Contents lists available at ScienceDirect



Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

# Enhanced photocatalysis and anticancer activity of green hydrothermal synthesized Ag@TiO<sub>2</sub> nanoparticles



D. Hariharan<sup>a</sup>, P. Thangamuniyandi<sup>b</sup>, A. Jegatha Christy<sup>c</sup>, R. Vasantharaja<sup>d</sup>, P. Selvakumar<sup>e</sup>, S. Sagadevan<sup>f</sup>, A. Pugazhendhi<sup>g</sup>, L.C. Nehru<sup>a,\*</sup>

<sup>a</sup> Department of Medical Physics, School of Physics, Bharathidasan University, Tiruchirappalli 620 024, Tamil Nadu, India

<sup>b</sup> School of Chemistry, Bharathidasan University, Tiruchirappalli 620 024, Tamil Nadu, India

<sup>c</sup> PG & Research Center of Physics, Jayaraj Annapackiam College for Women, Periyakulam, Tamil Nadu, India

<sup>d</sup> Centre for Ocean Research, Sathyabama Institute of Science and Technology, Chennai 600 119, Tamil Nadu, India

<sup>e</sup> Department of Physics, Coimbatore Institute of Technology, Coimbatore 641 014, Tamil Nadu, India

<sup>f</sup> Nanotechnology & Catalysis research Center, Institute of Advanced Studies, University of Malaya, Malaysia

<sup>8</sup> Innovative Green Product Synthesis and Renewable Environment Development Research Group, Faculty of Environment and Labour Safety, Ton Duc Thang University, Ho Chi Minh City, Viet Nam

	А	R	Т	I	С	L	Е	Ι	Ν	F	C
--	---	---	---	---	---	---	---	---	---	---	---

Keywords: Hydrothermal synthesis Photocatalyst Aloe vera Picric acid ROS Cancer cell lines-A549

# ABSTRACT

Titanium dioxide (TiO<sub>2</sub>) nanoparticles (NPs) have been doped with varying amounts (0.005, 0.010 and 0.015 M) of silver nanoparticles (Ag NPs) using hydrothermal method. Further, in this work, a green approach was followed for the formation of Ag@TiO<sub>2</sub> NPs using *Aloe vera* gel as a capping and reducing agent. The structural property confirmed the presence of anatase phase TiO<sub>2</sub>. Increased peak intensity was observed while increasing the Ag concentration. Further, the morphological and optical properties have been studied, which confirmed the effective photocatalytic behavior of the prepared Ag@TiO<sub>2</sub> NPs. The photocatalytic performance of Ag@TiO<sub>2</sub> has been considered for the degradation of picric acid in the visible light region. The concentration at 0.010 M of the prepared Ag@TiO<sub>2</sub> has achieved higher photocatalytic performance within 50 min, which could be attributed to its morphological behavior. Similarly, anticancer activity against lung cancer cell lines (A549) was also determined. The Ag@TiO<sub>2</sub> NPs generated a large quantity of reactive oxygen species (ROS), resulting in complete cancer cell growth suppression after their systemic *in vitro* administration. Ag@TiO<sub>2</sub> NPs was adsorbed visible light that leads to an enhanced anticancer sensitivity by killing and inhibiting cancer cell reproduction through cell viability assay test. It was clear that 0.015 M of Ag@TiO<sub>2</sub> NPs were highly effective against human lung cancer cell lines and showed increased production of ROS in cancer cell lines due to the medicinal behavior of the *Aloe vera* gel.

#### 1. Introduction

Generally, TiO<sub>2</sub> photocatalytic materials play potential roles in water treatment, catalysis, lithium-ion batteries, and sensors, anticancer and antibacterial applications [1,2]. TiO<sub>2</sub> absorbs only UV light that leads to low quantum yield [3]. Noble metals loaded on the surface of TiO<sub>2</sub> enable Visible light absorption-due to Schottky barrier effect. Among the noble metals, silver is easily available and has high work function, antibacterial property and more importantly, its SPR effect at the desired wavelength [4]. In Ag@TiO<sub>2</sub> formation, Ag (4d) promotes effective photo generated electron hole separation, which leads to narrow band gap that promotes visible light absorbance [5].

Visible light absorbance of TiO2 NPs not only promotes and

improves quantum yield but also generates excess free radicals that lead to enhanced photocatalytic activity. TiO<sub>2</sub> NPs generate free radicals such as superoxide radical (O<sub>2</sub><sup>--</sup>), hydroxyl radical (·OH), and singlet oxygen (<sup>1</sup>O<sub>2</sub>). Meanwhile, Picric acid (PA) is a well-known strong nitro aromatic compound and its salts are used as explosives even greater than many other polynitrated aromatic compounds such as trinitrotoluene [6]. Furthermore, the acquaintance to picric acid (PA) leads to adverse effects to our environment. In addition, PA also shows serious effects on human health such as eye and skin irritations, causes respiratory problems and affects the immune system [7]. Above all, the biodegradation products of PA are of concern due to their oncogenic properties, which allow them to easily affect our human society [8]. Though a large number of catalytic materials are reported on TiO<sub>2</sub>, a

\* Corresponding author. *E-mail addresses:* arivalagan.pugazhendhi@tdtu.edu.vn (A. Pugazhendhi), lcnehru@bdu.ac.in (L.C. Nehru).

https://doi.org/10.1016/j.jphotobiol.2019.111636

Received 24 June 2019; Received in revised form 6 September 2019; Accepted 19 September 2019 Available online 12 November 2019

1011-1344/ © 2019 Published by Elsevier B.V.

simple and practical approach to the degradation of PA still remains a challenge. In this context, the present study has focused on the effective photocatalytic degradation of such adverse PA using Ag@TiO<sub>2</sub> NPs.

In cell line studies, TiO<sub>2</sub> NPs induce the cell lines and ROS is produced, which damages DNA, leading to apoptosis, and necrosis. A549 cell line is an adenoma lung cancer cell line, and it is used to investigate the impact of NPs. Swarnalatha and Anjaneyulu, have reported picric acid degradation in aqueous TiO<sub>2</sub> suspension under UV light [9]. Tanaka et al. reported mono-, di- and trinitro phenol degradation in aqueous TiO<sub>2</sub> suspension under UV light irradiation [10]. The efficiency of picric acid degradation process in the visible light region has also been reported Hariharan et al. [11]. Kim et al. have reported that ZnO had shown an enhanced anticancer activity on A549 cell lines compared to TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, and CeO<sub>2</sub> [12]. There is no report on green hydrothermal synthesized Ag@TiO<sub>2</sub> nanostructures with anticancer activity against A549 cell lines.

In general, plant biomolecules act as capping and reducing agents for the synthesis of NPs [13]. Herein, *Aloe vera* gel could be generally used as an antioxidant due to the presence of different types of vitamins such as A, C and E [14]. Moreover, the gel extracted from the peel is able to retort and heal burns and wounds and can be a UV protecting material. Further, *Aloe vera* gel has been used as a capping and reducing agent for NPs, which aids to green synthesize NPs with high crystalline and optical properties compared to chemical synthesis [15]. With the medicinal importance of the *Aloe vera* gel mentioned above, the present study has focused on the synthesis, characterization and aimed to study the anticancer activity of the extracted Ag@TiO<sub>2</sub> NPs.

In this research, the green hydrothermal synthesized  $Ag@TiO_2$  NPs have been reported to be effective for visible light active picric acid degradation process and have shown anticancer activity against A549 cell lines. To the best of knowledge of the authors, this is the first report on using  $Ag@TiO_2$  NPs for degradation of picric Acid. It is clear that  $Ag@TiO_2$  NPs are highly effective against human lung cancer cell lines due to the medicinal properties of *Aloe Vera* gel and increased production of ROS in cancer cell lines. Herein, the present effective environmentally safe biogenic green synthesis of  $Ag@TiO_2$  NPs would be an appropriate way to obtain highly dispersed silver photocatalytic behavior over PA degradation activity and the as-prepared NPs also can be used in effective anticancer therapy.

#### 2. Materials and Methods

#### 2.1. Materials

Titanium (IV) isopropoxide (99.8%), silver nitrate ( $\geq$ 99%), 2,4,6trinitrophenol (C<sub>6</sub>H<sub>3</sub>N<sub>3</sub>O<sub>7</sub>, 98%) and (3-(4,5-dimethylthiazol-2-yl)-2,5Diphenyl tetrazolium Bromide ( $\geq$ 99%) have been procured from Sigma–Aldrich and used as such. Double Distilled (DD) water has been used to prepare the solutions. All the appliances have been rinsed with aquaregia and washed with DD water before use. *Aloe barbadensis (Aloe Vera*) plants have been collected at the Bharathidasan University campus, Bharathidasan University, Trichy, Tamil Nadu, India.

#### 2.2. Characterization Techniques

The ultraviolet-visible (UV–Vis) spectrum was recorded using, a UV-160 UV – Vis spectrophotometer (Shimazu Co., Japan). High-Resolution Transmission Electron Microscopy (HRTEM) Selected Area Electron Diffraction (SAED) pattern was collected at 200 kV using a JEM-2100F, JEOL, Japan. Energy Dispersive X-ray Spectrometry (EDS) has been performed with TEM. The samples utilized for TEM measurements have been prepared by dispersing the products in ethanol as well as placing several drops of the suspension on holey carbon films supported by copper grids. Further evidence for the composition of the product has been inferred from X-ray Photoelectron Spectroscopy (XPS), through an ESCA Lab MKII, UK. X-ray photoelectron spectrometer with MgK<sub> $\alpha$ </sub> X-rays as the excitation source. Powder X-ray diffraction (XRD) measurements of the samples have been performed with a Philips PW3040/60, USA X-ray diffractometer using CuK<sub> $\alpha$ </sub> radiation at a scanning rate of 0.06° s<sup>-1</sup>. Raman spectral studies were carried out using a laser confocal Raman microscope (Renishaw, UK, Model: Invia). The presence of functional groups was identified using Fourier transform infrared (FTIR) spectrum in the range of 4000–500 cm<sup>-1</sup> with an Alpha II Bruker FTIR spectrometer, USA.

# 2.3. Preparation of Aloe vera Gel

The preparation of *Aloe vera* gel was performed following the method of Ali et al. [16]. Herein, the *Aloe vera* leaf was rinsed twice using double distilled water in order to remove all the debris and visible unwanted particles from the surface. Then, the leaf was carefully peeled off using a sharp knife and peel was discarded. The leaf was slit long-itudinally, and the gel was scraped off from the leaf and then, transferred into a sterile beaker, using a sharp-edged spoon. Then, 10 ml of the gel was added to 100 ml distilled water and boiled for 2 h at 90 °C. The aqueous gel was filtered using Whatman filter paper. The gel was stored at 4 °C for further studies.

#### 2.4. Hydrothermal Synthesis of TiO<sub>2</sub> NPs

 $TiO_2$  nanostructures were prepared by the green hydrothermal method with slight modifications of the procedure reported in literature [11]. Ten milliliters of titanium tetra-isopropoxide (TTIP) was taken in a 60 ml Teflon beaker, 20 ml of distilled water was then added, and the above solution was kept in a stainless steel autoclave. It was then kept in a muffle furnace and heated at 180 °C for 24 h. The obtained homogeneous solution was kept in the autoclave and dried at 120 °C for 2 h. The final product was calcinated at 500 °C for 5 h.

# 2.5. Hydrothermal Synthesis of Ag@TiO<sub>2</sub> NPs

As per our previous report [17], different concentrations of (0.005 M, 0.010 M and 0.015 M) silver nitrate  $(\text{AgNO}_3)$  were added with 0.1 M Titanium (IV) isopropoxide, 5 ml of *Aloe Vera* gel and 100 ml of water. The above solution was stirred for 1 h then, it was kept in a stainless steel autoclave, which was placed on a muffle furnace and heated for one day at 180 °C. The obtained product was dried at 120 °C for 2 h. Finally, the obtained powder was calcinated at 500 °C for 5 h.

#### 2.6. Photocatalytic Experiments

Photocatalytic experiments were carried out according to the previously described method [18]. Picric acid degradation by the prepared Ag@TiO<sub>2</sub> nanostructures was evaluated. In each experiment, Ag@TiO<sub>2</sub> catalyst (20 mg) was incorporated in 100 ml of picric acid solution (500 mg/L). Then, the above solution was stirred in a dark place for 30 min for absorption-desorption equilibrium. The experiments were carried out in visible light irradiation on picric acid suspension using a high-pressure mercury lamp (543 nm). Picric acid suspension was drawn every 5 min and evaluated using UV–Visible spectrophotometer at  $\lambda_{max} = 353$  nm.

#### 2.7. Cell Culture and MTT Cell Viability Assay

Assay was carried out according to the previously described method of Vasantharaja et al. [19]. Human lung carcinoma cells, which were procured from the national center for cell sciences, Pune, India, were maintained in DMEM medium containing 10% FBS, 100 mg/ml streptomycin, 100 IU/ml penicillin and 2 mM glutamine. The cultures were maintained at 37 °C with 5% CO<sub>2</sub> in a humidified CO<sub>2</sub> incubator. The Ag@TiO<sub>2</sub> and TiO<sub>2</sub> NPs were evaluated for cytotoxic activities against human lung carcinoma (A549) cell lines. The cultural A549 cells were seeded separately on 96 well plates at a concentration of  $1\times10^4$  cells/ well. The cells were subjected to different concentrations of green synthesized Ag@TiO\_2 and TiO\_2 (0 to 200 µg/ml). After 24 h, 100 µl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added and incubated at 37 °C for 4 h in a CO\_2 incubator. After incubation, the purple colored formazan was dissolved by adding 100 µL of Dimethyl sulfoxide. After 30 min, the optical density was measured at 570 nm by using an ELISA multiwell plate reader for determining IC<sub>50</sub> values. All the tests were performed in triplicates to avoid any experimental error.

#### 2.8. Acridine Orange/Ethidium Bromide (AO/EtBr) Staining

AO/EB double staining assay was used to determine the ability of Ag@TiO<sub>2</sub> and TiO<sub>2</sub> NPs to induce apoptosis (A549 cells). The staining assay was carried out according to the previously described method [20]. A549 cells were seeded separately in six-well plates with the respective IC<sub>50</sub> concentrations of Ag@TiO<sub>2</sub> and TiO<sub>2</sub> NPs and were incubated for 24 h. Fluorescent dyes *viz.*, Ethidium bromide (100 µg/ml) and Acridine orange (100 µg/ml) solutions were mixed with respective cells. The cells were incubated at 37 °C for 30 min in dark. Later, AO/EB staining was observed through a fluorescence microscope. The mechanism of AO/EB double staining assay presents that the cells containing normal nuclear chromatin portray green nuclear staining (AO is taken up by viable cells). Apoptotic cells, which contained orange to red nuclei with condensed chromatin (EO is taken up by non-viable cells) were evaluated by fluorescence microscope at  $20 \times$  magnification.

# 2.9. Dichloro Dihydro Fluorescein Diacetate (DCFH-DA) Assay

The intracellular ROS generation by TiO<sub>2</sub> and Ag@TiO<sub>2</sub>NPs for A549 cells was detected by DCFH-DA staining. A549 cells were seeded in six-well plates and treated with TiO<sub>2</sub> and Ag@TiO<sub>2</sub> NPs. After 24 h of incubation, the cells were rinsed with  $1 \times$  cold PBS and stained with DCFH-DA ( $10 \,\mu g/10 \,\mu L$ ) in dark condition for 30 min. Finally, the ROS level was examined by fluorescence microscope (Floid cell imaging station, Life technologies, U.S.A). The assay was carried out according to the previously described method and in triplicates [21].

#### 3. Results and Discussion

# 3.1. FT-IR-Analysis of Plant Leaf Extract

FTIR spectrum of 500 °C calcinated Aloe vera gel (Supplementary file (Fig. S1)). Strong IR bands have been observed at 3459, 2085, 1634, 1550, 1409 and 662 cm<sup>-1</sup>. The spectrum for plant gel extract exhibited intense and distinct absorption bands at 3459 cm<sup>-1</sup>, which corresponded to N-H stretching. The bands at  $2085 \text{ cm}^{-1}$  were due to N=C=S bond stretching, and 1550 cm<sup>-1</sup> corresponded to N–O bond stretching. The low band at  $662 \text{ cm}^{-1}$  corresponded to C–X stretching (bending region). Generally, plant gel extract is known to contain biomolecules such as lignin, vitamins, amino acids, enzymes, anthraoquinones, polysaccharides, salicylic acids, minerals, monosaccharide, saponins, and sterols in different extracts of *Aloe Vera* aqueous gel [22]. The peak at 1409 cm<sup>-1</sup>was assigned to the COO<sup>-</sup> symmetric stretch from proteins with carboxyl side groups in the amino acid residues. Further, the peak that appeared at 1634 cm<sup>-1</sup> corresponded to the stretching modes of -C=O group. The above biomolecules might have contributed to the reduction of Ag<sup>+</sup> ions.

#### 3.2. HRTEM Analysis of TiO<sub>2</sub> and Ag@TiO<sub>2</sub>

The morphology properties of the pure  $TiO_2$  NPs and  $Ag@TiO_2$  nanostructures have been investigated by HRTEM. Fig. 1 (A) shows the HRTEM image of  $TiO_2$  NPs and Fig. 1 (B–D) shows the  $Ag@TiO_2$  nanostructures. In Fig. 1 (A), surface of the nanostructure was very

smooth compared to  $Ag@TiO_2$  NPs. After depositing different Ag concentrations on TiO\_2 NPs, the surface of TiO\_2 NPs was changed from Fig. 1(C), it could be observed that when Ag concentrations have been changed, morphologies of  $Ag@TiO_2$  NPs have also changed. Due to hydrothermal treatment, the rod shaped TiO\_2 NPs and small spherical Ag NPs were formed as shown in Fig. 1 (B & D). The doping concentration and the hydrothermal method of preparation could have been the two major factors influencing the morphological changes observed [23–25]. The calculated total size of Ag NPs was 38 nm and the size of TiO\_2 NPs was 57 nm. The SAED pattern lattice springs clearly showed the anatase phase that was matched with (101) crystallography plane. In Ag@TiO\_2 nanostructures, the percentage of Ag was 1.77% TiO\_2 was 69.05% and O was 29.24%, respectively as denoted by EDAX spectrum shown in supplementary file (Fig. S2).

#### 3.3. XPS Studies of TiO<sub>2</sub> and Ag@TiO<sub>2</sub> NPs

X-ray photoelectron spectroscopy was used to analyze the chemical state and elemental composition. Fig. 2 (A) (i-iv) shows the XPS spectra of TiO<sub>2</sub> and Ag@TiO<sub>2</sub>. Ti, C, Ag, and O and they are presented in green hydrothermal synthesized Ag@TiO2 (Table. S1, S2). Ti 2p spectrum shows peaks at 458.4 eV (Ti  $2p_{3/2}$ ) and 464.2 eV (Ti  $2p_{1/2}$ ). The position of peak was 5.2 eV indicating the  $Ti^{4+}$  [26] oxidation state for  $TiO_2$ nanostructure. The peak position for C1s in the Ag@TiO<sub>2</sub> samples could be decomposed into three chemically distinct components present in the biomolecules (Fig. 2(B)- ii). Besides the C1s peak at 284.5eVBE (major peak) which corresponded to the carbon atoms within amino acids, vitamins, lignin, anthraquinones, enzymes, polysaccharides, salicylic acids, minerals, monosaccharides, saponins and sterols, two other peaks at 284.4 and 288.2 eV BE have also been observed. The higher BE (binding energy) peak at 288.3 eV was attributed to the electron emission from carbons in carbonyl groups [27] (Carbonyl carbons of the polysaccharides or proteins-enzymes) and the lower peak at 284.4 eV BE would be most likely from carbons to the carbonyl carbon.

Compared to TiO<sub>2</sub> (Fig. 2 (A) - i), the peaks of Ag@TiO<sub>2</sub> shifted to higher binding energy. It confirms strong interaction between Ag and TiO<sub>2</sub> nanostructures. Using curve fitting program for Ag@TiO<sub>2</sub> nanostructures, Ti2P<sub>3/2</sub> peaks were further divided into two peaks, and they were present at 459.9 eV and 458.2 eV, caused by Ti<sup>4+</sup> and Ti<sup>3+</sup> species, respectively. Evidently, the presence of Ti<sup>3+</sup> species might be the reason for visible light absorption and enhanced photocatalytic activity in the visible light region [11].

XPS spectra of O1s TiO<sub>2</sub> NPs and Ag@TiO<sub>2</sub>NPs are presented in Fig. (2(A) - iii) and B(iii). O1s spectra were further fitted for TiO<sub>2</sub> and Ag@TiO<sub>2</sub> nanostructures, and they indicated ( $O_{Ti-O}$  at 529.6 eV for TiO<sub>2</sub> and Ag@TiO<sub>2</sub>), surface silver groups ( $O_{O-H}$ at 531.7 eV and 531.4 eV for TiO<sub>2</sub> and Ag@TiO<sub>2</sub>) [28], and adsorbed O<sub>2</sub> (at 531.7 eV and 533 eV for TiO<sub>2</sub> and Ag@TiO<sub>2</sub>) [29]. In Ag 3d spectrum, two peaks were observed for Ag 3d<sub>5/2</sub> (367.5 eV) and Ag3d<sub>3/2</sub> (373.5 eV) at low binding energies compared to bulk Ag (368.3 eV for Ag 3d <sub>5/2</sub>, and 374.3 eV for Ag 3d<sub>3/2</sub> [30], due to an electron moving from Ag to TiO<sub>2</sub> surface. Ag3d<sub>5/2</sub> indicated two peaks and they were Ag° (367.3 eV) and Ag<sub>2</sub>O (368.5 eV). After visible light irradiation, Ag ions were transferred to Ag<sub>2</sub>O, when Ag° was on the surface. It confirmed that Ag was strongly oxidized, during visible light exposure.

# 3.4. Structural Studies of TiO<sub>2</sub> and Ag@TiO<sub>2</sub> NPs

TiO<sub>2</sub> and Ag NPs anchored TiO<sub>2</sub> NPs were analyzed for crystallinity. Fig. 3 (A) shows that diffraction peaks were not associated with crystalline TiO<sub>2</sub>. It demonstrated that TiO<sub>2</sub> was amorphous in both the asdeposited intrinsic and NPs samples. After annealing at 500 °C, the diffraction peaks were observed at  $2\theta = 25.6^{\circ}$ ,  $38.0^{\circ}$ ,  $48.4^{\circ}$ ,  $54.2^{\circ}$ ,  $55.3^{\circ}$ ,  $63.0^{\circ}$ , and  $69.0^{\circ}$ , respectively to the (101), (112), (200), (105), (211), (204) and (220), crystal planes of the TiO<sub>2</sub> anatase phase (JCPDS file No.21–1272), which are presented in Fig. 3 (A) (b-d). All the



Fig. 1. TEM images: (A) Pure TiO<sub>2</sub>; (B) Ag@TiO<sub>2</sub>-0.005 M; (C) Ag@TiO<sub>2</sub>-0.010 M; (D) Ag@TiO<sub>2</sub>-0.015 M; (E) HRTEM image of Ag@TiO<sub>2</sub> nanostructures (Inset: selected area electron diffraction (SAED) from a single Ag nanocrystal).

Ag@TiO2 samples focused on the usual pure anatase phase (indicated A in XRD) and Rutile phase (indicated R in XRD) with similar peak intensities and shapes (like original TiO2). Even at the highest concentration (0.015 M) of the AgNO<sub>3</sub> solution utilized for the deposition of Ag, new diffraction peaks were not related to Ag species, which was because of the least amount of Ag and the amorphous state of Ag [31]. In addition, no changes were observed in the positions of diffraction peaks. It showed that Ag has been deposited on TiO2 surface by Ag@TiO2 formation, which was supported by TEM and XPS observations. They indicated that parts of Ag and TiO2were incorporated with the amorphous states, whereas a part of Ag aggregated into small crystals in the as-deposited samples. The peaks became more intense as the percentage of Ag increased. Further, the diffraction peaks of silver were not observed. It indicated that in the as-deposited samples, there was a very small amount of silver, which was below the instrument's detection limit.

#### 3.5. Raman Spectra of TiO<sub>2</sub> and Ag@TiO<sub>2</sub> NPs

Raman spectroscopy has been further employed in the characterization of the crystalline structure of TiO<sub>2</sub> NPs, and the results are shown in Fig. 3(B).  $TiO_2$  anatase structure was characterized by the tetragonal space group of I41/amd. TiO<sub>2</sub> NPs contained twelve atoms per unit cell with lattice parameters a = 3.784 Å and c = 9.514 Å. Anatase contained six Raman active modes  $(A_{1g} + B_{1g} + 4E_g)$  and they were allowed according to the factor group analysis. Ohsaka et al. have reported the Raman spectrum of an anatase single crystal [32]. It explained that the six allowed modes appeared at  $148 \text{ cm}^{-1}$  (E<sub>g</sub>),  $199 \text{ cm}^{-1}$  (E<sub>g</sub>),  $392 \text{ cm}^{-1}$  (B1<sub>g</sub>),  $445 \text{ cm}^{-1}$  (E<sub>g</sub>),  $517 \text{ cm}^{-1}$  (A1g), and  $635 \text{ cm}^{-1}$  (Eg), respectively. The Raman spectrum excited at 488 nmfor the three Ag@TiO<sub>2</sub> samples was compared with the pure TiO<sub>2</sub> NPs. The prime Raman bands of all the samples were attributed to the characteristic anatase phase of TiO2 NPs, dominated by the Raman spectra of the Ag@TiO<sub>2</sub> nanostructures. The observed result well matched with the previous report of Maury-Ramirez [33] that the prepared Ag@TiO<sub>2</sub> nanostructures confirmed the absence of signals related to Ag nanocrystals, which could be attributed to a relatively low content of Ag loaded onto the TiO2 backbone. Here, the most interesting phenomenon of the spectra was that the intensity of the TiO<sub>2</sub> related bands decreased with an increasing amount of Ag. It denoted that there was an interaction between the Ag and  $\text{TiO}_2$  NPs in order to influence the resonance of Raman effect for  $\text{TiO}_2$  NPs.

# 3.6. UV-Visible Spectra of TiO<sub>2</sub> and Ag@TiO<sub>2</sub> NPs

The UV-Vis absorption-diffusion reflectance spectra of pure TiO<sub>2</sub> NPs and different concentrations of Ag in Ag@TiO2 nanostructures are depicted in supplementary file (Fig. S3). The pure TiO<sub>2</sub> NPs showed the typical absorption of anatase with an intense transition in the UV region. There was no absorption in the visible light region whereas all the Ag@TiO2 NPs presented prominent absorption in the region of 400-600 nm, indicating that the absorption of Ag@TiO2 has significantly been red-shifted to the visible region due to the loading of the Ag nanocrystals on the surface of theTiO2 NPs. It was due to the promotion of the electron from the valence band to the conduction band. The Ag@TiO<sub>2</sub> NPs clearly showed the characteristic absorption of TiO<sub>2</sub> NPs in the UV region, which could be attributed to the Ag<sup>o</sup> Surface Plasmon Resonance (SPR) with the  $TiO_2$  inter-band transition at  $\lambda$  < 480 nm. Many of the factors such as quantity, particle size, dispersion and morphology of Ag NPs, can influence the SPR effect intensity. Meanwhile, a decrease in the band gap values could be observed for Ag@TiO<sub>2</sub> photo catalysts. The Ag@TiO<sub>2</sub> (AgNO<sub>3</sub> 0.015 M) had a strong absorption band at around 470 nm (magnet curve) and the observed result correlated with the previous report of Yu et al. on the absorption spectra of Ag doped TiO<sub>2</sub> [34]. It was also evident that the intensity of the Ag@TiO2 NPs was much higher than that of the other two Ag@TiO2 (0.005, 0.010 M) samples. It meant that the nanostructures had a larger silver distribution. This decrease happened because of the Ag NPs that have introduced localized energy levels in the band gap of TiO<sub>2</sub> NPs. The electrons could be excited with low energy from the valence band (VB) to these levels rather than to the conduction band (CB) of the semiconductor. It should be noted that the nanostructures of Ag@TiO2 with absorption in the visible region may involve in an increased activity of photo catalysis [16].

# 3.7. Photocatalytic Studies of TiO<sub>2</sub> and Ag@TiO<sub>2</sub> NPs

Shvadchina et al. have reported the photocatalytic degradation of



Fig. 2. XPS spectra: (A) TiO<sub>2</sub> samples: (i) Ti 2p; (ii) C1s; (iii) O 1 and (B) Ag@TiO<sub>2</sub> samples: (i) Ti 2p; (ii) C1s; (iii) O 1 s; (iv) Ag 3d.



PA using S-TiO<sub>2</sub> doped with sulphur and pure TiO<sub>2</sub>. They have observed the photocatalytic behaviors of both S doped and pure TiO<sub>2</sub> samples in the UV region, which were less effective at oxidation by picric acid compared to that of the oxidation by fulvic acids and humic acid [36]. However, the fermi energy level of Ag is higher than TiO<sub>2</sub>, and hence, the electron transfers from Ag to TiO<sub>2</sub>. During visible light irradiation on the photocatalytic process, Ag and TiO<sub>2</sub> form new fermi energy levels and adjust the same value by Schottky barrier effect mechanism, which [37,38] leads to a higher photocatalytic activity. Fig. 4, shows the photocatalytic degradation of PA using pure TiO<sub>2</sub> and 0.005, 0.015 M Ag@TiO2 NPs. Without using a catalyst, there was no degradation spectrum in PA. Fig. 4 (b, c, and d) show the PA degradation spectra within 100, 70, 60 min, respectively, when the pure TiO<sub>2</sub>,  $0.005\,\mathrm{M}$  and  $0.015\,\mathrm{M}$  Ag@TiO\_2 acted as catalysts. PA was not degraded when the catalyst was not active during visible light irradiation as shown in Fig. 4(a). A better result was achieved for 0.010 M Ag@TiO<sub>2</sub> NPs with PA degradation within 50 min (Fig. 4 (e)). According to the Schottky barrier effect, Ag concentration was increased, and the visible light adsorption was increased. The increased visible light adsorption is usually directly proportional to the enhanced photocatalytic activity. This confirmed the increased concentration of Ag being directly proportional to the increased photocatalytic activity. In the present study, 0.010 M Ag@TiO<sub>2</sub> has increased photocatalytic activity compared to 0.015 M Ag@TiO<sub>2</sub> NPs due to photocatalytic activity depending on the shape of the NPs. The shape of 0.010 M Ag@TiO2 NPs was different compared to 0.005 M and 0.015 M Ag@TiO2 as depicted by HRTEM. Fig. 4(g) describes that acid is not degraded in acetic nature (pH. 1–7). Hence, pH value was increased, and PA was degraded in basic conditions (pH =10). The order of photocatalytic activity of picric acid

degradation can be summarized as follows.

 $Ag@TiO_2(0.010M) > Ag@TiO_2(0.015M) > Ag@TiO_2(0.005M) > TiO_2$ 

Photocatalytic charge separation process under visible light is listed in Eqs. (1)–(7) as given below;

$Ag@TiO_2 (e^-) + O_2 Ag@TiO_2 + O_2^{}$	(1)
$O_2^{} + H^{(+)} HO_2^{}$	(2)
$HO_2^{\cdot} + H^{(+)} e^- H_2O_2$	(3)
$H_2O_2 + O_2^{} OH^{-} + OH^{} + O_2$	(4)
$H_2O_2^{hv}$ 2OH	(5)

$$Ti^{3+} + H_2O_2 Ti^{4+} + OH^- + OH^-$$
 (6)

$$OH'(or O_2^{-}) + PA$$
 degraded or mineralized products (7)

#### 3.8. Determination of Photodegradation Products

The total molecular picric acid was being degraded for 0, 10, 20, 30, 40 and 50 min, respectively. As seen in Fig. 5 (a), for '0' min, there were two small peaks of m/z, 113, and 181 except for a strong peak at m/z 91, 228 corresponding to phenol and PA. The peak of m/z 181 could be attributed to the loss of one NO<sub>2</sub> from PA. The LC-MS spectrum of an ion at m/z 181 yielded the fragment ions corresponding to [M-3H]<sup>+</sup>. Since these peaks existed in the samples before the visible light irradiation, they might be attributed to the limited degradation of PA in room light during the LC-MS operating procedures.

After 30 min irradiation, it was found that two new peaks (m/z 194 and 175) were clearly observed (shown in Fig. 5 (b)). The peak of m/z 194 always existed during the photo degradation of PA for 30 min; perhaps it belonged to the impurity in the solution. The parent molecule with m/z 228 lost one nitro group corresponding to m/z 181 (Fig. 5 (c)) LC-MS peak of m/z 175 yielded the fragment ions corresponding to [M + CH<sub>3</sub>OH + 4H]<sup>+</sup>. Additional peak appear m/z 157 yielded the fragment peak of m/z 97 The compound of m/z 66 was the cyclopentadiene product of picric acid.

Another possible fragmentation of the LC-MS spectrum of an ion at m/z 181 yielded the fragmentations corresponding to  $[M-3H]^+$ . The compound of m/z 228 could be attributed to degradation and it had lost one nitro group from picric acid. The LC-MS spectrum of an ion at m/z 133 yielded the fragmentations corresponding to  $[M-6H]^+$  The peak at m/z 97 yielded the fragmentations corresponding to  $[M]^+$  and mechanism for the final product of picric acid was cyclopentadiene  $[M-C_5H_6]^+$  at m/z 66 given in Fig. 5 (d)).

# 3.9. Reactive Oxygen Species (ROS) Analysis of Ag@TiO2 and TiO2 NPs

The reactive species level has been determined by the DCFH-DA stain for Ag@ TiO<sub>2</sub> NPs and TiO<sub>2</sub> NPs. Control cells showed (Fig. 6a) low light emission of green fluorescence compared to TiO<sub>2</sub> NPs (Fig. 6b). Very bright light was observed for various concentrations of Ag@TiO<sub>2</sub> NPs (Fig. 6c-e). This was due to the non-fluorescent DCFH-DA that penetrated the cell, and the intracellular oxidation converting the fluorescent dye [39]. It confirmed that when ROS was increased the bright light would also increase. The highest bright light (increased ROS production) has been achieved for 0.015 M Ag@TiO2 NPs. This was because of the ability of the production of free radical elements by NPs and the induction of reactive and nonreactive free radical elements in the cell cytoplasm. These were major crucial regulators or mediators of cell cycle arrest and apoptosis induction. The increased concentration of silver nitrate for doping (0.005, 0.010, 0.015 M), showed dramatically increasing production of ROS in cellular metabolism. The unbalanced metabolic rate is a real path leading to cell death [40].



Fig. 4. Absorption spectra of PA (a) without catalyst; (b) Pure-TiO<sub>2</sub>, (c) Ag@TiO<sub>2</sub>–0.005 M (d) Ag@TiO<sub>2</sub>–0.015 M, (e) UV-vis absorption spectra for degradation of picric acid Ag@TiO<sub>2</sub> (0.010 M) kinetic linear simulation curves of PA photocatalytic degradation with (f) pH variation (g) Catalyst variation.



Fig. 5. LC-MS spectrum of picric acid degradation at (a) 0 min (b) 30 min, (c) Picric acid degradation products; (d) Mechanism of the proposed degradation products of picric acid.



Fig. 6. ROS images for A549 cell lines using (a) Control; (b) TiO<sub>2</sub>; (c) 0.005 M of Ag@TiO<sub>2</sub>; (d) 0.010 M of Ag@TiO<sub>2</sub>; (e) 0.015 M of Ag@TiO<sub>2</sub>.



Fig. 7. Stain analysis image of A549 cell lines using (a) Control; (b) TiO<sub>2</sub>; (c) 0.005 M Ag@TiO<sub>2</sub>; (d) 0.010MAg@TiO<sub>2</sub>; (e) 0.015 M Ag@TiO<sub>2</sub>.

# 3.10. Cell Growth Inhibition of Ag@TiO2 and TiO2 NPs

The anticancer activities of Ag@TiO<sub>2</sub> and TiO<sub>2</sub> have been evaluated *in vitro* against A549 human lung carcinoma cell lines. After 24 h, cell viability has been measured in the concentration range of 10–200 µg. When the doping concentration of Ag@TiO<sub>2</sub> was increased, the cell viability was decreased. IC<sub>50</sub> values of green synthesized Ag@TiO<sub>2</sub> and TiO<sub>2</sub> were found to be 115 µg/mL (0.015 M), 138 µg/mL (0.010 M), 155 µg/mL (0.005 M) and 200 µg/mL, respectively as shown in supplementary file (Fig. S4). The IC<sub>50</sub> value was low only for 0.015 M Ag@TiO<sub>2</sub>.

When doping concentration increased, green synthesized Ag@TiO<sub>2</sub> exhibited good anticancer activities compared to TiO<sub>2</sub>. The excess electron production was directly proportional to the enhanced production of ROS (given in Fig. 6(a)), and it was used to break the A549 cell walls. Fig. S4 anticancer activity of (A) TiO<sub>2</sub> (B) 0.005, 0.010 and 0.015 M Ag@TiO<sub>2</sub> for A549 lung cancer cell lines.

# 3.11. Morphological Evidence of Apoptosis Analysis of Ag@TiO\_ and TiO\_ NPs

The treated A549 cell morphology has been observed by AO/EB double staining assay as shown in Fig. 7. In the abovementioned figure, green color represents live cells (early apoptotic cells) in the range of 500 to 530 nm in the fluorescent microscope, red and orange colors (late apoptotic) represent dead cells in the range of 510 to 595 nm in a fluorescent microscope. Green synthesized TiO<sub>2</sub> NPs have been added to A549 cell lines. A small number of cells was dead when treated with TiO<sub>2</sub> NPs and it represented that a high amount of green color would be emitted (Fig. 7b) compared to treatment with green synthesized Ag@TiO<sub>2</sub> NPs wherein high amounts of red light was emitted due to increased cell death (Fig. 7(c-e)). Non- treated cell line had emitted only green color (Fig. 6a), which represented that all the cells were viable. High percentage of cell death was achieved at the concentration of 0.015 M Ag@TiO<sub>2</sub> (Fig. 7e). This result was well matched with the observed ROS production (Fig. 6e). Above all, ROS, MTT and AO/EtBr results confirmed an enhanced anticancer activity against A549 cell lines using Ag@TiO2 compared to that of the previous similar report [41].

### 4. Conclusions

Visible light active Ag@TiO2 NPs have been prepared biogenically

through hydrothermal method using *Aloe vera* gel. Structural study confirmed the presence of Ag@TiO<sub>2</sub> NPs. The photocatalytic performance of Ag@TiO<sub>2</sub> has been considered for the degradation of picric acid in the visible light region, which is due to their UV absorption phenomena and Ti<sup>3+</sup> oxidation state confirmed through XPS result. 0.010 M of the prepared Ag@TiO<sub>2</sub> has achieved a higher photocatalytic performance within 50 min, which attributed to the morphological impacts observed. Compared to the other concentrations, 0.015 M of Ag@TiO<sub>2</sub> showed better optical property having high visible light absorption that led to an excess electron transfer to cell lines. This resulted in the increased production of ROS in the cell lines increasing the cell death, confirming the enhanced anticancer activity. The simple and biogenic method proposed in the present work to prepare highly visible light active Ag@TiO<sub>2</sub> NPs can be used in industrial effluent treatment as well as in biomedical applications.

# Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jphotobiol.2019.111636.

# References

- B. Wang, J.S. Chen, H.B. Wu, Z. Wang, X.W. Lou, Quasiemulsion-templated formation of α-Fe2O3 hollow spheres with enhanced lithium storage properties, J. Am. Chem. Soc. 133 (43) (2011) 17146–17148.
- [2] H. Chen, J. He, H. Tang, C. Yan, Porous silica nanocapsules and nanospheres: dynamic self-assembly synthesis and application in controlled release, Chem. Mater. 20 (18) (2008) 5894–5900.
- [3] E. Grabowska, A. Zaleska, S. Sorgues, M. Kunst, A. Etcheberry, C. Colbeau-Justin, H. Remita, Modification of titanium (IV) dioxide with small silver nanoparticles: application in photocatalysis, J. Phys. Chem. C 117 (4) (2013) 1955–1962.
- [4] P.V. Kamat, Photophysical, Photochemical and Photocatalytic Aspects of Metal Nanoparticles, ACS Publications, 2002.
- [5] D. Wang, Z.-H. Zhou, H. Yang, K.-B. Shen, Y. Huang, S. Shen, Preparation of TiO 2 loaded with crystalline nano ag by a one-step low-temperature hydrothermal method, J. Mater. Chem. 22 (32) (2012) 16306–16311.
- [6] H. Östmark, S. Wallin, H.G.J.P. Ang, Vapor pressure of explosives: a critical review, Explos. Pyrotech. 37 (1) (2012) 12–23.
- [7] K.-M. Wollin, H. Dieter, Toxicological guidelines for monocyclic nitro-, amino-and aminonitroaromatics, nitramines, and nitrate esters in drinking water, Arch. Environ. Contam. Toxicol. 49 (1) (2005) 18–26.
- [8] B. Pramanik, N. Singha, D. Das, Sol-, gel-, and paper-based detection of picric acid at femtogram level by a short peptide gelator, ACS Appl. Polym. Mater. 1 (4) (2019) 833–843.
- [9] B. Swarnalatha, Y. Anjaneyulu, Studies on the heterogeneous photocatalytic oxidation of 2, 6-dinitrophenol in aqueous TiO2 suspension, J. Mol. Catal. A Chem. 223 (1–2) (2004) 161–165.
- [10] K. Tanaka, W. Luesaiwong, T. Hisanaga, Photocatalytic degradation of mono-, di-

Journal of Photochemistry & Photobiology, B: Biology 202 (2020) 111636

and trinitrophenol in aqueous TiO2 suspension, J. Mol. Catal. A Chem. 122 (1) (1997) 67–74.

- [11] D. Hariharan, A.J. Christy, J. Mayandi, L. Nehru, Visible light active photocatalyst: hydrothermal green synthesized TiO2 NPs for degradation of picric acid, Mater. Lett. 222 (2018) 45–49.
- [12] I.-S. Kim, M. Baek, S.-J. Choi, Comparative cytotoxicity of Al2O3, CeO2, TiO2 and ZnO nanoparticles to human lung cells, J. Nanosci. Nanotechnol. 10 (5) (2010) 3453–3458.
- [13] R.B. Shafreen, S. Seema, A.P. Ahamed, N. Thajuddin, S.A. Alharbi, Inhibitory effect of biosynthesized silver nanoparticles from extract of Nitzschia palea against curlimediated biofilm of Escherichia coli, Appl. Biochem. Biotechnol. 183 (4) (2017) 1351–1361.
- [14] A. Chaudhary, N. Kumar, R. Kumar, R.K. Salar, Antimicrobial activity of zinc oxide nanoparticles synthesized from Aloe vera peel extract, SN Appl. Sci. 1 (1) (2018).
- [15] A. Ayeshamariam, M. Kashif, V. Vidhya, M. Sankaracharyulu, V. Swaminathan, M. Bououdina, M. Jayachandran, Biosynthesis of (ZnO-Aloe vera) nanocomposites and antibacterial/antifungal studies, Int. J. Nanoelectron. Mater. 9 (1) (2016).
- [16] K. Ali, S. Dwivedi, A. Azam, Q. Saquib, M.S. Al-Said, A.A. Alkhedhairy, J. Musarrat, Aloe vera extract functionalized zinc oxide nanoparticles as nanoantibiotics against multi-drug resistant clinical bacterial isolates, J. Colloid Interface Sci. 472 (2016) 145–156.
- [17] D. Hariharan, A. Jegatha Christy, S. Pitchaiya, S. Sagadevan, P. Thangamuniyandi, U. Devan, L.C. Nehru, Green hydrothermal synthesis of gold and palladium doped titanium dioxide nanoparticles for multifunctional performance, J. Mater. Sci. Mater. Electron. 30 (13) (2019) 12812–12819.
- [18] V.M. Ramakrishnan, M. Natarajan, A. Santhanam, V. Asokan, D. Velauthapillai, Size controlled synthesis of TiO<sub>2</sub> nanoparticles by modified solvothermal method towards effective photo catalytic and photovoltaic applications, Mater. Res. Bull. 97 (2018) 351–360.
- [19] V. Raguraman, D. MubarakAli, G. Narendrakumar, R. Thirugnanasambandam, R. Kirubagaran, N. Thajuddin, Unraveling rapid extraction of fucoxanthin from Padina tetrastromatica: purification, characterization and biomedical application, Process Biochem. 73 (2018) 211–219.
- [20] V. Raguraman, J. Jyotsna, S. Palaniappan, S. Gopal, R. Thirugnanasambandam, R. Kirubagaran, Sulfated polysaccharide from Sargassum tenerrimum attenuates oxidative stress induced reactive oxygen species production in in vitro and in zebrafish model, Carbohydr. Polym. 203 (2019) 441–449.
- [21] R. Vasantharaja, L. Stanley Abraham, V. Gopinath, D. Hariharan, K.M. Smita, Attenuation of oxidative stress induced mitochondrial dysfunction and cytotoxicity in fibroblast cells by sulfated polysaccharide from Padina gymnospora, Int. J. Biol. Macromol. 124 (2019) 50–59.
- [22] M.S. Ali, M.A. Sayeed, M.M. Nabi, M.A.A. Rahman, In vitro antioxidant and cytotoxic activities of methanol extract of Leucas aspera leaves, J. Pharm. Phytochem. 2 (1) (2013).
- [23] A. Kathalingam, K. Kesavan, A. Rana, J. Jeon, H.-S. Kim, Analysis of Sn concentration effect on morphological, optical, electrical and photonic properties of spray-coated Sn-doped CdO thin films, Coatings 8 (5) (2018) 167.
- [24] C. Aydin, Synthesis of Pd: ZnO nanofibers and their optical characterization

dependent on modified morphological properties, J. Alloys Compd. 777 (2019) 145–151.

- [25] H. Liu, Y. Zhang, R. Li, X. Sun, S. Désilets, H. Abou-Rachid, M. Jaidann, L.-S. Lussier, Structural and morphological control of aligned nitrogen-doped carbon nanotubes, Carbon 48 (5) (2010) 1498–1507.
- [26] F.B. Li, X.Z. Li, M.F. Hou, Photocatalytic degradation of 2-mercaptobenzothiazole in aqueous La3+-TiO2 suspension for odor control, Appl. Catal. B 48 (3) (2004) 185–194.
- [27] S. Li, Y. Shen, A. Xie, X. Yu, L. Qiu, L. Zhang, Q. Zhang, Green synthesis of silver nanoparticles using Capsicum annuum L. extract, Green Chem. 9 (8) (2007) 852–858.
- [28] G.B. Hoflund, Z.F. Hazos, G.N. Salaita, Surface characterization study of Ag, AgO, and Ag 2 O using x-ray photoelectron spectroscopy and electron energy-loss spectroscopy, Phys. Rev. B 62 (16) (2000) 11126.
- [29] X. Quan, Q. Zhao, H. Tan, X. Sang, F. Wang, Y. Dai, Comparative study of lanthanide oxide doped titanium dioxide photocatalysts prepared by coprecipitation and sol-gel process, Mater. Chem. Phys. 114 (1) (2009) 90–98.
- [30] H.W. Chen, Y. Ku, Y.L. Kuo, Photodegradation of o-Cresol with Ag Deposited on TiO2 under visible and UV light irradiation, Chem. Eng. Technol. 30 (9) (2007) 1242–1247.
- [31] P. Tippayawat, N. Phromviyo, P. Boueroy, A. Chompoosor, Green synthesis of silver nanoparticles in aloe vera plant extract prepared by a hydrothermal method and their synergistic antibacterial activity, PeerJ 4 (2016) e2589.
- [32] T. Ohsaka, F. Izumi, Y. Fujiki, Raman spectrum of anatase, TiO2, J. Raman Spectrosc. 7 (6) (1978) 321–324.
- [33] A. Maury-Ramirez, Photocalytic Coatings for Air-Purifying, Self-Cleaning and Antimicrobial Properties, MDPI AG2015, 2019.
- [34] B. Yu, Y. Zhou, P. Li, W. Tu, P. Li, L. Tang, J. Ye, Z. Zou, Photocatalytic reduction of CO2 over Ag/TiO2 nanocomposites prepared with a simple and rapid silver mirror method, Nanoscale 8 (23) (2016) 11870–11874.
- [36] Y.O. Shvadchina, M. Cherepivskaya, V. Vakulenko, A. Sova, I. Stolyarova, R. Prikhodko, The study of properties and catalytic activity of titanium dioxide doped with sulphure, J. Water Chem. Technol. 37 (6) (2015) 283–288.
- [37] S.A. Ansari, M.M. Khan, M.O. Ansari, J. Lee, M.H. Cho, Biogenic synthesis, photocatalytic, and photoelectrochemical performance of ag–ZnO nanocomposite, J. Phys. Chem. C 117 (51) (2013) 27023–27030.
- [38] W. Liang, T.L. Church, A.T. Harris, Biogenic synthesis of photocatalytically active Ag/TiO 2 and Au/TiO 2 composites, Green Chem. 14 (4) (2012) 968–975.
- [39] N. Krishnakumar, N. Sulfikkarali, N. RajendraPrasad, S.J.B. Karthikeyan, P. Nutrition, Enhanced anticancer activity of naringenin-loaded nanoparticles in human cervical (HeLa) cancer cells, Biomed. Prevent. Nutr. 1 (4) (2011) 223–231.
- [40] A.S.H. Hameed, C. Karthikeyan, A.P. Ahamed, N. Thajuddin, N.S. Alharbi, S.A. Alharbi, G. Ravi, In vitro antibacterial activity of ZnO and Nd doped ZnO nanoparticles against ESBL producing Escherichia coli and Klebsiella pneumoniae, Sci. Rep. 6 (2016) 24312.
- [41] M. Ahamed, M.A.M. Khan, M.J. Akhtar, H.A. Alhadlaq, A. Alshamsan, Ag-doping regulates the cytotoxicity of TiO2 nanoparticles via oxidative stress in human cancer cells, Sci. Rep. 7 (1) (2017) 17662.