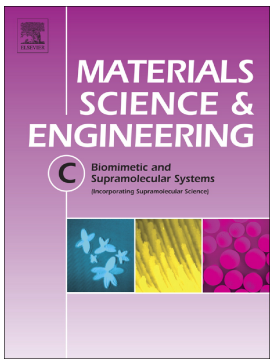

Bio-modified TiO2 nanoparticles with Withania somnifera, Eclipta prostrata and Glycyrrhiza glabra for anticancer and antibacterial applications

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Bio-modified TiO_2 nanoparticles with *Withania somnifera*, *Eclipta prostrata* and *Glycyrrhiza glabra* for anticancer and antibacterial applications

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Abstract:

Titanium dioxide nanoparticles exhibit good anticancer and antibacterial activities. They are known to be environmentally friendly and stable, less toxic and excellent biocompatibility nature. Due to these properties, they are well suited for biological applications particularly in

biomedical applications such as drug delivery and cancer therapy. In this paper Pure TiO_2 nanoparticles modified with *Withania somnifera* (Ashwagandha), *Eclipta prostrata* (Karisalankanni) and *Glycyrrhiza glabra* (Athimathuram) are examined for their biological applications. X-ray diffraction results revealed the anatase nature of the samples. From the TEM analyses, it is observed that there is an increase in the particle size of the bio modified samples. UV results show the red shift for the bio modified samples when compared with the pure samples. The samples are then subjected to MTT assay to determine the cell viability. KB oral cancer cells are used for the determination of anticancer nature of the Pure and bio modified nanoparticles. It is observed that *Withania somnifera* - *Eclipta prostrate* modified TiO_2 nanoparticles exhibit excellent anticancer activities among other bio modified and pure samples. The samples are then examined for their antibacterial activities against three Gram-negative bacterial strains namely, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and two Gram-positive bacterial strains namely, *Staphylococcus aureus* and *Streptococcus mutans.* Among the modified and pure samples, *Withania somnifera* - *Eclipta prostrata* showed good antibacterial nature against Gram-positive and Gram-negative bacteria.

Keywords: TiO_2 nanoparticles, anticancer, Ashwagandha, Karisalankanni and Athimathuram.

1. Introduction:

In the present millennia, nanomaterials have gained special attention due to their attractive phenomena [1 - 4]. Titanium dioxide (TiO_2) is an excellent photocatalyst [5 - 8] having a large bandgap of 3.2 eV and widely used in optoelectronic devices [9 - 12] and dye-sensitized solar cells [13 - 17]. There is increasing usage of this material mainly due to its biocompatible nature and excellent photocatalytic activity [18-19]. They play a major role in abolishing the

growth of bacteria due to their production of ROS in the presence of UV light [20 - 21]. They serve as an excellent antibacterial agent [22 - 23]. Reactive oxygen species formed during the reduction of oxygen or oxidation of H_2O was found to be the most important step in many reactions. This is evidence 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) [24 - 26] and the deposition of bioproducts on to TiO_2 surface can greatly increase the amount of ROS production which results in the enhanced photocatalytic and biological activity [27]. Due to their tendency to generate excessive reactive oxygen species in cancer cells, they also serve as an efficient anticancer agent [10]. Various metal oxide nanoparticles particularly TiO_2, ZnO, MgO, CaO, CuO, Al_2O_3 and Ag_2O are investigated for their antimicrobial [28 - 33] and anticancer [34 - 39] nature. TiO_2 nanoparticles are synthesized by several techniques like sol-gel, chemical vapor deposition and reversed micelle methods [40 - 42] and many scientists have tried to prepare the material in the form of nanospheres, nanowires, nanorods, nanostars and nanodisks [43].

Among the synthesis methods, the hydrothermal method is a better technique for the sake of easy procedure with inexpensive chemicals as starting materials [44]. When TiO_2 NPs are modified with chemical agents, the biological activities are enhanced [12]. Cheng H [45] observed that Selenium modified TiO_2 nanoarrays which comprise about 6.43 wt % of Se showed good anticancer activities against MC3T3-E1 cells and antibacterial activities against Staphylococcus *aureus* and *Escherichia coli*. The TiO_2 nanoarrays are fabricated by the seed-mediated hydrothermal technique and the Se-modification is done through an electrochemical process. He also reported that the samples tend to suppress the growth of MG63 (a kind of osteosarcoma) cells.

Shah M S A S [46] studied the polyethylene glycol-polyurethane-titania known as PEG-PU-TiO_2 polymer nanocomposite films that are prepared by using a simple solution casting method. Silver is then incorporated into the film by the photochemical reduction of silver nitrate solution. *E.coli* and *B.subtilis* are the test pathogens used for the experimental observations. The sample incorporated with silver indicated the efficient antibacterial activity against Gram-positive and Gram-negative bacteria. Zahid M [47] studied the antibacterial nature of Mn - TiO_2 by the sol-gel method. The antibacterial nature of the Mn-TiO_2 NPs coated cotton fabrics is examined through a bacteria reduction test against two bacterial pathogens namely *Staphylococcus aureus* and *Klebsiella pneumoniae*. He recorded that the Mn -TiO_2 NPs coated cotton fabrics exhibit antibacterial nature when compared with the untreated fabrics.

Ali T [48] observed the antibacterial activity of silver doped Titanium dioxide nanoparticles by the disc diffusion method. The test pathogens used are *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Enterobacter Cloacae*. The results from the experiments reveal that as the concentration of silver doping increases in the TiO_2 nanoparticles, it increases the zone of inhibition and for particularly 8.0 mole % doping of Ag a nanoparticle showed excellent bactericidal activity against the pathogens and produces twice the percentage of the zone of inhibition when compared with pure TiO_2 nanoparticles. Nithya N [49] prepared Pure and Neodymium (Nb) doped TiO_2 nanoparticles using sol-gel method. The samples are then subjected to Kirby - Bauer disk diffusion method against human pathogenic bacteria such as *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). The synthesized samples exhibit good antibacterial agents and it is observed that as the concentration of the Nb doping increases, the zone of inhibition also increases which results in the enhancement of antibacterial activity. Lu Z [50], observed the antibacterial activity of G

(glass substrate), G-PDA-TiO_2 (PDA - glass substrate of Polydopamine), and G - PDA - Ag/TiO_2 against Gram-negative *E. coli* and Gram-positive *B. subtilis* bacteria using the spread plate method under visible light. The results indicate that G - PDA - Ag/TiO_2 showed long term antibacterial activity. Senerathna U L N H [51] observed the antibacterial nature of the TiO_2 nanoparticles modified with *Garcinia zeylanica* extract. He prepared 21 nm anatase TiO_2 nanoparticles using Garcinia *zeylanica* extract and then examined the antibacterial nature against Methicillin-resistant *Staphylococcus aureus* and it is found to exhibit antibacterial nature. The Turmeric, Ginger, Garlic, Aqua Rosa extracts modified with Pure Titanium dioxide nanoparticles showed good antibacterial and anticancer nature [52 - 53]

For cost-effective and less toxic, plant extracts can be used as dopants. Ashwagandha, Karisalankanni and Athimathuram are the medicinal plants with pharmacological applications [54 - 56]. Ashwagandha, popularly known as Indian ginseng was scientifically known as *Withania somnifera* which was used in the treatment of tuberculosis, arthritis and cancer [57]. Alkaloids and steroidal lactones are the important chemical constituents which comprised of withanolides and withaferin. Sharma H et al. [58] reported that the Ashwagandha plant extracts damaged the cancer cells which were studied on human, colon and lung cancer cell lines. False Daisy, bhringraj were the common names of Karisalankanni whose botanical name is found out to be *Eclipta prostrata*. According to Chung I [59], *Eclipta prostrata* contains coumestans, thiophene derivatives, steroids, terpenes, and flavonoids.

Dalal S et al. [60] observed that the wedelolactone, a major constituent of Karisalankanni is responsible for the significant antimicrobial nature against bacterial pathogens. *Glycyrrhiza glabra* is the scientific name for Athimathuram, and it is commonly known as licorice which is found to be one of the most important medicinal plants. Glycyrrhizin, the major constituent of

licorice is responsible for the sweet taste. Other constituents are glucose, sucrose, starch, flavanoids which collectively give licorice its pharmacological properties. Their bioactive components exhibit antiviral, anticancer, antifungal, antibacterial and anti-ulcer activities [61 - 62]. The chemical structure of withanolides, wedelolactone, glycyrrhizin, glabridin and glabrene was shown in **Figure 1.**

In the present work, pure TiO_2 nanoparticles, Ashwagandha modified, Karisalankanni modified and Athimathuram modified TiO_2 NPs are synthesized by hydrothermal method. The samples are studied using XRD, FTIR and UV analyses. The anticancer activities of Pure and the modified samples are determined using cell viability assay against the KB Oral cancer cell line and the antibacterial nature is evaluated against the bacterial strains using well diffusion technique. The modified samples exhibited excellent anticancer and antibacterial activities when compared with the Pure samples.

2. Materials and Methods:

Titanium tetra-isopropoxide (97%) and isopropanol (99%) were purchased from Sigma - Aldrich Chemicals. All the chemicals used were of analytical grade without any purification TiO_2 nanoparticles were synthesized by a hydrothermal technique using 5 mL of Titanium tetra-isopropoxide and 10 mL of isopropanol. The mixture was stirred using magnetic stirrer for 2 hours and then kept in a furnace at 200 °C for about 2 h, and then the solution was washed several times with distilled water and ethyl alcohol for the removal of impurities. The obtained solution was then dried in an oven at 100 °C and then annealed at 350 °C, the obtained powder were used for further characterization.

10 g of Ashwagandha roots were weighed and then added with 100 mL of deionised water and then heated around 60 °C for nearly half an hour. Then the solution was filtered and then obtained as Ashwagandha extract and stored in the refrigerator for further use. A similar methodology was applied for Karisalankanni and Athimathuram extracts. The extracts are named as **E1, E2, E3, E4, E5** and **E6** for Ashwagandha, Karisalankanni, Athimathuram, Ashwagandha - Karisalankanni, Ashwagandha - Athimathuram and Athimathuram - Karisalankanni **[Table 1]**. 0.2 mg is present in the 4 mL of the extract.

4 mL of Ashwagandha extract was added drop by drop to 0.2 g of TiO_2 powder which was mixed with 20 mL of deionised water and then stirred for about 2 hours. Then the obtained solution was centrifuged and kept in an oven at 100 °C. The resultant dried sample was then collected as Ashwagandha modified TiO_2 (**S1**) powder. In the similar way, using 4 mL of Karisalankanni, Athimathuram, Ashwagandha - Karisalankanni, Ashwagandha - Athimathuram and Athimathuram - Karisalankanni extracts, the Ashwagandha modified TiO_2, Karisalankanni modified TiO_2 (**S2**), Athimathuram modified TiO_2 (**S3**), Ashwagandha - Karisalankanni modified TiO_2 (**S4**), Ashwagandha - Athimathuram modified TiO_2 (**S5**) and Athimathuram - Karisalankanni modified TiO_2 (**S6)** nanoparticles were synthesized respectively.

The pure and the modified samples crystalline nature was determined by X-ray diffraction (XRD) analyses, and for spectral analyses, UV-Vis spectrophotometer was used in the range from 200 - 800 nm. TEM (Transmission Electron Microscopy) measurements were performed using HRTEM - JEOL - 3010, operated at an accelerating voltage of 300 kV. The functional groups of the samples were analyzed using Perkin Elmer Infrared Spectrometer. The samples were then subjected to Agar diffusion method for the determination of antibacterial nature [63 - 65] against five different microorganism namely *Escherichia coli* (MTCC 443),

Klebsiella pneumoniae (MTCC 530), *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 737) and *Streptococcus mutans* (MTCC 890) which were purchased from Microbial Type culture collection and gene bank (MTCC) at Chandigarh. KB cells were purchased from National Centre for Cell Science (NCCS) Pune.

The obtained bacterial cultures were maintained on nutrient agar. The bacterial test suspension containing 1.5×10^{8} cells were spread on a nutrient agar plate. The samples were added to the wells of 6 mm and then kept in the incubator for 24 hours at 37 °C. Thus, the zone of inhibition was measured in millimetres. The standard antibiotic used was streptomycin. For cell viability assay, KB Oral cancer cells were seeded in 96 well plates, and the plate was kept in the incubator for nearly about one day around 37 °C. Then MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] reagent was added to the plates after removing from the incubator. The plate was then again wrapped with aluminium to avoid exposure from light. After incubating the plate again for three hours, the reagent was removed, and 100 μL of DMSO (Dimethyl sulfoxide) was added to the mixture. The absorbance was recorded using 570 nm and 630 nm as standard wavelength. The viable percentage of the pure and the modified samples were determined.

3. Results and discussion:

3.1. XRD determination of pure and modified NPs:

The Pure and modified samples are analyzed using XRD method. **Figure 2** displayed the XRD pattern of the nanoparticles. The XRD pattern exhibited peaks at 25.3°, 37.8°, 48°, 54.7°, 63°, 70° and 75.7°. These peaks are corresponding to the crystal planes (1 0 1), (0 0 4), (2 0 0), (1 0 5), (2 0 4), (2 2 0) and (2 1 5) respectively which is found to be in agreement with the

JCPDS No: 21-1272 [66 - 67]. The sharp peak around the crystal plane (1 0 1) indicates that they are anatase in nature [68]. From the **Figure 3**, it is observed that the modified samples are found to be less intense than the Pure samples. This clearly showed that pure nanoparticles are more crystalline than the modified samples. From the XRD pattern, a peak observed around 30.7° is assigned to (2 1 1) plane which was confirmed to be brookite phase. **Figure 4** represents the XRD pattern of the Pure TiO_2 Nanoparticles.

3.2 UV analyses and TEM analyses for Pure and modified TiO_2 NPs:

Figure 5 showed the UV analyses for the Pure and modified samples. It is observed that the maximum absorbances for **S1**, **S2, S3, S4, S5** and **S6** are found to be 315 nm, 382 nm, 380 nm, 392 nm, 399 nm and 366 nm respectively. According to Trivedi et al. [71], Ashwagandha extract showed maximum absorbance around 208 nm. The maximum absorbance for the Pure sample has been observed at 264 nm which is in good agreement with M.M Karkare [69]. Hasan N et al. [70] reported that the maximum absorbance for pure nanoparticles was found to be 337 nm. The maximum absorbances for modified TiO_2 are found to be red shifted when compared with that of the pure NPs. This was due to the chemisorption of Ashwagandha, Karisalankanni and Athimathuram molecules on the TiO_2 surface.

From the results, it is observed that there is a good interaction between the dopants and the TiO_2 nanoparticles and hence the dopants have modified the surface of the TiO_2. The red shift also showed that there is an increase in the particle size of the modified TiO_2 nanoparticles when compared with that of Pure samples. 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 [100, 101]. This is responsible for the production of superoxide radicals which will enhance photocatalytic activity further [102]. **Figure 6** represents the UV absorbance for the Extracts.

Figure 7 shows the TEM, HRTEM images and d spacing for Pure TiO_2 samples. **Figure 8 and 9 showed** the TEM images of Pure and modified TiO_2 samples at 50 nm and 100 nm resolution respectively. **S3** and **S5** samples exhibited more agglomeration when compared with other TiO_2 samples. **Figure 10** displayed the HRTEM images of Pure and modified samples. The lattice fringes are seen clearly which confirm the crystalline nature of the modified samples. **Figure 11** showed the particle size distribution of the Pure and modified NPs. From the diagram, it is found that the average particle size is found to be 7.5 nm for Pure and 9.5, 12.5, 12.5, 12.5, 12.5 and 11.5 nm for **S1**, **S2**, **S3**, **S4**, **S5** and **S6** respectively. The particle size for the modified samples increases when compared with that of the pure samples, and this is confirmed from the TEM analyses. These decrease the band gap of the material and hence, enhance the biological activity further.

3.4. FTIR investigations of Pure TiO_2 and modified nanoparticles:

Figure 12 represents the FTIR Spectra of the Pure TiO_2 Nanoparticles. **Figure 13 and Figure 14** showed the characteristic peaks of the FTIR spectra of the extracts (**E1, E2** and **E3**) and the modified samples. From the spectra, it is observed that **E1** showed peaks at 3342 (O-H stretching), 1376 (C-H alkane bending), 1617 (C=O stretching), 1022 (C-O alkoxy stretching) and 634 cm^{-1} (C-H aromatic bending) which is in good agreement with Trivedi et al. [71] , **E2** showed characteristic absorption bands at 3322 (O-H stretching), 1739 (C=O stretching), 1617 (C=C alkene stretch), 1405 (aromatic stretch C=C), 1031 (C-O ether stretch) and 673 cm^{-1} (N-H amine stretch) which is in good agreement with Kamble [72]. According to Dinesh [73], spectral peaks observed at 2953 (C-H stretching vibration), 1739 (C=O), 1602 (C=C), 1370 (C-O), 1027 (C-O-C) and 727 cm^{-1} confirm the peaks with E3 sample.

The peak at 1739 cm^{-1} present in **E2**, vanishes when combined with **E1**. Similarly, the peak at 1739 cm^{-1} present in the **E2** and **E3** extract vanishes when both extracts are combined as **E6** extract. Withanolide, an active ingredient of Ashwagandha, had the characteristic band at 1617 and 1022 cm^{-1} (C=O and C-O stretching vibrations) [74]. Wedelolactone, an important component of Karisalankanni whose prominent peaks are at 1617 cm^{-1} (lactone carbonyl) and 1739 cm^{-1} (C=C) [75]. Glycyrrhizin, constituent of athimathuram exhibited peaks at 2953 (C-H aromatic stretch) and 1602 cm^{-1} (C=C stretch) [76]. **Figure 14** showed the FTIR Spectra for the modified samples in the range 1000 - 400 cm^{-1}. A sharp peak is observed in all the modified samples at 1634 and 1635 cm^{-1} which indicate clearly that the dopants have reacted with titanium carboxylate from TTIP.

3.5. Anticancer activities of pure and modified samples:

KB oral cancer cell lines are subjected for the determination of anticancer activities. The pure and modified samples are analyzed for their anticancerous activity with different concentrations of 10, 20, 30, 40 and 50 µg / mL respectively. Camptothecin is the standard drug used for the analysis. As the concentration of the sample increases, the anticancerous activity also increases. It is reported that for a particular concentration there was variation in the cell viabilities for different samples. It is observed that pure TiO_2 nanoparticles showed more viability percentage when compared with the modified samples, which proved that plant dopants when treated with TiO_2 act as good anticancer agents. **Figure 15** showed the concentration vs cell viability graph of the KB cells treated with seven different TiO_2 samples for five different concentrations**.**

From the **Figures,** it is observed that for 10 µg / mL of Pure TiO_2, **S1, S2, S3, S4, S5** and **S6** samples showed 98.28% , 32.07%, 39.977% , 47.66%, 39.08%, 57.41 % and 56.04 % viabilities, for 20 µg / mL showed 77.32 %, 31.29 %, 31.06 %, 31.84 %, 28.06 %, 49.44 % and 41.81 % viabilities, for 30 µg / mL showed 69.73 %, 20.26 %, 24.94 %, 18.15 %, 16.59 %, 26.56 % and 35.04 % viabilities , for 40 µg / mL showed 60.07 %, 18.70 %, 7.68 % , 8.017 %, 11.69 , 21.85 % and 20.05 % viabilities and for 50 µg / mL showed 50.67 %, 5.34 %, 2.33 % , 4.342 % , 3.34 % , 14.65 % and 7.88 % viabilities respectively which clearly indicated that as the concentration increases, cell viability decreases which in turn increases the anticancer activity. Karisalankanni modified TiO2 (**S2**) and Ashwagandha - Karisalankanni modified TiO_2 (**S4**) exhibit excellent anticancer activity on KB oral cancer cell line. From the experimental data, one can conclude that Karisalankanni is able to destroy maximum cancer cells when modified with Pure TiO_2. The increase in the particle size of the **S2** and **S4** modified samples decreases the band gap which is responsible for the production of superoxide radicals that destroys the cancer cells.

Rai M et al. [77] reported that withanolide; a component present in *Withania somnifera* is very effective against various cell lines. Yang Z et al. [78] observed that the root extracts of *Withania somnifera* inhibited breast cancer in rats with lesser side effects. Jha et al. [79] studied the activity of *Azadirachta indica, Withania somnifera and Ocimum sanctum* with ethanol solvent against the cancer cells and finally reported that *Withania somnifera* showed potent activity against cancer. Yadav N K et al. [80] studied *Eclipta alba* against seven different cell lines such as MDA - MB-231, HeLa, SK-OV-3, SW 620, Du145, A549 and PANC-1 but among them *alba* species inhibited all the seven cell lines, and inhibition rate was more for MDA-MB-

231 (breast) cancer cell line. *Eclipta alba* showed anticancer activity on HepG2 cells [81], SMMC - 7721 [82], Ovarian cells [83 - 84].

According to Priya M M et al. [55] wedelolactone, the major constituent of *Eclipta prostata* is responsible for the anticancer activity against Oral cancer cell line. She also pointed that 100, 200, and 300 microgram of the sample is able to inhibit 30 %, 50 % and 70 % of the cells respectively. From the diagram, it is observed that Pure Karisalankanni have 30 % of inhibition rate for 100 µg compound whereas the doped particles revealed 60 % of inhibition rate for 10 µg test compound. As the concentration of the sample increases, the ROS production increases [49] which in turn might destroy the cancer cell wall and therefore the activity increases. This is explained with the phenomena as the concentration increases the number of nanoparticles easily enters inside the cells and generates excess free radicals which are responsible for the destruction of the cancer cells [85].

3.6. Antibacterial activities using TiO_2 nanoparticles and bioagents:

The synthesized Pure and modified TiO_2 nanoparticles are subjected for their antibacterial activity using Agar diffusion technology against three Gram-negative bacteria such as *Escherichia coli* (*E.coli*), *Pseudomonas aeruginosa* (*P.aeruginosa*), *Klebsiella pneumonia (K. pneumoniae*) and two Gram- positive bacteria named as *Staphylococcus aureus* (*S.aureus*) and *Streptococcus mutans* (*S.mutans*). Streptomycin is used as a standard antibiotic. **Figure 16** showed the zone of inhibition against Gram-negative and Gram-positive bacteria. From **Figure 16,** it is observed that **S4** and **S6** samples exhibited antibacterial activities against *E.coli*. **S6** sample showed antibacterial activity with *K.pneumoniae*. Pure TiO_2, **S3 and S4** samples exhibited activities against *S.aureus* and *S.mutans*. The maximum zone of inhibition is seen for **S6** samples against *E.coli*. Bhudhiraja et al. [86] observed that the pharmacological properties of

the *Withania somnifera* are due to the presence of with anolides, a major constituent present in the medicinal herb.

Saidulu C H [87], studied the acetone, aqueous and petroleum ether extract against four pathogens namely *S.aureus, B.subtilis, E.coli* and *P.aeruginosa* and he found that acetone, aqueous and petroleum ether showed largest, intermediate and no zone of inhibition respectively. It is also reported that leaves showed more activity than roots. According to Bhatt S et al. [88], no activity is observed for aqueous extract of Ashwagandha against bacterial strains using disc diffusion technology. Kaur S et al. [89] reported methanolic extracts of *Withania somnifera* showed the highest antibacterial activity when compared with other extracts.

According to Pandey M K [90], the hexane extracts of *Eclipta alba* showed the highest antibacterial activity whereas the aqueous extracts have moderate antibacterial nature against *S.aureus, E.coli, K.pneumoniae, S.pyogenes* and *P.aeruginosa*. Dalal et al. [60] also pointed out that the antibacterial activity of *Eclipta alba* is due to the presence of wedelolactone, a component present in *Eclipta alba* species. Gurrapu S [91] observed that alcoholic extract has more antibacterial nature against bacterial strains and also pointed out that the OH group present in the alkaloid enhances the antimicrobial nature. Gupta V K et al. [92] observed that the *Glycyrrhizin glabra* showed antimicrobial nature due to the presence of glabridin. Anagha K et al. [93] showed that antibacterial activity is better in aqueous and ethanolic extracts of the species.

It is observed that the **S3** samples showed the highest zone of Inhibition for *S.aureus* and *S.mutans*, **S4** sample for *S.aureus* and **S6** sample for *E*.coli. It is observed that addition of Ashwagandha and Karisalankanni to the pure sample decreases the activity against Gram-

positive bacteria, whereas addition of Athimathuram and Ashwagandha - Karisalankanni to the Pure TiO_2 enhances the activity further for *S.mutans* and *S.aureus.* This might be due to the phytochemicals found in these extracts. The combined effect of Athimathuram - Karisalankanni and Ashwagandha - Karisalankanni with Pure TiO_2 enhances the antibacterial activity further when compared with Pure samples against *E.coli*. There is no activity for Pure and modified samples against *P.aeuroginosa*. Overall the maximum zone of inhibition is found in **S6** against *E.Coli*. Glycyrrhizin, glabrene and glabridin (Athimathuram) and wedelolactone (Karisalankanni) may be responsible for the generation of Reactive Oxygen Species (ROS) which leads to the maximum damage in the bacterial cell walls. The minimum zone of inhibition is observed for Pure TiO_2 against bacterial strains.

Singh J et al. [94] reported that TiO_2 has three phases and among them, the anatase phase exhibited excellent antibacterial activity due to its photocatalytic property. Silver nanoparticles synthesized from plant materials such as curciligo orchioides [95] and aloe vera [96] exhibit excellent antimicrobial activity against bacterial strains. According to Zhang H et al. [97], the TiO_2 nanoparticles act as a positive charge and test organism act as negative charge, and an electrostatic attraction takes place between them which leads to the damage of the bacterial cell wall. Niazi J H and Arré G [98 - 99] explained in their findings that there is a disruption in the bacterial cell wall due to the increase in the membrane fluid caused by the lipid peroxidation and this observation is mainly due to the photocatalytic behaviour of the TiO_2 nanoparticles.

4. Conclusion:

Titanium dioxide nanoparticles modified with bioagents such as Ashwagandha, Karisalankanni and Athimathuram have been prepared using the hydrothermal method. X-ray

diffraction results revealed the anatase nature of the samples. From the TEM analyses, it is observed that there is an increase in the particle size of the bio modified samples. The samples are then examined for their antibacterial activities against three Gram negative bacterial strains namely, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and two Gram positive bacterial strains namely, *Staphylococcus aureus* and *Streptococcus mutans.* Among the modified and pure samples, *Withania somnifera* - *Eclipta prostrata* showed good antibacterial nature against Gram positive and Gram negative bacteria. The samples are then subjected to MTT assay to determine the cell viability. KB oral cancer cells are used for the determination of anticancer nature of the Pure and bio modified nanoparticles. It is observed that [S3] *Withania somnifera* - *Eclipta prostrata* modified TiO_2 nanoparticles exhibit excellent anticancer activities among other bio modified and Pure samples. Thus the experimental overall results indicate bio modified samples showed better activities when compared with pure TiO_2 nanoparticles.

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Figure caption:

Figure 1 : Chemical structure of Withanolide , Wedelolactone, Glycyrrhizin , Glabridin and Glabrene

Figure 2 : XRD patterns of (a) Pure TiO_2 (b) S1 (c) S2 (d) S3 (e) S4 (f) S5 (g) S6

Figure 3 : Expanded XRD patterns of (a) Pure TiO_2 (b) S1 (c) S2 (d) S3 (e) S4 (f) S5 (g) S6 (Between $2\theta = 24°$ and $27°$)

Figure 4 : XRD pattern of Pure TiO_2 Nanoparticles

Figure 5 : UV Spectra of (a) Pure TiO_2 (b) S1 (c) S2 (d) S3 (e) S4 (f) S5 (g) S6 nanoparticles.

Figure 6 : UV Spectra of (a) E1 (b) E2 (c) E3 (d) E4 (e) E5 (f) E6 extracts.

Figure 7 : (a) TEM, (b) HRTEM images and (c) d spacing for Pure TiO_2 nanoparticles.

Figure 8 : TEM images of (a) S1 (b) S2 (c) S3 (d) S4 (e) S5 (f) S6 nanoparticles for 100 nm

Figure 9 : TEM images of (a) S1 (b) S2 (c) S3 (d) S4 (e) S5 (f) S6 nanoparticles for 50 nm

Figure 10 : HRTEM images of (a) S1 (b) S2 (c) S3 (d) S4 (e) S5 (f) S6 nanoparticles.

Figure 11 : Distribution of average particle size of Pure TiO2 , S1, S2, S3, S4, S5 and S6 samples

Figure 12 : FTIR Spectra of Pure TiO_2 and Ashwagandha modified Nanoparticles

Figure 13 : FTIR Spectra of (a) E1 extract (b) E2 extract (c) E3 extract (d) E4 extract (e) E5 extract (f) E6 extract

Figure 14 : FTIR Spectra (i) 4000 - 400 cm^{-1} and (ii) 1000 - 400 cm^{-1} for (a) S1 (b) S2 (c) S3 (d) S4 (e) S5 (f) S6 samples

Figure 15 : Toxicity profiles (concentration vs cell viability) of the KB cells treated with Seven different TiO_2 samples for various concentrations [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] - All the experimental results were performed in triplicate and the results were expressed as mean ± Standard Deviation (SD). Data represented here shows the statistical difference (p-value < 0.005) between the treated and control samples.

Figure 16 :

Graph showing the antibacterial activity of (a) Pure TiO_2 (b) S1 (c) S2 (d) S3 (e) S4 (f) S5 (g) S6 nanoparticles against *E.coli*, *K.pneumoniae*, *P.aeruginosa*, *S.aureus* and *S. mutans* . All the experimental results were performed in triplicate and the results were expressed as mean ± Standard Deviation (SD). Data represented here shows the statistical difference (p-value < 0.05) between the treated and control samples.

Table 1: Volume of the plant extracts

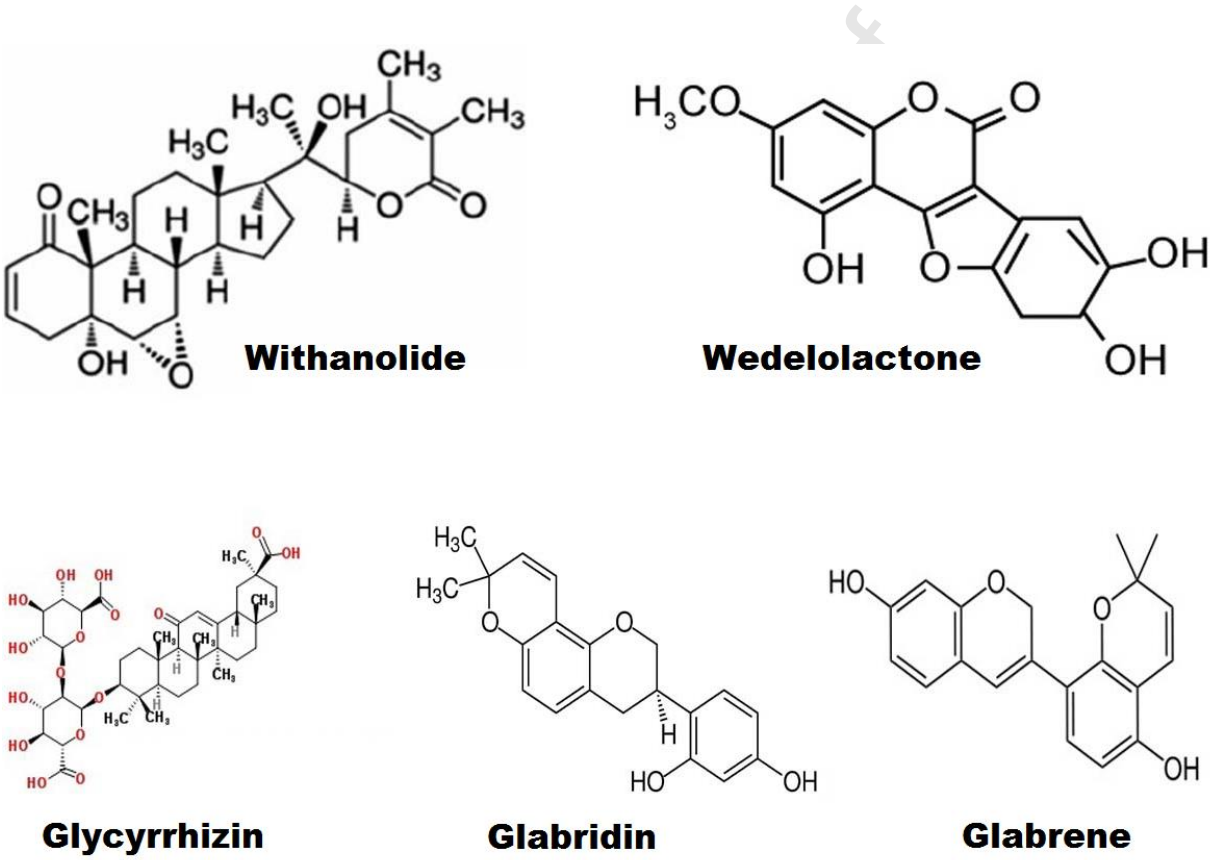


Figure 1

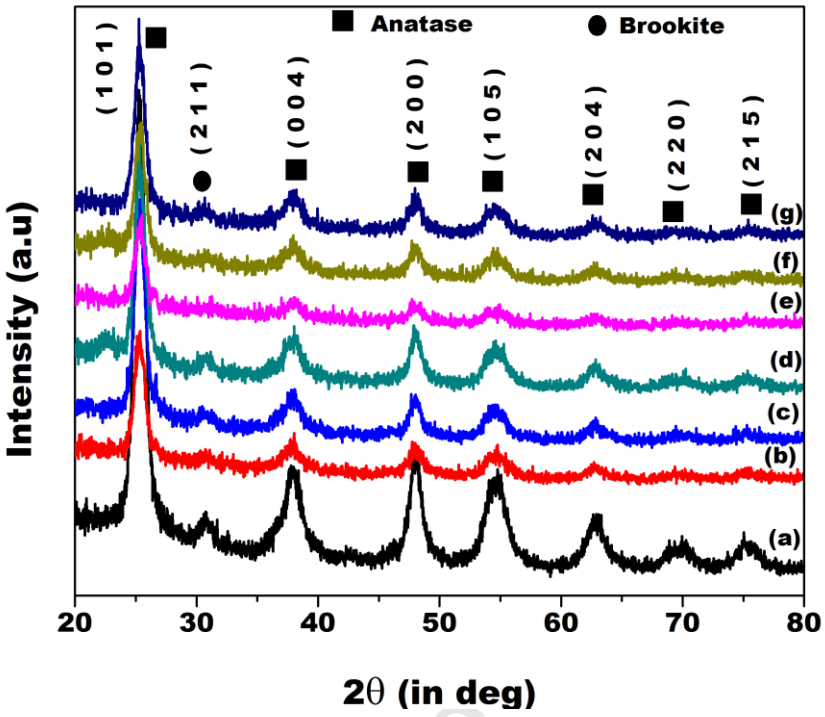
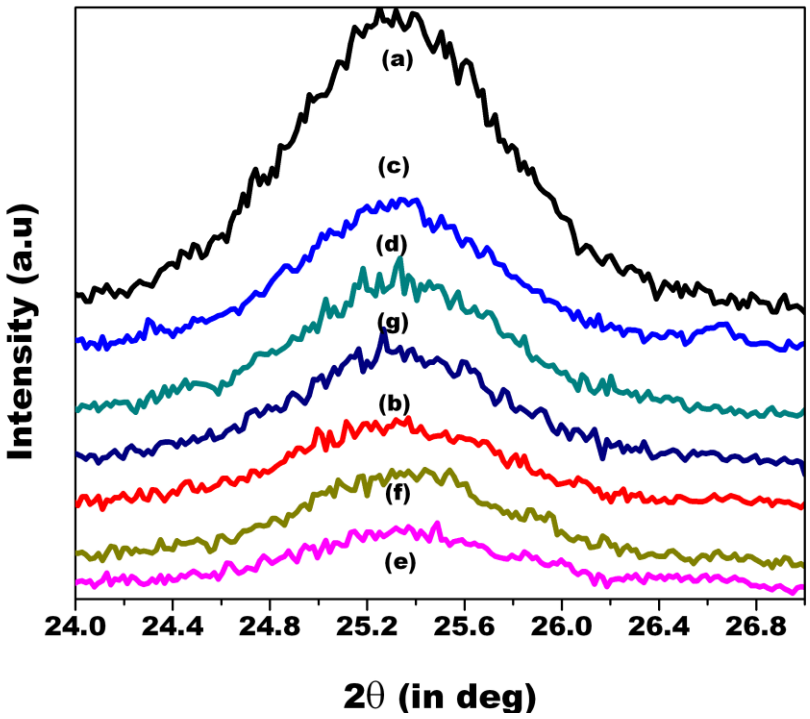


Figure 2



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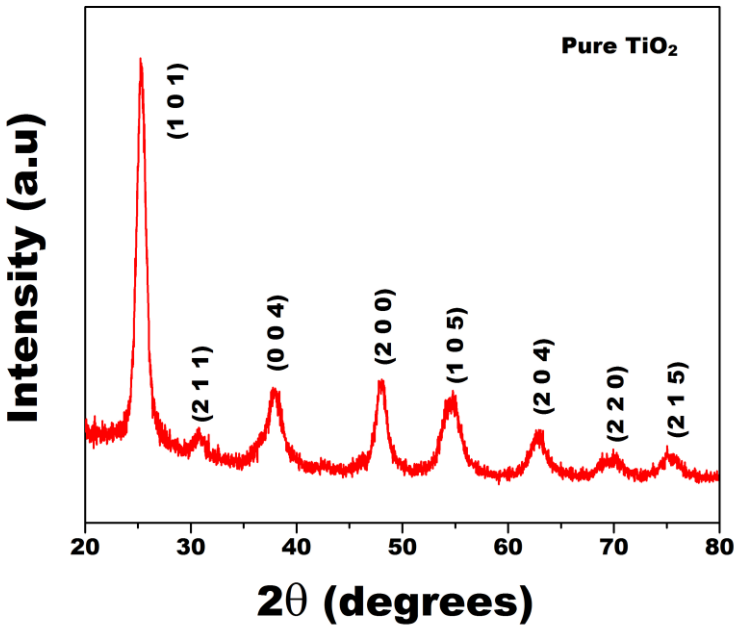

Figure 4

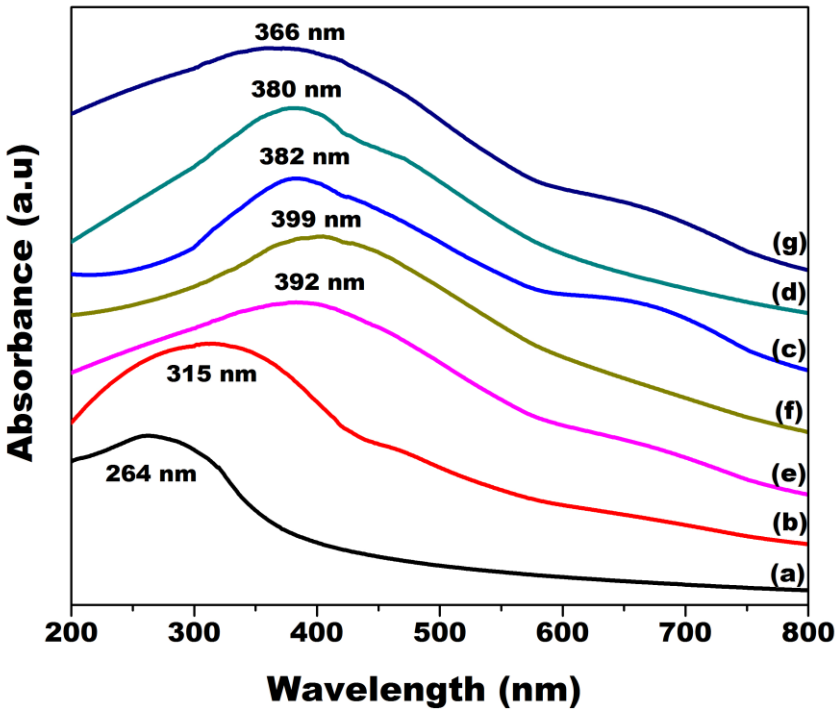

Figure 5

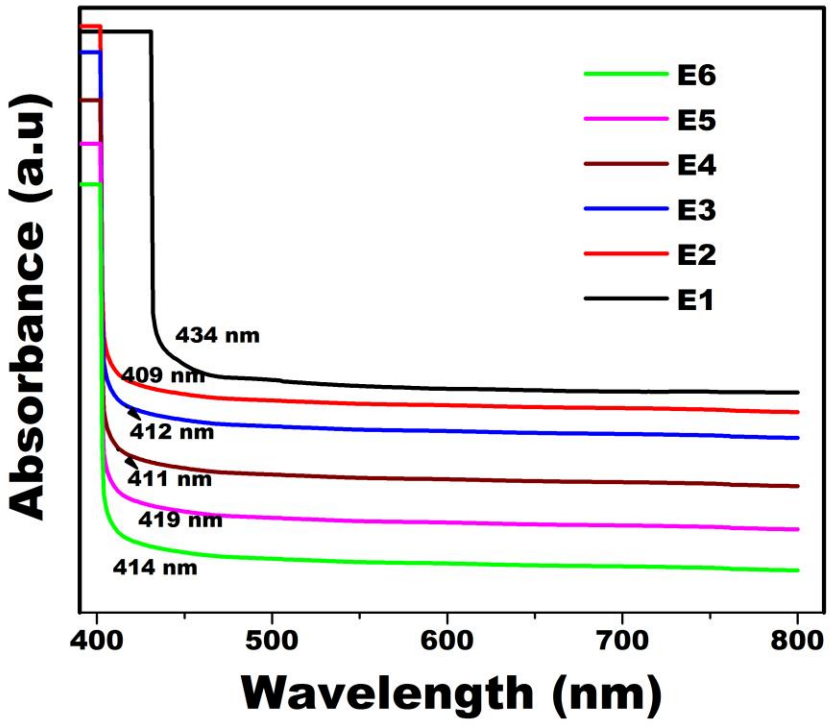

Figure 6

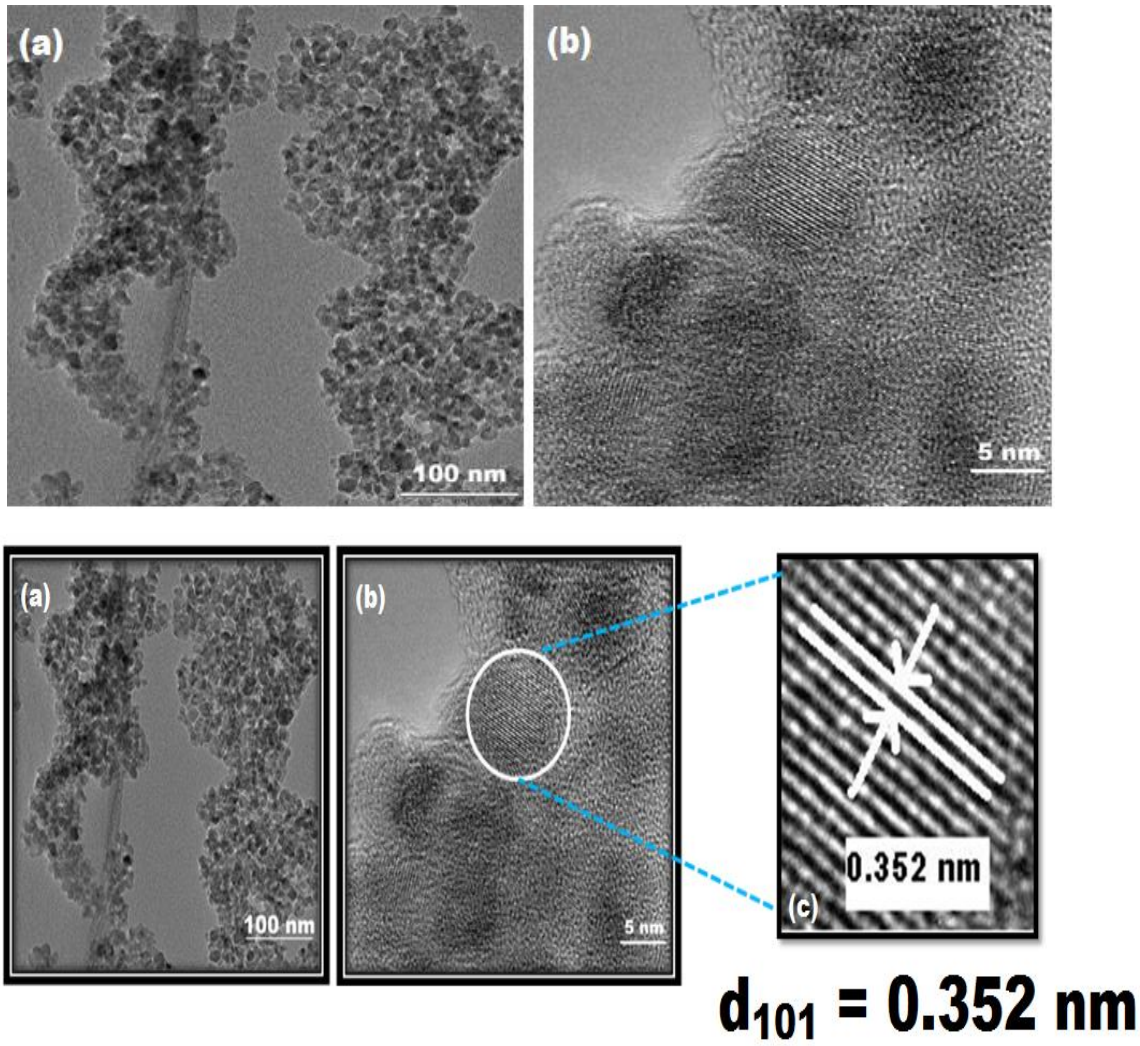

Figure 7

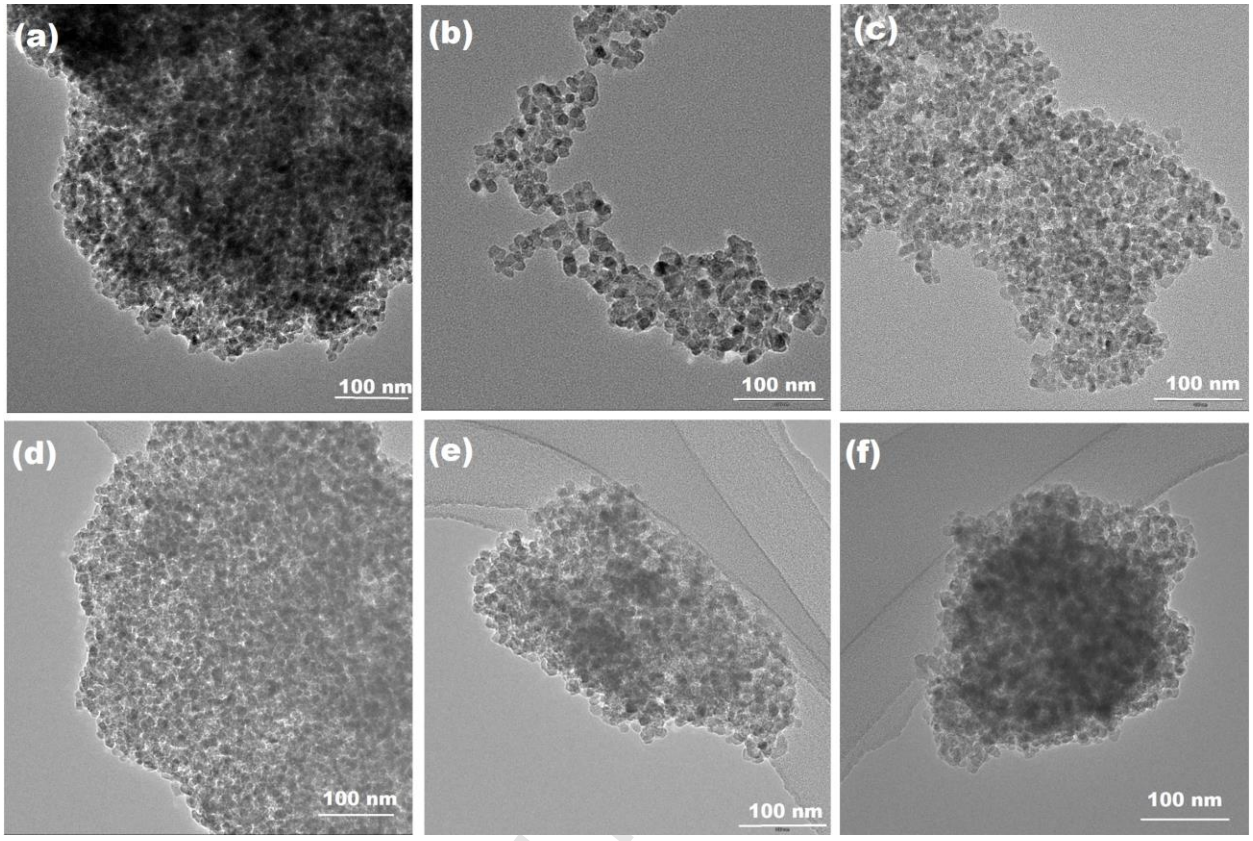

Figure 8

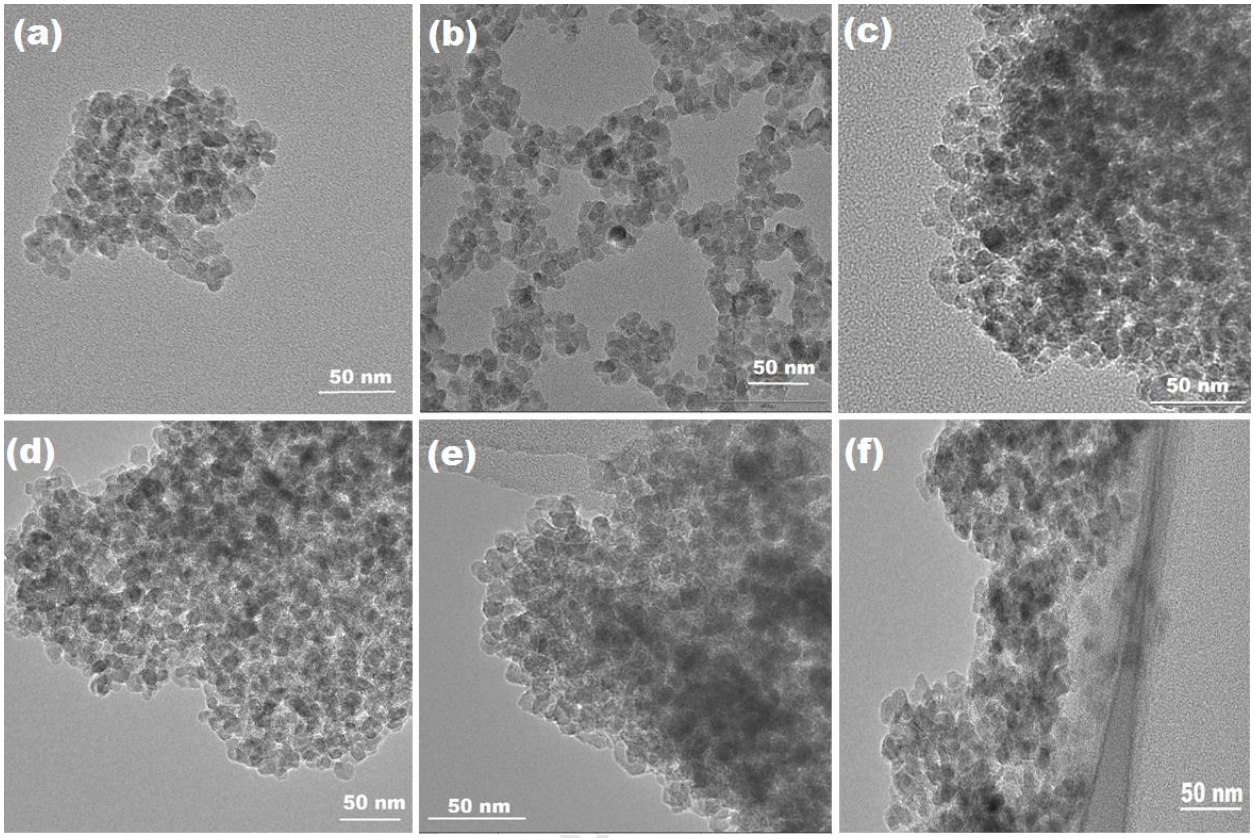

Figure 9

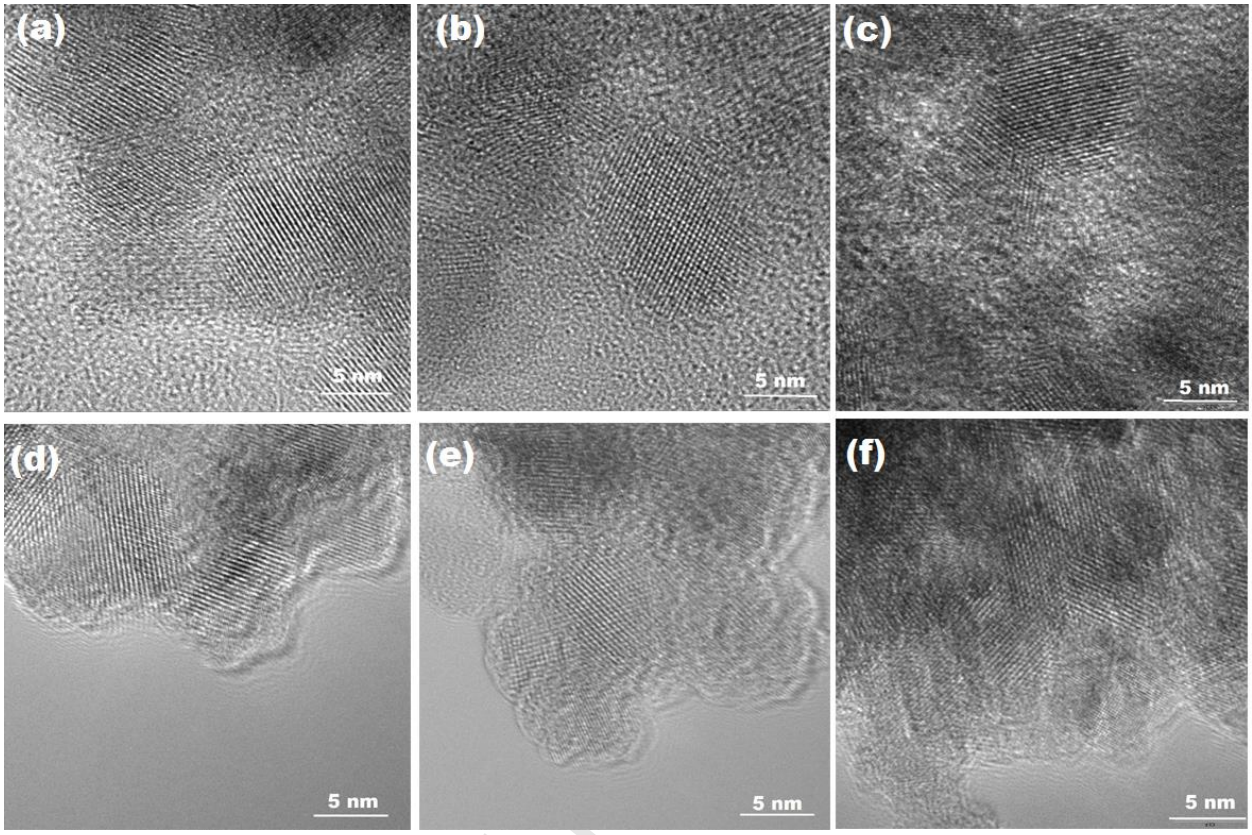

Figure 10

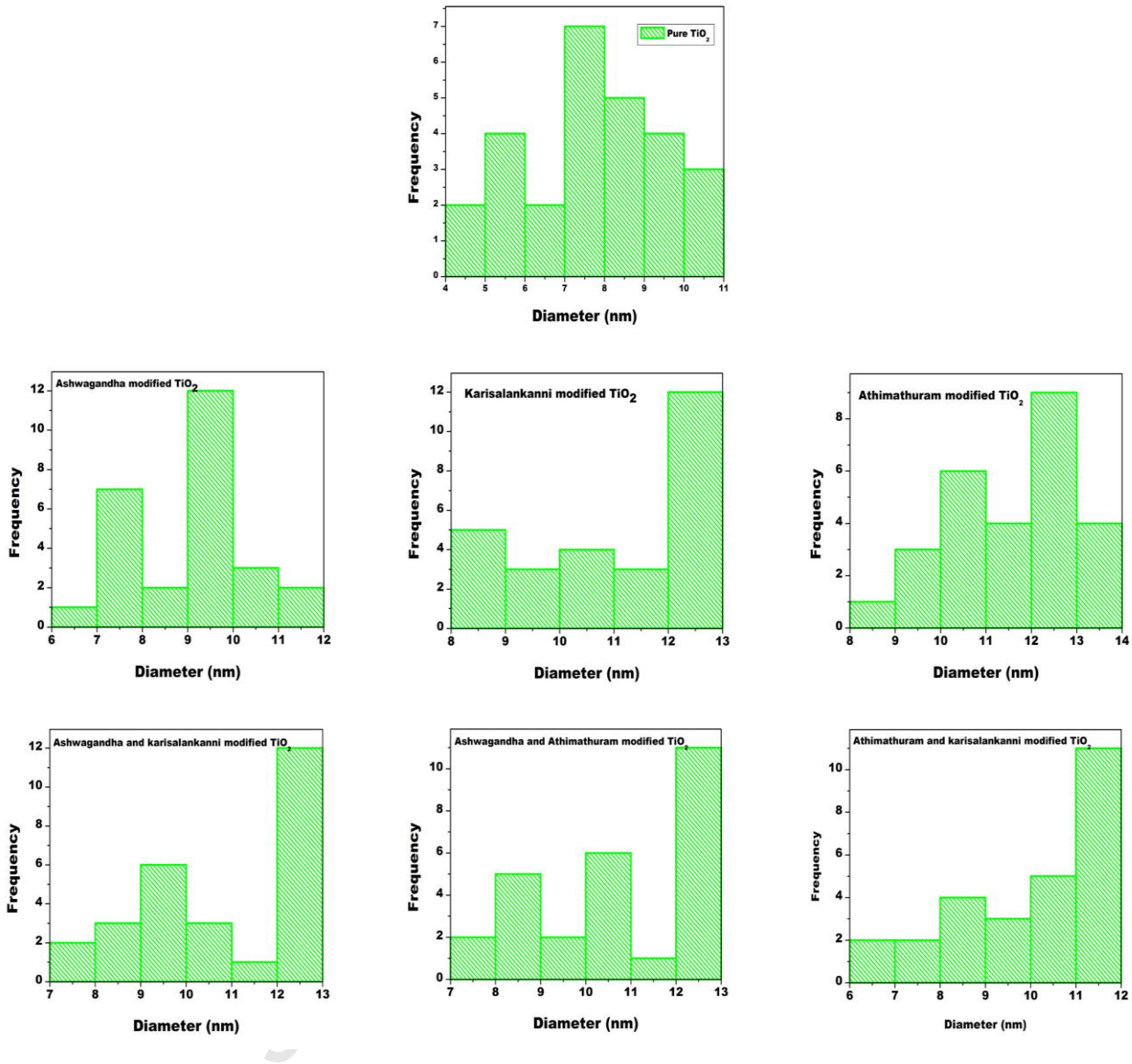
Pure TiO2
Frequency
Diameter (nm)
Ashwagandha modified TiO2
Karisalankanni modified TiO2
Athimathuram modified TiO2
Ashwagandha and karisalankanni modified TiO2
Ashwagandha and Athimathuram modified TiO2
Athimathuram and karisalankanni modified TiO2

Figure 11

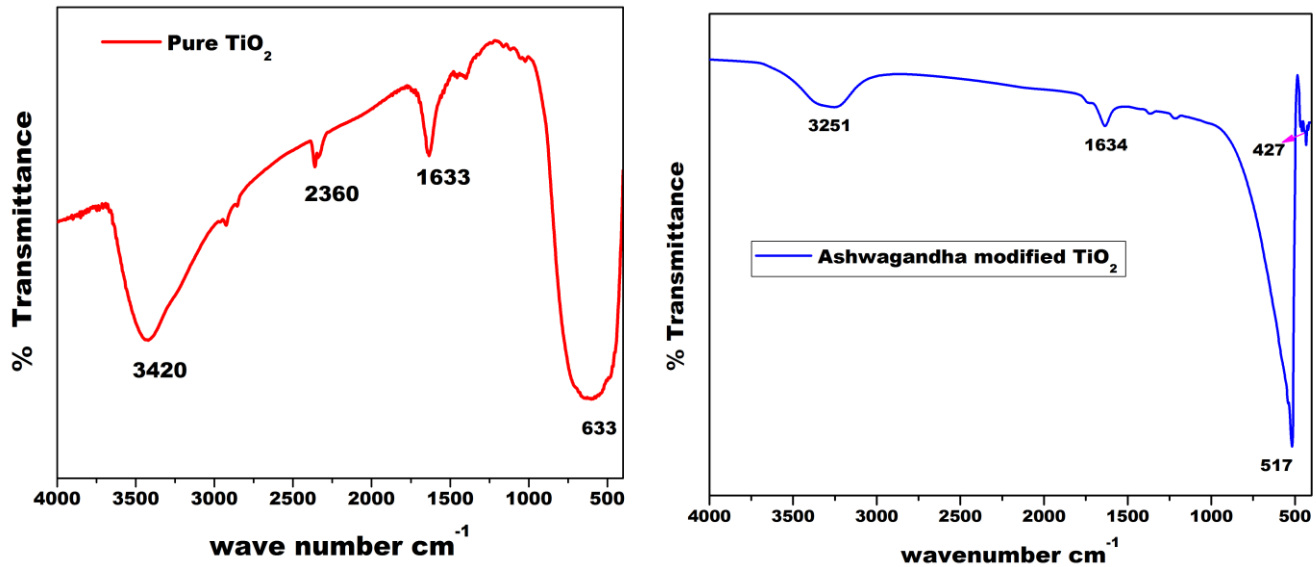

Figure 12

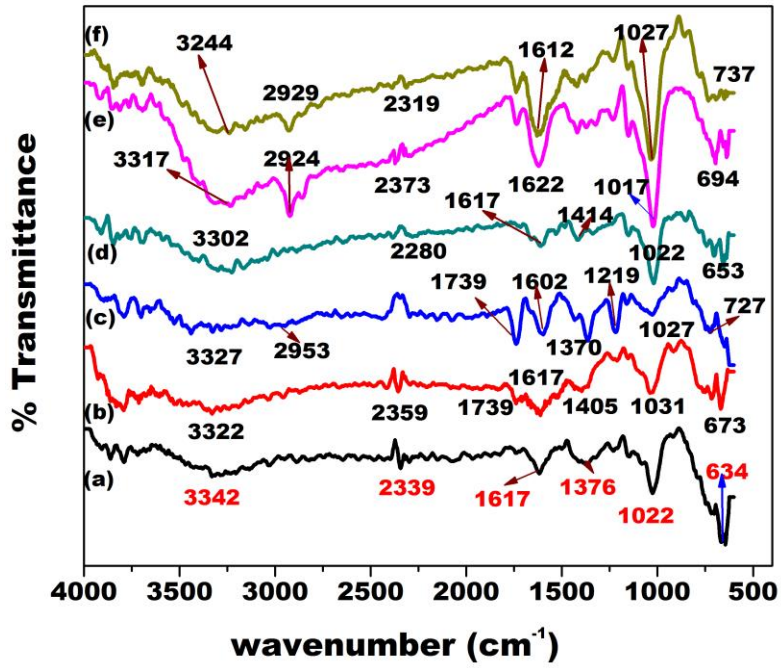

Figure 13

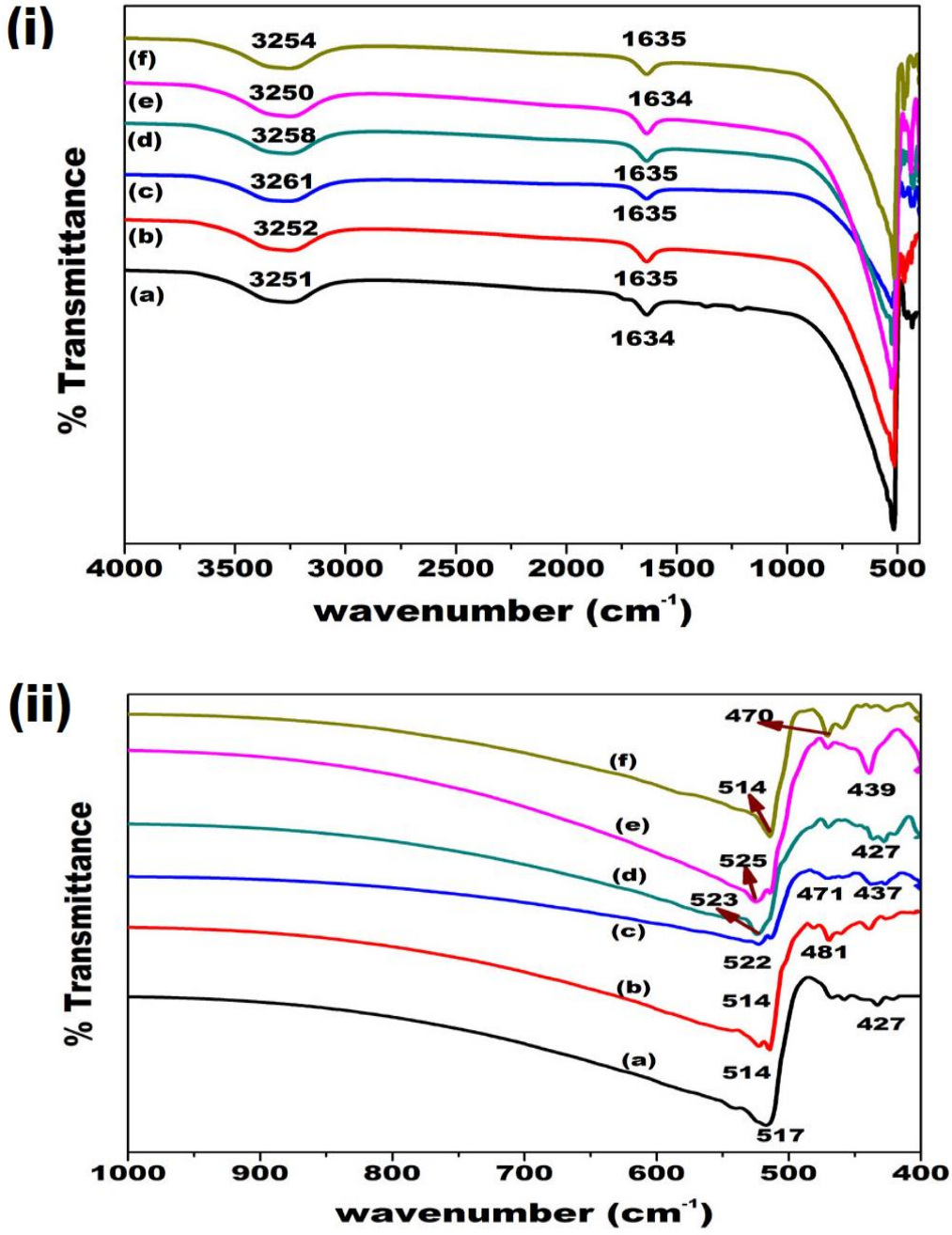
(i)
3254
1635
(f)
3250
1634
(e)
3258
(d)
3261
1635
(c)
1635
3252
(b)
3251
1635
(a)
1634
% Transmittance
4000 3500 3000 2500 2000 1500 1000 500
wavenumber (cm-1)
(ii)
470
(f)
514
439
(e)
525
427
(d)
523
471 437
(c)
522
481
(b)
514
427
(a)
514
517
% Transmittance
1000 900 800 700 600 500 400
wavenumber (cm-1)

Figure 14

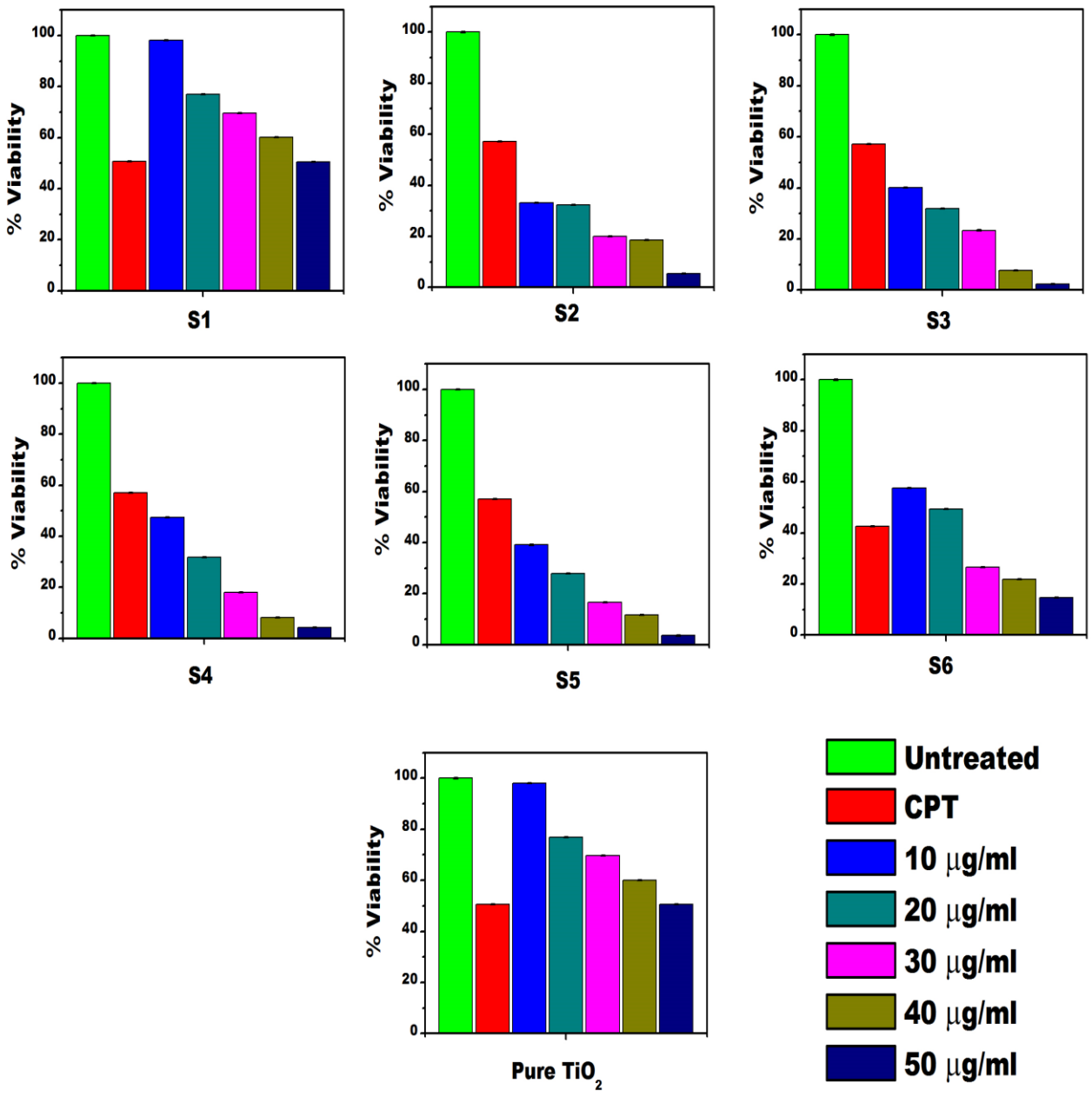
% Viability
S1
S2
S3
S4
S5
S6
Pure TiO2
Untreated
CPT
10 μg/ml
20 μg/ml
30 μg/ml
40 μg/ml
50 μg/ml

Figure 15

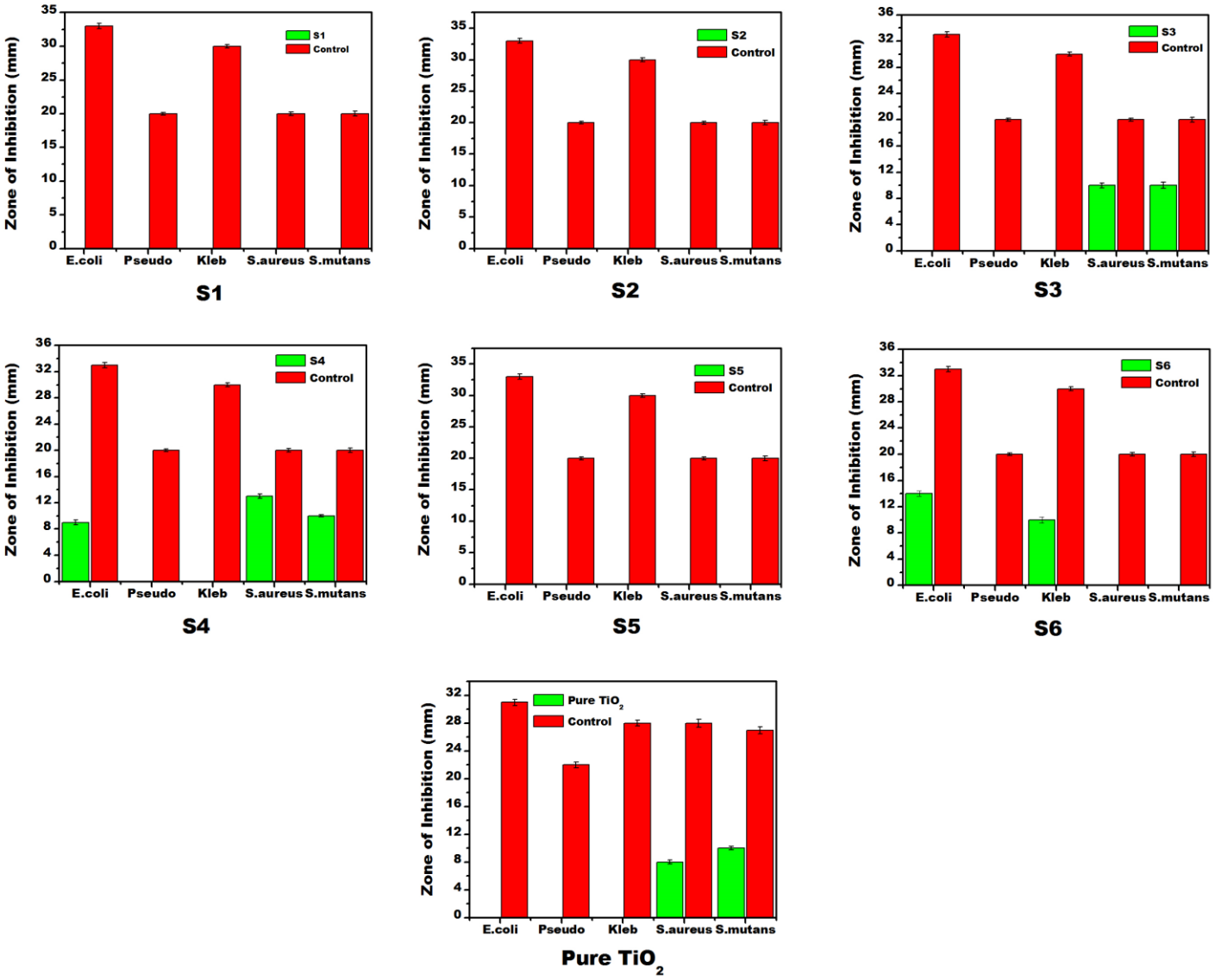
Zone of Inhibition (mm)
S1
Control
E.coli
Pseudo
Kleb
S.aureus
S.mutans
S1
S2
S3
S4
S5
S6
Pure TiO2
Pure TiO2

Figure 16

S. No	Extract	Volume	Ratio of volume		
			E1	E2	E3
1	E4	4 mL	2 mL	2 mL	
2	E5	4 mL	2 mL		2 mL
3	E6	4 mL		2 mL	2 mL

Table 1

Research Highlights

- Pure and Bio-modified TiO_2 NPs were synthesized by hydrothermal method.
- ***Withania somnifera*** (ashwagandha), ***Eclipta prostrata*** (karisalankanni) and ***Glycyrrhiza glabra*** (athimathuram) were used to modified TiO_2 NPs.
- 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.