac sodium.

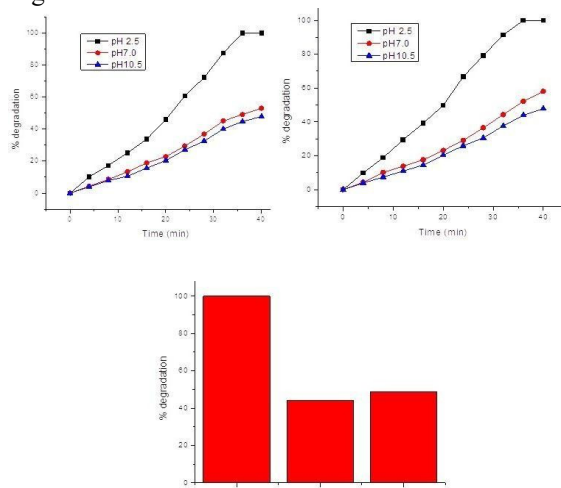


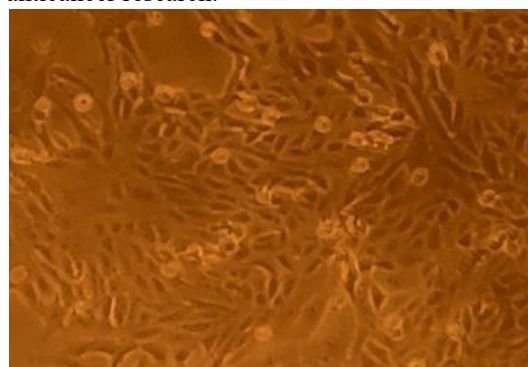
Fig 7 Drug degradation of Ag/ TiO₂ Nanoparticles using Beta vulgaris leave extract - visible and UV light in three different pH

3.3. Cytotoxicity and Anticancer Activity

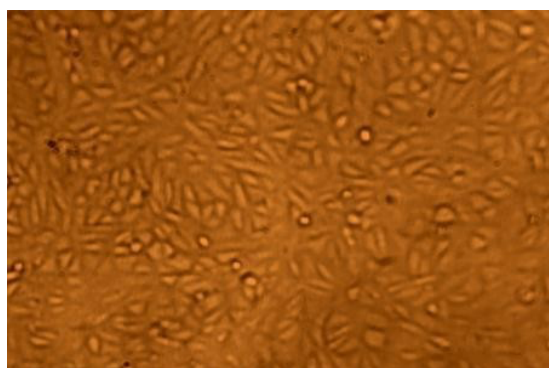
The in vitro cytotoxicity of green-synthesized Ag/TiO₂ nanoparticles mediated by Beta vulgaris leaf extract was evaluated against Vero and MCF-7 cell lines using the MTT assay. The results are presented in Figure 8, while the corresponding cell viability values are summarized in Table 1. The synthesized Ag/TiO₂ nanoparticles exhibited a concentration-dependent cytotoxic effect on both Vero and MCF-7 cell lines. As the nanoparticle concentration increased from 7.8 to 1000 µg/mL, cell viability gradually decreased, indicating enhanced cytotoxic activity. In the Vero cell line, cell viability decreased from 96.51% at 7.8 µg/mL to 54.06% at 1000 µg/mL, demonstrating moderate

toxicity toward normal cells. The concentration corresponding to approximately 50% cell viability was 31.2 µg/mL, indicating an IC₅₀ value of approximately 31.2 µg/mL.

In contrast, the synthesized nanoparticles exhibited greater cytotoxicity against the MCF-7 human breast cancer cell line. Cell viability decreased from 65.38% at 7.8 µg/mL to 24.78% at 1000 µg/mL, suggesting a stronger inhibitory effect on cancer cells than on normal Vero cells. Approximately 50% cell viability was observed at 31.2 µg/mL (51.70%), indicating an IC₅₀ value close to 31.2 µg/mL for the MCF-7 cell line. The greater reduction in cell viability observed in MCF-7 cells compared with Vero cells suggests that Ag/TiO₂ nanoparticles possess selective cytotoxic activity against cancer cells. According to the toxicity classification proposed by Geran and the U.S. National Cancer Institute (NCI), compounds with an IC₅₀ value below 20 µg/mL are considered highly toxic, 21–200 µg/mL are moderately toxic, 201–500 µg/mL are weakly toxic, and values above 500 µg/mL are regarded as non-toxic. Based on these criteria, the synthesized Ag/TiO₂ nanoparticles, with an estimated IC₅₀ value of approximately 31.2 µg/mL, can be classified as moderately toxic toward Vero cells. The observed cytotoxicity against MCF-7 cells indicates promising anticancer potential while maintaining comparatively higher viability in normal cells at lower concentrations. The concentration-dependent decrease in cell viability may be attributed to the generation of reactive oxygen species (ROS), oxidative stress, mitochondrial dysfunction, and apoptosis induced by Ag/TiO₂ nanoparticles. The phytochemicals present in Beta vulgaris leaf extract may also contribute to the enhanced biological activity by acting as reducing and stabilizing agents during nanoparticle synthesis. Similar concentration-dependent cytotoxic effects of green-synthesized metal nanoparticles against Vero and cancer cell lines have been reported in previous studies, supporting the potential application of plant-mediated Ag/TiO₂ nanoparticles in biomedical and anticancer research.



Control cells
1000 µg/ml



7.8µg/ml

Fig 8 Anticancer studies of Ag/ TiO₂ Nanoparticles using *Beta vulgaris* leave extract

Table:1 Average optical density and cell viability of cytotoxicity (Vero cell lines) and anticancer activity (MCF-7 cell line) of silver doped titanium oxide nanoparticles using *Beta Vulgaris* leaf extract

S. No	Concentration (µg/ml)	Vero cell lines		MCF-7 cell line	
		Optical density (OD)	Cell Viability (%)	Optical density (OD)	Cell Viability (%)
1	1000	0.279	54.06	0.174	24.78
2	500	0.310	60.07	0.211	30.05
3	250	0.342	66.27	0.250	35.61
4	125	0.374	72.48	0.287	40.88
5	62.5	0.405	78.48	0.326	46.43
6	31.2	0.438	84.88	0.363	51.70
7	15.6	0.468	90.69	0.401	57.12
8	7.8	0.498	96.51	0.459	65.38
9	Cell control	0.516	100	0.702	100

The in vitro anticancer activity of green-synthesized Ag/TiO₂ nanoparticles mediated by *Beta vulgaris* leaf extract was evaluated against the human breast cancer (MCF-7) cell line using the MTT assay. The anticancer efficacy of the nanoparticles was assessed at different concentrations (7.8, 15.6, 31.2, 62.5, 125, 250, 500, and 1000 µg/mL) and compared with the standard anticancer drug cyclophosphamide. The results are presented in Fig 9, while the corresponding cell viability data are summarized in Table 1.

Fig 9 % degradation of cytotoxicity and Anticancer studies of Ag/ TiO₂ Nanoparticles using *Beta vulgaris* leaves extract

The synthesized Ag/TiO₂ nanoparticles exhibited a concentration-dependent cytotoxic effect against MCF-7 cells. As the nanoparticle concentration increased, cell viability progressively decreased, indicating enhanced anticancer activity. The percentage of viable cells declined from 65.38% at 7.8 µg/mL to 57.12% at 15.6 µg/mL, 51.70% at 31.2 µg/mL, 46.43% at 62.5 µg/mL, 40.88% at 125 µg/mL, 35.61% at 250 µg/mL, 30.05% at 500 µg/mL, and reached the lowest value of 24.78% at 1000 µg/mL. These findings demonstrate that increasing the concentration of Ag/TiO₂ nanoparticles significantly enhanced their inhibitory effect on MCF-7 cell proliferation. Approximately 50% cell viability was observed at 31.2 µg/mL, indicating an IC₅₀ value close to this concentration. This result suggests that Ag/TiO₂ nanoparticles possess considerable cytotoxic potential against human breast cancer cells. The dose-dependent reduction in cell viability may be attributed to nanoparticle-induced oxidative stress, generation of reactive oxygen species (ROS), mitochondrial dysfunction, DNA damage, and activation of apoptosis pathways. Compared with the standard drug cyclophosphamide, the synthesized Ag/TiO₂ nanoparticles exhibited promising anticancer activity, particularly at higher concentrations. Although cyclophosphamide remains an effective chemotherapeutic agent, its clinical use is often associated with adverse side effects and the development of drug resistance. Therefore, green-synthesized Ag/TiO₂ nanoparticles represent a potential alternative therapeutic strategy because of their significant cytotoxic activity, eco-friendly synthesis, and the presence of bioactive phytochemicals from *Beta vulgaris* leaf extract, which may enhance their biological efficacy.

The results demonstrate that Ag/TiO₂ nanoparticles synthesized using *Beta vulgaris* leaf extract exhibit significant dose-dependent anticancer activity against MCF-7 cells, highlighting their potential application as a novel nanomaterial for breast cancer therapy. Further in vivo studies and mechanistic investigations are required to confirm their therapeutic efficacy and safety before clinical

application.

3.4. Antioxidant Activity

The antioxidant potential of the synthesized Ag/TiO₂ nanoparticles was assessed using the DPPH radical scavenging assay, and the results are presented in Figure 11. The nanoparticles exhibited a concentration-dependent increase in antioxidant activity, demonstrating their ability to effectively neutralize free radicals. Previous studies have similarly reported significant antioxidant properties for TiO₂-based nanomaterials, supporting the findings of the present investigation. The DPPH assay was conducted at nanoparticle concentrations ranging from 200 to 1000 µg/mL. The control sample exhibited an optical density (OD) of 0.597, representing the maximum DPPH radical activity in the absence of nanoparticles. A progressive increase in radical scavenging activity was observed with increasing nanoparticle concentration. The percentage inhibition values were 31.99%, 45.05%, 57.62%, 71.18%, and 82.91% at concentrations of 200, 400, 600, 800, and 1000 µg/mL, respectively. Simultaneously, the optical density decreased from 0.406 to 0.102 as the nanoparticle concentration increased, indicating effective reduction of DPPH radicals. The inverse relationship between absorbance and scavenging activity confirms the strong antioxidant capacity of the Ag/TiO₂ nanoparticles. The highest antioxidant activity was recorded at 1000 µg/mL, with a scavenging efficiency of 82.91%. These findings demonstrate that Ag/TiO₂ nanoparticles synthesized using *Beta vulgaris* leaf extract possess significant antioxidant activity and exhibit a dose-dependent free radical scavenging effect. Such properties highlight their potential utility in biomedical and pharmaceutical applications where oxidative stress mitigation is desirable.

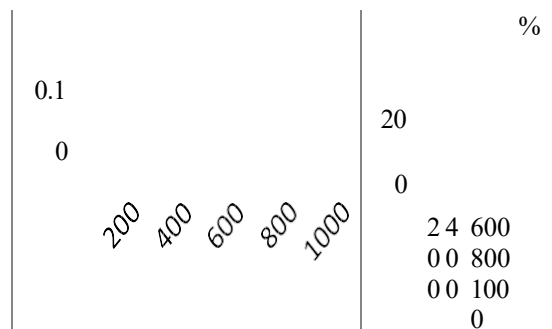
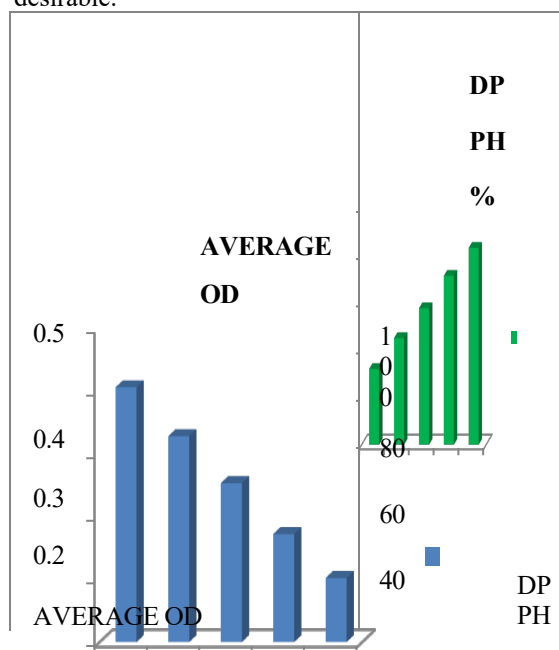


Fig 10 Antioxidant activity of Ag/ TiO₂ Nanoparticles using Beta vulgaris leaves extract

Table 2. Antioxidant activity of silver doped titanium oxide nanoparticles using Beta Vulgaris leaf extract

S.No	Concentration (µg/ml)	AVERAGE Optical density	Antioxidant %
1	200	0.406	31.99
2	400	0.328	45.05
3	600	0.253	57.62
4	800	0.172	71.18
5	1000	0.102	82.91
Control		0.597	

3.5. Anti -Microbial activity

The antibacterial activity of green-synthesized Ag/TiO₂ nanoparticles (Ag/TiO₂ NPs) mediated by *Beta vulgaris* leaf extract is presented in Figure 13 (Petri plate images), while the corresponding cluster column chart is shown in Figure 12. The antibacterial efficacy was evaluated against both Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative (*Escherichia coli* and *Pseudomonas* spp.) bacterial pathogens at concentrations of 500, 750, and 1000 mg/mL. The zones of inhibition are summarized in Table 3.

The results revealed that the antibacterial activity of Ag/TiO₂ nanoparticles was concentration dependent for most of the tested bacterial strains. Among the Gram-positive bacteria, *Bacillus cereus* exhibited the highest susceptibility, with zones of inhibition increasing from 16 mm at 750 mg/mL to 18 mm at 1000 mg/mL, indicating excellent antibacterial activity. In contrast, *Staphylococcus aureus* showed a constant zone of inhibition of 9 mm at all three tested concentrations (500, 750, and 1000 mg/mL), suggesting that increasing the nanoparticle concentration did not improve its antibacterial effect against this organism.

Table 3. Anti-Microbial activity of Ag/ TiO₂ Nanoparticles by Beta Vulgaris leaf extract

S.No	Zone of inhibition values(mm)	
	Antibacterial activity	Antifungal

	Concentrations µg/ml	Gram positive		Gram negative		activity	
		<i>aureus</i> Staphylococcus	<i>cereus</i> Bacillus	<i>coli</i> Escherichia	<i>Pseudomonas</i>	<i>albicans</i> Candida	<i>viride</i> Trichoderma
1	500	9	-	-	-	7	8
2	750	9	16	8	15	7	8
3	1000	9	18	10	15	8	8
4	Standard	15	17	9	10	20	17

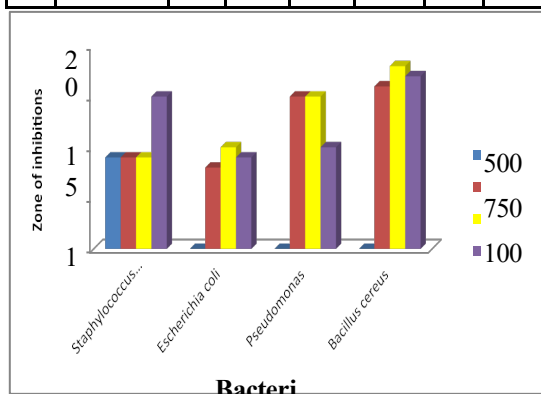


Fig 11 Antibacterial activity of Ag/ TiO₂ Nanoparticles using Beta Vulgaris leaf extract

Among the Gram-negative bacteria, *Pseudomonas* spp. demonstrated strong antibacterial susceptibility, with a zone of inhibition of 15 mm at both 750 mg/mL and 1000 mg/mL, while no inhibition was observed at 500 mg/mL. *Escherichia coli* showed moderate antibacterial activity, with inhibition zones of 8 mm at 750 mg/mL and 10 mm at 1000 mg/mL, whereas no inhibition was detected at 500 mg/mL. These findings indicate that higher concentrations of Ag/TiO₂ nanoparticles are more effective against Gram-negative bacterial pathogens.

The antifungal activity of Ag/TiO₂ nanoparticles synthesized using *Beta vulgaris* leaf extract was evaluated against *Candida albicans* and *Trichoderma viride*. The antifungal results are presented in Figure 12, with the inhibition zones summarized in Table 3. Overall, the nanoparticles exhibited poor antifungal activity at all tested concentrations. Against *Candida albicans*, inhibition zones of 7 mm were observed at 500 mg/mL and 750 mg/mL, with a slight increase to 8 mm at 1000 mg/mL. Similarly, *Trichoderma viride* showed inhibition zones of 8 mm at all three concentrations, indicating that increasing the

nanoparticle concentration had little or no effect on antifungal activity. Compared with the standard antimicrobial agent, Ag/TiO₂ nanoparticles demonstrated comparatively lower antifungal efficacy but showed promising antibacterial activity, particularly against *Bacillus cereus* and *Pseudomonas* spp. The standard drug produced inhibition zones of 15 mm, 17 mm, 9 mm, 10 mm, 20 mm, and 17 mm against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas* spp., *Candida albicans*, and *Trichoderma viride*, respectively, confirming the comparatively lower antimicrobial efficacy of the synthesized nanoparticles, especially against fungal pathogens.

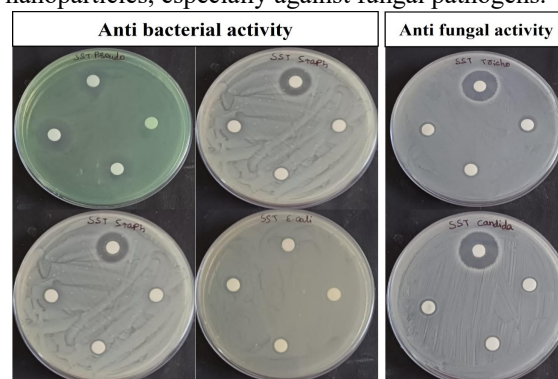


Figure:12 Plates of Anti-microbial Activity of Ag/ TiO₂ Nanoparticles using Beta vulgaris leave extract

Conclusion

The present study successfully synthesized Ag-doped TiO₂ nanoparticles (Ag/TiO₂ NPs) using *Beta vulgaris* leaf extract through a simple, eco-friendly, and cost-effective green synthesis approach. The synthesized nanoparticles were characterized by UV-Visible spectroscopy, FTIR, XRD, DLS, FESEM, EDS, and TEM, confirming their successful formation and physicochemical properties. The Ag/TiO₂ nanoparticles exhibited excellent photocatalytic activity, achieving 97.69% and 98.65% degradation of diclofenac under optimized conditions within 20 and 40 min, respectively. Biological evaluation demonstrated moderate cytotoxicity toward Vero cells and significant dose-dependent anticancer activity against MCF-7 breast cancer cells. The nanoparticles also showed antioxidant activity and strong antibacterial activity, particularly against *Bacillus cereus* and *Pseudomonas* spp., while exhibiting comparatively weak antifungal activity against *Candida albicans* and *Trichoderma viride*. The findings indicate that green-synthesized Ag/TiO₂ nanoparticles possess multifunctional properties with promising applications in environmental remediation, biomedical research, drug delivery, pharmaceutical formulations, and the food industry. Further in vivo studies are required to confirm their safety and therapeutic potential.

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