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Original article

Hydrothermal synthesis of pure and bio modified TiO_2: Characterization, evaluation of antibacterial activity against gram positive and gram negative bacteria and anticancer activity against KB Oral cancer cell line

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Highlights

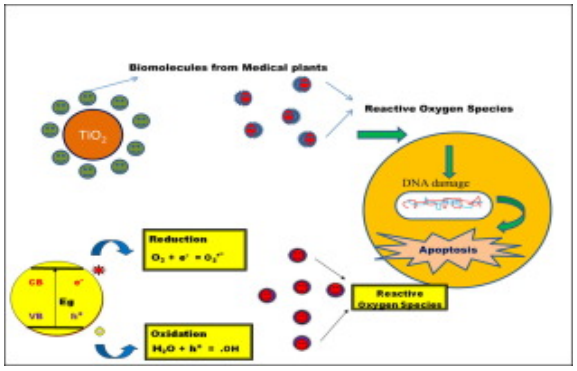
- Pure Titanium dioxide (TiO_2 NPs) and Turmeric, Ginger, Garlic modified TiO_2 NPs were synthesized by Hydrothermal method using Titanium tetra isopropoxide as precursor.
- Antibacterial and anticancer activities of pure TiO_2, modified (turmeric, ginger and garlic) TiO_2 nanoparticles were investigated.
- Antibacterial activities were performed against five bacterial strains namely *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus mutans*.
- Anticancer activities for the samples were performed in KB Oral cancer cell line.

- The modified TiO_2 NPs indicate a greater efficiency on anticancer and antibacterial properties when compared with the pure TiO_2 NPs.

Abstract

Titanium dioxide nanoparticles were found to be good anticancer and antibacterial agents. In this study, the antibacterial and anticancer activities of pure TiO_2, turmeric, ginger and garlic modified TiO_2 nanoparticles were investigated. X-ray diffraction (XRD), Transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR) and UV–visible spectroscopy were used to analyze the samples. Antibacterial activities were performed against five bacterial strains namely *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus mutans*. The modified TiO_2 nanoparticles exhibited enhanced antibacterial activity when compared with pure TiO_2 samples and anticancer activities for the samples were performed in KB Oral cancer cell line. The results of the modified TiO_2 NPs indicate a greater efficacy on anticancer and antibacterial properties compared to the pure TiO_2 NPs.

Graphical abstract



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Keywords

TiO_2 nanoparticles; XRD; TEM; Antibacterial; Anticancer; Ginger; Garlic and turmeric

1. Introduction

The human environment has been deteriorating due to various communicable diseases which spread mainly due to the microbes. Microbes damage the environment, health industry, food industry, textile industry. The best way to eradicate this damage was to invent suitable and best antimicrobial agent in this field. The nanoparticles in particular metal oxide nanoparticles as antimicrobial agents help to eradicate the damage due to microbes (Sadiq et al., 2009). These agents were categorized into two groups namely, organic and inorganic agents (Fu et al., 2005). Among the two groups the inorganic materials were showing excellent resistance towards microbes (Fu et al., 2005, Makhluf et al., 2005). Metal oxide nanoparticles exhibit excellent activity against bacteria even at smaller concentrations (Rai et al., 2009).

Various inorganic oxides such as TiO_2, ZnO, MgO, CaO, CuO, Al_2O_3 and Ag_2O exhibited good antimicrobial activity (Rai et al., 2009, Shi et al., 2012, Stankovic et al., 2013, Tang et al., 2012, Wei et al., 1994, Bellantone et al., 2002). Titanium dioxide (TiO_2) is an excellent photocatalyst (Fujishima and Honda, 1972, Liu et al., 2010, Chen and Mao, 2007) having large band gap of 3.2 eV, and are being used in optoelectronic devices (Chen et al., 2007, Xia et al., 2003, Jang et al., 2008) and dye-sensitized solar cells (Qiu et al., 2010, Luo et al., 2009, Kim et al., 2009). They play a major role in abolishing the growth of bacteria due to their production of ROS in the presence of UV light (Hu et al., 2006, Battin et al., 2009). They serve as an excellent antibacterial agent (Fu et al., 2005). TiO_2 has been increasingly used for its better biocompatibility and photocatalytic property (Fujishima and Rao, 2000, Qiang et al., 2011). Reactive Oxygen Species formed during the reduction of oxygen or oxidation of H_2O was found to be the most important step in many photocatalytic reaction. This is clearly true with the case of TiO_2 nanoparticles.

The antibacterial and photocatalytic activities of TiO_2 nanoparticles have been attributed to their ability to produce Reactive Oxygen Species (ROS) (Nosaka and Nosaka, 2017, Huang et al., 2016b, Huang et al., 2015b) and the deposition of bio products on to TiO_2 surface can greatly increase the amount of ROS production which result in the enhanced photocatalytic and biological activity (Etacheri et al., 2013). Due to their tendency to generate excessive reactive oxygen species in cancer cells, they also serve as an efficient anticancer agent (Hu and Lan, 2006, Battin and Kammer, 2009).

Iron oxide, Cerium oxide, Zinc oxide, Copper oxide, etc., are known to be excellent nanoparticles which act against cancer cells (Bhattacharyya et al., 2011, Vinardell and Mitjans, 2015). Shilpa Chakra et al. (2017) reported that ZnO/TiO_2 nanocomposite exhibit excellent anticancer activity against HeLa cells, CHO cells, MD-231 Cells and B-16F10 cells. Hariharan et al. (2013) reported that TiO_2 nanoparticles with Cynodon Dactylon leaf extract exhibited excellent anticancer activity at low concentration against A549 cells. Lotfian and Nemati (2016b) investigated the dose and time dependent activity of TiO_2 nanoparticles in MCF-7 cancer cells. He observed that these nanoparticles suppressed the growth of MCF-7 cancer cells effectively. He et al. (2016) showed that the Ag NPs using Dimocarpus Longon peel extract had excellent antibacterial and anticancer activity against prostate cancer (PC-3) cells.

Rajesh kumar (2016) reported that few micrograms of gold nanoparticles showed excellent activity against HepG2 and A549 cells. Rhizomes are known to possess excellent anticancer property. In ancient time, people used plant derivatives for the treatment of various tumors and in cancer therapy. The use

of plant derivatives results in complete healing and reducing the side effects associated with cancer (Joseph and Nair, 2013, Chanda and Nagani, 2013) as well as improving the immunity of the body. The use of antibiotics increases in the day to day world. In the present day, use of antibiotics was increasing steadily and hence there was a great demand for alternative drugs, especially with least side effects (Khulbe and Sati, 2009) were practiced in many areas of the universe for so many years (Sofowora, 1984). Turmeric, ginger and garlic showed excellent antimicrobial and medicinal characteristics.

The major component in turmeric is Bisdemethoxycurcumin. The active components in ginger contain terpenes and phenols (Grzanna and Lindmark, 2005) which include gingerol and shogaol. Gingerol is found in higher quantities (Prasad, 2015). Garlic contains flavonoids and sulphur-containing compounds: diallyl sulphate, alliin, ajoene, allicin. Curcumin in turmeric, allicin in garlic and gingerol in ginger are reported to be strong antioxidants (Menon and Sudheer, 2007, Rahman and Fazlic, 2012, Masuda and Kikuzaki, 2004). The major constituents of turmeric ginger and garlic was found to be curcumin, gingerol and allicin and the chemical structure are shown in Fig. 1.

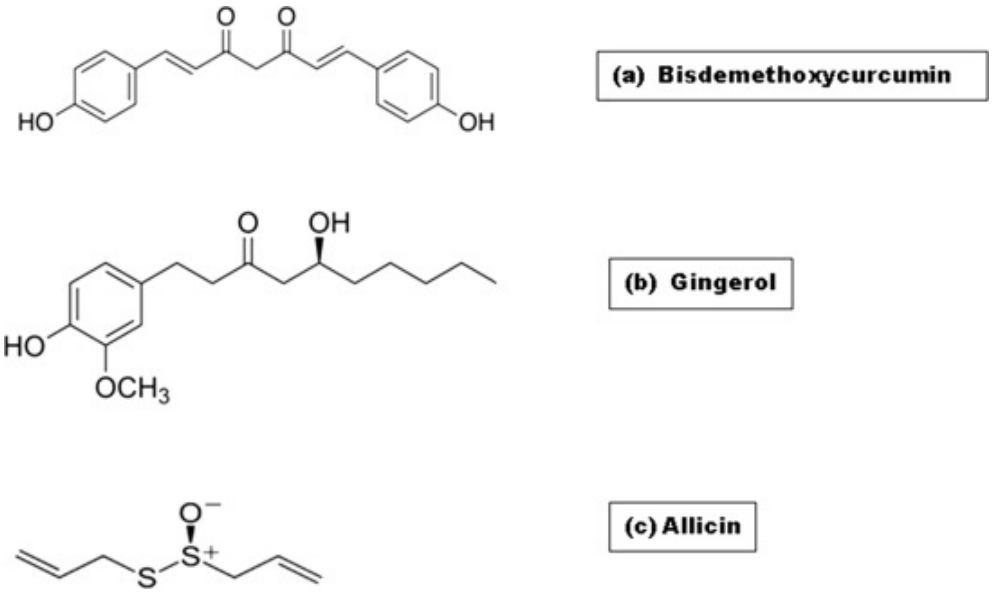

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Fig. 1. Chemical structure of curcumin, gingerol and allicin.

The biomolecules present in the turmeric, ginger and garlic exhibit excellent anticancer property. Hence, this motivated to investigate the anticancerous activity of the TiO_2 nanoparticles modified with these extracts.

In the present work, pure TiO_2 nanoparticles and turmeric, ginger, garlic modified NPs were synthesized. The nanoparticles were characterized using XRD, FTIR, UV–Vis spectroscopy, and Transmission electron microscopy (TEM). The antibacterial nature of the pure and modified TiO_2 nanoparticles were tested against gram positive and gram negative bacteria using well diffusion method. The cytotoxicity of the pure and modified samples was tested against KB (KERATIN – forming

tumor cell line HeLa) Oral cells using MTT assay. The modified TiO_2 nanoparticles showed enhanced anticancer activity and as well as improved the antibacterial activity.

2. Materials and methods

Titanium tetra isopropoxide and Isopropanol were acquired from Sigma - Aldrich Chemicals. Fresh Turmeric, Ginger and Garlic were purchased and stored in air tight light-proof container.
(a) Preparation of extract:

Ginger was cleaned with deionised water. 10 g of ginger was crushed and then boiled with 100 ml of deionised water at 60 °C for 30 min. Then, it was filtered with Whatman filter paper. Thus, ginger extract was obtained and was stored at 4 °C for future use. In similar manner, turmeric and garlic extracts were also prepared.
(b) Preparation of TiO_2 samples:

Titanium dioxide NPs were prepared from 5 ml of Titanium (IV) isopropoxide and 10 ml of isopropanol using hydrothermal method. The obtained mixture were transferred to Teflon autoclave and then subjected to temperature of about 200 °C for 2 h. Then, the solution was centrifuged and then rinsed with water and ethanol to get rid of impurities and then kept in oven at 100 °C. The obtained TiO_2 powder was annealed at 350 °C for further preparation.
(c) Preparation of modified TiO_2 nanoparticles:

0.2 g of TiO_2 powder was mixed with 20 ml of deionised water and stirred vigorously for half an hour. Then to this mixture, 4 ml of turmeric extract was added drop by drop and stirred for 90 min. The final products were centrifuged and then rinsed with water and ethanol to remove the impurities and then kept in an oven at 100 °C. Thus, turmeric – TiO_2 samples were obtained. The same procedure was repeated by using 4 ml of ginger, garlic and ginger-garlic extracts and obtained the ginger – TiO_2, garlic – TiO_2 and ginger-garlic modified TiO_2 NPs respectively.

2.1. Characterization techniques

The solutions of the pure TiO_2 and modified TiO_2 were analyzed using UV–Vis spectrophotometer in the range from 200 to 800 nm for spectral analysis. A Perkin Elmer Infra red spectrophotometer was used for the determination of the surface functional groups over the range of 400–4000 cm^{-1}. The crystalline nature of the sample was analyzed by X-ray diffraction (XRD) analysis using XPERT-PRO operated at 45 kV and 40 mA at 2° angle pattern. TEM measurements were performed using HRTEM – JEOL – 3010, operated at an accelerating voltage 300 kV.

2.2. Antibacterial assay

The antibacterial activities of the pure and modified TiO_2 nanoparticles were determined by agar well diffusion method (Cernik, 2013, Naika et al., 2014, Vilas et al., 2016). The test organisms used for antimicrobial analysis namely *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 530),

Pseudomonas aeruginosa (MTCC 1688), *Staphylococcus aureus* (MTCC 737) and *Streptococcus mutans* (MTCC 890) were purchased from Microbial Type culture collection and gene bank (MTCC) at Chandigarh. The bacterial strains were maintained on nutrient agar.

2.2.1. Nutrient broth preparation

Pure Culture from the plate were inoculated in to the nutrient agar plate and sub cultured at 37 °C for 24 h. Inoculum was prepared by aseptically adding the fresh culture to the saline tube and cell density was adjusted to Mc Farland standard so that the suspension becomes 1.5×10^8 cfu/ml.

2.2.2. Antimicrobial test

38 g of Muller Hinton agar (Hi media) was dissolved in 1000 ml of distilled water for preparation of medium. Then it was autoclaved at 15 Lbs pressure at 121 °C for one-fourth of an hour (pH = 7.3). It was cooled and later mixed well and then poured in to the petriplate. The plates were swabbed with pathogenic bacterial cultures viz namely *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *S. mutans* and then finally the samples were loaded on to the wells which were bored for 6 mm on the surface of the Muller Hinton medium and then kept at incubator for nearly about 24 h at 37 °C. Then the zone of inhibition was measured in millimeters. *Streptomycin* was used as a standard control.

2.3. Cell viability assay

KB cells were seeded in a 96-well plate and incubated the plate for 24 hrs at 37 °C. After the incubation period, the plates were taken out from incubator and MTT reagent was added to it. The plate was covered with aluminium foil to avoid light exposure. The plates were again incubated for 3 h. The reagent was removed and then 100 μl of DMSO was added to it. The mixture so obtained was stirred nicely. The absorbance was read on a spectrophotometer or an ELISA reader at 570 nm and 630 nm used as reference wavelength. The optical density was calculated for the viable cells using the given formula:

$$\%\,of\ cell\ viability = \frac{(Absorbance\ O.D\ by\ sample)}{(Absorbance\ O.D\ by\ control)} \times 100$$

3. Results and discussion

3.1. Structural investigation of TiO_2 NPs:

The nanoparticles synthesized in this method were characterized using powder XRD. Fig. 2 exhibit the XRD pattern of TiO_2 NPs. The XRD pattern show peaks at 25.3°, 37.8°, 48°, 54.7°, 63°, 70° and 75.7° emanating from the crystal planes (101), (211), (004), (200), (105), (204), (220) and (215) respectively, of anatase TiO_2 lattice. This was matched to JCPDS file No: 21-1272 of anatase TiO_2 (Wei and Zhu, 2013, Nainani and Thakur, 2012). Ba-abbad et al., reported the intense peak at $2\theta = 25.3°$ confirms the TiO_2 anatase structure (Ba-abbad and Kadhum, 2012). The peak 30.5° corresponds to the brookite phase of TiO_2. Fig. 3 shows XRD peaks from (211) plane of TiO_2 for the modified TiO_2 lattice. It

was observed that the peak intensity and the FWHM increases for ginger and garlic modified samples. This indicates the well crystalline nature of ginger and garlic modified TiO_2 samples.

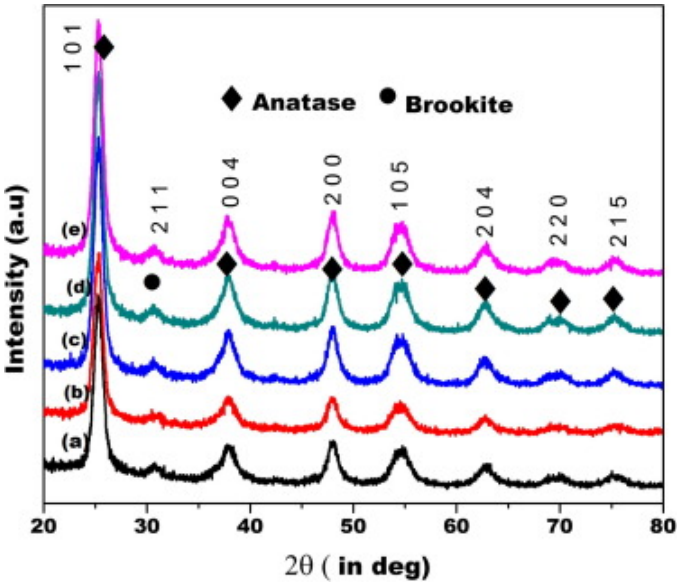

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Fig. 2. XRD patterns of (a) unmodified TiO_2 (b) turmeric modified TiO_2 (c) ginger modified TiO_2 (d) garlic modified TiO_2 (e) ginger and garlic modified TiO_2.

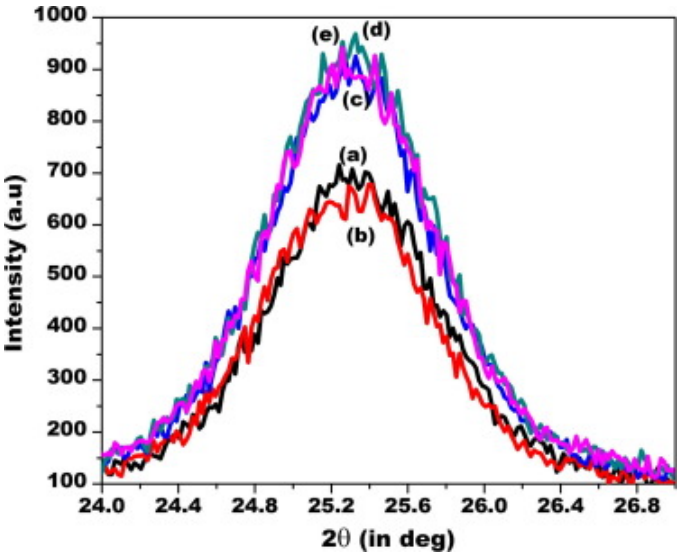

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Fig. 3. Expanded XRD patterns of (a) unmodified TiO_2 (b) turmeric modified TiO_2 (c) ginger modified TiO_2 (d) garlic modified TiO_2 (e) ginger and garlic modified TiO_2 (between $2\theta = 24^\circ$ and 27°).

3.2. UV investigations of pure and modified TiO_2 NPs

Fig. 4 shows the UV spectra of the synthesized samples. The absorption profile slightly differed for the TiO_2 samples. For Pure TiO_2, the maximum absorbance was observed at 264 nm. Similar observation was found in the literature. Karkare et al. and Hasan and Wu (2010) observed at 265 nm and 337 nm for Pure TiO_2 nanoparticles respectively. For the modified TiO_2 samples, the absorbance maximum is slightly red shifted due to the chemisorption of turmeric, ginger and garlic molecules on the TiO_2 surface. The maximum absorption of the turmeric modified, ginger modified, garlic modified and ginger-garlic mixture modified TiO_2 is observed to be 284 nm, 312 nm, 325 nm and 278 nm respectively. This shift indicates that there is a strong interaction between the dopants and TiO_2 nanoparticles. Hence, it is clear that the dopants have modified the surface of the TiO_2.

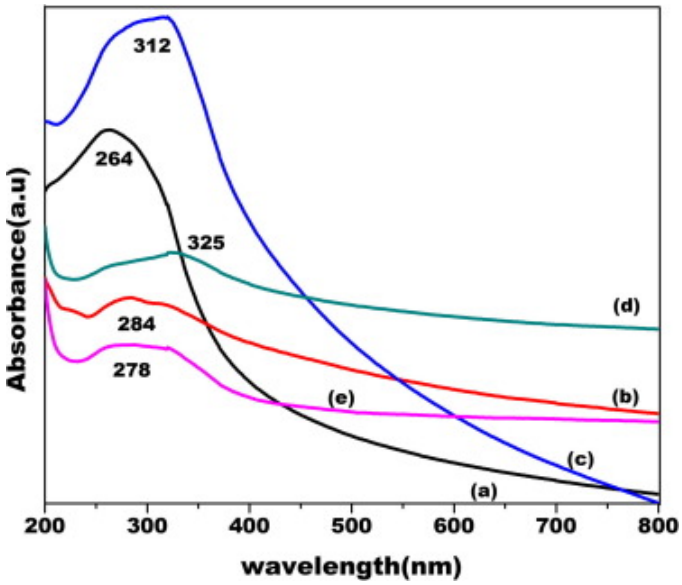

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Fig. 4. UV Spectra of (a) pure TiO_2 (b) turmeric modified TiO_2 (c) ginger modified TiO_2 (d) garlic modified TiO_2 (e) ginger and garlic modified TiO_2 nanoparticles.

The absorption edge of TiO_2 showed red shift which clearly indicate that there is an increase in the particle size of the modified TiO_2 nanoparticles when compared with that of pure samples. This increase in particle size is also observed in the TEM analysis. The increase in the particle size attributes to the decrease in the band gap which in turn results for more generation of electron and hole pairs (Huang et al., 2015a, Huang et al., 2016a). This is responsible for the production of superoxide radicals which will enhance photocatalytic activity further (Huang et al., 2017).

3.3. Size and morphological investigations of pure and modified Samples:

Fig. 5 shows TEM images of pure and modified TiO_2 samples. It was observed that the TiO_2 nanoparticles modified with turmeric and ginger-garlic combination show a high level of

agglomeration when compared to the other TiO_2 samples. Fig. 6 shows the HRTEM images and Fig. 7 shows the particle size distribution of the Pure and modified NPs. Crystalline fringes in the HRTEM images show the crystalline nature of the TiO_2 samples. From the size distribution histogram, it was observed that average particle size were found to be 7.5 nm for pure and 9.5 nm, 10 nm, 10.5 nm and 9 nm for turmeric, ginger, garlic and ginger-garlic mixture respectively.

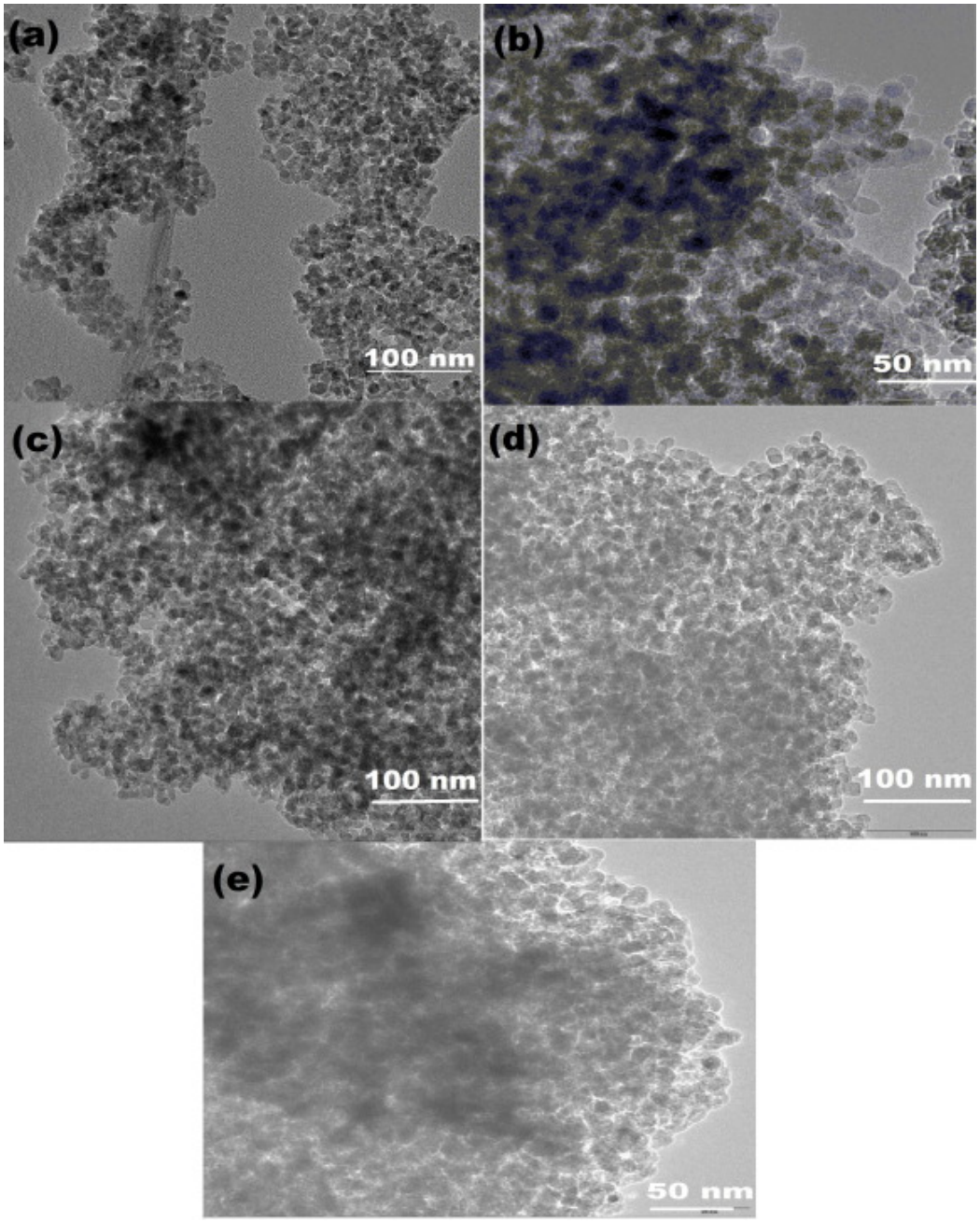

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Fig. 5. TEM images of (a) pure TiO_2 (b) turmeric modified TiO_2 (c) ginger modified TiO_2 (d) garlic modified TiO_2 (e) ginger and garlic modified TiO_2 nanoparticles.

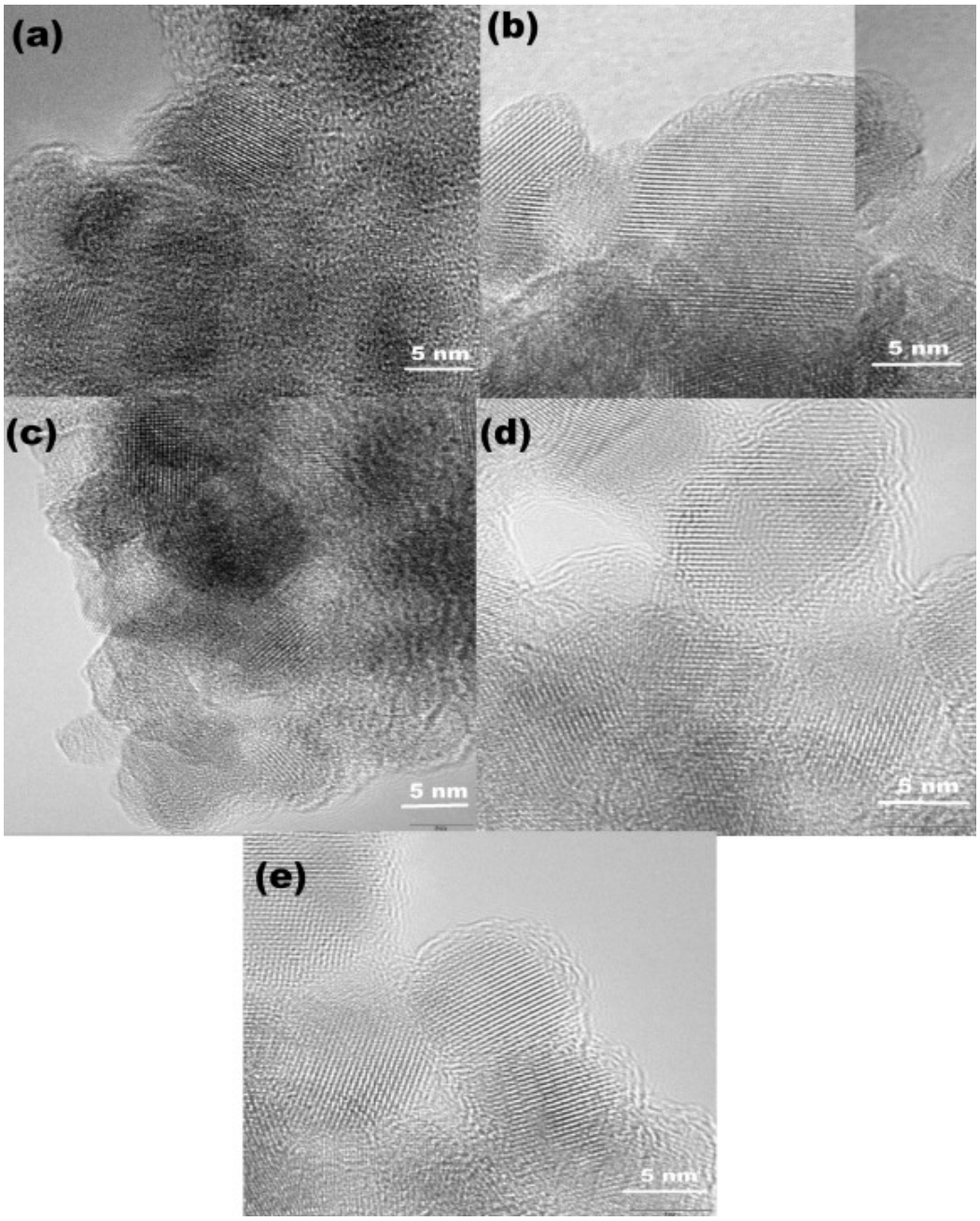

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Fig. 6. HRTEM images of (a) pure TiO_2 (b) turmeric modified TiO_2 (c) ginger modified TiO_2 (d) garlic modified TiO_2 (e) ginger and garlic modified TiO_2 nanoparticles.

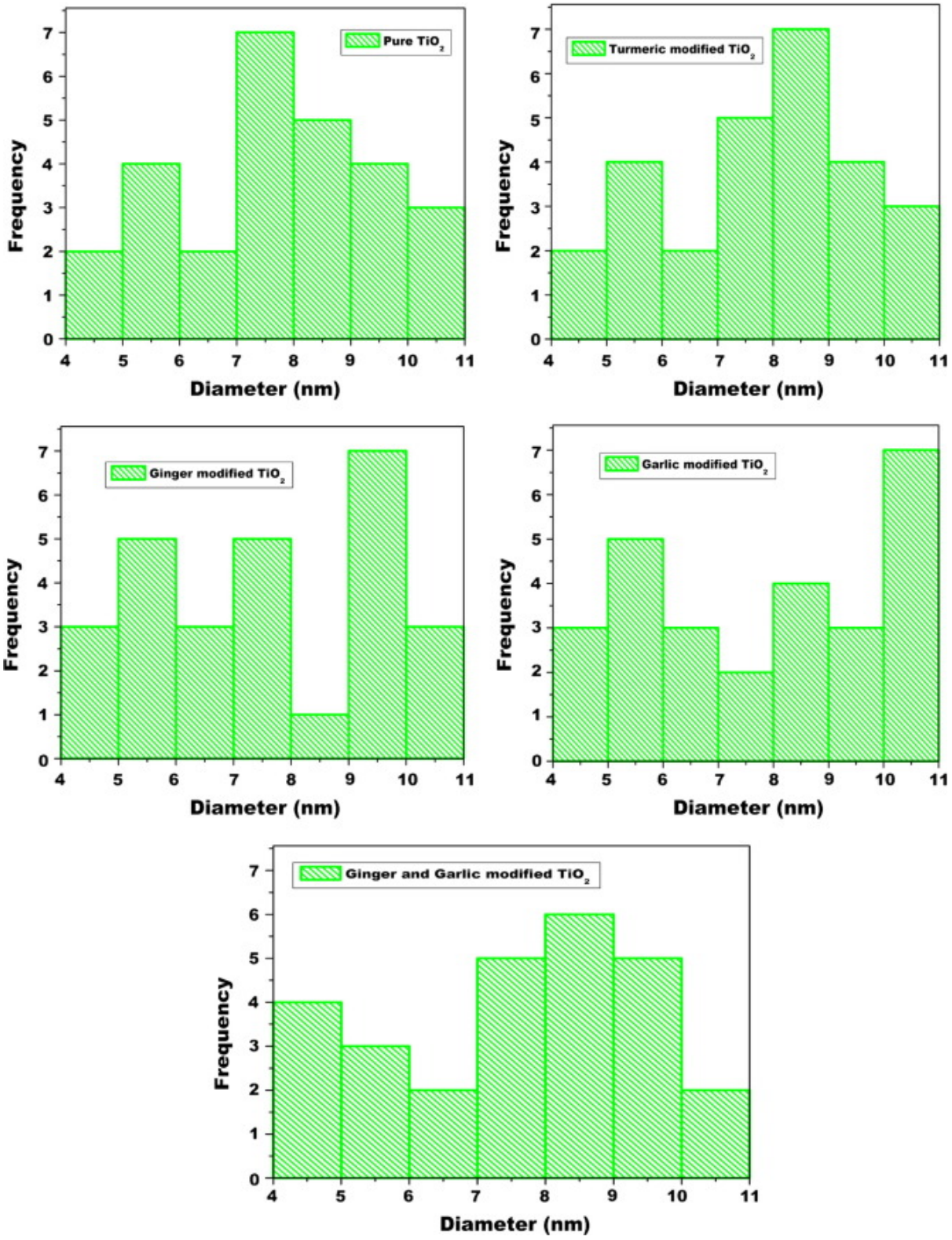

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Fig. 7. Distribution of average particle size of (a) pure TiO_2 (b) turmeric modified TiO_2 (c) ginger modified TiO_2 (d) garlic modified TiO_2 (e) ginger and garlic modified TiO_2 nanoparticles.

3.4. FTIR investigations of pure TiO_2 and modified TiO_2 nanoparticles:

Fig. 8, Fig. 9 shows the FTIR spectra of the extracts (ginger, garlic and turmeric) and the characteristic peaks due to the dopants were indicated. The key component of turmeric in curcumin, showed the characteristic band at 1629 cm^{-1} due to the C═O vibration (Bich and Thuy, 2009). In case of ginger and garlic, the main components are gingerol and allicin respectively. The characteristic band of gingerol and allicin occurs at 1642 and 1636 cm^{-1} which represent C═O (Shinde and Sachin, 2017) and C═C (Lu and Lu, 2014, Songsungkan and Chanthai, 2014) vibrations respectively. It was noticed that the characteristic band due to the main components has been shifted in the FTIR analysis.

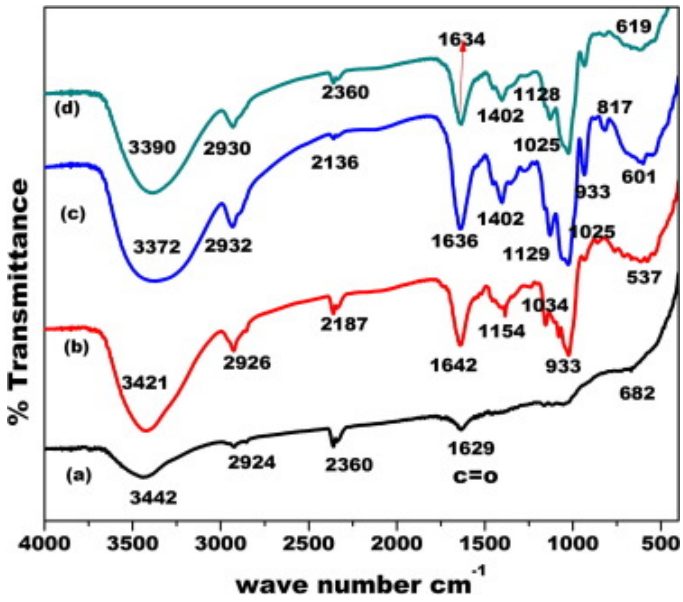

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Fig. 8. FTIR spectra of (a) turmeric extract (b) ginger extract (c) garlic extract (d) ginger and garlic extract.

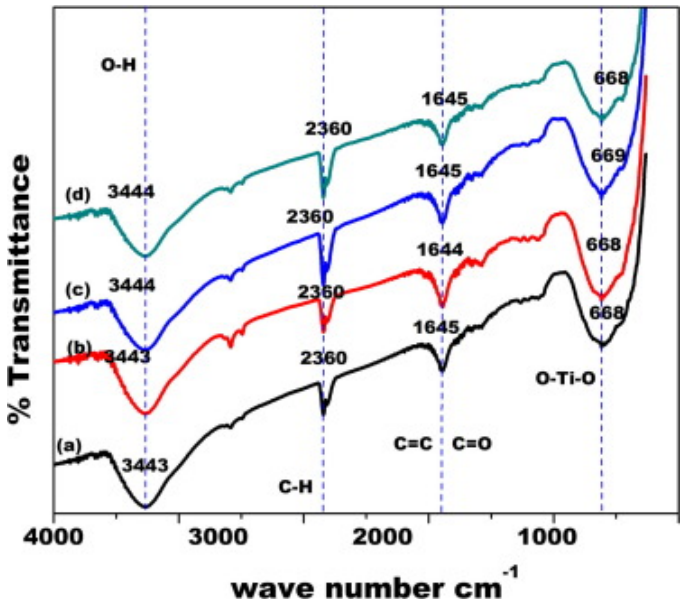

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Fig. 9. FTIR spectra of (a) turmeric modified TiO_2 (b) ginger doped TiO_2 (c) garlic modified TiO_2 (d) ginger and garlic modified TiO_2 nanoparticles.

The other peaks observed are 3372 and 2932 cm^{-1} which indicate the symmetric and asymmetric stretching vibrations of CH bond, 1636 cm^{-1} shows the stretching band of C═C, and 1025 cm^{-1} exhibit strong stretching vibration at S═O bond and it is in agreement with the reported literature (Ilic et al., 2010). A slight shift in the peak at 1645 cm^{-1} for turmeric extract when compared with 1633 cm^{-1} in pure TiO_2 is the signature of the reaction between aromatic ring of the bisdemethoxycurcumin in turmeric and titanium carboxylate obtained from TTIP. Similar observation was seen for ginger and garlic at 1644 and 1645 cm^{-1} respectively. A strong stretching vibration of TiO_2 was found out to be around 633 cm^{-1} which denotes O—Ti—O (Cheyne et al., 2011), and this peak becomes broader when doped with turmeric, ginger and garlic.

3.5. Anticancer activities

Anticancer activity of KB Oral cancer cell line was studied using cell viability assay. The synthesised TiO_2 nanoparticles of different concentrations, namely 0.000001, 0.00001, 0.0001, 0.001, 0.01, 0.1 and 10 mg/ml were used for anticancer study for a duration 24 h. Concentration of nanoparticles vs cell viability graph was plotted for each of the sample. Dose-dependent cell viability was observed. It was also observed that for a given concentration, different samples exhibited varied cell viability. Wells containing pure TiO_2 nanoparticles showed higher cell viability when compared with the samples modified with garlic, ginger and turmeric in KB cell line. More cell death occurred in the presence of modified TiO_2 samples, this is attributed to the presence of ginger, garlic and turmeric.

The antioxidant nature of dopants turmeric, ginger, garlic has imparted anticancer activity to TiO_2 nanoparticles. Excess free radicals/Reactive oxygen species (ROS) generated in the cells will lead to the

damage of the cell as well as RNA. Valko et al. (2007) reported that DNA damage may lead to the development of cancer and poor health conditions. Among the modified TiO_2 samples, ginger modified TiO_2 nanoparticles showed maximum anticancerous activity and the combination of ginger-garlic exhibited the least activity.

It was observed that 10 mg/ml concentration of the, turmeric modified TiO_2, ginger modified TiO_2, garlic modified TiO_2 and ginger-garlic modified TiO_2 shows 60.6%, 57.17%, 60.76% and 65.72% viability whereas 0.01 mg/ml concentration shows, 67.98%, 63.59%, 67.14% and 83.85% viability. Fig. 10, Fig. 11 represents the toxicity profile and inverted microscopic images of KB cells treated with pure and modified TiO_2 nanoparticles. The antioxidant activity of garlic, ginger and turmeric might be the reason for the enhanced anticancer activity (Tanvir and Sakib Hossen, 2017, Masuda and Kikuzaki, 2004, Rahman and Fazlic, 2012) of the modified TiO_2 samples. Zhang et al. (2016) in his work proposed an effective therapeutic strategy for preventing and treating Inflammatory Bowel Disease and colitis-associated cancer using ginger-derived nanoparticles. Hariharan et al., reported in the literature that the biomolecules present in the Cynodon Dactylon extracts gives excess electrons to NPs and this in turn generate ROS in KB cells which is responsible for damaging the cell wall as shown in Fig. 12.

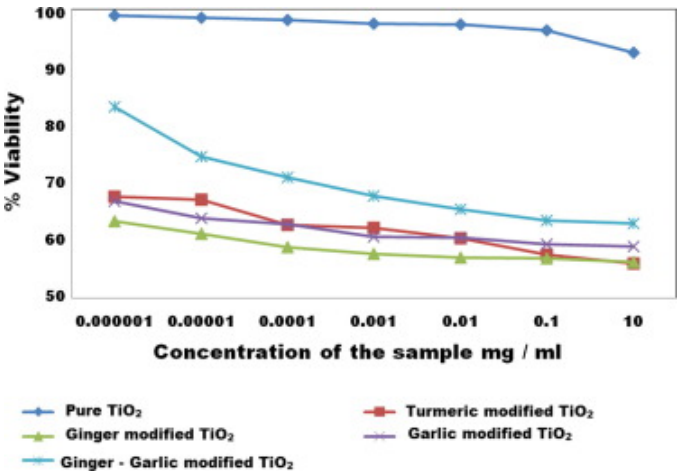

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Fig. 10. Toxicity profiles (concentration vs cell viability) of the KB cells treated with five different TiO_2 samples (pure TiO_2, turmeric modified TiO_2, ginger modified TiO_2, garlic modified TiO_2 and ginger-garlic modified TiO_2 nanoparticles. [Cells were treated with different concentrations of the samples for 24 h. At the end of the incubation period, cell viability was determined by MTT assay.]

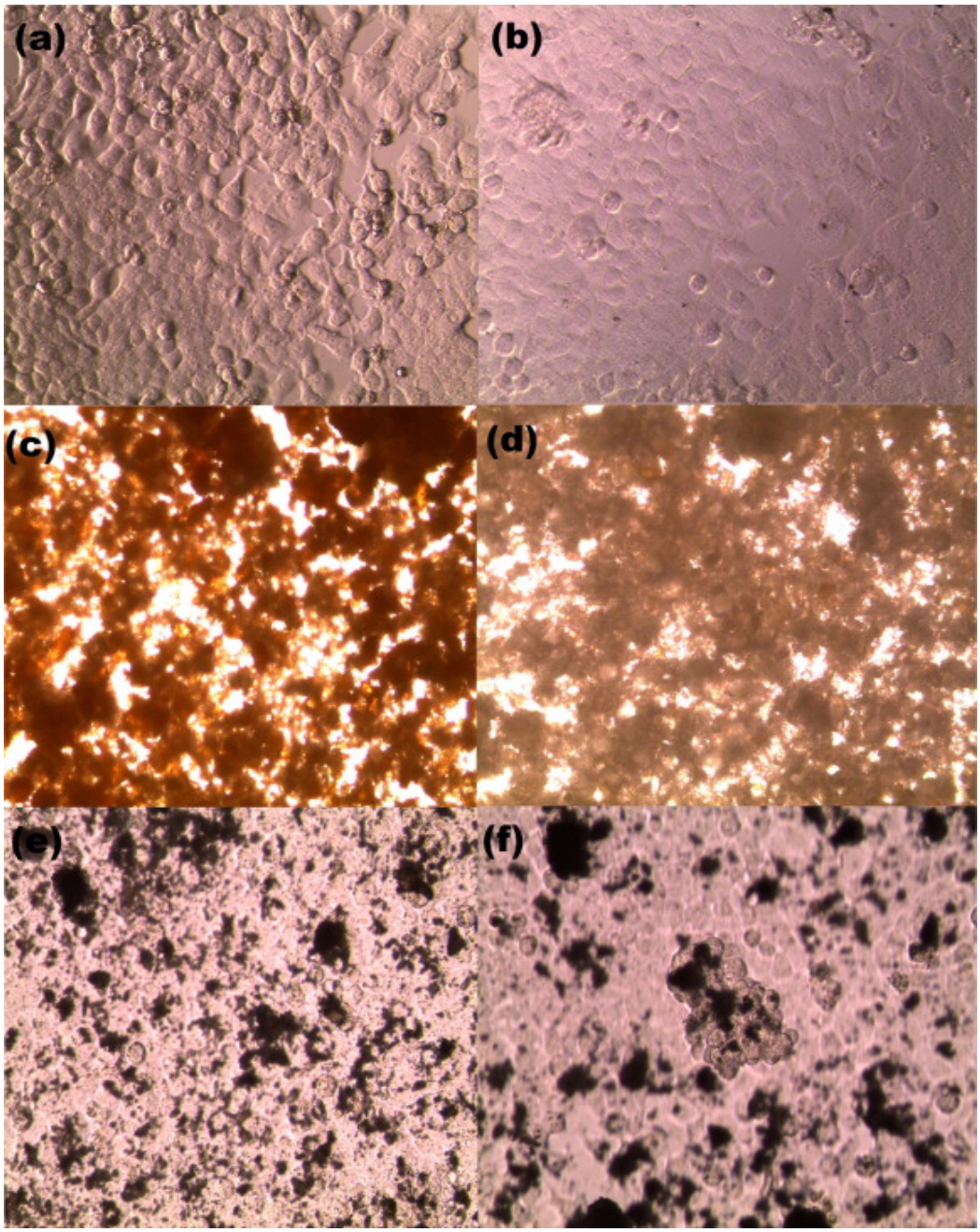

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Fig. 11. Inverted microscope images of the KB oral cancer cells treated with 10 mg concentration of TiO_2 nanoparticles (incubated for 24 h at 37◦C in a 5% CO_2 atmosphere, at the end of 24 h exposure) (a) control (b) pure TiO_2 (c) turmeric modified TiO_2 (d) ginger modified TiO_2 (e) garlic modified TiO_2 and (f) ginger-garlic modified TiO_2 nanoparticles.

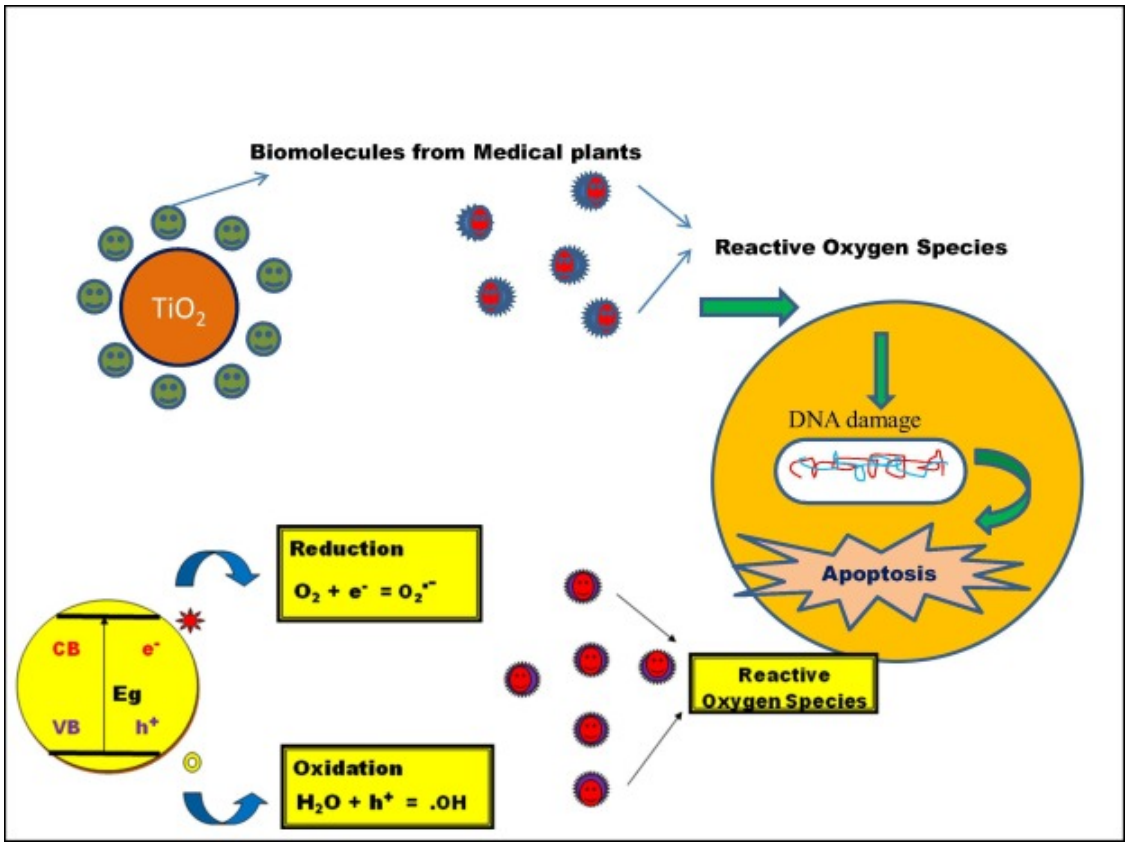

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Fig. 12. Mechanism in anticancer activity.

As the concentration of the sample increases the production of ROS increases which in turn damages the cancer cell wall and hence anticancer activity increases for ginger modified TiO_2 nanoparticles. Cell viability decreases with increase in the concentration of the TiO_2 nanoparticles which explains when the concentration of the sample increases more nanoparticles penetrate inside the cells which was responsible for excess generation of free radicals and in turn results in the cell death (Alishah and Pourseyedi, 2017). The TiO_2 nanoparticles were subjected for anticancer activity on various cell lines (Lotfian and Nemati, 2016a, Murugan and Dinesh, 2016, Hu et al., 2012, Govindhan and Pragathiswaran, 2016).

Hu and Lan (2006) reported that graphene/TiO_2 is responsible for the reduction process due to the antioxidant nature and apoptosis nature in HeLa cells, and there by leads to induced apoptotic death. Shilpa Chakra et al. (2017) reported that ZnO/ TiO_2 nanocomposite exhibit excellent anticancer activity against HeLa cells, CHO cells, MD-231 cells and B-16F10 cells. Rezaei-Tavirani et al. (2013) reported that the TiO_2 nanoparticles had a high effect on Breast cancer cell. Hariharan et al. (2013) reported that TiO_2 nanoparticles with Cynodon Dactylon leaf extract exhibited excellent anticancer activity at low concentration against A549 cells.

Lotfian and Nemati (2016b) reported that TiO_2 nanoparticles were able to suppress the MCF-7 Cancer cells effectively. Thevenot et al. (2008) examined the anticancer effect on TiO_2 nanoparticles and

confirmed the cell viability depend on nanoparticles concentrations and showed activity whereas the synthesized TiO_2 nanoparticles along with their dopants give better results. Further, animal studies were needed to determine the in vivo toxicity of TiO_2 NPs before clinical applications can be considered.

3.6. Antibacterial activity using TiO_2 nanoparticles and bio dopants

In the present work, the antibacterial activity of synthesized pure TiO_2 nanoparticles and modified TiO_2 nanoparticles were studied using agar well diffusion method against gram negative and gram positive bacterial strains The antibacterial activities were carried out with 1 mg/ml concentration of TiO_2 and modified TiO_2 nanoparticles. Fig. 13, Fig. 14, Fig. 15, Fig. 16, Fig. 17 shows the zone of inhibition for the Pure TiO_2, turmeric modified TiO_2, ginger modified TiO_2, garlic modified TiO_2 and ginger-garlic mixture modified TiO_2 samples against *Escherichia coli* (*E. coli* – Gram negative), *Pseudomonas aeruginosa* (*P. aeruginosa* – Gram negative), *Klebsiella pneumonia (K. pneumoniae* – Gram negative), *Staphylococcus aureus* (*S. aureus* – Gram positive) and *Streptococcus mutans* (*S. mutans* – Gram positive) respectively.

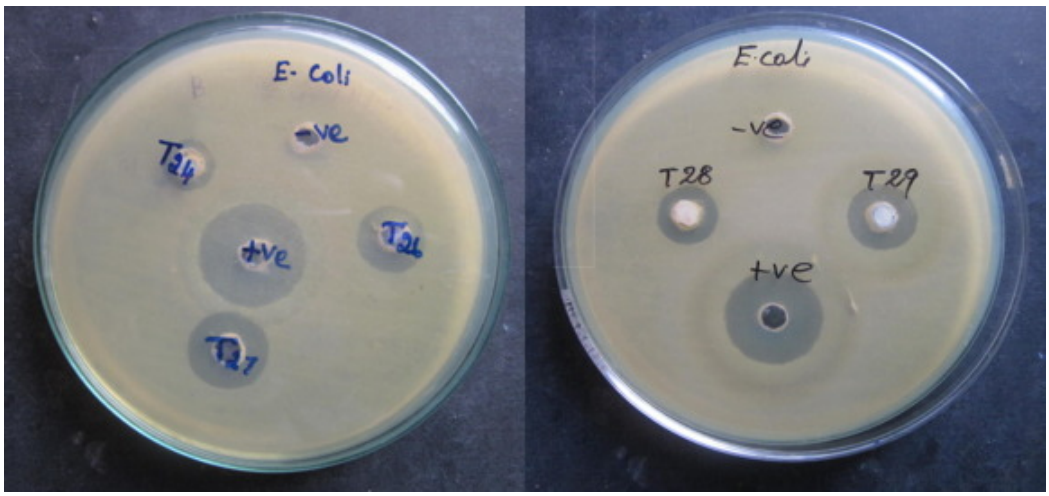

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Fig. 13. Zone of inhibition of (a) pure TiO_2 – T 24 (b) turmeric modified TiO_2 – T 26 (c) ginger modified TiO_2 – T 27 (d) garlic modified TiO_2 – T 28 (e) ginger-garlic modified TiO_2 – T 29 against *Escherichia coli* (gram negative bacteria).

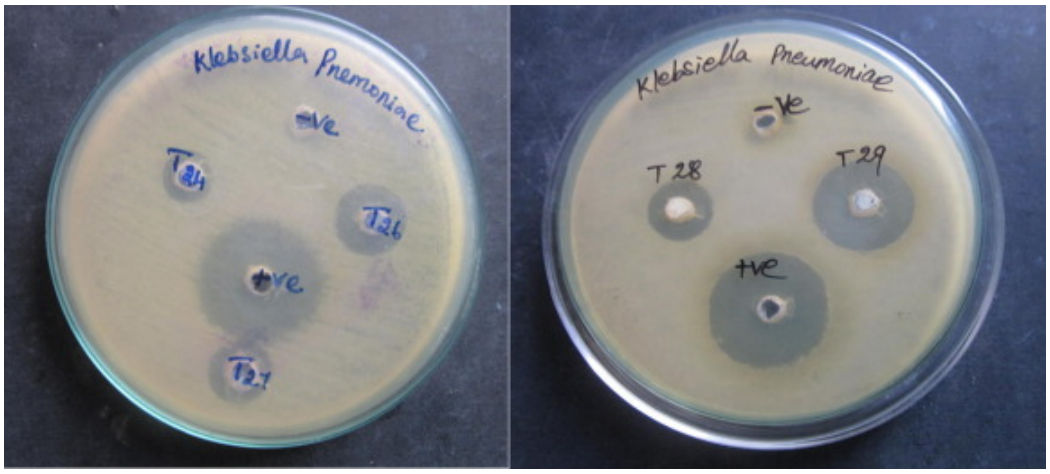

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Fig. 14. Zone of inhibition of (a) pure TiO_2 – T 24 (b) turmeric modified TiO_2 – T 26 (c) ginger modified TiO_2 – T 27 (d) garlic modified TiO_2 – T 28 (e) ginger-garlic modified TiO_2 – T 29 against *Klebsiella pneumoniae* (gram negative bacteria).

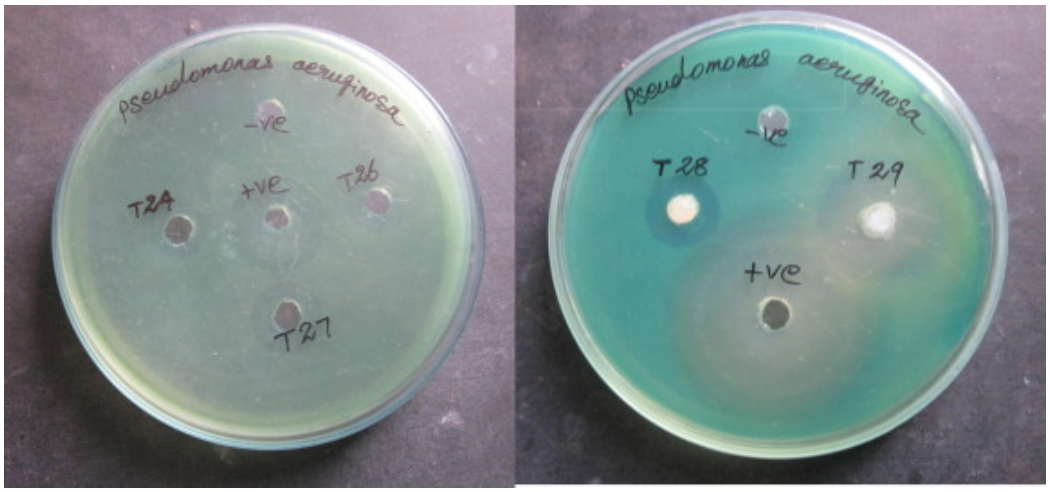

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Fig. 15. Zone of inhibition of (a) pure TiO_2 – T 24 (b) turmeric modified TiO_2 – T 26 (c) ginger modified TiO_2 – T 27 (d) garlic modified TiO_2 –T 28 (e) ginger-garlic modified TiO_2 – T 29 against *Pseudomonas aeruginosa* (gram negative bacteria).

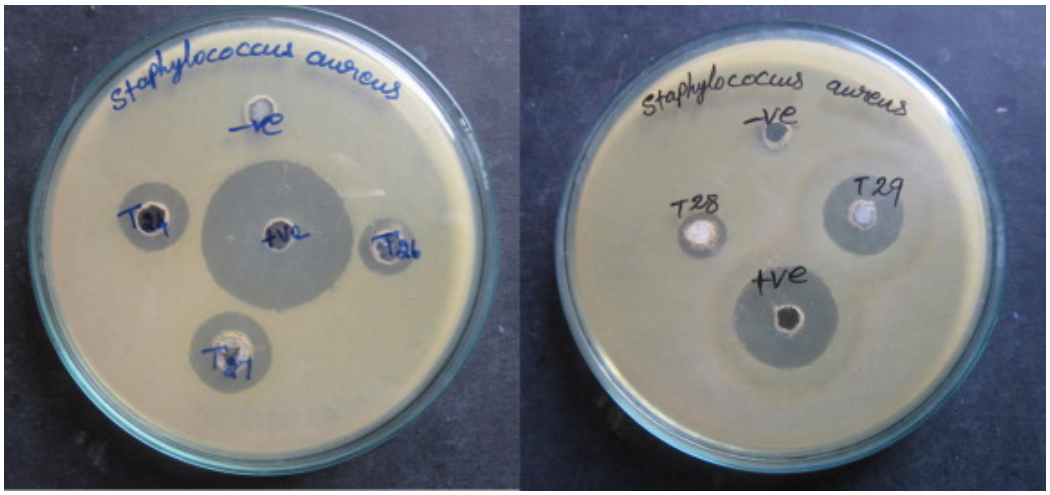

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Fig. 16. Zone of inhibition of (a) pure TiO_2 – T 24 (b) turmeric modified TiO_2 – T 26 (c) ginger modified TiO_2 – T 27 (d) garlic modified TiO_2 – T 28 (e) ginger – garlic modified TiO_2 – T 29 against *Staphylococcus aureus* (gram positive bacteria).

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Fig. 17. Zone of inhibition of (a) Pure TiO_2 – T 24 (b) turmeric modified TiO_2 – T 26 (c) ginger modified TiO_2 – T 27 (d) garlic modified TiO_2 – T 28 (e) ginger-garlic modified TiO_2 – T 29 against *Streptococcus mutans* (gram positive bacteria).

Pure samples showed maximum activity against *Staphylococcus aureus*. Ahamad and Sardar (2013) reported that the antibacterial activity of TiO_2 nanoparticles synthesized by Sol gel method against *E. coli* using Disc diffusion method and the zones of inhibition were measured to be 17 mm for 100 µg/ml concentration of TiO_2 nanoparticles. According to Piskin et al. (2013) TiO_2 nanoparticles synthesized by Sonochemical method using well diffusion method showed excellent zone of inhibition against *E. coli*.

Parham et al. (2016) investigated the antimicrobial activities of different metal oxide nanoparticles and showed that the photocatalytic nature of the TiO_2 nanoparticles were responsible for the resistivity against bacterial strains.

Among the three phases of TiO_2 nanoparticles, anatase phase showed excellent photocatalytic activity and hence showed more bactericidal activity (Singh and Mohapatra, 2015) when compared with other two phases. Report were shown using silver nanomaterials synthesized using different plant species namely curciligo Orchioides (Kayalvizhi et al., 2016) and aloe vera (Dinesh et al., 2015). From the literature it was evident that the antibacterial activity increases due to the addition of the dopants. Naz et al. (2010) reported water extracts of turmeric showed the antibacterial nature against gram positive and gram negative bacteria. The maximum zone was observed for methanol extracts of turmeric against *S. aureus*. For Ginger modified particles maximum activity was seen for *E. coli* and *S. aureus*.

Maximum zone of inhibition was observed for garlic modified samples against the bacterial strains of *P. aeruginosa* and *S. mutans*. Similarly for ginger-garlic modified samples, maximum activity was observed in *P. aeruginosa*. Islam et al. (2014) observed that ginger exhibit maximum zone against salmonella species and minimum zone against *E. coli*. According to Sah et al. (2012), garlic and ginger showed maximum activity against *S. aureus*. Pankaj sah also observed that antibacterial property reduces as the temperature increases. Ranjan et al. (2012) pointed out that garlic sustains the antibacterial activity till 120 °C. Avato et al. (2000) reported that the antibacterial nature of garlic was due to the chemical compound allicin. Chester (1944) reported that allicin showed more bacteriostatic character than bactericidal property. Curcumin and bisdemethooxy curcumin in turmeric, gingerol in ginger and allicin in garlic plays an important role in antibacterial activity (Ankri and Mirelman, 1999, Tyagi et al., 2015) and they may be responsible for the enhancement of bacterial activity further when compared with the pure samples.

Fig. 18 shows the Graph of Zone of Inhibition for Pure and modified samples against bacterial strains. Ginger modified TiO_2 showed maximum activity against *Escherichia coli*. Turmeric modified and ginger-garlic modified samples showed maximum activity against *Pseudomonas aeruginosa*. Ginger modified and ginger-garlic modified samples showed maximum activity against *Klebseilla pneumoniae*.

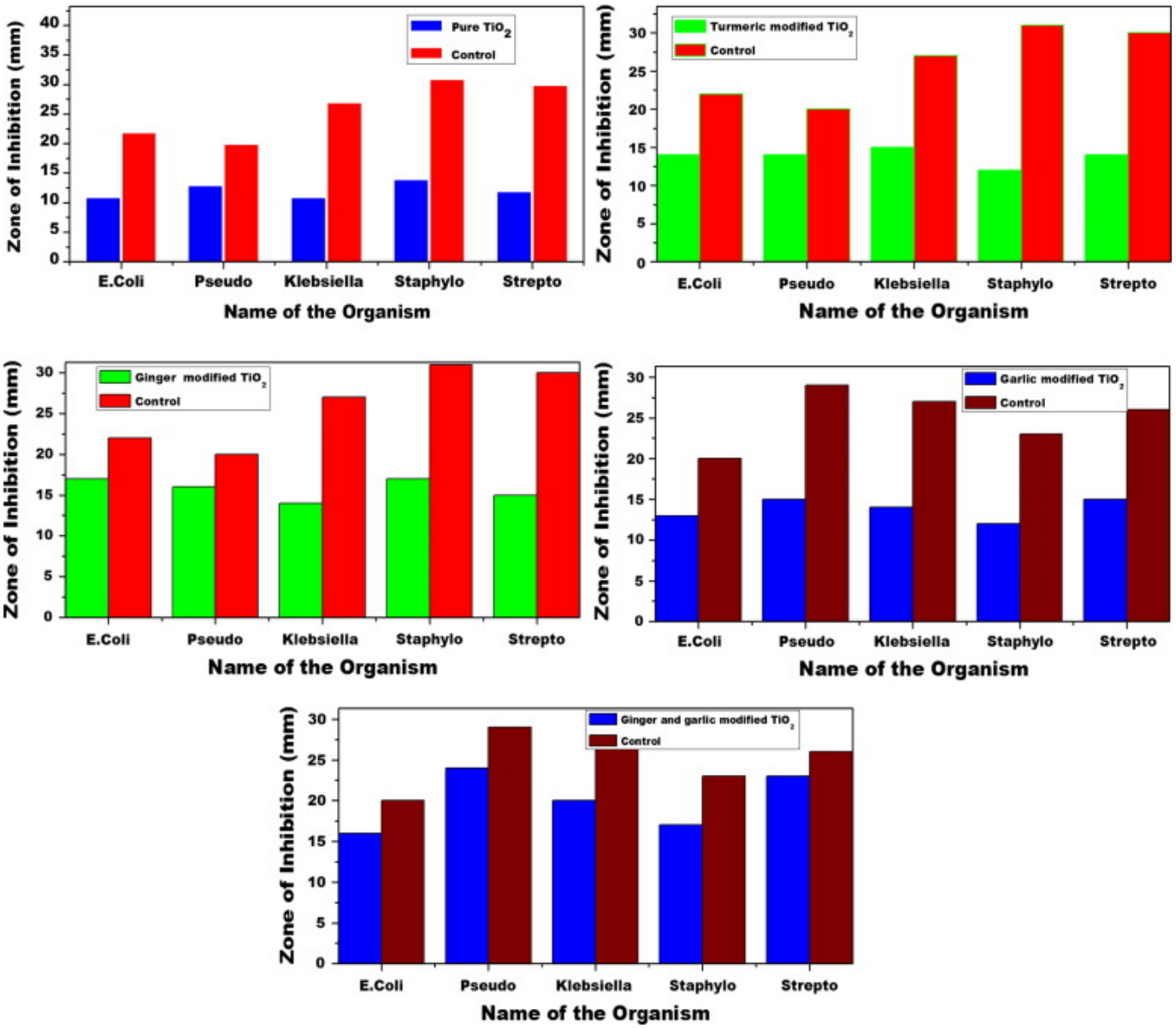

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Fig. 18. Graph showing the antibacterial activity of (a) pure TiO_2 (b) turmeric modified TiO_2 (c) ginger modified TiO_2 (d) garlic modified TiO_2 (e) ginger-garlic modified TiO_2 against *E. coli* (G−), *K. pneumoniae* (G−), *P. aeruginosa* (G−), *S.aureus* (G+) and *S. mutans* (G+) where G+: gram positive and G−: gram negative.

Ginger-garlic modified nanoparticles showed activity against *Staphylococcus aureus* and *Streptococcus mutans*. As observed from the experimental findings, the ginger-garlic modified TiO_2 nanoparticles exhibit maximum zone against the test organisms. The activity was found to increase with the addition of ginger and garlic with TiO_2 which shows that the gingerol in ginger and allicin in garlic may be responsible for the generation of reactive oxygen species that enhances the antibacterial nature. Titanium nanoparticles can easily react with sulphur groups which leads to the bacterial cell death (Bai and Rai, 2011). The mechanism was reported as TiO_2 nanoparticles and the modified samples act as positive charge and the test bacterial strains act as negative charge which leads to the electrostatic

attraction between them, and in turn results in the damage of the cell wall and similar mechanism was observed by Zhang and Chen (2009). The photocatalytic nature of the TiO_2 nanoparticles causes peroxidation which in turn increases the fluid in the membrane and finally leads to the disruption of the cell wall (Niazi and Gu, 2009, Arré et al., 2014).

4. Conclusion

Titanium dioxide nanoparticles have been synthesized using hydrothermal method. Anatase TiO_2 nanoparticles were modified with bio agents such as turmeric, ginger and garlic. XRD Spectra showed that prepared nanoparticles were crystalline in nature. UV–visible spectra of modified TiO_2 nanoparticles showed red shift for the maximum absorbance of the samples. The Ginger-garlic modified TiO_2 samples showed maximum zone of inhibition against *Pseudomonas aeruginosa* and minimum zone of inhibition against *Streptococcus mutans*. The modified samples exhibited enhanced antibacterial activity when compared with the pure TiO_2 samples. The Ginger modified TiO_2 shows the minimum viability % for 10 mg/ml concentration of the samples and exhibit significant increase in the anticancer activity than the pure TiO_2 nanoparticles.

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