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SHORT COMMUNICATIONS

Eco friendly synthesis of TiO_2 nanoparticles using aqueous *Ocimum americanum* L. leaf extracts and their antimicrobial, anti-proliferative and photocatalytic activities

S. Vijayakumar[1] · E. Vidhya[1] · G. Chinnu Anand[1] · M. Nilavukkarasi[1] · V. N. Punitha[1] · B. Sakthivel[2]

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Abstract

Bio-synthesis of TiO_2 nanoparticles was accomplished with *Ocimum americanum* L leaf extracts. The morphology, optical and functional properties of synthesized TiO_2 nanoparticles were characterized by XRD, UV–Vis spectroscopy, FT-IR, and FE-SEM with EDAX respectively. The aqueous extracts of these nanoparticles were evaluated for its antimicrobial activities against *Bacillus cereus, Clostridium perfringens, Salmonella paratyphi, Klebseilla pnemoniae, Candida albicans and Aspergillus niger* using an agar well method. The antiproliferative activity was analyzed by MTT assay. Photocatalytic analysis against methylene blue dye was investigated. The biosynthesized TiO_2 nanoparticles was analyzed by SEM with an average size of the particle was 25 nm. The synthesized TiO_2 nanoparticles were exhibited excellent antimicrobial and antiproliferation activities against selected pathogens and human skin cancer cell line (431) respectively and expression of admirable photocatalytic activity with reduction of 91.1%. Based on the above studies, it is concluded that these nanoparticles are eco-friendly, inexpensive and pollution free material which can be applied for multifaceted environmental applications.

Keywords Green synthesis · *Ocimum americanum* · TiO_2 · Antimicrobial · Antiproliferative · Photodegradation

Introduction

Today, nanotechnology has gathered impending importance in various sectors viz., industrial, pharmaceutical and pollution free environment owing to its unique properties (Debjani and Pratyusha 2013). Among metal oxides, Titanium dioxide (TiO_2) is not a hazardous material according to the United Nations (UN). The green synthesis of titanium oxide nanoparticles has more advantage due to their less consumption of chemicals (Baskar et al. 2017). Titanium is a stable metal and has proven to have high photo catalytic and strong antimicrobial activities (Subhapriya and Gomathipriya 2018). The particular method and material was chosen for their ability to act as good reducing and stabilizing agent (Dobrucka and Długaszewska 2016). In this study, Titanium nanoparticles are synthesized using the leaf extract of *Ocimum americanum*. To the best of our knowledge, this type of synthesis is the first of its kind.

Ocimum americanum is commonly known as American basil or hoary basil, which is widely distributed in Asia, Africa, Central and South America. It is an annual herb belonging to the family Lamiaceae. In Indian traditional system of Ayurveda, it has been used as a medicine for diarrhea, diabetes and skin infection (Reddy et al. 2016). Therefore the aim of the present study is to biosynthesize TiO_2 nanoparticles using leaf extract of *O. americanum* and to explore the potential applications for antimicrobial, antiproliferative and photo degradation activities of the said nanoparticles.

✉ S. Vijayakumar
svijaya_kumar2579@rediff.com

[1] Computational Phytochemistry Laboratory, PG and Research Department of Botany, AVVM Sri Pushpam College (Autonomous) Poondi, (affiliated with Bharathidasan University), Thanjavur, Tamil Nadu, India

[2] PG and Research Department of Physics, AVVM Sri Pushpam College (Autonomous) Poondi, (affiliated with Bharathidasan University), Thanjavur, Tamil Nadu, India

Material and methods

Chemicals, Specimen collection and preparation of plant extract

AR grade of all the chemicals were obtained from Sigma Aldrich, India. Fresh leaves of *Ocimum americanum* were gathered from the Botanical garden of A.V.V.M. Sri Pushpam College, Poondi, Tamil Nadu, India in August 2019 and taxonomically identified by Jonn Brito, Rapinat herbarium, St. Joseph College, Tiruchirappalli, India.

Ten gram of fresh leaves was rinsed with tap water followed by Milli Q water and powdered using an electric blender. This powdered sample was mixed with 100 mL of Milli pore water and stirred for 30 min. Then, the plant extract was filtered using Whatman filter paper (No.1).

Synthesis of TiO_2 nanoparticles

10 mL leaf extract of *O. americanum* was added to 0.5 M solution of Titanium Oxy sulphate and stirred for 30 min at 60 °C. After that, 1 M of NaOH solution was added slowly until the pH reaches the values of 8. The precipitate was often washed with Milli Q water for the removal of the excess of NaOH. Then, the precipitate was filtered and calcinated at 800 °C for 3 h which directs the formation of well crystalline nano TiO_2.

Characterization of TiO_2 nanoparticles

The crystallinity of TiO_2 nanoparticles was characterized using XRD. The X-ray diffraction (XRD) technique is used to identify the crystalline size and phase purity of the TiO_2 nanoparticles. The optical characteristics of TiO_2 nanoparticles were observed at diverse wavelength ranges from 300 to 700 nm. The functional group of nanoparticles was examined by Fourier transform infrared spectroscopy (FTIR). The field emission scanning electron microscope (FE-SEM) was used to analyze the morphology of the TiO_2 nanoparticles. The energy dispersive X-ray (EDX) technique was used to analyze the composition of the TiO_2 nanoparticles.

Biological behaviors

The human skin cancer cell lines (431) were acquired from National Centre for Cell Sciences (NCCS, Pune, India). Cytotoxicity analysis was followed by MTT assay and agar well method was used for antimicrobial activity against pathogens. Two Gram Positive (*Bacillus cereus* MTCC430*, Clostridium perfringens* MTCC450), two Gram Negative (*Klebsiella pneumoniae MTCC618, Salmonella paratyphi* MTCC735) bacteria and two fungi (*Candida albicans* MTCC227 and *Aspergillus niger* MTCC281) were obtained from Microbial Type Culture Collection (MTCC, Chandigarh India). The above bacterial and fungal cultures were sub-cultured on MHA (Muller-Hinton Agar) and SDA (Sabouraud dextrose agar) medium at 35 °C and 30 °C respectively. The well size of 6 mm was punched in MHA and SDA plates. Then 20 µl of sample was infused with wells of all plates through micropipette. Gentamycin (5 µg) was used as positive controls for bacteria and fungus accordingly. Pure solvent alone served as negative control. The bacterial and fungal cultures were incubated at 30 ± 2 °C for 24 h and 37 ± 2 °C for 72 h respectively. The assays were measured for their diameter in millimeters and each experiment was done thrice.

Photo catalytic behavior

The photo degradation of Methylene Blue (MB) dye was carried out using TiO_2 in aqueous solution under annular type photo-reaction, With 100 W halogen lamp, as the UV light source. The catalyst loaded in the experiment was 50 mg of sample dispersed in 150 ml of MB solution (1×10^{-5}). Then the solution was mixed by sonication for 30 min in dark to reach an adsorption—desorption equilibrium. The catalyst loaded MB solution was illuminated under visible light irradiation for different time intervals. At given time intervals, the photo-catalyst was removed from the irradiated solution by centrifugation. The dye degradation was recorded by the changes in the absorption spectrum. This reaction was observed at various time intervals Viz., 15, 30, 45, 60, 75, 90, 105, 120, 135, 150 min.

Results and discussion

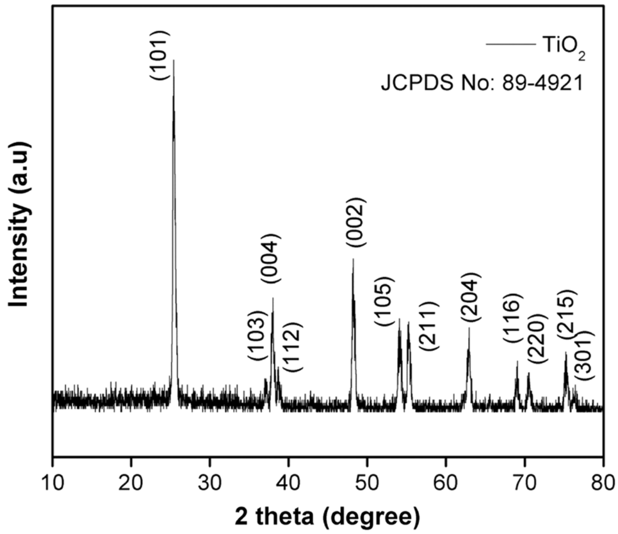
Structural features

The XRD diffraction patterns (Model-D8 Advance BRVKER, GERMANY) of bio-synthesized TiO_2 nanoparticle are shown in Fig. 1a. The peak positions with 2θ values of 24.4, 37.1, 37.9, 39.1, 48.1, 54.2, 55.5, 62.9, 68.9, 70.6, 75.4, 76.9 are indexed as (101), (103), (004), (112), (002), (105), (211), (204), (116), (220), (215) and (301) diffraction planes which are in good agreement with the standard pattern of powder TiO_2 Wurtzite structure (JCPDS File no 89-4921). The XRD patterns showing strong and narrow diffraction peaks indicate the synthesized TiO_2 nanoparticles have good crystalline nature. There is no impurity peak present in the XRD pattern. The average particle size of the synthesised nanoparticles was around 21.4 nm calculated from the Debye Scherrer's formula (Abisharani et al. 2019)

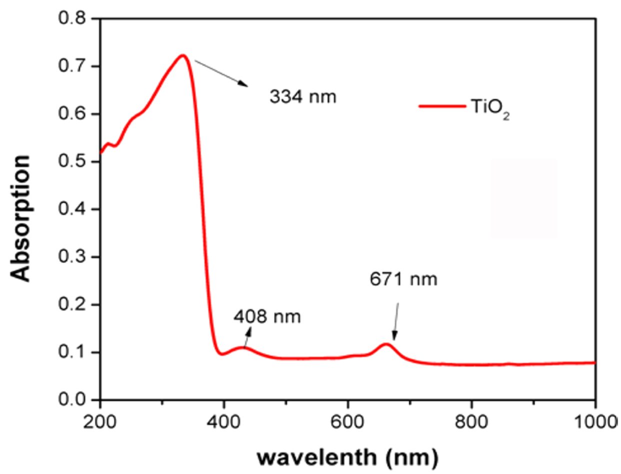
$$D = \frac{0.9}{\beta \cos \theta} \lambda$$

UV–visible absorption and emission spectrum analysis

Figure 1b shows the UV–Vis absorption spectra (Shimadzu spectrometer—Model UV1700) of the sample indicating strong absorption peak at 334 nm, which is in good



(a) **XRD spectrum of biosynthesized TiO₂ nanoparticles**



(b) **UV Vis absorption spectrum of biosynthesized TiO2 nanoparticles**

Fig. 1 **a** XRD spectrum of biosynthesized TiO_2 nanoparticles. **b** UV Vis absorption spectrum of biosynthesized TiO_2 nanoparticles

agreement with the previous work (Mobeen Amanulla and Sundaram 2019).

FT-IR analysis

The FT-IR spectrum of the sample in the range of 500–4000 cm^{-1} was recorded using Shimadu 84,003 spectrometer (Fig. 2). The presence of peaks at 3420 cm^{-1} and 1628 cm^{-1} were ascribed for water molecules (Mobeen Amanulla and Sundaram 2019). The sharp peak observed at 2921 cm^{-1} indicated the C=C stretching (Abisharani et al. 2019). The band positioned at 2851 cm^{-1} is characteristic of the C–H bond stretching vibrations (Bagheri et al. 2013).

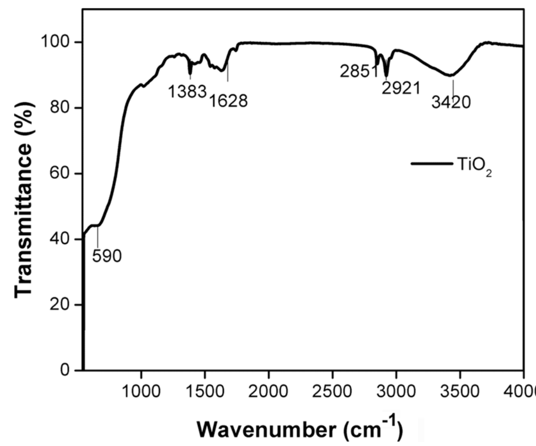


Fig. 2 FTIR spectrum of biosynthesized TiO_2 nanoparticles

The peak at 1383 cm^{-1} is owing to the presence of C–H rock alkenes (Dobrucka 2017).

FE-SEM with EDAX

Figure 3a shows the FE-SEM micrograph (TEM, Hitachi H-7100) of the sample and the corresponding EDAX (Energy dispersive X-ray) pattern is given as Fig. 3b. The average particle size of the TiO_2 nanoparticles was calculated around 25 nm which matches XRD data. EDAX pattern helps to exhibit the presence of TiO_2 in the synthesized nano-material with weight percentage of 67.45%. Figure 2a shows the spherical shape of TiO_2 nanoparticle and are distributed evenly throughout the prepared sample. The average particle size of the TiO_2 nanoparticles were measured using histogram method (Fig. 3c) and the value is around 25 nm.

Antimicrobial activity

Antimicrobial activities of aqueous extract of biosynthesized TiO_2 nanoparticles were investigated by agar well diffusion method against selected pathogens and their results are shown in Table 1 and Fig. 4. For the antibacterial activity, maximum zone of inhibition was examined against *B. cereus* (36 ± 1.08 mm) was followed by *S. paratyphi* (28 ± 2.06 mm), *C. perfrigens* (23 ± 1.52 mm) and *K. pnemoniae* (21 ± 1.82 mm). For the antifungal activity, maximum zone of inhibition was observed against *C. albicans* (30 ± 0.84 mm) followed by *A. niger* (22 ± 1.20 mm). The acquired zones of inhibition of aqueous extract of biosynthesized TiO_2 nanoparticles were compared with leaf extract, Titanium Oxy sulphate and standard drug of Gentamycin (50 µg).

TiO_2 nanoparticles are capable of dissolving the outer membrane of bacteria due to the occurrence of hydroxyl groups prompting the death of the organism (Rajakumar

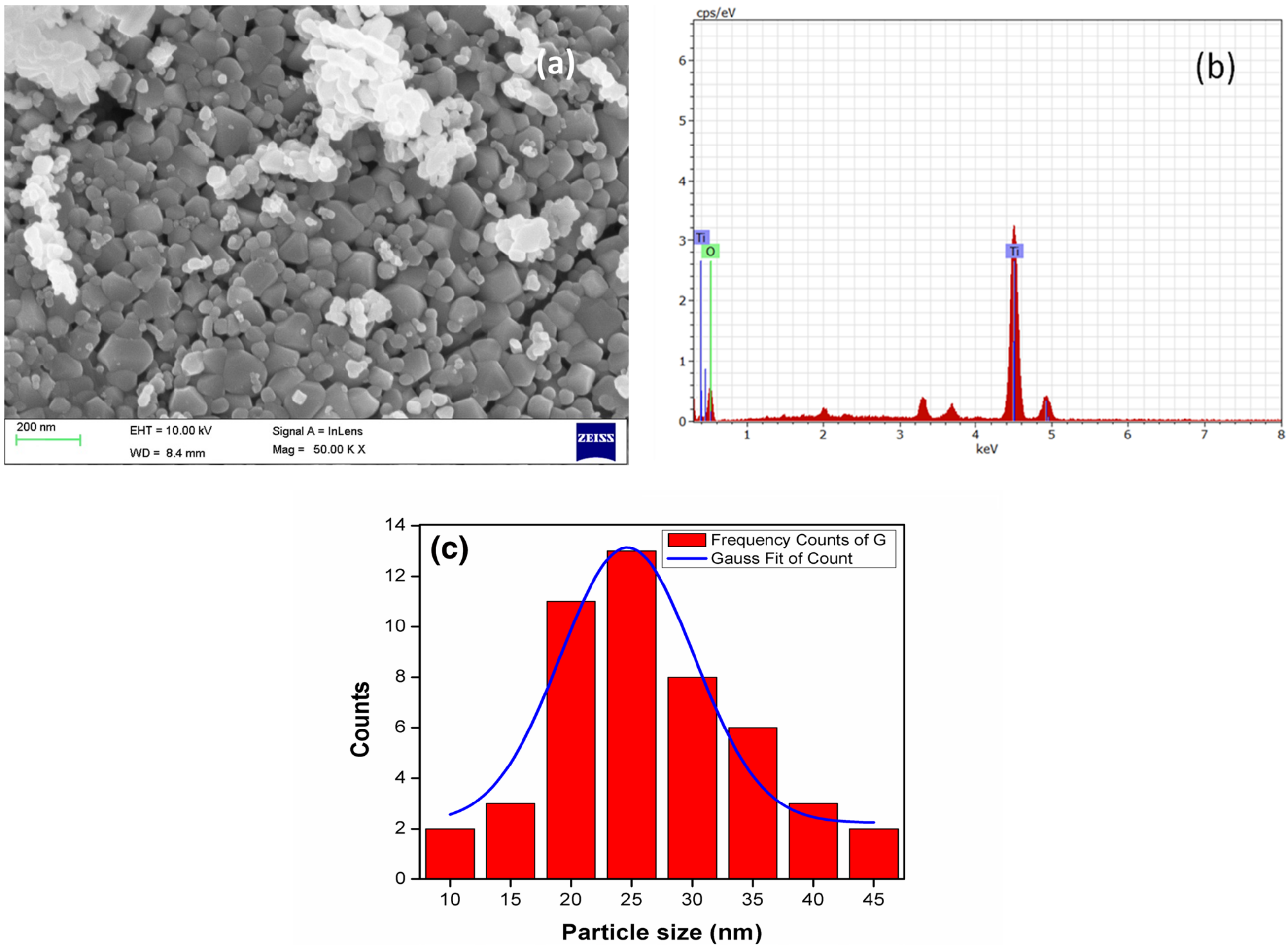


Fig. 3 **a** FE-SEM micrograph, **b** EDAX analysis and **c** frequency histogram of TiO_2 nanoparticles distributions

Table 1 Antimicrobial activities of synthesized TiO_2 nanoparticles against pathogenic microorganisms

Name of the microorganisms	Zone of inhibition (µg/ml^{-1})[a]			
	PE	$TiOSO_4$	TiO_2 nps	Control
Bacillus cereus	20 ± 1.02	18 ± 1.45	36 ± 1.08	20 ± 0.94
Clostridium nefrigens	16 ± 0.90	15 ± 0.86	23 ± 1.52	18 ± 1.64
Salmonella paratyphi	15 ± 1.24	14 ± 1.43	28 ± 2.06	20 ± 1.89
Klebseilla pnemoniae	16 ± 2.12	14 ± 1.48	21 ± 1.82	18 ± 0.86
Candida albicans	22 ± 0.92	20 ± 2.42	30 ± 0.84	20 ± 1.82
Aspergillus niger	18 ± 2.43	16 ± 1.63	22 ± 1.20	22 ± 2.56

PE plant extract, *TiOSO4* Titanium Oxy sulphate, *TiO_2-* Biosynthesized TiO_2 Nanoparticles, *Control* Gentamycin (50 µg)

[a]Mean values of three triplicates ± standard deviation

2012). The antibacterial impact was further conspicuous against Gram positive bacteria due to the presence of peptologlycon layers (Subhapriya, and Gomathipriya 2018). Still now, there is no dependable description for antimicrobial mechanism of TiO_2 nanoparticles. For more than hundred years, TiO_2 are being utilized in the treatment of burn injuries in medical sector (Tristram et al. 2007). The antibacterial impact of TiO_2 is generally attributed to the disintegration of bacterial outer membranes by the ROS (Reactive Oxygen Species), that leads to phospholipids per oxidation and ultimately cell will die (Nadtochenko et al. 2006).

Antiproliferation activity

The in vitro antiproliferation activity was confirmed by MTT assay on the cell line of human skin cancer cells (A431) using biosynthesized TiO_2 nanoparticles. Figure 5 shows the cell viability (50.72%) of A431 cells at lowest concentrations which can be proved by IC_{50} = 31.2 µL. The inhibition of cell proliferation activity was performed

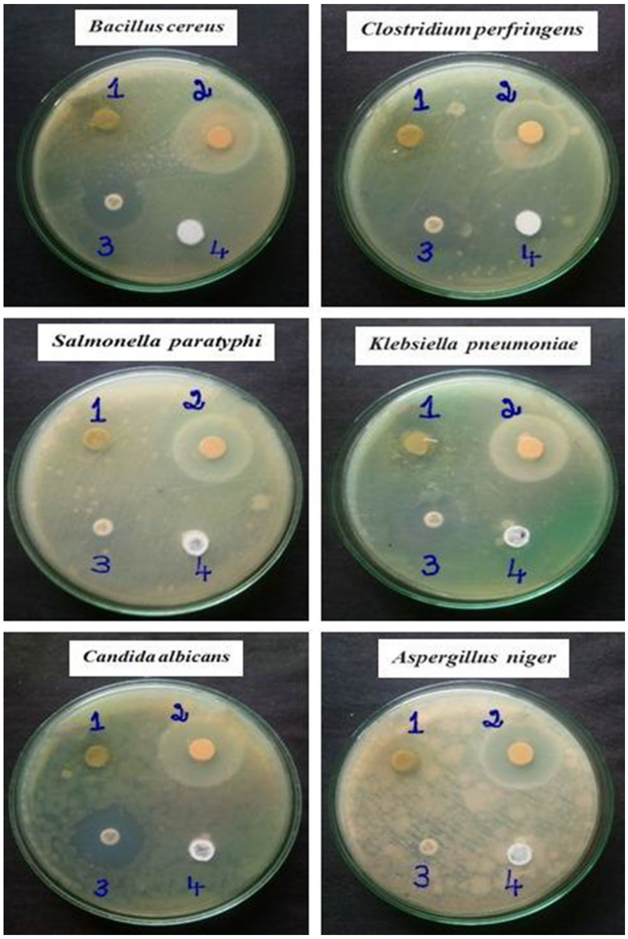


Fig. 4 Antimicrobial activity of TiO_2 nanoparticles against pathogenic microorganisms. 1. Plant extract. 2. Synthesized TiO_2 nanoparticles. 3. Control—Gentamycin (50 µg). 4. Titanium Oxy sulphate

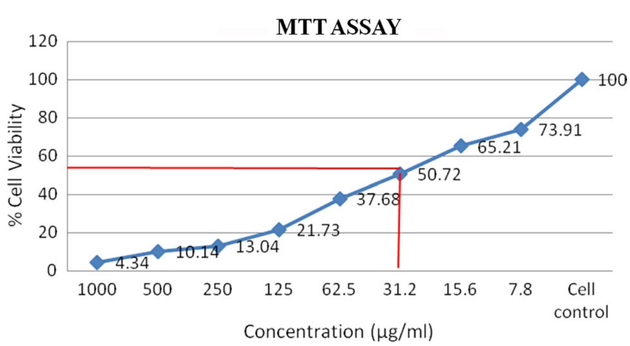


Fig. 5 Cytotoxic effect of TiO_2 nanoparticles using MTT assay

by time and dose dependent manner (Emam et al. 2019). The present investigation with green TiO_2 nanoparticles on A431 cells worked with dose and time dependent strategy and similar trend was observed by He et al. (2017).

Photocatalytic activity

The photocatalytic activity of synthesized TiO_2 nanoparticles was analyzed using methylene blue dye. The Fig. 6 shows that characteristic absorption peak occurred at 664 nm is due to the pi–pi* transition of MB. The degradation of methylene blue in the presence of TiO_2 nanoparticles with various time intervals was studied and it indicates that the MB solution was started to degrade at 15 min and totally degrade after an irradiation of 135 min which derives from the flat absorption spectrum of TiO_2 loaded MB solution. The photo-degradation efficiency of MB solution was calculated from the following relation. The synthesized Tio2 nanoparticle helps to degrade the MB solution with 91.1% effectively.

Photo-degradation efficiency $(\%) = (C_0 - C_{150} / C_0 \times 100) = (A_0 - A_{150} / A_0) \times 100$ where C_0 and C_{150} are the concentrations of MB solution at $t = 0$ and $t = 135$ min, respectively, while A_0 and A_{150} indicates the absorption intensity of MB solution (Khade et al. 2015; Mustapha et al 2017).

Conclusion

Bio-synthesized TiO_2 nanoparticles were successfully synthesized using *O. americanum* leaf extracts. The crystal structure and phase purity of the bio TiO_2 nanoparticles was analyzed by XRD technique. The optical property of the nanoparticles was investigated through UV Vis absorption spectroscopy. The spherical shape morphology of TiO_2

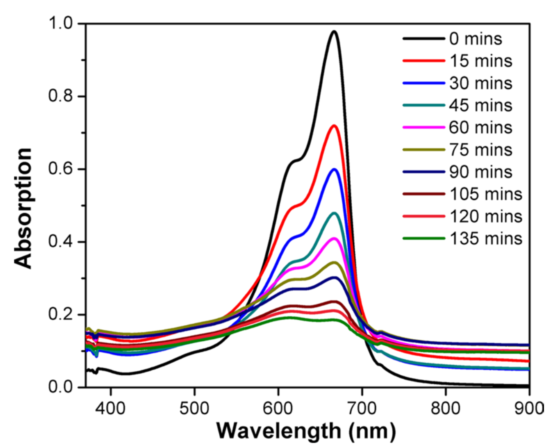


Fig. 6 Photo degradation of MB with bio synthesised TiO_2 nanoparticles

nanopaticles was confirmed by FESEM analysis. The TiO_2 nanoparticles exhibit excellent antimicrobial, antiproliferative and phocatalytic activities. The biosynthesized TiO_2 nanoparticles are eco-friendly, cost-effective and free from toxic chemicals. Therefore, it has definite multifunctional relevance in environmental applications.

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