

Biosynthesis and characterization of titanium dioxide nanoparticles using pithecellobium dulce and lagenaria siceraria aqueous leaf extract and screening their free radical scavenging and antibacterial properties

S. Kalyanasundharam*, M. Jeevan Prakash

* Department of Chemistry, Poompuhar College(Autonomous), Melaiyur, Tamilnadu, India-609 107

*E-mail address: skalyanasundharam@gmail.com

Keywords: Biosynthesis, titanium dioxide

ABSTRACT . The recent development and implementation of new technologies have led to new era, the nano-revolution which unfolds role of plants in bio and green synthesis of nanoparticles which seem to have drawn quite an unequivocal attention with a view of synthesizing stable nanoparticles. In this present work, we have compared titanium dioxide nanocrystals synthesized by both chemical method and biological method from Titanium tetra isopropoxide as precursor. The biological method was performed by using the aqueous plant extract of Pithecellobium dulce and Lagenaria siceraria. The synthesized nanocrystals were characterized by FTIR Spectroscopy, Scanning Electron Microscopy (SEM), Energy Dispersive Xray (EDAX), Transmission Electron Microscope (TEM) and X-ray diffraction (XRD) techniques and also Antioxidant assay and antimicrobial test were performed to evaluate its biological behaviour. The nanocrystals synthesized by biological method were found to have higher antioxidant potential, antimicrobial activity than chemically synthesized.

1. INTRODUCTION

Biological synthesis of nanoparticles by plant extract is at present under exploitation for the reason that to develop environmentally benign nanoparticles synthesis to avoid adverse effects in biomedical applications (M. Sundrarajan et al 2011). The manipulation of matter on an atomic, molecular, and supramolecular scale. The earliest, widespread description of nanotechnology, Nanoparticles are usually ≤ 100 nm in each spatial dimension or less have attracted great attention due to their unusual and fascinating properties, Nanoparticle research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields and applications advantageous over their bulk counterparts [M. C. Daniel et al, 2004]. Nanoparticles are classified primarily into two types, viz organic and inorganic nanoparticles. The nanoparticles of carbon are called the organic nanoparticles. Magnetic nanoparticles, noble metal nanoparticles (platinum, gold and silver) and semiconductor nanoparticles (titanium dioxide, zinc oxide and zinc sulfide) are classified as inorganic nanoparticles (Kathiresan et al, 2013).

Titanium dioxide (TiO_2), an important semiconductor nanoparticle has been interesting attention for its wide range of applications, such as electronics, optics, optoelectronics, transparent, UV-light emitters, piezoelectric devices, chemical sensors, spintronics. Further, TiO_2 NPs exhibit size and shape-dependent properties which are of interest for applications ranging from biosensing and catalysts to optics, antimicrobial activity, computer transistors, electrometers, chemical sensors, and wireless electronic logic and memory schemes. These particles also have many applications in different fields such as medical imaging, nanocomposites, filters, drug delivery, and hyperthermia of tumors (D. Bhattacharya et al, 2005; D. Goodsell, 2004; R. Paull et al, 2003; O. V. Salata, 2004). Nanoparticles are usually 0.1 to 1000 nm in each spatial dimension and are commonly synthesized using two strategies: top down and bottom up. In top-down approach, the bulk materials are

gradually broken down to nanosized materials, whereas in bottom-up approach, atoms or molecules are assembled to molecular structures in nanometer range. Bottom-up approach is commonly used for chemical and biological synthesis of nanoparticles, by chemical methods leads to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects in medical applications. Due to the obvious disadvantages of the chemical reduction method, a biological synthesis of nanomaterials is an important aspect of nanotechnology. There is a growing need to develop simple, low-cost, eco-friendly and size control approaches for the synthesis of metal nanoparticles. Biological route synthesis of nanoparticles has received much focused attention from researchers in order to elucidate the mechanism of synthesis. To circumvent this many biological systems like bacteria [Kumar CG et al, 2011], fungi [Rodrigues AG et al, 2012], yeast, cyanobacteria, actinomycetes and plants have been used. But the best one appears to be the use of plants.

However, review of literature revealed that the synthesis of metal oxides and semiconductors nanoparticles using plant has been explored in number of few literatures [Mahdavi M, 2013], which aroused our interest in the present study the synthesis of TiO_2 nanoparticles by utilizing the aqueous leaf extract of *P. dulce* and *Lagenaria siceraria*. *Pithecellobium dulce* (*P. dulce*) Benth (Fabaceae) is a small to medium sized, evergreen, spiny tree, up to 18 m height, native of tropical America and cultivated throughout the plains of India and in the Andamans. It is known as 'Kodukkapuli' in Tamil. The bark of the plant is reported to be used as astringent in dysentery, febrifuge and it is also used in dermatitis and eye inflammation. In traditional medicine practice, the leaves of *P. dulce* are used for the treatment to fearache, leprosy, pepticulcer, toothache, venereal diseases and also act as emollient, anodyne, larvicidic and abortifacient, and antidiabetic properties in folk medicine. The presences of steroids, saponins, lipids, phospholipids, glycosides, glycoli. And the *Lagenaria siceraria* leaf, Bottle gourd (*Lagenaria siceraria* (Mol.) standley, family Cucurbitaceae) commonly called as Dudhi or Ghiya is widely cultivated in the tropical and subtropical regions of the world, *Lagenaria siceraria*, which has diuretic and anti-swelling effects, is used as food. A decoction of *Lagenaria siceraria* is employed in the treatment of anasarca, ascites and beriberi [Deshpande *et al.*, 2008]. So far, there is no report on the synthesis of TiO_2 by utilizing the aqueous leaf extract of *Pithecellobium dulce* and *Lagenaria siceraria*. Hence, the present study involves the synthesis and characterization of *Pithecellobium dulce* and leaf mediated TiO_2 and evaluating the anti-bacterial effect 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.

2. MATERIAL AND METHODS

2.1. Materials

Titanium tetraisopropoxide [$\text{Ti}(\text{OCH}(\text{CH}_3)_2)_4$], Isopropanol ($\text{C}_3\text{H}_7\text{OH}$), 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.

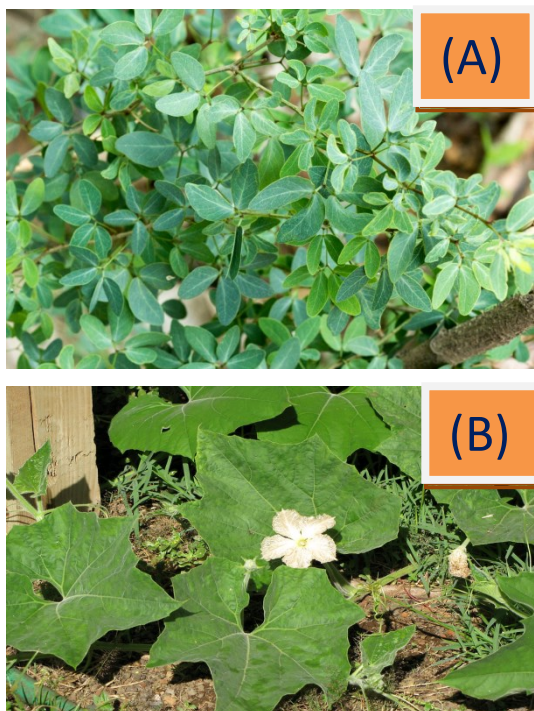


Figure 1: Plant Images of (A) Pithecellobium dulce leaf (B) Lagenaria siceraria leaf

Preparation of aqueous leaf extract

Lagenaria siceraria and Pithecellobium dulce leaf were collected from rural areas of Chidambaram, Tamil Nadu, India. Leafs were washed thoroughly with tap water and dried in the shade. Dried leafs were cut into small pieces and ground coarsely using pulverizer. Soxhlet extraction was then performed to obtain the crude aqueous extract which is stored in the refrigerator for further use.

Synthesis of titanium dioxide nanocrystals

By chemical method

TiO₂ nanocrystals 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 450°C for 30 minutes to obtain nanocrystalline TiO₂.

Biosynthesis of TiO₂ nanoparticles

For the synthesis of titanium dioxide nanoparticles, the Erlenmeyer flask containing 0.4M of titanium tetraisopropoxide in ethanolic leaf extract was reacted under stirring at 50°C. After four hours of continuous stirring, the formed titanium dioxide nanoparticles was 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 nanopowder was used for further analytical techniques.

3. ANALYTICAL METHODS

High resolution Scanning Electron Microscopy (HRSEM) and Elementary Dispersive X-ray (EDX) analysis experiments were carried out on a FEI Quanta FEG 200 instrument with EDX analyzer facility at 25 8C. TEM images of metal oxide nanoparticles were obtained using a transmission electron microscope (PHILIPS CM200 model) at an Operating voltages : 20-200kv Resolution :2.4 Å. XRD spectra was recorded on the X'PERT PRO model X-ray diffractometer

from Pan Analytical instruments operated at a voltage of 40 kV and a current of 30 mA with Cu Ka radiation. Fourier transform infrared spectroscopy. FT-IR spectroscopic studies were carried out to identify the possible functional groups in the leaf extract responsible for capping leading to efficient stabilization of the TiO₂. The FT-IR spectra of powdered TiO₂ were mixed with KBr pellets and are recorded in the 4,000–400 cm⁻¹ range on a Shimadzu FTIR-8400s

Antimicrobial activity

In the present study, in vitro antimicrobial activities were carried out by the using of disk-diffusion method [H.W. Seely et al, 1975; A. L. Barry, 1976]. This method followed the following procedure: 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 TiO₂ 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°C. The sensitivity was recorded by measuring the clear zone of growth inhibition on agar surface around the disks in millimeter.

Free radical scavenging activity

Biosynthesized Au-NPs and *S. nigrum* were tested for the scavenging effect on DPPH radical according to the method of [Blois, 1958] Different concentrations (50, 100 and 150 µL) of *S. nigrum* and biosynthesized Au-NPs were added, in equal volume, to 0.1 mM metabolic DPPH solution. The reaction mixture was incubated for 30 min at room temperature under shaking condition and the absorbance was recorded at 517 nm. The synthetic antioxidant butyl hydroxyl toluene (BHT) was used as positive control. All determinations were performed in triplicate. The DPPH radical scavenging activity (RSA) was expressed in percentage of inhibition using the following formula.

$$RSA = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{sample} is the absorbance of the blank control and A_{control} is the absorbance of the test sample.

In this study we have synthesized TiO₂ nanoparticles from titanium tetraisopropoxide solution using *Lagenaria siceraria* and *Pithecellobium dulce* leaf extract. The green synthesised TiO₂ nanoparticles were characterised using XRD, SEM, EDX and FTIR and investigated for antibacterial activity, in comparison with chemically synthesized TiO₂ nanoparticles.

4. RESULTS AND DISCUSSION

Powder X-ray diffraction (XRD)

The XRD pattern of TiO₂ nanoparticles obtained using leaf extract of *Lagenaria siceraria* and *Pithecellobium dulce* and chemically synthesized TiO₂ nanoparticles are shown in Figure 2a, 2b and 2c. In the green synthesized TiO₂ using *Pithecellobium dulce* show slightly broad diffraction peaks emerge at 2θ angles of 25.41°, 37.77°, 47.82°, 54.52° and 62.92° corresponds to Bragg's reflection from 101, 004, 200, 105 and 204 crystal planes(JCPDS files # 21-1272), suggesting the anatase phase of nanocrystalline TiO₂ this also confirm with angles 25.47°, 37.87°, 48.02°, 54.62° and 63.07° obtained from synthesized TiO₂ nanoparticles from leaf extract of *Lagenaria siceraria* (Vijayalakshmi, R et al, 2012) .

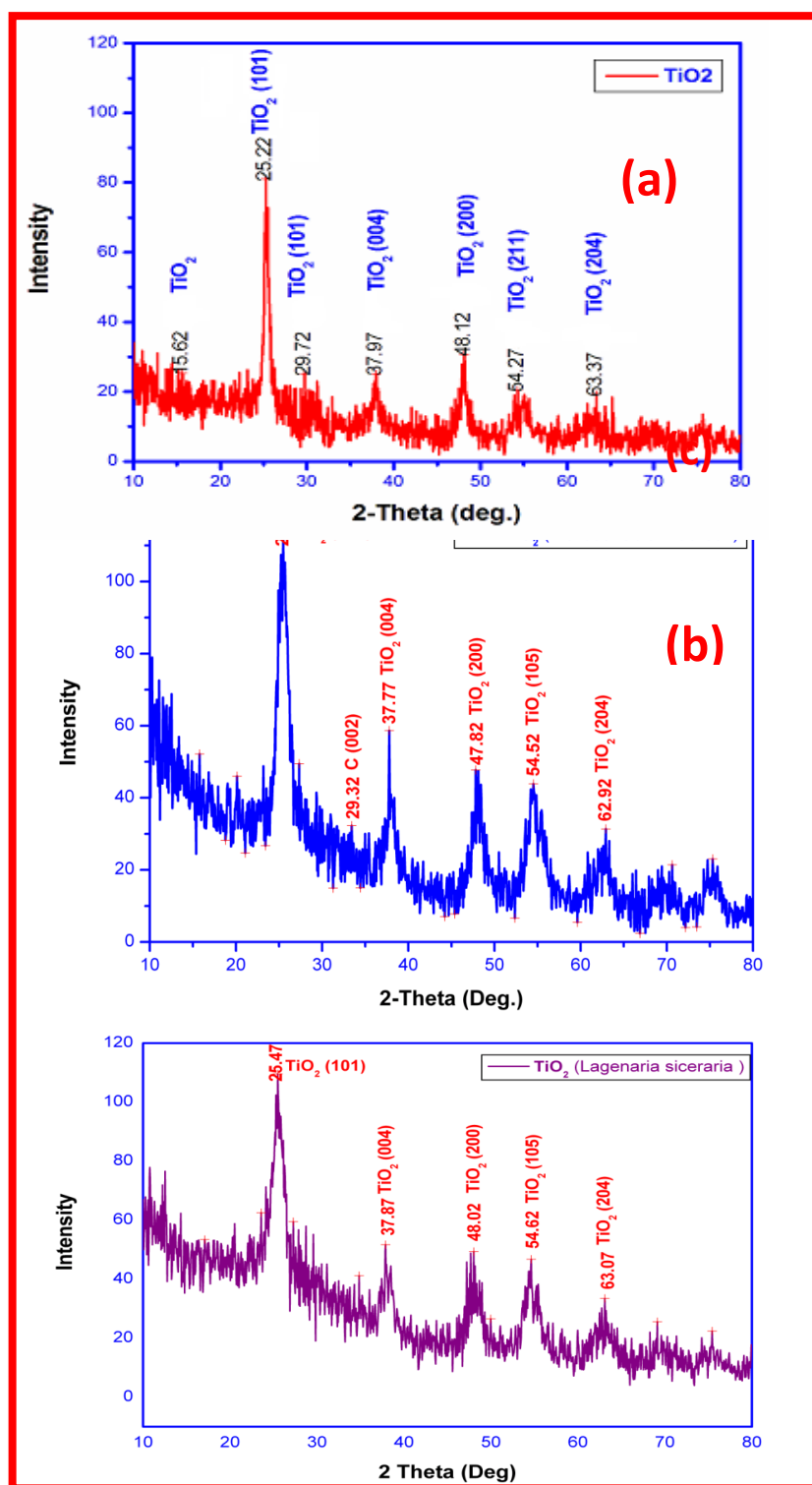


Fig.2. XRD patterns of (a) Pithecellobium dulce leaf Extract synthesized TiO_2 (b) Lagenaria siceraria leaf Extract synthesized TiO_2 (c) Chemically synthesized TiO_2

In the chemically synthesized TiO_2 also, sharp diffraction peaks emerge at 2θ angles 25.22° , 37.97° , 47.12° , 54.27° and 63.37° corresponds to Bragg's reflection from 101, 004, 200, 105 and 204 crystal planes. The result of XRD analysis confirms the presence of TiO_2 nanoparticles in the green and chemically synthesized sample. The XRD peaks of green synthesized TiO_2 nanoparticles obtained using leaf extract of *Lagenaria siceraria* and *Pithecellobium dulce* and chemically synthesized TiO_2 nanoparticles differ in the broadening and intensity. The diffraction peak of the green synthesised TiO_2 nanoparticles is slightly broadened, whereas the peak of chemically synthesized TiO_2 nanoparticles is comparatively sharp. Thus, the broadening of XRD peak of green synthesized 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 synthesized TiO_2 nanoparticles (Naheed Ahmad et al. 2012).

The average crystalline size (L) of the TiO_2 nanoparticles can be calculated from the Debye–Scherrer formula, $L = 0.89\lambda / b \cos \theta$ where L is the crystalline size (in nm), λ is the wavelength (in nm), b is the full width at half maximum intensity (FWHM-in radian), and θ is the Bragg diffraction angle. The average crystalline size of TiO_2 obtained from leaf extract of *Pithecellobium dulce*, *Lagenaria siceraria* and chemically synthesized was figured out to be about 7.5 nm, 8.6nm and 18.5nm. As can be understood, these values are very close to the TEM value.

FT-IR

Fig. 3a, 3b and 3c shows the FT-IR spectrum of leaf Extract synthesized TiO_2 of *Pithecellobium dulce*, leaf Extract synthesized TiO_2 of *Lagenaria siceraria* and chemically synthesized TiO_2 nanoparticles. In green synthesized TiO_2 nanoparticles from leaf extract of

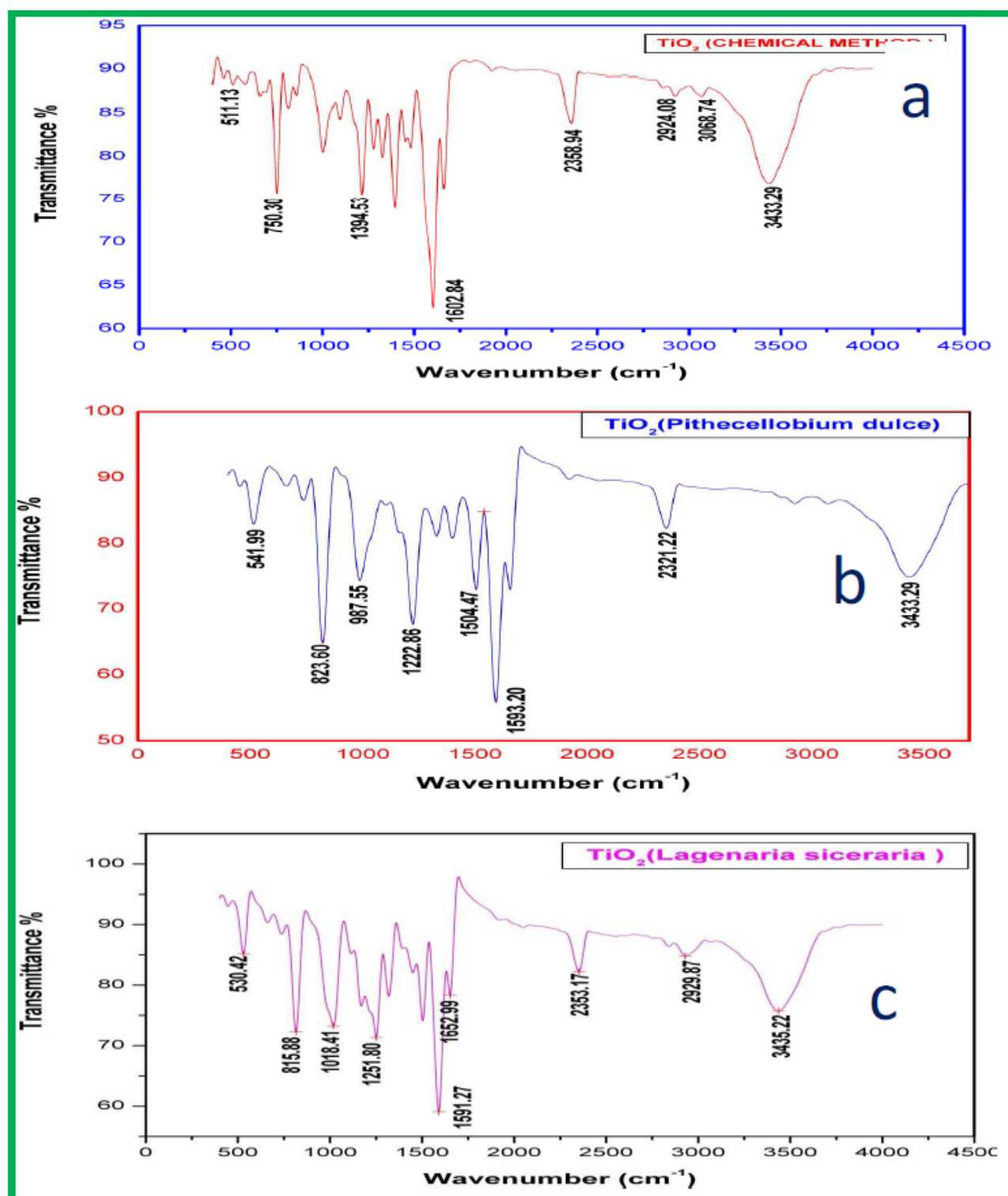


Figure 3: (a) IR Spectrum of Pithecellobium dulce leaf Extract synthesized TiO_2 (b) Lagenaria siceraria leaf Extract synthesized TiO_2 (c) Chemically synthesized TiO_2

Pithecellobium dulce shows IR spectrum covers an absorption band at 3433.29 cm^{-1} , 2321.22 cm^{-1} , 1593.20 cm^{-1} , 1504.47 cm^{-1} , 1222.86 cm^{-1} , 823.60 cm^{-1} and 541.99 cm^{-1} . IR spectrum of Lagenaria siceraria shows absorption band at 3435.22 cm^{-1} , 2353.17 cm^{-1} , 1591.27 cm^{-1} , 1251.80 cm^{-1} , 815.88 cm^{-1} and 530.45 cm^{-1} . Strong absorption peaks at 3433.29 and 3435.22 cm^{-1} refers to O-H stretch of water and phenolic compounds which are present in the FTIR spectrum of Pithecellobium dulce and Lagenaria siceraria extract too that indicates a strong interaction between leaf extracts and green synthesized TiO_2 nanocrystals. The peak at 1593.20 and 1591.27 cm^{-1}

shows the aryl substituted C=C stretching of *Pithecellobium dulce* and *Lagenaria siceraria* leaf extract. The intense peak between 823, 815 and 541, 530 cm^{-1} describes the Ti-O stretching bands of *Pithecellobium dulce* and *Lagenaria siceraria* leaf extract synthesized TiO_2 nonmaterial. At the same time chemically synthesized TiO_2 nanoparticles a broad absorption peak around 3433cm^{-1} is due to the stretching vibration of -OH groups on the TiO_2 surface. The broad band from 511 and 750 cm^{-1} were ascribed to the strong stretching vibrations of Ti-O-Ti bonds. In IR study, the phytochemicals present in the crude extract of the flower may interact with the surface of the TiO_2 nanoparticles, thus forming a cap. Phenol and amide capped nanoparticles were reported to possess significantly higher biomedical activities than uncapped nanoparticles. Therefore, the green synthesized TiO_2 nanoparticles may possess potential biomedical benefits than uncapped TiO_2 Nano particles (Thiyagarajan Devasena et al,2014)

SEM AND EDX

Fig. 4a, 4b and 4c shows the SEM images of biostabilised TiO_2 nanoparticles obtained using *Pithecellobium dulce* and *Lagenaria siceraria* leaf extract and chemical route respectively. The leaf extract mediated synthesized TiO_2 nanoparticles show monodispersity without aggregation when compared to that of chemically synthesized TiO_2 nanoparticles. \

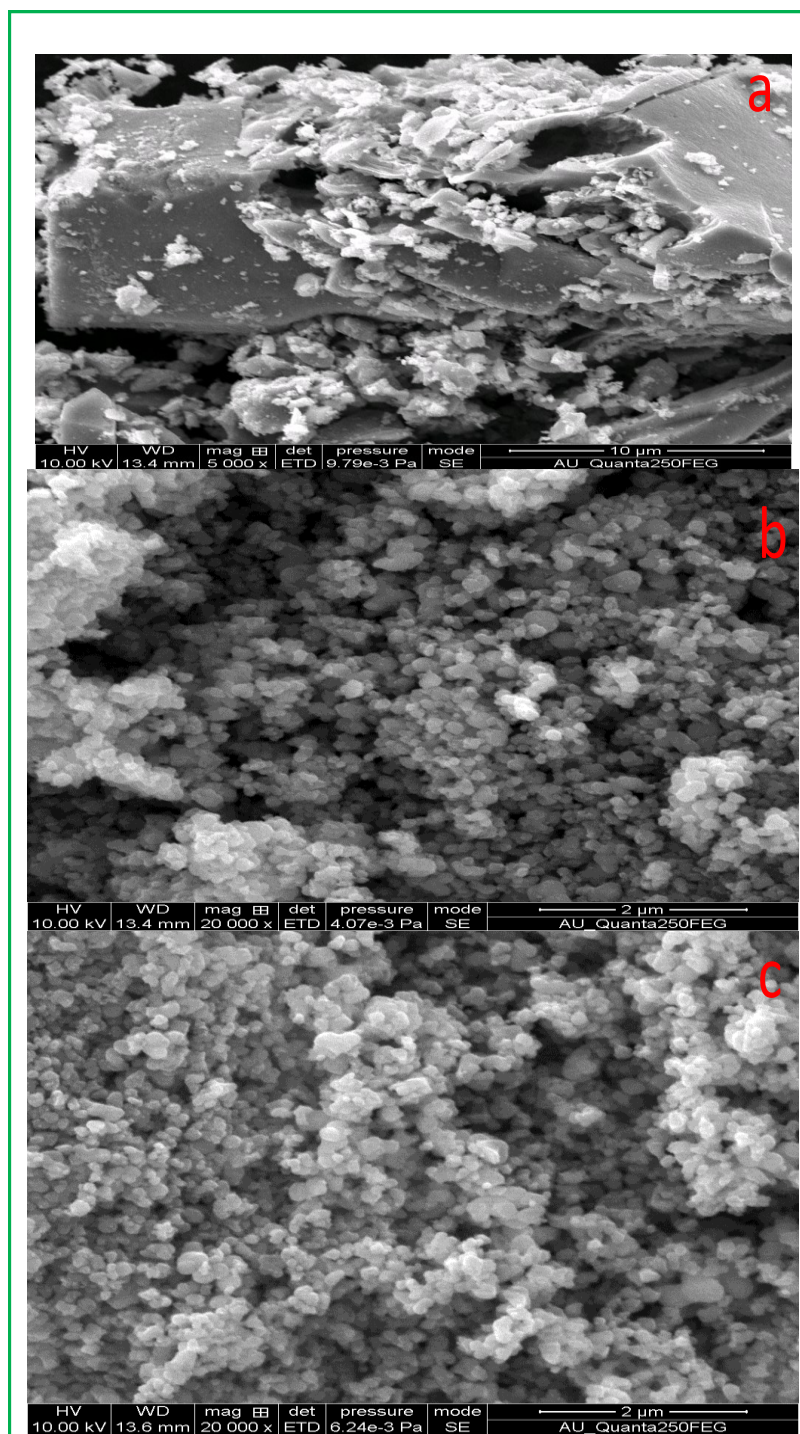


Figure 4: (a) SEM Images of Pithecellobium dulce leaf Extract synthesized TiO_2 (b) Lagenaria siceraria leaf Extract synthesized TiO_2 (c) Chemically synthesized TiO_2

This is due to the capping of TiO_2 nanoparticles with the compounds present in the Pithecellobium dulce and Lagenaria siceraria leaf extract. Previous report that plant phytochemical functionalized nanoparticles are disaggregated and stable with good dispersibility (Archana et al. 2012). EDX spectrum of green synthesized TiO_2 shown in Figure 6a, 6b and 6c, in which peaks of titanium and oxygen elements proves TiO_2 NPs prepared is essentially free from impurities.

TEM

Transmission electron microscopy was used to examine the morphological characteristics of the TiO_2 nanoparticles obtained using Pithecellobium dulce and Lagenaria siceraria leaf extract and chemical route respectively (Figure 5a, 5b, 5c). TEM images showed that sample was composed of agglomerated and isolated particles and it reveals that small particles aggregate into secondary particles because of their extremely small dimensions and high surface energy. Therefore the diameter and the size distribution of the nanoparticles are difficult to be determined precisely by

simply viewing the TEM image. The TEM image of the TiO_2 sample obtained from *Pithecellobium dulce* (Figure 5a) shows the presence of spherical shape with average diameter of ~ 6.3 nm. The morphology of the TiO_2 sample synthesized from *Lagenaria siceraria* is also spherical shape (Figure 5b). These particles have an average diameter of about 6.6 nm. The morphology of the TiO_2 sample synthesized from chemical method spherical and hexagonal shape (Figure 5c), but the particles are greater than those found green method. These particles have an average diameter of about 14.4 nm. The TEM images of both samples show large agglomeration of the TiO_2 particles.

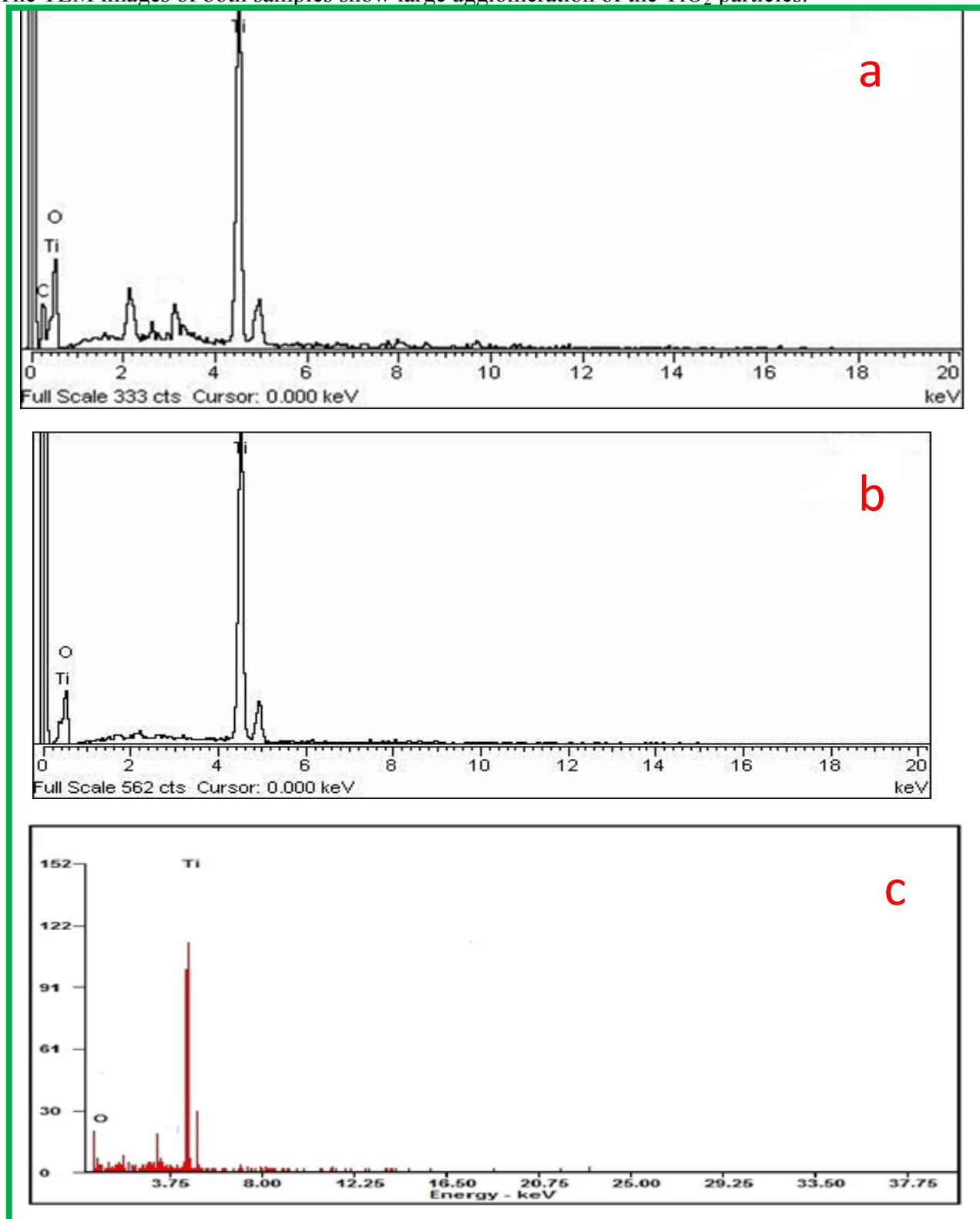


Figure 6: (a) EDX Spectrum of Pithecellobium dulce leaf Extract synthesized TiO_2 (b) Lagenaria siceraria leaf Extract synthesized TiO_2 (c) Chemically synthesized TiO_2

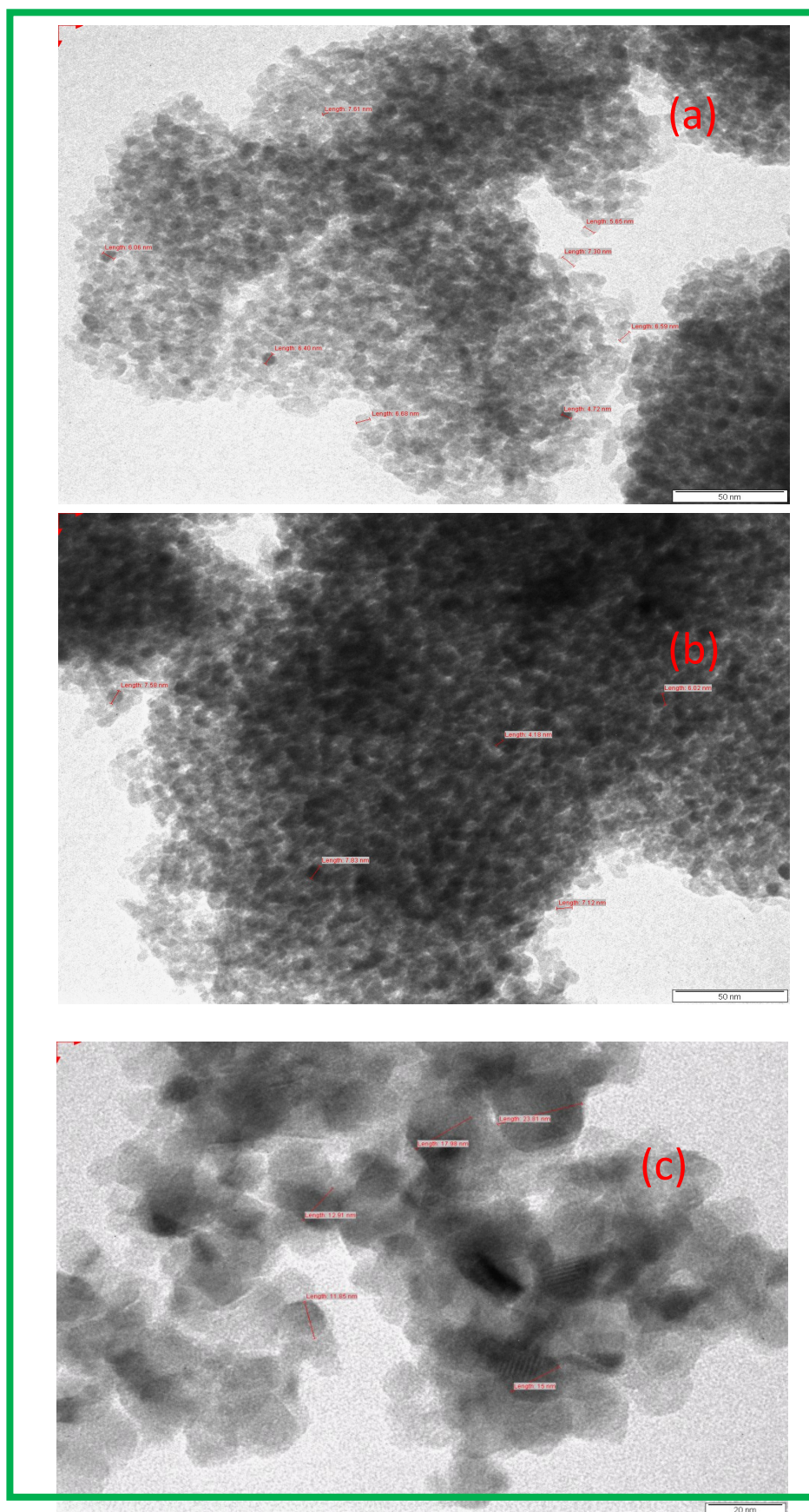


Figure 5: (a) TEM Images of *Pithecellobium dulce* leaf Extract synthesized TiO_2 (b) *Lagenaria siceraria* leaf Extract synthesized TiO_2 (c) Chemically synthesized TiO_2

ANTIBACTERIAL PROPERTIES

Antibacterial activity results revealed that *Pithecellobium dulce* and *Lagenaria siceraria* leaf extract mediated synthesized TiO_2 nanoparticles acted as excellent antibacterial agents against both Gram-positive and Gram-negative bacteria when compared chemically synthesized TiO_2 nanoparticles. It is clear from the XRD and SEM results that leaf extract mediated synthesized TiO_2 nanoparticles are smaller in size compared to chemically synthesized TiO_2 nanoparticles. Figure 7 shows the zone of inhibition produced by green and chemically synthesized TiO_2 nanoparticles against both Gram-positive and Gram-negative bacterial strains. *Pithecellobium dulce* TiO_2 nanoparticles exhibited maximum (14 mm) bacterial growth inhibition against *B. subtilis* and *Lagenaria siceraria* TiO_2 nanoparticles exhibited maximum (11 mm) bacterial growth inhibition against *B. subtilis*, in the form of zone-of-inhibition studies, where diffusion of nanoparticles on nutrient agar plates inhibits growth. In contrast, chemically synthesized TiO_2 nanoparticles showed zones of inhibition of 12 mm against *B. subtilis*. In the case of *E. coli* maximum growth, inhibition zones were found to be the following; 19, 17, and 14 mm for *Pithecellobium dulce*, *Lagenaria siceraria* leaf extract, and chemical synthesized TiO_2 respectively (Figure 7 & Table 1). Similar patterns were observed in the case of *P. aeruginosa*, *Streptococcus pyogenes* and *S. aureus*, where the maximum zone of inhibition was exhibited by *Pithecellobium dulce* followed by *Lagenaria siceraria* and Chemical TiO_2 . It appears that the antibacterial activity of the nanomaterials increased with increase in surface-to-volume ratio due to the decrease in size of nanoparticles.

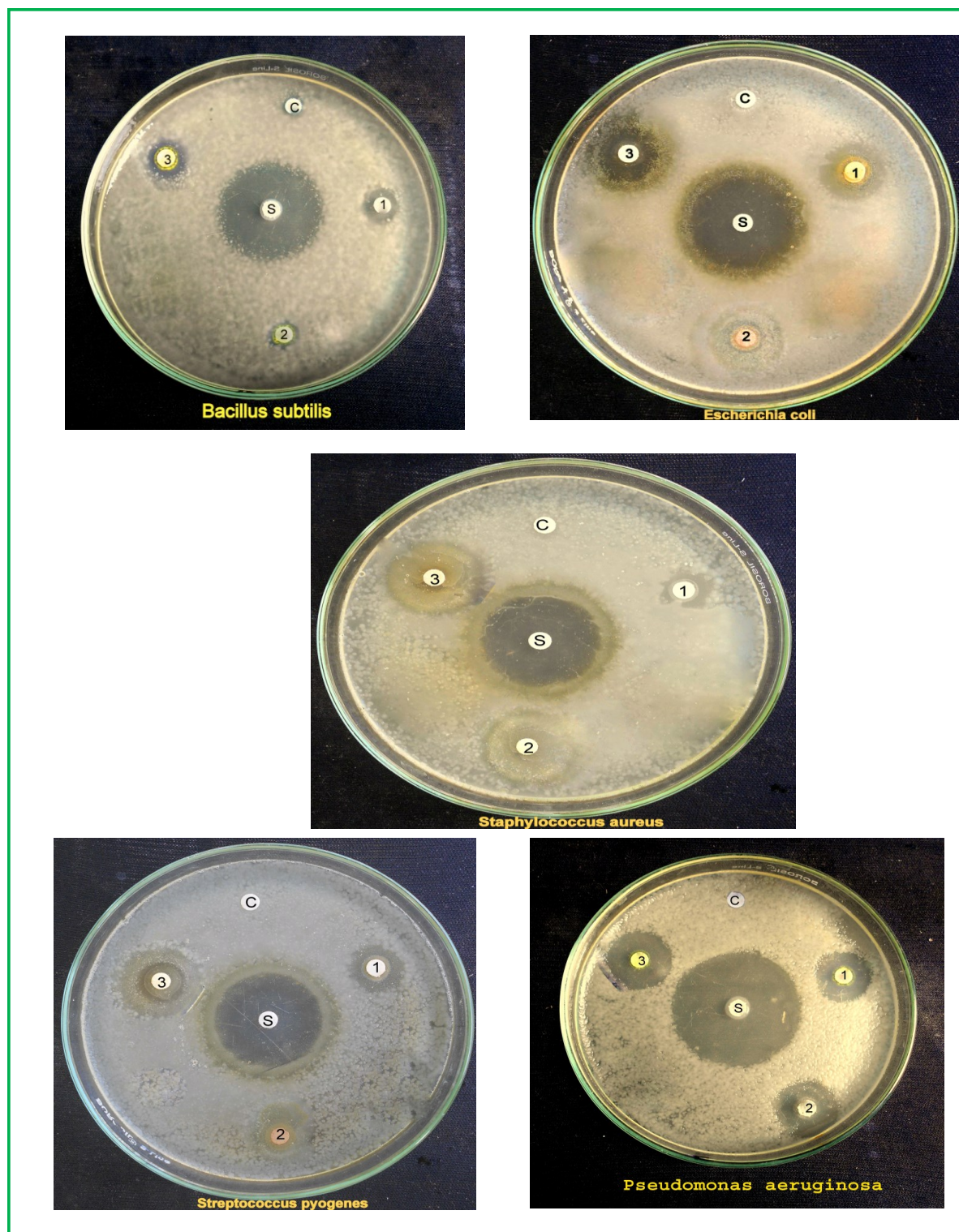


Figure 7: The antibacterial effect of (3) Pithecellobium dulce leaf Extract synthesized TiO_2 (2) Lagenaria siceraria leaf Extract synthesized TiO_2 (1) Chemically synthesized TiO_2

DPPH radical scavenging activity

Free radical is unstable atoms or molecules with outer most electrons unpaired and it is highly reactive. The free radicals always strive to form stable bond, due to gaining or losing of an unpaired electron. DPPH is a stable compound and it will be reduced by accepting the hydrogen or electrons. The reducing activity of TiO₂ nanoparticles synthesized from chemical and leaf extract of *Pithecellobium dulce* and *Lagenaria siceraria* was quantified spectrophotometrically by changing the DPPH color from purple to yellow. Percent of inhibition of DPPH radical scavenging activity was presented on Table 2. The DPPH activity of the TiO₂ nanoparticles synthesized from green and chemical method were found to increase in a dose dependent manner. The DPPH radical scavenging activities were 19.02 ± 0.52 , 28.76 ± 0.15 and $37.56 \pm 0.60\%$ in 50, 100 and 150 μL for chemically synthesized TiO₂ nanoparticles and 31.18 ± 0.42 , 45.48 ± 0.19 and $58.20 \pm 0.25\%$ in 50, 100 and 150 μL for *Lagenaria siceraria* mediated synthesized TiO₂ nanoparticles whereas value obtained 40.78 ± 0.57 , 53.68 ± 0.49 , and $65.57 \pm 0.32\%$ in 50, 100 and 150 μL for *Pithecellobium dulce* mediated TiO₂ nanoparticles, respectively. The results obtained in the DPPH assay showed effective free radical inhibition by chemically and biosynthesized TiO₂ nanoparticles. The average percentage inhibition of *Pithecellobium dulce* mediated synthesized TiO₂ nanoparticles was showed maximum of 52% as compared to that of chemically synthesized TiO₂ nanoparticles and *Lagenaria siceraria* synthesized TiO₂ nanoparticles (26% and 45%) at different concentrations used in this study and the activity increased with increasing concentrations of TiO₂ nanoparticles. The radical scavenging potential is dependent on various factors such as particle size, morphology, defects etc. Thus, the antioxidant assays suggest that *Pithecellobium dulce* and *Lagenaria siceraria* mediated synthesized TiO₂ nanoparticles are good radical scavengers than chemically synthesized TiO₂ nanoparticles due to decrease particle size and increase surface area. The free radical scavenging activity is a surface reaction since only the surface comes in contact with the free radicals.

Table – 1
Antibacterial activity [disc diffusion method]

S. No.	Bacteria	Standard Antibiotic Disk*	Zone of inhibition (mm)			
			1	2	3	C
			Chemical Method TiO ₂	<i>L. siceraria</i> TiO ₂	<i>P.dulce</i> TiO ₂	Control DMSO
1	<i>Bacillus subtilis</i>	25	12	11	14	-
2	<i>Escherichia coli</i>	25	14	17	19	-
3	<i>Pseudomonas aeruginosa</i>	31	13	15	17	-
4	<i>Staphylococcus aureus</i>	28	11	16	19	-
5	<i>Streptococcus pyogenes</i>	28	12	15	18	

*ciprofloxacin

Table – 2

Antioxidant activity of Pithecellobium dulce leaf Extract synthesized TiO₂, Lagenaria siceraria leaf Extract synthesized TiO₂ and Chemically synthesized TiO₂

s. no	Concentrations (μL)	chemically synthesized TiO ₂ nanoparticles	<i>Lagenaria siceraria</i> mediated synthesized TiO ₂ nanoparticles	<i>Pithecellobium dulce</i> mediated TiO ₂ nanoparticles
1	50 μL	19.02 ± 0.52	31.18 ± 0.42	40.78 ± 0.57
2	100 μL	28.76 ± 0.15	45.48 ± 0.19	53.68 ± 0.49
3	150 μL	37.56 ± 0.60	58.20 ± 0.25	65.57 ± 0.32

5. CONCLUSION

In this study we have prepared TiO₂ nanoparticles by using leaf extract of Pithecellobium dulce and Lagenaria siceraria and by a chemical method. Both nanoparticles were characterized for their size, and crystallinity using XRD, FTIR and SEM. The TiO₂ nanoparticles which was capped and stabilized by phenolic and amine moieties of leaf extract was found to be lesser in size and more dispersed than the TiO₂ nanoparticles prepared by chemical method. Moreover the leaf extract prepared TiO₂ nanoparticles exhibited considerable antimicrobial activity against pathogenic bacteria, which is comparable with that of standard antibiotic and also perform antioxidant activity showed green synthesized TiO₂ nanoparticles were effective free radical inhibition than chemically synthesized TiO₂ nanoparticles. Based on these results we conclude that the leaf extract-stabilized TiO₂ nanoparticles may have potential biomedical applications when compared to chemically synthesized TiO₂ nanoparticles due to its enhanced dispersibility, stability and surface coatings.

Reference

- [1] A. L. Barry., *The antimicrobial susceptibility test. Principle and Practice*, Lea and Febiger, Philadelphia, 180 (1976).
- [2] Archana Maurya, Pratima Chauhan, Amita Mishra, and Abhay K. Pandey, Surface functionalization of TiO₂ with plant extracts and their combined antimicrobial activities against *E. Faecalis* and *E. Coli*, *Journal of Research Updates in Polymer Science*, 1, 43-51(2012).
- [3] Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. *Nature*. 29:1199 1200.
- [4] D. Bhattacharya and R. K. Gupta, "Nanotechnology and potential of microorganisms," *Critical Reviews in Biotechnology*, vol. 25, no. 4, pp. 199–204, 2005.
- [5] D. Goodsell, *Bionanotechnology: Lessons from Nature*, Willey- Less, New Jersey, NJ, USA, 2004.
- [6] Deshpande, J.R., A.A. Choudhari, M.R. Mishra, V.S. Meghre, S.G. Wadodkar and A.K. Dorle, 2008. Beneficial effects of *Lagenaria siceraria* (Mol.) Standley fruit epicarp in animal models. *Indian J. Exp. Biol.*, 46: 234-242.
- [7] H.W. Seely and P. J. Van Demark., *A Laboratory Manual of Microbiology*, Taraporewala Sons and Co., Mumbai, 55 (1975).
- [8] Kathiresan K, Asmathunisha N (2013) A review on biosynthesis of nanoparticles by marine organisms. *Coll Surf B* 103:283–287
- [9] Kumar CG, Mamidyala SK. Extracellular synthesis of silver nanoparticles using culture supernatant of *Pseudomonas aeruginosa*. *Colloids and Surfaces B: Biointerfaces*. 2011; 80:462-466.

-
- [10] M. C. Daniel and D. Astruc, "Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology," *Chemical Reviews*, vol. 104, no. 1, pp. 293–346, 2004.
- [11] M. Sundrarajan et al, Green synthesis of titanium dioxide nanoparticles by nyctanthes arbor-tristis leaves extract Chalcogenide Letters Vol. 8, No. 8, August 2011, p. 447-451
- [12] Mahdavi M, Namvar F, Bin Ahmad M, Mohamad R. Green biosynthesis and characterization of magnetic iron oxide(Fe_3O_4) Nanoparticles using seaweed (*Sargassum muticum*)Aqueous Extract. *Molecules* 2013; 18:5954-5964.
- [13] Naheed Ahmad and Seema Sharma, Green Synthesis of Silver Nanoparticles Using Extracts of *Ananas comosus*, *Green and Sustainable Chemistry*, 2, 141-147(2012).
- [14] O. V. Salata, "Applications of nanoparticles in biology and medicine," *Journal of Nanobiotechnology*, vol. 2, no. 1, article 3, 2004.
- [15] R. Paull, J. Wolfe, P. H'ebert, and M. Sinkula, "Investing in nanotechnology," *Nature Biotechnology*, vol. 21, no. 10, pp. 1144– 1147, 2003.
- [16] Rodrigues AG, Ping LY, Marcato PD, Alves OL, Silva MCP, Ruiz RC, Melo IS, Tasic L, DeSouza AO. Biogenic antimicrobial silver nanoparticles produced by fungi. *Applied Microbiology Biotechnology*. 2012; DOI:10.1007/00253-012-4209-7.
- [17] Thiagarajan Devasena et al, Comparative Studies on Green Synthesized and Chemically Synthesized Titanium Oxide Nanoparticles. A Validation for Green Synthesis Protocol using Hibiscus Flower. *J. Environ. Nanotechnol.*, Vol. 3(4), 78-85, (2014)
- [18] Vijayalakshmi, R. and Rajendran, V., Synthesis and characterization of nano- TiO_2 via different methods, *Arch of App Sci Res.*, 4, 1183- 1190(2012).