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# Synthesis, characterization, structural and optical properties of titanium-dioxide nanoparticles using Glycosmis cochinchinensis Leaf extract and its photocatalytic evaluation and antimicrobial properties

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#### **ABSTRACT**

The present study reports on the development of an Eco-friendly, nontoxic, inexpensive and low cost effective method for green synthesis of titanium dioxide nanoparticles, using Glycosmis cochinchinensis leaf extract. X-Ray diffraction (XRD), Ultraviolet spectral studies (UV-Vis), Fourier transform infrared (FTIR) spectroscopy, Scanning electron microscopy and Energy dispersive spectrometry (SEM-EDS) and Transmission electron microscopy (TEM) were used to characterize the TiO<sub>2</sub> nanoparticles, while the application of TiO<sub>2</sub> nanoparticles was assessed and antimicrobial activity was evaluated photocatalytically. XRD studies indicated that the titanium dioxide nanoparticles were crystalline in nature and in the anatase phase. According to our derived data, TiO<sub>2</sub> nanoparticles exhibited maximum absorbance peak at 430 nm in UV-Vis spectroscopy and the band gap energy was to be found to be 3.02 eV. FTIR spectral studies confirm that flavonoids and proteins are the stabilizing agents of the TiO<sub>2</sub> nanoparticles. The SEM and TEM images indicate that the morphology of the product is spherical nanoparticles with an average particle size of 40 ±5 nm with standard deviation. The antibacterial activity of the TiO<sub>2</sub> nanoparticles was tested against gram positive bacteria (S. saprophyticus and B. subtilis) and gram negative bacteria (E. coli and P. aeruginosa). Accordingly, maximum zone of inhibition was observed against gram negative bacteria. The antifungal activity of the TiO<sub>2</sub> nanoparticles was then tested against Aspergillus niger and Trichoderma reesei. Herein, maximum zone of inhibition was found to be 60 mm against T.reesi. Hence, Glycosmis cochinchinensis leaf extract green synthesized TiO<sub>2</sub> nanoparticles can be considered to be an effective antimicrobial agent.

**Keywords:** Titanium dioxide nanoparticles, *Glycosmis cochinchinensis*, Photocatalytic evaluation and Antimicrobial properties

#### 1. INTRODUCTION

Day to day large amount of unconsumed dyes are generally used in several industries consisting of printing procedure, textile, plastic, cosmetics, overload dyes are launched into the effluent stream as waste after colouring and are released into the environment [1].

Photocatalysis, as an environment-friendly technique, have excellent potential to remove hazadours residues from the environment. Photocatalysis in waste water treatment technique, it is used for the overall mineralization of organics. Nanoparticles residential or commercial properties of such as increased surface, dimension and also morphology are various as well as improved when as compared to the bulk equivalents, a frustrating interest on the research studies of nanotechnology, advancement of rapid straightforward, cost-effective, and environment-friendly procedures for the synthesis of nanoparticles is worth [2].

Metal and metal oxide nanoparticles may supply remedies to technological and also environmental challenges in the areas of solar power conversion and high portion of atoms which is in charge of their interesting residential or commercial properties such as antimicrobial, magnetic, electronic, catalytic, medication, water treatment, air filtration and photocatalysis to eliminate various pollutants and hazardous dyes. Using metal semiconductor photocatalysis, such as ZnO, CdS, SnO<sub>2</sub>, and TiO<sub>2</sub>, to purify environment is exactly emphasized. As one of the most important semiconductor photocatalysts, Titanium oxide (TiO<sub>2</sub>) has been taken into consideration as an important nanomaterial because of their photocatalytic residential properties, chemical stability and non-toxicity, most of these applications, and much more specifically, the photocatalytic based ones, is greatly studied in the field of environmental waste water treatment technique, depend upon the capacity of the material to generate under lighting electron-hole sets in the surface area these hole-electron sets respond with adsorbed particles at semiconductor surface area.

Amongst the numerous photocatalytic materials that have actually been used, the majority of interest has focused on titanium dioxide (TiO<sub>2</sub>) as a photocatalytic activity is used for cleaning contaminated air and water. Various sorts of approaches are readily available for synthesis of titanium dioxide nanoparticles, those are solution Combustion [3], Sol-Gel [4], Hydrothermal [5], Solvothemal [6], Microwave Assisted [7], co-precipitation [8], Chemical Vapour Deposition [9] and Environment-friendly synthesis.

Although chemical technique of synthesis needs short time period for synthesis of large amount of nanoparticles, this technique needs topping representatives for size stabilizing of the nanoparticles. Chemicals used for nanoparticles synthesis and stablizing are toxic hazardous chemicals which may present environmental dangers. The need for environmental non-toxic synthetic methods for nanoparticles synthesis results in the creating interest in biological approaches which are economical and do not involve using toxic chemicals, high stress, power and temperature levels.

Therefore, there is an increasing need for environment-friendly nanotechnology is useful over chemical agents due to their less environmental effects. The non- toxic and biocompatible properties of Titanium find its applications in biomedical sciences such as bone tissue engineering as well as in pharmaceutical industries

The metal nanoparticles are synthesized using different materials like plant extracts, microorganisms, fungis and enzymes, reports are readily available for the Biosynthesis of metal oxide nanoparticles. In the biosynthesis method, extracts from plant may function as both reducing and capping agents in synthesis of nanoparticles. Earlier writers reported that

the TiO<sub>2</sub> nanoparticles were synthesized from *Annona squamosa peel* extract [10], *Catharanthus roseus* leaf aqueous extract, and environment-friendly TiO<sub>2</sub> nanoparticles have been synthesized using all-natural products like *Nyctanthes arbor-tristis* extract [11].

*Glycosmis cochinchinensis* is (Lour) Pierre, coming from Rutaceae family members, is dispersed throughout the Indian-Malayan region. The plants in this category usually produce a variety of alkaloids including acridones, quinolones, quinazolines, furoquinolines, carbazoles, indoles and amide byproducts.

Hence the aim of the here and now research was to investigate the photocatalytic activity of synthesized TiO<sub>2</sub> NPs using aqueous leaf extract of *Glycosmis cochinchinensis* extrac and also characterized by XRD, UV-Visible and FTIR spectroscopic techniques. Scanning electron microscopy and energy dispersive spectrometry (SEM–EDS), Transmission electron microscopy (TEM), Photocatalytic efficiency was carried out for Rhodamine B (RhB) dye and its kinetic behavior was additionally studied and antimicrobial activity.

#### 2. MATERIALS AND METHODS

# 2. 1. Materials



Figure 1. Photograph of G. cochinchinensis leaf

Fresh leaves of *G. cochinchinensis* (Figure 1.) were collected from rural areas of Chidambaram, Tamil Nadu, India. TiO(OH)<sub>2</sub> (99.9%) was procured from Sigma-Aldrich, Bangalore, India and used as received. Lyophilized cultures of target strains were procured from the Microbial Type Culture Collection Center (MTCC) located at the Institute of Microbial Technology (IMTECH) Chandigarh, India. Nutrient media used for antibacterial activity were purchased from Hi-Media, Mumbai, India. All other reagents used in the reaction were of analytical grade with maximum purity. All aqueous solutions were prepared using deionized water.

# 2. 2. Preparation of leaf extract

The fresh and healthy leaves of *G. cochinchinensis* were washed several times with deionized water to remove the dust particles on their surface. A healthy and undamaged leaf of *G. cochinchinensis* ten grams of leaf was finely cut and stirred with 100 mL of de-ionized water at 85 °C for 20 min, using microwave irradiation. The leaf extract was allowed to cool and filtered through Whatman No.1 filter paper. The filtered leaf extract was used for further experiments as reducing agent and stabilizer of synthesis nanoparticle, being usable for within 2 weeks.

# 2. 3. Synthesis of TiO<sub>2</sub> nanoparticles using G. cochinchinensis leaf extracts

For the green synthesis of TiO<sub>2</sub> nanoparticles, the Erlenmeyer flask containing 100 mL of TiO(OH)<sub>2</sub> (0.1 mM) was stirred for 2 h. 25 mL of the filtered aqueous leaf extract of *G. cochinchinensis* was added the solution at room temperature under the mixture was then incubated for 12 h. The pure TiO(OH)<sub>2</sub> and aqueous leaf extract of *G. cochinchinensis* show any color change primarily confirmed the production of TiO<sub>2</sub> nanoparticles. After the reaction of *G. cochinchinensis* extract with TiO (OH)<sub>2</sub>, the synthesized nanoparticles turned light green in color. The nanoparticle pellet was dried and used for characterization.

#### 2. 4. Characterization of synthesized TiO<sub>2</sub> nanoparticles

# 2. 4. 1. X-Ray diffraction

The green synthesized titanium nanoparticles was monitored by the crystalline form of TiO<sub>2</sub> stability and particle size distribution were characterized X-Ray diffraction (XRD) measurements of the *G. cochinchinensis* leaf extract broth reduced TiO<sub>2</sub> nanoparticles were carried out at 2 V.

# 2. 4. 2. UV-Vis Absorbance Spectroscopy

The bioreduction of titanium ions in solution was monitored periodically by measuring the UV-Vis spectrophotometer analysis. UV-visible spectroscopy analysis was carried out by a computer controlled in a wavelength range from 200–800 cm<sup>-1</sup>.

# 2. 4. 3. Fourier transform infrared spectroscopy

The presence of functional groups and the binding property of the  $TiO_2$  nanoparticles were determined by Fourier transform infrared spectroscopy (FT-IR) were recorded in the range of 400–4000 cm<sup>-1</sup>.

# 2. 4. 4. Scanning Electron Microscope (FESEM)

Using SEM technique, the size, shape, and morphology of the  $TiO_2$  nanoparticles were examined. Dried suspension of  $TiO_2$  nanoparticles is synthesized by reduction between titanium ions, and leaf extract of G. cochinchinensis plant was used for analysis. The SEM was applied at an accelerating voltage of 25 kV.

# 2. 4. 5. Transmission electron microscopy

The size and morphology of the  $TiO_2$  nanoparticles were measured with Transmission Electron Microscope (TEM) using operating at an accelerating voltage of 200 kV. A specimen for TEM sample was made by placing a drop of the suspension on a carbon coated copper grid and the excess solution was removed from tissue paper and allowed to air dry at room temperature for overnight

#### 2. 5. Antibacterial activity of TiO<sub>2</sub> Nanoparticles

The antibacterial effect of TiO<sub>2</sub> nanoparticles were examined by disc diffusion analyse against a gram positive bacteria (*Staphylococcus saprophyticus* and *Bacillus subtilis*) and gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria by Kirby-Bauer disc diffusion method 6 mm wells were cut on Mueller-Hinton agar swabbed with individual pathogenic bacteria 4 wells were cut in each plate where twenty-five micrograms of ciprofloxacin was used as positive control (PC). The test plates were incubated at 37 °C for 24 h. After the incubation period, the zone of inhibition (in mm diameter) was observed and tabulated.

#### 3. RESULTS AND DISCUSSIONS

# 3. 1. X-Ray Diffraction (XRD) analysis

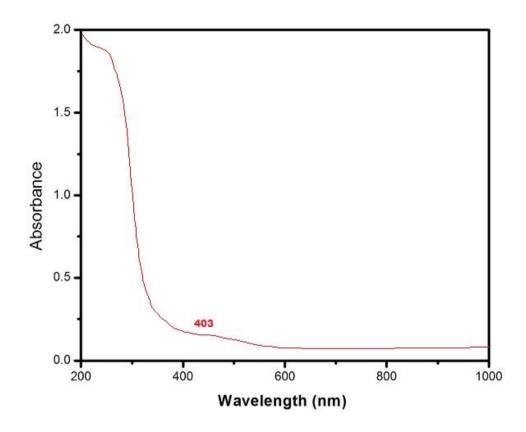
The crystallinity nature and phase purity of titanium dioxide nanoparticles green synthesized using G. cochinchinensis leaf extract was confirmed by  $Cu K\alpha - X$ -Ray Diffractometer for confirming the presence of  $TiO_2$  and analyze the structure and shown in Fig. 1 As can be observed from the XRD analysis showed six distinct diffraction peaks at 20 with 25.30, 38.04, 48.13, 54.71, 62.77 and 75.22° corresponds to the crystal planes of (101), (004), (200), (105), (204) and (215) respectively, to the anatase structure of the titanium oxide nanoparticles and these results confirmed using Joint Committee on Powder Diffraction Standards. The peaks of the graph are in good agreement with the literature report [12]. Useful it has high intensity sharp peak and absence of unidentified peaks confirmed the crystallinity and higher purity of prepared  $TiO_2$  was successfully formed. From the XRD pattern, the average sizes of the green synthesized  $TiO_2$  nonmaterial were estimated using Debye-Scherrer's formula [13].

#### 3. 2. Optical properties

# 3. 2. 1. UV-Vis Absorbance Spectroscopy

The UV-Visible absorption spectrum which has been extensively studied is one of the most important methods to reveal the energy structures and optical properties of

semiconductor nanostructures. The reduction of titanium ions and leaf extract lead to the formation of nanoparticles at room temperature. During this reaction, synthesis of titanium oxide nanoparticles reduced by *G. cochinchinensis* leaf extract made the color of leaf extract change. Changing the color of the reaction mixture and formed in the reaction media after 30 minutes appearance the maxima around 403 nm was recorded by means of the UV-Vis spectrophotometer (Figure 2). Absorption spectrum of TiO<sub>2</sub> nanoparticles indicates the reduction process and formation of nanoparticles which shows excellent agreement with those reported in literatures.



**Figure 2.** UV-Vis absorption spectra for T<sub>1</sub>O<sub>2</sub> nanoparticles.

# 3. 2. 2. Band gap energy

The electron is transferred from the valence band to the conduction band the place there takes place an abrupt increase in the absorbency of the materials to the wavelength corresponding to the band gap energy, The band gap energy was determined based on the numerical derivative of the optical absorption coefficient, for the study of the optical properties of the green synthesized TiO<sub>2</sub> nanoparticles using *G. cochinchinensis* leaf extract, the band gap and the type of electronic transition were determined, which were calculated by means of the fundamental absorption method refers to band to band transitions by using energy relation [14]

where: C is the speed of light in vacuum (3 × 10<sup>8</sup> m/s),  $\lambda$  is the wave length of the  $\lambda = c/v$  ( $\lambda = 403$  nm), h is Plank's constant (6.626 × 10<sup>-34</sup> J·s), v is the frequency.

Band gap energy is calculated as:

$$E = (6.6025 \times 10^{-34}) (3 \times 10^{8}) / 403 = 3.23 \text{ eV}.$$

The band gap energy for the green synthesized  $TiO_2$  nanoparticles to be the 3.02 eV (Figure 3). The band gap was increases with particle size decreased in the confirmed to the XRD spectra.

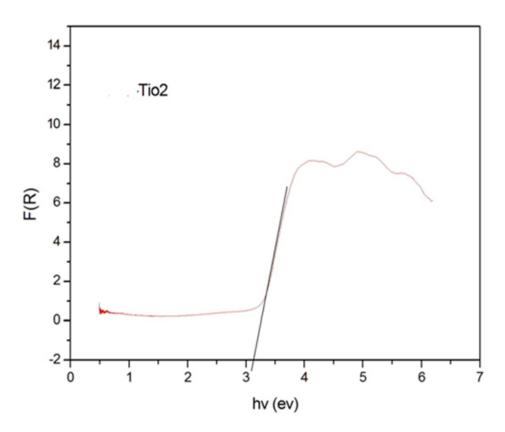


Figure 3. Band gap energy of green synthesis of TiO<sub>2</sub> nanoparticles

# 3. 3. Fourier Transform Infrared (FTIR) analysis

FTIR analysis was used to determine the functional groups and capping of the bioreduced of TiO<sub>2</sub> synthesized by the *G. cochinchinensis* leaf extract. In green synthesized titanium dioxide nanoparticles a broad band was observed between 3800 to 3000 cm<sup>-1</sup> which is due to hydroxyl (O-H) stretch, representing the water as moisture.

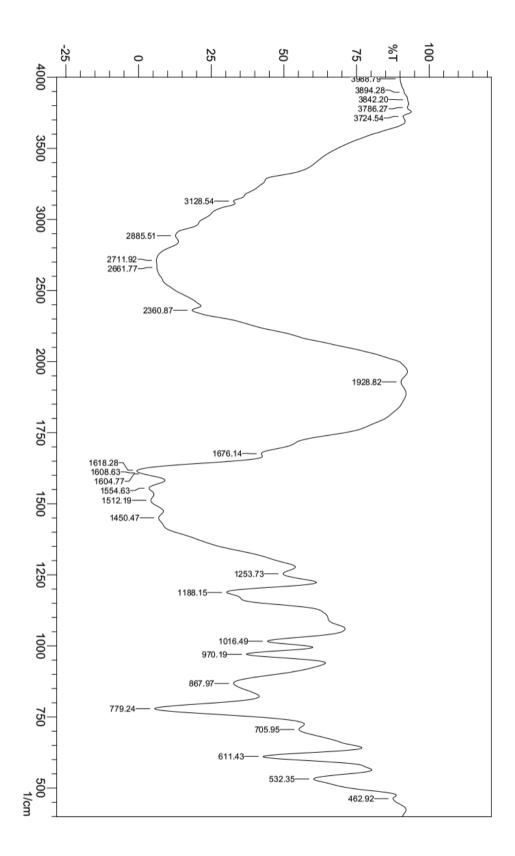


Figure 4. FTIR spectra of titanium nanoparticles and G. cochinchinensis leaf extract

The peak at 2885 cm<sup>-1</sup> indicated the secondary amines and 2360 cm<sup>-1</sup> confirmed to the C–C. The peak at 1676 cm<sup>-1</sup> was due to O– H bending vibration of adsorbed water molecule on the surface of TiO<sub>2</sub> which may have crucial role in photocatalytic activity. The peak at 1618 cm<sup>-1</sup> can be assigned to the amide I band of the proteins and aromatic rings. The strong peak noticed at 1554 cm<sup>-1</sup> represents aliphatic of the nitro compound with stretching of N-O. The peak at 1450 cm<sup>-1</sup> corresponding to the C–N stretching mode of the aromatic amine group.

The reported by synthesized TiO<sub>2</sub> nanoparticles using *Calotropis gigantean* extract the similar peak presented at 1016 cm<sup>-1</sup> shows aliphatic amines with stretches of C-N [16]. The peaks corresponding to the broadband centered at 543 cm<sup>-1</sup> characteristic of Ti–O bending mode of vibration which confirms the formation of metal oxygen bonding. The intense peak between 800 and 450 cm<sup>-1</sup> describes the Ti-O stretching bands [17].

FTIR spectrum reveals the information about the interaction between the functional groups of the plant phytochemicals and the nanoparticles. The variations in the peak positions indicated, presumably, some metabolites such as tannins, avonoids alkaloids, carotenoids, terpenoids, flavonoids and proteins the *G. sochinchinensis* leaf broth acted as both reducing and capping agents for the green synthesis of TiO<sub>2</sub> nanoparticles (Figure 4).

# 3. 4. Scanning Electron Microscope (SEM) analysis

The grain size, shape and surface properties like morphology were investigated by the Scanning Electronic Microscopy. Figure 5 (a and b) represent the SEM image of  $TiO_2$  nanoparticles synthesized using G. cochinchinensis leaf extract and it is observed the SEM image was observed within the magnification of 2  $\mu$ m and 5  $\mu$ m, the  $TiO_2$  nanoparticles were agglomerated and they formed irregular Shape. Few particles with were spherical in shape and without aggregation. We could therefore speculate that the phytochemicals of G. Cochinchinens leaf extract coats the surface of the  $TiO_2$  nanoparticles thus preventing their aggregation, the size was ranging from 15 nm to 45 nm.

The elemental composition was determined by EDX analysis as the results shown in Figure 5 (c) the spectrum has prominent peaks of Ti and O, confirmed that the nanoparticles synthesized using G. Cochinchinensis leaf extract, indicates the amount of Titanium and Oxide present in the TiO<sub>2</sub> Nanoparticle is 84.02% & 15.98% and no characteristic peaks of impurities or other precursor compounds are observed [18,19]

# 3. 5. Transmission Electron Microscope (TEM) analysis

Transmission electron microscope (TEM) was used to study the crystal structure, morphology, shapes and particle size, Figure 6 (a and b) shows the TEM images of green synthesized titanium nanoparticles.

The TEM images exhibit that it was clear that the morphology of titanium nanoparticles is almost spherical shape with smooth surfaces and the Figure. 6 (c) showed the size distribution histogram of the nanoparticles. The particle distributions indicate mean grain size values from 15 to 45 nm with an average particle size of  $40 \pm 5$  nm synthesized. The crystalline nature of the TiO<sub>2</sub> Nanoparticle was confirmed by the selected area electron diffraction (SAED) pattern with bright circular spots corresponding to (101), (004), (200), (105), (204) and (215) respectively planes of the anatase lattice of TiO<sub>2</sub> Nanoparticle [20,21].

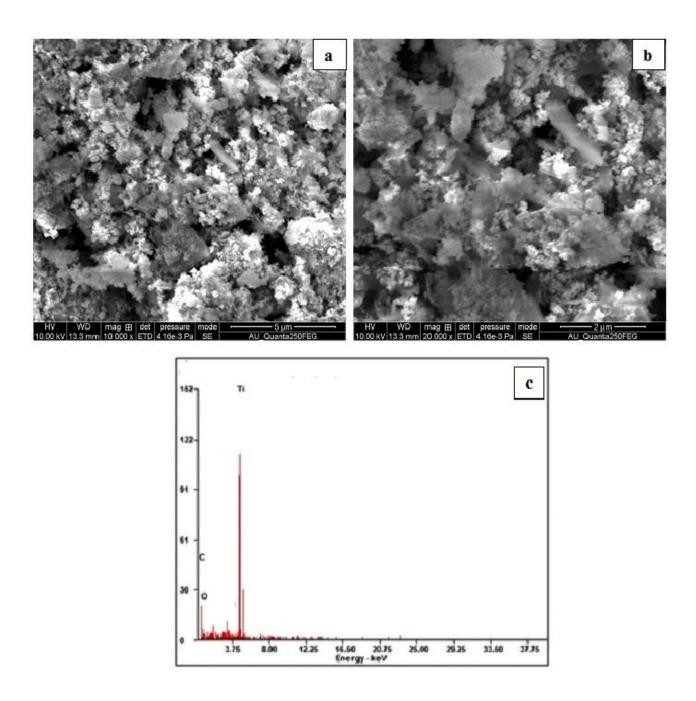
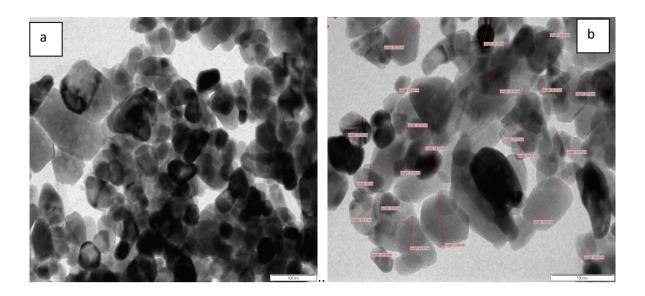
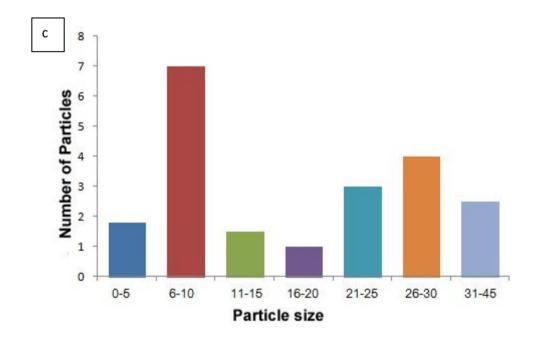


Figure 4. The SEM images of the synthesized  $TiO_2$  nanomaterials (a)  $5\mu m$ , (b)  $2\mu m$ , (c) EDAX spectrum of titanium nanoparticles





**Figure 5. a**: transmission electron microscopy (TEM) images of TiO<sub>2</sub> nanoparticles; **b**: selected area electron diffraction (SAED) pattern; **c**: histogram showing the particle sizes of green synthesized TiO<sub>2</sub> nanoparticles corresponding to TEM images

# 3. 6. Antibacterial activity of TiO<sub>2</sub> nanoparticles

The antibacterial action of green synthesized TiO<sub>2</sub> nanoparticles was investigated towards different pathogenic organisms this kind of as *S. saprophyticus*, *B. subtilis*, *E. coli* and *P. aeruginosa*. The diameter of inhibition zone (mm) with *G. cochinchinensis* leaf extract, chemically synthesized TiO<sub>2</sub> nanoparticles and green synthesized TiO<sub>2</sub> nanoparticles using

 $G.\ cochinchinensis$  leaf extract solution was shown in Table 1. As can be observed from Fig. 10, the final results of antibacterial scientific studies clearly suggest that the  $TiO_2$  nanoparticles synthesized using  $G.\ cochinchinensis$  leaf extract has a far greater antibacterial activity even at a lower dose when compared to the chemically synthesized  $TiO_2$  nanoparticles and  $G.\ cochinchinensis$  leaf extract.

**Table 1.** Inhibitory action of control, *G. cochinchinensis* leaf extract, chemically synthesized TiO<sub>2</sub> and green synthesis of TiO<sub>2</sub> nanoparticles against human pathogenic bacteria

	Cultures	Standard	Concentrations (100 μL)		
Plates			G. cochinchinensis leaf extract	Chem TiO <sub>2</sub>	Green - TiO <sub>2</sub>
1	Staphylococcus saprophyticus	25	14	17	21
2	Bacillus subtilis	25	10	15	19
3	Escherichia coli	27	13	17	23
4	Pseudomonas aeruginosa	29	15	19	25

The chemical synthesis may possibly even now lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects in health care applications. It is effectively identified that the particle size and surface region plays a important position in their interactions with the biological cells and the in vivo fate of a particulate drug delivery technique. Due to their size and large surface region, green synthesized TiO<sub>2</sub> nanoparticles create electronic effects and these effects can increase the binding strength of the nanoparticles with the bacteria. Thus, the green synthesized TiO<sub>2</sub> nanoparticles nanoparticles get connected very easily to the cell membrane and also penetrated inside the bacteria. We speculated that the following mechanisms can account for the increased antibacterial activity of green synthesized TiO<sub>2</sub> nanoparticles compared to G. Cochinchinens leaf extract, chemically synthesized TiO<sub>2</sub> nanoparticles. It was observed that the negative charge on the cell surface of Gram-negative bacteria was higher than that the Gram-positive bacteria.

This consequence is possible due to difference in the structure of the cell wall between gram-positive and gram-negative bacteria. The cell wall of the gram-positive bacteria is composed of a thick layer of peptidoglycan, consisting of linear polysaccharide chains cross-linked by short peptides as a result forming more rigid structure leading to difficult penetration of the green synthesized TiO<sub>2</sub> nanoparticles compared to the gram-negative bacteria the place the cell wall possesses a thinner layer of peptidoglycan. The increase in surface region determines the prospective quantity of reactive groups on the particle surface, which are expected to present higher antibacterial action. From the current results, it is clearly

evident that the greater inhibitory action of green synthesized TiO<sub>2</sub> nanoparticles depends not only on size of the nanoparticles, but also on the capping agent (proteins) of the nanoparticles.

The surface charge and chemical properties of nanoparticles are established by capping agents, which perform an important part for the duration of nanoparticles and bacterial interactions.

#### 4. CONCLUSIONS

 $TiO_2$  nanoparticles were synthesized using the environmental friendly process of synthesizing method known as green synthesis. In this method has various advantages over other methods and due to low cost and is done at low temperature. XRD analysis showed that the  $TiO_2$  nanoparticles are anatase structure with higher crystallinity and purity. FT-IR results exhibited the presence of various functional biomolecules that acted as reducing and capping agent for conversion of  $TiO_2$  nanoparticles. The UV-Visible absorption appearance the maxima around 403 nm and band gap energy for the  $TiO_2$  nanoparticles to be the 3.02 eV. The spherical shaped particles were confirmed through the SEM and TEM analysis an average particle size of  $40 \pm 5$  nm. Moreover, anti-bacterial activity of green synthesized  $TiO_2$  nanoparticles showed better activity towards negative bacteria (*E. coli* and *P. aeruginosa*) compared to chemically synthesized  $TiO_2$  nanoparticles.

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