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Biosynthesis, Characterization of Tio$_2$ Nanoparticles by Using Solanum Xanthocarpum Berry Extract and Their Biomedical and Photocatalytic Activity

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Abstract: Green synthesis of nanoparticles using plant extract is the novel method to develop environmentally benign nanoparticles which can be used in numerous biomedical applications. The present study deals with the green synthesis of titanium dioxide nanoparticles (TiO_2) using Solanumxanthocarpum Berry Extract without any catalyst, template, and surfactant. Colloidal TiO_2 were synthesized by reacting aqueous titanium (IV) is protoxide with Solanumxanthocarpum Berry Extract. The structural, morphological and optical properties of the synthesized nanoparticles have been characterized by using XRD, FTIR, TEM and HR-SEM with EDX analysis. The XRD pattern with sharp peaks describes the crystallinity and purity of titanium dioxide nanoparticles. FT-IR confirmed the presence of functional groups of both leaf extract and TiO_2 NPs. The morphological characterization of synthesized nanoparticles was analyzed by FE – SEM. The intense, narrow width of Titanium and Oxygen have high purity and crystalline were identified using EDX. This report also explains the efficient antibacterial activity of biostabilized TiO_2 nanoparticles when compared to chemically synthesized TiO_2. These nanoparticles were used for photocatalytic degradation of Methylene blue (MB) dye under solar light irradiation at room temperature. Effect of the amount of catalyst on the rate of photo degradation was investigated.

Keywords: Green synthesis, Berry Extract, TiO_2 nanoparticles, antibacterial and photo catalytic activity

I. INTRODUCTION

Nanotechnology is a field of science which deals with production, manipulation and use of materials ranging in nanometers. With the advancement of technologies and improved scientific knowledge a way for research and development in the field of herbal and medicinal plant biology towards intersection of nanotechnology has been observed. One such interference is applying plants source in the green synthesis of nanoparticles. A green synthesis route for the production of silver nanoparticles using methanol extract from Solanumxanthocarpum berry (SXE) is reported in the present investigation. Nanoparticles can be easily synthesized using various methods by various approaches available for the synthesis of silver nanoparticles include chemical [1], electrochemical [2], radiation [3], photochemical methods [4] and Langmuir-Blodgett [5-6] and biological techniques [7]. But most of the chemical methods used for the synthesis of nanoparticles involve the use of toxic, hazardous chemicals that create biological risks and sometime these chemical processes are not eco friendly. This enhances the growing need to develop environmentally friendly processes through green synthesis and other biological approaches. Sometimes the synthesis of nanoparticles using various plants materials and their extracts can be beneficial over other biological synthesis processes which involve the very complex procedures of maintaining microbial cultures [8-9].

Green synthesis of nanoparticles has been an emerging research area now a day. The advancement of green syntheses over chemical and physical methods is: environment friendly, cost effective and easily scaled up for large scale syntheses of nanoparticles, furthermore there is no need to use high temperature, pressure, energy and toxic chemicals.

The use of plants for the production of TiO_2 nanoparticles has received lots of attention due to its rapid, eco-friendly, non-pathogenic, economical protocol and providing a single step technique for the green synthesis processes [10]. The reduction and stabilization of silver ions by combination of bimolecular such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpinoids and vitamins which are already established in the plant extracts having medicinal values and are environmental benign, yet chemically complex structures [11].

Solanumxanthocarpum, commonly known as yellow-berried nightshade, is a prickly plant, which grows wild in different regions of the Indo-Pakistan subcontinent. It has been reported that this plant contains several steroidal alkaloids like solanacarpine, solanacarpidine, solancarpine, solasonine, solamargine and other constituents, such as caffeic acid, coumarins (aesculetin and aesculin), steroids carpesterol, diosgenin, campesterol, daucosterol) and triterpenes (cycloartanol and cycloartenol) [12].

Fig 1: Solanumxanthocarpum Berries

The fruit from S. xanthocarpum has flavonoids quercitrin and apigenin glycosides as the major chemical constituents. Various medicinal properties have been ascribed to different parts of this multipurpose herb. For example, the root is an expectorant, and is employed in folk medicine systems for the treatment of cough, asthma and chest pain as well as wound healing [13–16]. Fruits are edible, act as an anthelmintic, and are used as a remedy for the treatment of different ailments. A recent study appraises the ant hyperglycemic and antioxidant activities of leaf extracts from S. xanthocarpum on alloxan-induced diabetic rats [17].

The wastewater discharged from the industries like textiles, dye, paper and some other industries contains residual dyes. It has been estimated that more than 10% of the total dyestuff used in dyeing processes is released into the environment which are hazardous to aquatic system and surrounding ecosystem. It clearly delivers that necessary steps would have to be taken for the degradation of such a hazardous dyes. For that there are numerous technologies involving physical, chemical and biological method. The above methods are cost effective, produce secondary sludge, simply transfer pollutants to another phase where it does not destroys and are not completely degrade the dyes. The advanced oxidation process is the efficient technology to degrade the recalcitrant dye pollutants. This technology is highly potent and produces strongly oxidizing radical to destruct the organic pollutant with no selectivity. Among AOPs, the heterogeneous photocatalyst degradation processes are the efficient technology to degrade both aquatic and atmospheric organic contaminants. Methylene blue (MB) (3,7-bis(Dimethylamino)phenothiazin5iumchloride) is a thiazine dye, that causes difficulties in breathing, when consumed it results in vomiting, diarrhea and nausea.

Nowadays semiconductors such as TiO_2, ZnO, CdS, MgO, CdS and Fe_2O_3 are effectively employed in the AOP. In this consortium TiO_2 are most widely used semiconductors for the advanced oxidation processes to degrade pollutants under UV irradiation.

Titanium dioxide has been modern the novel applications in wide areas of science and technology as well as has multiple properties like semiconducting, piezoelectric, pyroelectric, catalysis and optoelectronics [18]. TiO_2 is one of the most widely used material owing to its many applications in the field of photocatalysis, gas and humidity sensors, water treatment, selfcleaning, solar cells, photo electrochemical cells, protective coatings on optical elements and bioanalytical chemistry [19-23]. It is also widely employed as a pigment to provide whiteness and opacity to products such as paints, coatings, plastics, papers, inks, sunscreen, foods, medicines as well as most toothpastes[24-26].

So in the present work very stable Titanium metal oxide nanoparticles (TiO_2Nps) were synthesized using S. xanthocarpum berry extract (SXE). SXE acted both as a reducing as well as a capping agent and evaluating the anti-bacterial effect and photocatalytic activity of biosynthesized TiO_2 in comparison to chemically synthesized TiO_2 and plant extract against human pathogens such as gram-positive and gram- negative bacteria

II. MATERIAL AND METHODS

Titanium tetraisopropoxide [Ti (OCH $(CH_3)_2)_4$], Isopropanol (C_3H_7OH), Acetic acid (CH_3COOH) and ethanol were purchased from Sigma Aldrich, India. All the chemicals were used without any further purification. Nutrient media used for antibacterial activity were purchased from Hi-Media, Mumbai, India. All aqueous solutions were prepared using de-ionized water. All glass wares were cleaned with chromic acid followed by thorough washing with de-ionized water and then acetone for prior use.

A. Preparation of aqueous Berry extract

Solanumxanthocarpum Berries were collected in and around Chidambaram, Cuddalore district, Tamil Nadu, India. Solanumxanthocarpum Berries were washed, shade dried and finely powdered. The powder 100g, was suspended in 250 ml of water for two hours and then heated at 60-65ºC for 30 minutes. The extract was collected separately and the processes were repeated thrice with the residual powder, each time collecting the extract. The collected extracts were pooled and passed through a fine cotton cloth. The filtrates were evaporated at 40-50ºC in a rotavapour under reduced pressure. The dark semisolid material (yield-14%) obtained was stored at 0-4ºC until use[27].

B. Synthesis of Titanium Dioxide Nanoparticles

1) *By chemical method:* TiO_2 nanoparticles were prepared by the sol gel technique. Metal precursor Titanium tetraisopropoxide (2 mL) was added to solvents Isopropanol (2 mL) and acetic acid (1 mL). It was rapidly stirred at room temperature and distilled water was added drop wise to obtain sol gel transformation. It was then air dried and calcined at 500°C for 30 minutes to obtain nanocrystallineTiO_2.
2) *Biosynthesis of TiO_2nanoparticles :*For the synthesis of titanium dioxide nanoparticles, the Erlenmeyer flask containing 0.4M of titanium tetraisoproxide in aqueous Solanumxanthocarpum Berry extract was reacted under stirring at 50°C. After four hours of continuous stirring, the formed titanium dioxide nanoparticles were acquired by centrifugation at 10000 rpm for 15 minutes. Then the centrifuged particles were washed with ethanol and again subjected to centrifugation at 5000rpm for 10 minutes. Separated titanium dioxide nanoparticles were dried and grinded to calcinate at 500°C in muffle furnace for about 3 hours. The calcined titanium dioxide nano powder was used for further analytical techniques.

C. Antimicrobial Activity

In the present study, in vitro antimicrobial activities were carried out by the using of disk-diffusion method. First of all, Petri plates were prepared with 20 mL of sterile Muller Hinton Agar . Then, the 24 h prepared test cultures of inoculums were swabbed on the top of the solidified media and allowed to dry for 10 min. chemically and biosynthesized TiO_2 nanoparticles impregnated disks at the concentrations of 50 µg/mL for bacteria was placed aseptically on sensitivity plates with appropriate controls. The loaded disks were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Ciprofloxacin (5 µg/disk) was used as positive control for bacteria. All the plates were then incubated at 37^0C. The sensitivity was recorded by measuring the clear zone of growth inhibition on agar surface around the disks in millimeter.

D. Photocatalytic Activity

The photocatalytic activity of biosynthesized TiO_2 nano particles was studied by degradation of methylene blue (MB) under solar irradiation. The dye solution was prepared by dissolving 1 mg powder of MB in 100 ml distilled water. 10 mg TiO_2 nanoparticles was added to 50 ml of prepared MB dye solution and the mixer was stirred magnetically for 30 min in shadow before exposing to sunlight. The colloidal suspension was then put under solar irradiation with constant stirring. The average temperature of the atmosphere during the experiment found to be 30 °C with 3.30 hrs mean shine duration. At every 30 min, 5 ml of suspension was collected from the colloidal mixer. The collected suspension was then scanned at different wavelength from 200 to 800 nm using the Shimadzu -1800, UV – Vis Spectroscope to study the dye degradation in presence of TiO_2 NPs.

III. RESULTS AND DISCUSSION

A. Xrd Pattern Of Tio_2

The XRD pattern of TiO_2 nanoparticles obtained using extract of Solanumxanthocarpum Berry and chemically synthesized TiO_2 nanoparticles are shown in Fig 2. A sharp diffraction peak was observed in chemically synthesized TiO_2 nanoparticles, whereas, the intensity of diffraction peak of green synthesized TiO_2 nanoparticles is less with slight broadening. The lattice parameters obtained were close and consistent with standard data for TiO_2 (JCPDS 21-1272). XRD spectra was recorded on the X'PERT PRO model X-ray diffract meter from Pan Analytical instruments operated at a voltage of 40 kV and a current of 30 mA with Cu Ka radiation.

We have calculated the average crystallite size of TiO_2 nanoparticles synthesized by green route and chemical route using the Scherrer'sformula($d = 0.89\lambda/\beta\cos\theta$) The calculated crystallite was found to be 18 nm and 27 nm for green and chemically synthesized TiO_2 nanoparticles respectively. The XRD peaks of green synthesized TiO_2 nanoparticles obtained using extract of Solanumxanthocarpum Berry and chemically synthesized TiO_2 nanoparticles differ in the broadening and intensity. The diffraction peak of the green synthesised TiO_2 nanoparticles is broadened, whereas the peak of chemically synthesized TiO_2 nanoparticles is comparatively sharp. Thus, the broadening of XRD peak of green synthesised TiO_2 nanoparticles observed in our study confirms the size reduction. The XRD peak of chemically synthesized TiO_2 nanoparticles is sharp, thus indicating that their size is still larger than the green synthesised TiO_2 nanoparticles. The intensity of the diffraction peak of green synthesized TiO_2 nanoparticles is less when compared to chemically synthesized nanoparticles.

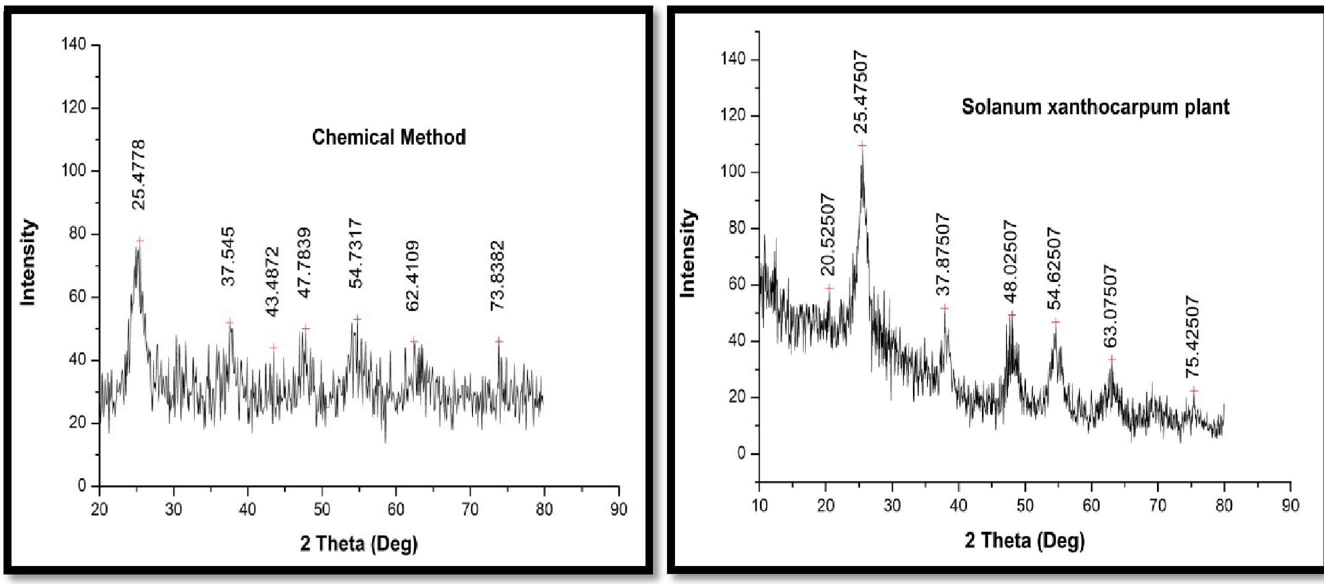


Fig 2: XRD pattern of a) Chemically synthesized TiO_2 NPs (b) Biosynthesized TiO_2 NPs

Therefore, we suggest that the phytochemicals present in the Solanumxanthocarpum Berry Extract would have coated the surface of the TiO_2 nanoparticles, resulting in decreased intensity in XRD peak. This phytochemical coating may enhance the stability and the dispersibility of the nanoparticles, which in turn may enhance their bioavailability, making them suitable for biological applications. The chemically synthesized TiO_2 nanoparticles on the other hand showed comparatively high intense peaks, clearly indicating that they are bare and uncapped.

B. EDX analysis of TiO2 nanoparticles

Energy dispersive X-ray analysis is employed to establish the chemical identity of the synthesized particles. The presence of titanium and oxygen atoms without any other material is observed in the EDX spectrum which also supports XRD results. Absence of any other elements other than titanium and oxygen indicates the elimination of water molecules and other organic residues of Solanumxanthocarpum Berry Extract during calcination of the as-prepared sample. This also confirms the complete conversion of starting material, titanium tetra (IV) is protoxide to TiO2 nanoparticles after calcination at 500°C.

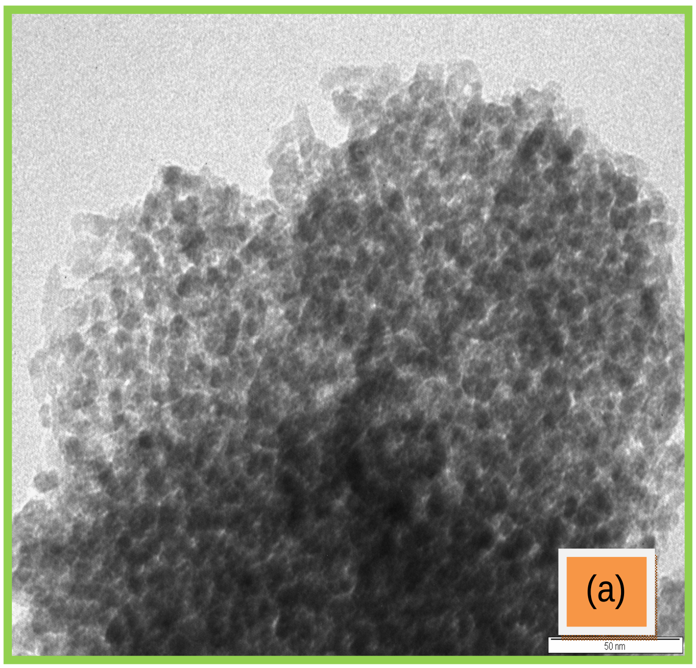
C. Ft-Ir Analysis

The FT-IR analysis was carried out to identify the possible biomolecules responsible for the reduction of $Ti(OCH(CH_3)_2)_4$ and capping them. The FT-IR spectrum of the crude extract depicted some significant peaks at 3350, 1615 and 1435 cm^{-1} demonstrating free OH in molecule and OH group forming hydrogen bonds, carbonyl group (C=O) and stretching C=C aromatic ring, respectively. The hydroxyl groups of phenolics in Solanumxanthocarpum Berry Extract were not only responsible for the reduction of titanyl hydroxide but also function as capping ligands to the surfaces of TiO_2 NPs, as confirmed by FT-IR spectroscopy

Furthermore, the FT-IR spectrum of TiO2 NPs is shown in Figure 3B. The FT-IR of TiO2 NPs shows demonstrative differences in the shape and location of signals indicating the interaction with involved sites of phytochemicals for production of nanoparticles. The peaks at 3420, 1610 and 1486, 1300 and 1100 cm-1 represent the OH functional groups, carbonyl group (C=O), stretching C=C aromatic ring and C-OH stretching vibrations, respectively. This revealed that polyphenolics could be adsorbed on the surface of metal nanoparticles, possibly by interaction through -electrons interaction in the absence of other strong ligating agents. In fact the -electrons in a Red/Ox system can transfer to the free orbital of metal ion and convert that to the free metal.
Moreover, some absorbance bands are lattice vibration modes indicating the functional groups of samples in which the sharp band around 1000 cm-1 is Ti-O-Ti stretching bond. Also, band at 3450 cm-1 represents the primary OH stretching of hydroxyl functional group and the broad band around 1420 cm-1 could be generally attributed to the bending vibration of H-OH groups for TiO_2.

D. TEM

Transmission electron microscopy was used to examine the morphological characteristics of the TiO_2 nanoparticles obtained using Solanumxanthocarpum Berry extract and chemical route respectively (Figure 3). Biosynthesized TiO_2 nanoparticles display a size between 40–60 nm and spherical morphology. It is clear from Figure 3 that the sizes of TiO_2NPs synthesized from green route are almost uniform, and all of the particles are spherical in shape



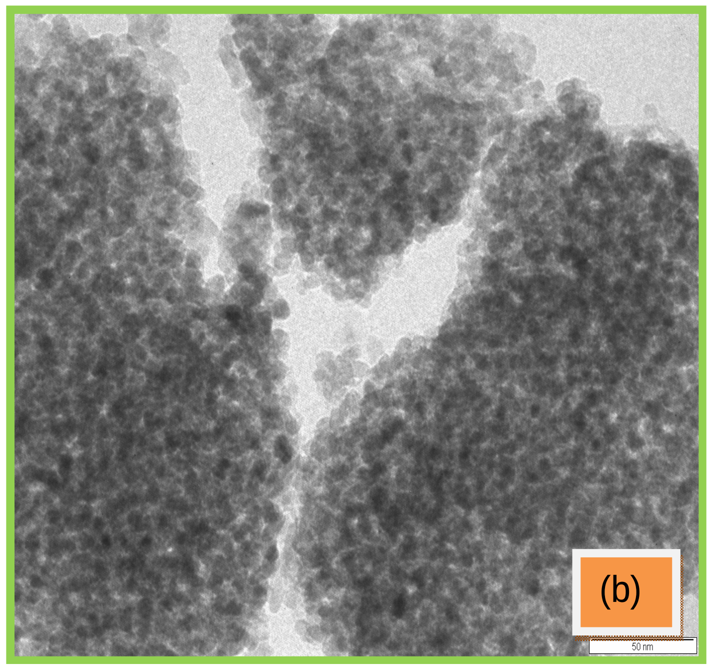


Figure 3: (a) TEM Images ofSolanumxanthocarpum Berry Extract synthesized TiO_2 and(b) Chemically synthesized TiO_2

E. Sem

The SEM analysis was used to determine the structure ofthe reaction products that were formed**.** SEM image Figure-4 by green synthesis methoddogged the particle size and external morphology of the TiO_2nanoparticle SEM image has showed individual particles aswell as a number of aggregates. The SEM image showedrelatively uneven spherical shape nanoparticle. It can be seen from the image that the Titanium dioxide nanoparticles range from 50-
100 nm. The morphology of TiO_2 nanoparticle prepared bychemical method is shown in the Figure-4represent that the obtained products are composed of near spherical shapemorphology with the average size in the range of 100-150nm.

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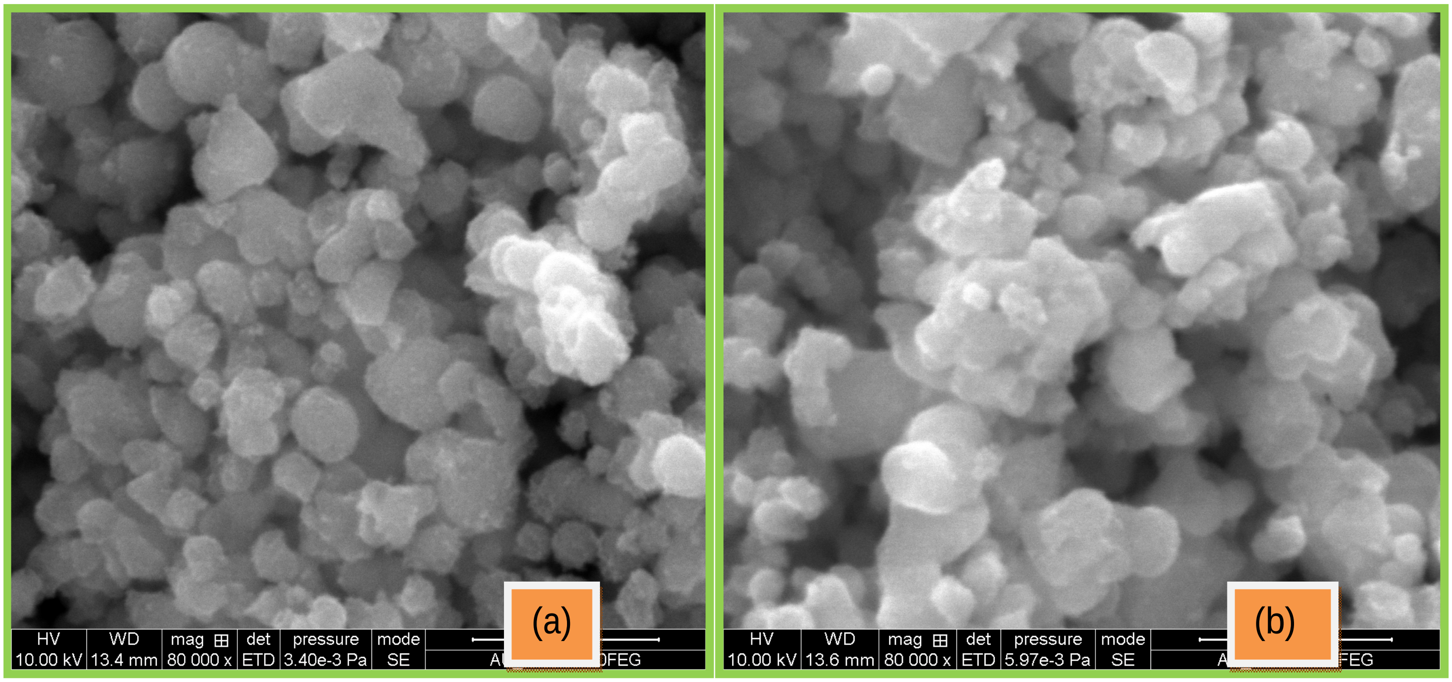

Fig 4: (a) SEM Images ofSolanumxanthocarpum Berry Extract synthesized TiO_2 and (b) Chemically synthesized TiO_2

E. Photocatalytic Activity

The photodegradability of methylene blue with TiO_2nano particles obtained using Solanumxanthocarpum Berry Extract and chemically synthesized TiO_2nano particles under solar light irradiation is presented in Figure 6. Almost 89% of degradation of the dye takes place at a time of 60 min with using Solanumxanthocarpum Berry Extract under solar light.

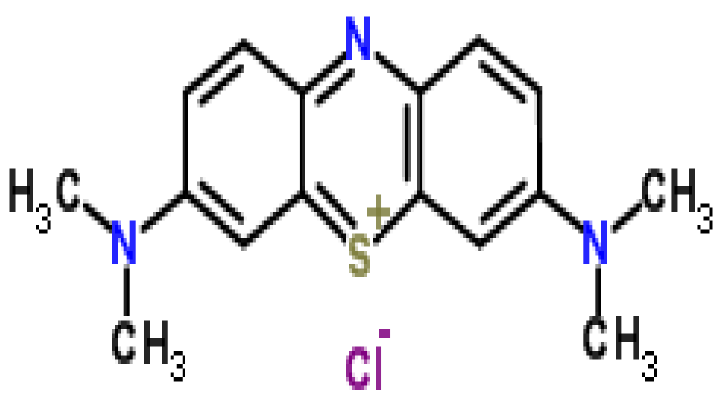

Fig 5: Structure of Methylene Blue

Methylene Blue (MB) is an inexpensive and common dye used in textile industry. Methylene blue (MB) is a cationic thiazine dye that is deep blue in the oxidized state while it is colour less in its reduced form (leucomethylene blue).When chemically synthesized TiO_2 was used under the same conditions, 81% of degradation occurred, at a time of 60 min. This shows that TiO_2nano particles obtained using Solanumxanthocarpum Berry Extract is more efficient in Methylene blue dye degradation than other catalysts. It was observed that there was a decrease in absorbance of Methylene blue dye solution with increasing time of exposure. In the present case, the degradation followed the first-order reaction kinetics.

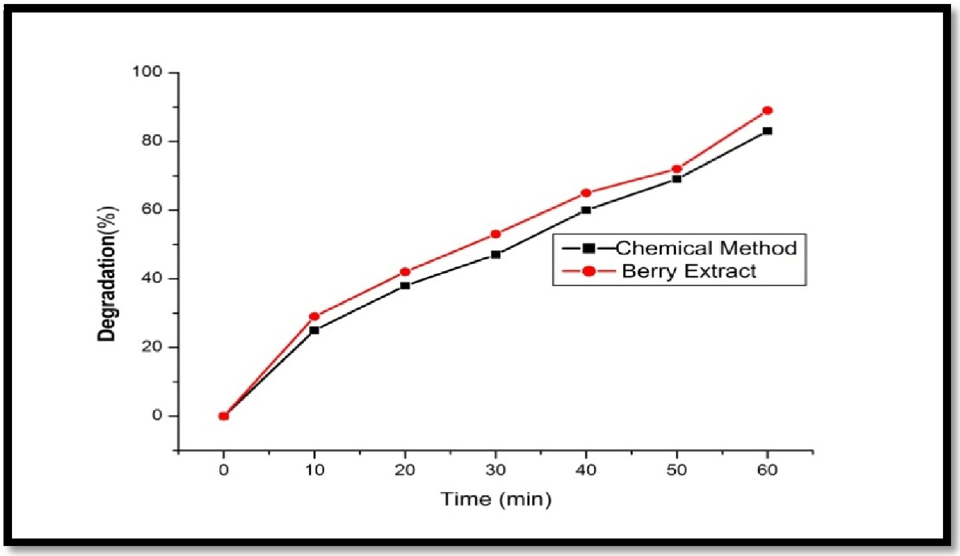

Fig 6: Changes of UV-Vis spectra during the degradation of MB on Chemically synthesized TiO_2 NPs and Biosynthesized TiO_2 NPs10 mg catalyst; 50 ml MB (10 mgl^{-1}).

The samples prepared with plant extracts exhibit higher photocatalytic activity. During 60 min irradiation, the photo catalytic activities of TiO_2nano particles prepared using two different methods can be ordered as follows: TiO_2 (Solanumxanthocarpum Berry Extract) $\geq$ TiO_2 (Chemical method)

Table 1: Antibacterial activity of TiO_2nano particles prepared from chemical and green method

S. No.	Bacteria	Ciprofloxacin	Zone of inhibition (mm)		
			Solanumxanthocarpum Berry Extract TiO_2	Chemical method TiO_2	DMSO
1	Bacillus subtilis	25	16	13	-
2	Escherichia coli	25	21	15	-
3	Staphylococcus aureus	28	19	14	-

TiO_2 nanoparticles were tested for antibacterial activity using Bacillus subtilis, Escherichia coli and Staphylococcus aureusby disc diffusion method[28-30]. The antibacterial activity of TiO_2 nanoparticles was compared with the positive control, Ciprofloxacin. The zone of the inhibition is summarized in the Table-1. The bactericidal activity exhibited by 15 µg/ml of biostabilized TiO_2 nanoparticles was comparable with that of the positive control, also the activity of biostabilized TiO_2 nanoparticles at a concentration of 15 µg/ml was found to be greater than that of the positive control. However the zone of inhibition produced by chemically synthesized TiO_2 nanoparticles is lesser than green synthesized TiO_2 nanoparticles studied against Bacillus subtilis ,Escherichia coli and Staphylococcus aureus. Nanomaterials were reported to exhibit broad-spectrum biocidal activity towards various microorganisms like bacteria, fungi, and viruses this can suggest that the biostabilized TiO_2 nanoparticles capped by polyphenols has better activity than chemically synthesized TiO_2 nanoparticles to induce membrane damage and cell death of Bacillus subtilis ,Escherichia coli and Staphylococcus aureus. In comparison with the chemically synthesized TiO_2nanocrystals, the green synthesised ones exhibit greater antimicrobial activity against bacterial organisms. The metal oxides carry a positive charge while the microorganisms carry negative charge; this causes electromagnetic attraction between microorganisms and the metal oxides which leads to oxidization and finally the death of microorganism [31]. Nanomaterials also could deactivate the cellular enzymes and DNA by coordinating to electron-donating groups, such as thiols, carbohydrates, amides, indoles, and hydroxyls. They cause pore in bacterial cell walls, leading to increase in permeability and cell death [32].

IV. CONCLUSION

In this study we have prepared TiO_2 nanoparticles by 1)using Solanumxanthocarpum Berry Extract as capping agent and 2)chemical method. Both nanoparticles were characterized for their size, and crystallinity using XRD,SEM,IR, EDX and TEM analysis. The TiO_2 nanoparticles which was capped and stabilized by phenolic and amine moieties of Solanumxanthocarpum Berry Extract was found to be lesser in size and more dispersed than the TiO_2 nanoparticles prepared by chemical method. Moreover theBerry extract stabilized TiO_2 nanoparticles exhibited considerable antimicrobial activity against pathogenic bacteria, which is comparable with that of standard antibiotic. Based on these results we conclude that the Solanumxanthocarpum Extract stabilized TiO_2 nanoparticles may have potential biomedical applications and photo catalytic property when compared to chemically synthesized TiO_2 nanoparticles due to its enhanced dispersibility, stability and surface coatings.

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