Chapter 3

Synthesis and Characterization of Pure TiO_2-Nanoparticles using *Syzygium cumini* (Jamun) Leaf Extract: A Green Approach and Antibacterial Properties

This chapter deals with the green synthesis of TiO_2 nanoparticles (NPs) using different fractions of *Syzygium cumini* (Jamun**)** leaf extract. Synthesized NPs are characterized using Dynamic light scattering (DLS), X-ray diffraction (XRD), high-resolution electron microscopy (HR-SEM) with energy dispersive X-ray spectroscopy (EDXS), Bruner Emmet Teller (BET) surface area analysis techniques. Fourier Transform Infrared (FTIR) and zeta potential analysis of leaf extract and synthesized particles is also carried out to investigate the biomolecule present in extract and their function during particle synthesis. HRSEM analysis revealed the formation of non-agglomerated spherical shaped NPs with moderate grain size for 1:1 ratio of aqueous leave extract. The aqueous extract of *Syzygium cumini* (Jamun**)** acted as capping/stabilizing agent and prevented the NPs from agglomeration. Diffuse reflectance spectroscopy (DRS) is used to calculate the band gap energy of the synthesized particle. The antibacterial study of the synthesized TiO_2 against gram negative (*E. coli*) and gram positive (*B.cereus*) is also performed successfully.

3.1 Introduction

Titanium dioxide (TiO_2) is being extensively used as clean photo catalyst, because of its unique physicochemical properties and wide band gap energy (Chen et al., 2006). Different temperatures influence the phases of the particle in nano range i.e. anatase (tetragonal), rutile (tetragonal) and brookite (orthorhombic) (Behpour et al., 2012). Many inherent properties of TiO_2 nanoparticles like non toxicity, photo induced superhydrophobicity, antifogging effect (Borhani and Taghdisi, 2011) and low cost productivity have led their usage in diversified applications like photo-catalytic degradation (Ahmed et al., 2013) and splitting, electro-chromic devices (Tian et al., 2012), photovoltaic cells, sensing instruments, hydrogen storage and environmental remediation.

Basically, synthesis of TiO_2 nanoparticle is done by one of the two approaches i.e. chemical approach and green approach. Chemical approach includes the methods such as chemical vapor deposition (Swihart, 2003), sol processes, sol-gel processes (Mackenzie and Bescher, 2007), laser pyrolysis (Figgemeier et al., 2007), spray pyrolysis (Mueller et al., 2003), atomic or molecular condensation, aerosol processes (Xia et al., 2001), supercritical fluid synthesis (Byrappa et al., 2008), spinning and use of templates. It also involves the rigorous use of harsh and toxic chemicals (Singh et al., 2018). So, an alternative approach known as ‘Green synthesis' is evolved and is the prime interest of many researchers worldwide. It is an important and eco-friendly synthesis technique which comes under the bottom-up approach and requires either plants or microorganisms as one of the material for synthesis. It helps in prevention / minimization of waste, minimal use of non-toxic solvents (Singh et al., 2018) and reduction of pollution. Contrary to the chemically synthesized ones, nanoparticles from green synthesis route poses lesser hazards to the environment and avoid the production of unwanted or harmful by-products through the build-up of stable, sustainable, and eco-friendly synthesis procedures.

Plant extracts may act as reducing agents, stabilizing agents, or capping agent in the synthesis of nanoparticles. For the synthesis of nanoparticles using plant extracts, parameters like temperature, precursor, time, concentration, pH of reactant, etc influences the size, shape, and properties as mentioned in the literature. In addition, various bio molecules present in leaves extract also influence the size and properties of the synthesized particles.

In this chapter, synthesis of TiO_2 NPs using different amounts of *Syzygium cumini* (Jamun**)** leaf extract is performed. Effect of dosage of leaves extract on the synthesis of TiO_2 NP is studied. Experimental methodology, characterization and obtained results are thoroughly discussed.

3.2 Experimental

3.2.1 Materials

Titanium-isopropoxide (TTIP Sigma-Aldrich, AR grade, purity>97%) is used as purchased for the synthesis of TiO_2 nano particles. Fresh leaves of *Syzygium cumini* (Jamun**)** are collected from IIT (BHU) Varanasi campus and are further processed for use as bio-template. Nutrient Agar (NA) and Nutrient Broth (NB) are purchased from High Media, Bombay, India.

3.2.2 Green synthesis of TiO_2 NPs

(A) Preparation of Plant extract

First, *Syzygium cumini* (Jamun**)** leaves collected from the IIT (BHU) Varanasi campus are washed thoroughly with distilled water to remove dust and then subjected to drying in a laboratory dryer. Next, laboratory grinder is used to make the dry leaves into powder form. Now extract is prepared by taking 40g of leaf powder in 200ml of distilled water and heating the mixture at 80°C for one hour. Schematic representation of extract preparation is shown in the Figure 3.1. At the end, the extract is filtered with whatman filter paper (Grade 42) and is stored at 4°C in the refrigerator for further use.

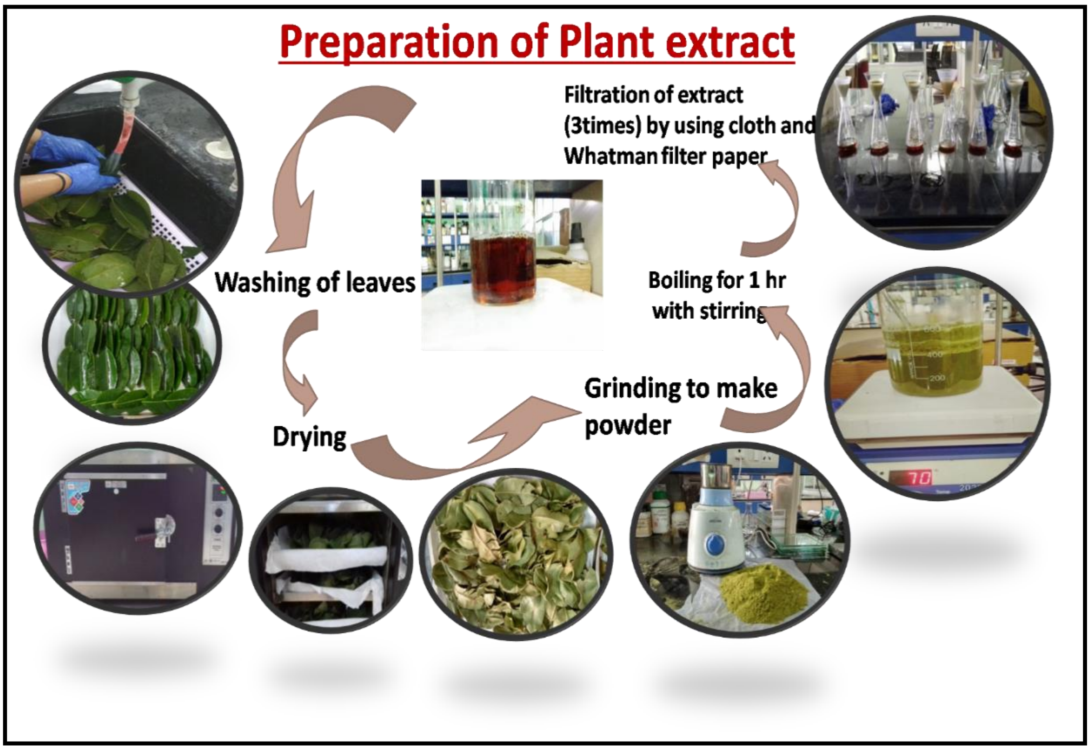


Figure 3.1 Preparation of *Syzygium cumini* (Jamun**)** leaves extract

(B) Synthesis of Titanium dioxide (TiO_2) Nano-particle

Green synthesis of TiO_2 nanoparticle is done by use of previously synthesized leaf extract as stabilizing or capping agent**.** Figure 3.2 depicts the steps involved in the formation of TiO_2 nanoparticles. In the step 1, a fixed concentration of TTIP solution (5mM) is added to the leaves extract in a volumetric ratio mentioned in the Table 3.1. Here different ratios are taken to study its effect on the synthesis of the particles. In the step 2, the mixture solution is allowed to stirrer for 8-10 h at room temperature. A change in colour to light orange is observed in the solution mixture**.** In the step 3, synthesized particles are separated from the solution using centrifugation at 9000rpm for 15 min. In the step 4, recovered particles are cleaned using distilled water thrice and then allowed to dry overnight at room temperature. In the step 5, to remove any unwanted impurities left, calcination is done at 570°C for 3h. White colour TiO_2 NPs powder obtained post calcination is shown in step 6 which are then stored in the air tight bottles for further usage. The effect of extract ratio on the synthesized particles is discussed in results and discussion part of this chapter. Possible reactions mechanism involved in the green synthesis of TiO_2 NPs is shown in equations 3.1, 3.2 and 3.3. When Ti^{4+} from the metal precursor diffuses and form complexes with the molecules present in the leaves extract. The van der waals interaction between the surface molecules act as driving force for the formation of the TiO_2 nanoparticles. The reactions involve in the production of anatase TiO_2 by using TTIP as metal precursor

Hydrolysis: $Ti(OCH(CH_3)_2)_4 + 4H_2O \longrightarrow Ti(OH)_4 + 4(CH_2)_2CHOH$ (3.1)

Condensation: $Ti(OH)_4 \longrightarrow TiO_2.nH_2O + (2\text{-}n)\ H_2O$ (3.2)

Crystallization via calcination: $TiO_2.nH_2O \longrightarrow TiO_2$ (3.3)

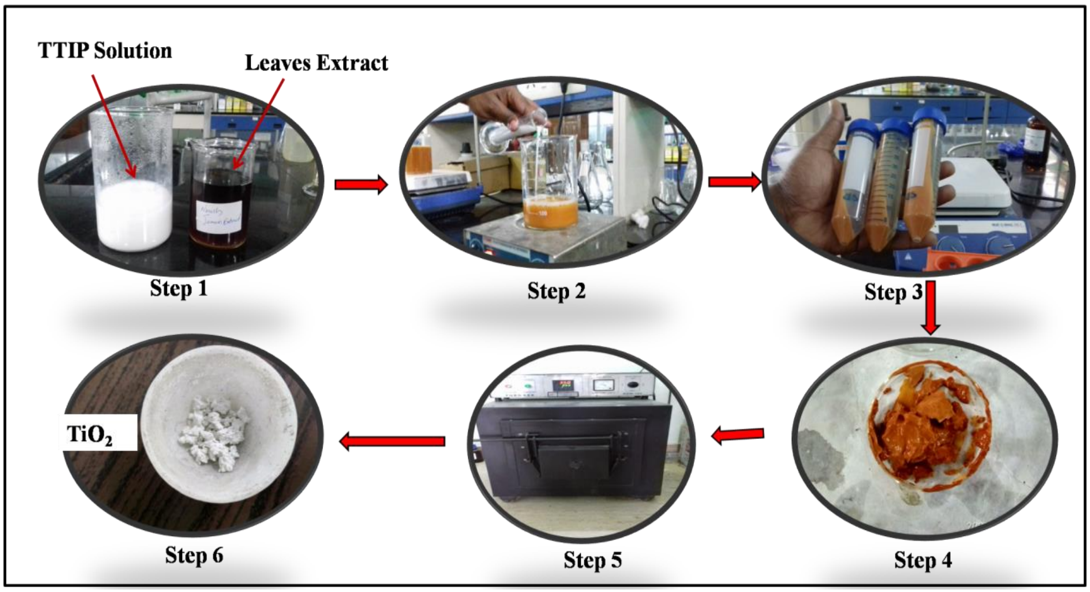


Figure 3.2 Green synthesis of TiO_2 nanoparticles

Table 3.1 Ratios of the leaves extract to metal precursor taken for TiO_2 synthesis

Serial Number	**Precursors**	**Ratio of Leaves Extract (Leaf Extract: Precursors)**
1	Titanium isopropoxide	100ml:100ml(1:1)
2	Titanium isopropoxide	66.7ml:133.4ml(1:2)
3	Titanium isopropoxide	133.4ml:66.7ml(2:1)

3.2.3 Antibacterial test: Halo zone Test

To evaluate the antifouling property by halo zone test, the particles are immobilized in a polymer matrix (due to their easy film processability) for the formation of inhibition zone around the film. Prior to the experiment all the glasswares, petri plates and Nutrient Agar (NA) + Nutrient Broth (NB) solution capped in a conical flask (2.3 g NB, 1.8 g NA in 100 ml distilled water) are autoclaved for 1 h. After specified time all the materials are taken out from autoclave and kept it in laminar bench with UV lights on to bring it to the room temperature for 30 min. In the mean-time the synthesized nanoparticles which is immobilized in an inert polymeric film is also placed under UV light for sterilization. The NB+ NA solution is then poured on the petri plate and is allowed to solidify. After solidification, 100µl of bacterial solutions (*E.coli, B. cereus*) are added separately and finely spread on the agar plates. UV-treated nanocomposite film is carefully placed on the plate; incubated it for one day at 35°C to all zone formation as shown in the Figure 3.3.

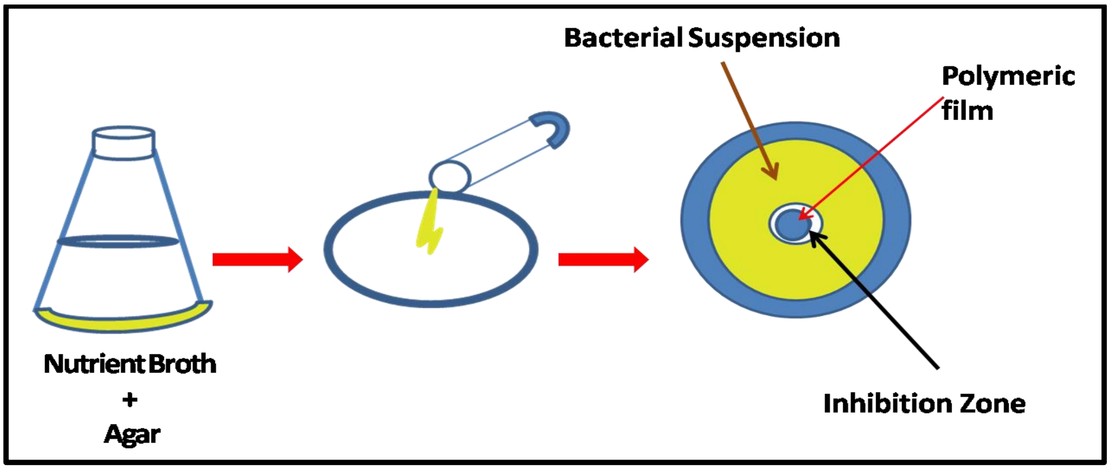


Figure 3.3: Antibacterial property test

3.2.4 Characterization of synthesized TiO_2 NPs

Green synthesized TiO_2 NPs are characterized by powder X-ray diffraction to know its crystal structure and phase. X-ray diffractometer (Ultima IV, Rigaku) equipped with a Cu-Kα radiation (λ = 0.154 nm) within a 2θ range of 10°–80° at 40 kV/15 mA is used. The distribution of TiO_2 nanoparticles and morphological structure are investigated using high-resolution scanning electron microscopy (HRSEM) (Nova Nano SEM 450) equipped with an energy dispersive X-ray spectroscopy system (EDAX Inc.). Particle diameter and selected area electron diffractions (SAED) patterns are recorded by utilizing a high-resolution transmission electron microscope (TEM, Model: Tecnai G2 20 TWIN, FEI Company of USA). Fourier Transform-Infrared spectrometer (FTIR) (Model Nicolet 560) is used to characterize the functional groups involved in the green synthesis of TiO_2 NPs. Brunauer-Emmett-Teller surface area (ASAP 2020, Micromeritics) is done to know the surface area, pore size distribution and pore volume of the green synthesized TiO_2 NPs. The average particle size and charge on the surface of the particle is analysed by a dynamic light scattering (DLS, Nano plus common). The band gap is studied using CARY-100 Bio UV – Visible spectrophotometer.

3.3 Results and Discussion

3.3.1 X-ray diffraction (XRD) analysis of TiO_2 NPs

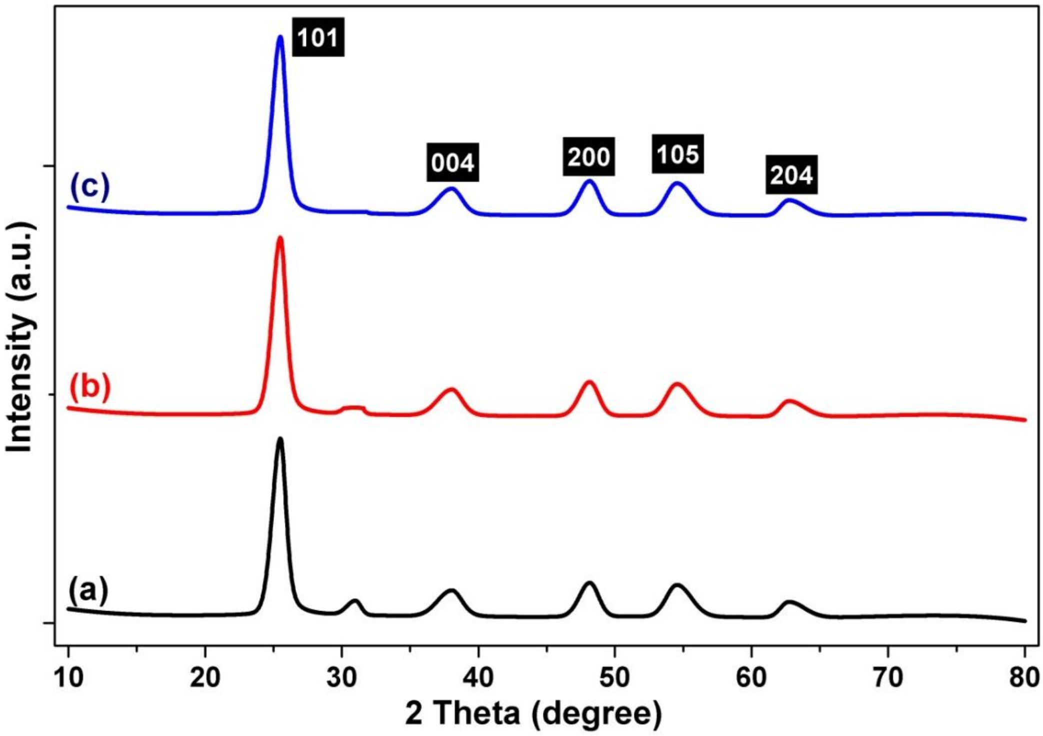


Figure 3.4 X-ray patterns of TiO_2 NPs at (a) 2:1, (b) 1:1, (c) 1:2 ratio of leaf extract to metal precursor

XRD patterns of the green synthesized TiO_2 NPs at different ratios of leaf extract to metal precursor are shown in Figure 3.4, which successfully unfolded its crystalline nature and anatase phase. Various peaks in a wide range of 2θ angle (10 <2θ <80) revealed that the distinct peaks recorded at 25.49, 38.04, 48.12, 54.49 and 62.65^0 are well corresponded to the miller indices (hkl) values, (1 0 1), (0 0 4), (2 0 0), (1 0 5) and (2 0 4) respectively, confirming the anatase phase (calcined at 570 °C for 3 h) of the crystalline TiO_2 NPs. Obtained results also closely match with the Joint Committee on Powder Diffraction Standards (Anatase XRD, JCPDS Card No. 78-2486) (Chen et al., 2009). The sharp peaks clearly indicate the high crystallinity of the nanosized TiO_2, which is considered to be favorable for photocatalytic activity. Crystalline size of the green synthesized TiO_2 NPs is calculated by Debye Scherrer's equation 3.4.

$$d = 0.89\lambda/\beta \cos\theta \qquad (3.4)$$

where d is the crystalline size of NPs (nm), λ is the wavelength of X-ray radiation source, 0.89 is a constant crystalline shape factor, θ is the Bragg's diffraction angle, and β is the angular full width at half maximum (FWHM) of XRD peaks recorded at diffraction angle 2θ. From the Figure 3.4 the average crystalline size of the green synthesized TiO_2 NPs is found to be 10 nm, 11 nm and 13 nm as the concentration of the metal precursor increased from 66.7ml to133.4ml as listed in the Table 3.2. At diffraction angle 2θ equal to 31° in Figure 3.4 (a) and (b) a weak diffraction peak is observed due presence of (121) face of brookite phase with orthorhombic crystalline structure. Literature mentions that samples containing both the phase exhibit high photo catalytic activity (Allen et al., 2018).

Table 3.2 Crystallographic parameters for synthesized TiO_2 NPs at different leaf extract ratio

Ratio of Leaves Extract: Precursor	2θ(Degree)	d Spacing	FWHM	Crystallite size in nm
1:1	25.56	3.48	0.846	11
2:1	25.49	3.46	0.987	10
1:2	25.87	3.42	0.835	13

3.3.2 SEM-EDX analysis of TiO_2 NPs

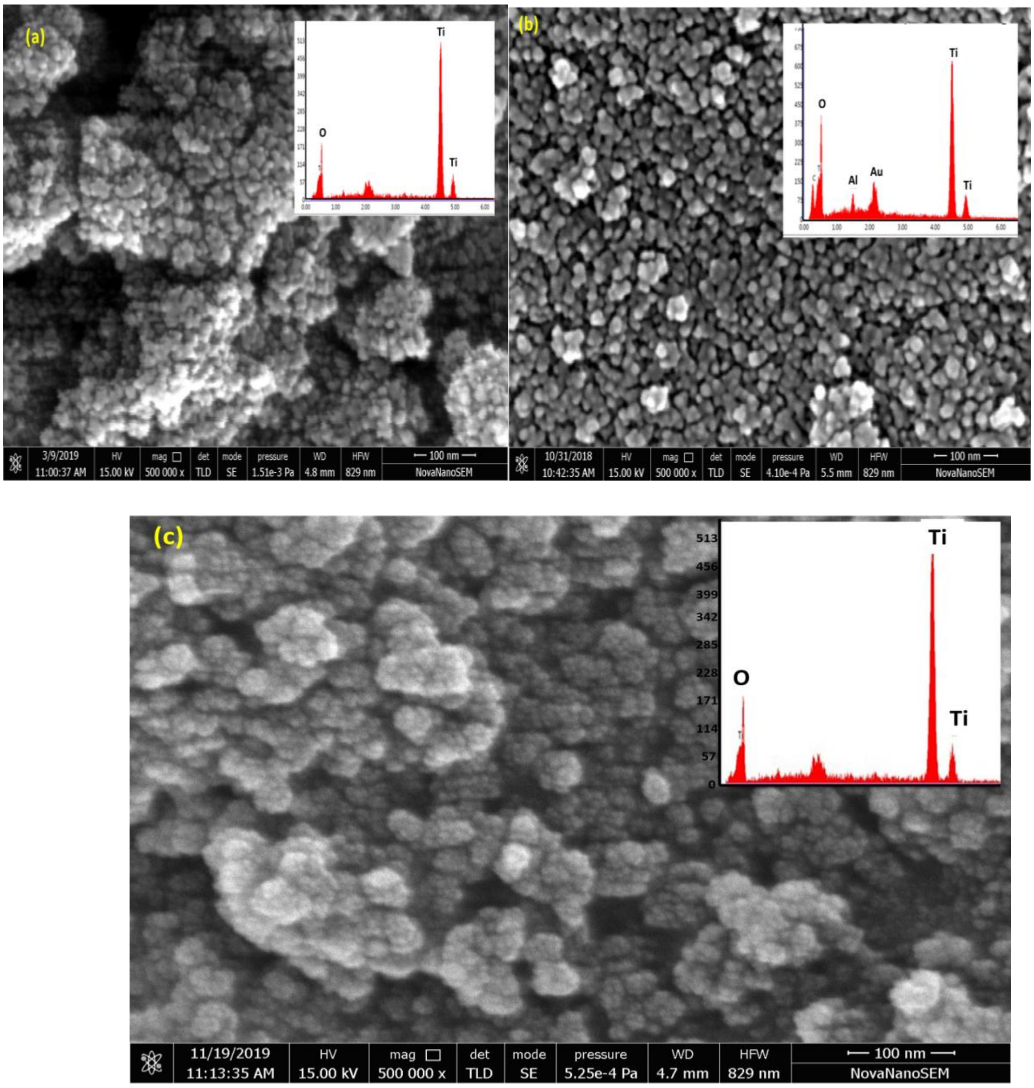


Figure 3.5 HRSEM-EDX of synthesized TiO_2 NPs (a) TiO_2 (2:1), (b) TiO_2 (1:1), and (c) TiO_2 (1:2)

The morphology of the particles obtained under different ratios of leaf extract to metal precursor was shown by HRSEM images in Figure 3.5(a-c). In Figure 3.5a, the particle is synthesized by taking 2:1 ratio of jamun extract to TTIP solution. The particles are spherical in shape and small in size due to high concentration of capping/stabilizing agent available in the reaction mixture compared to others. The particles size is observed in the range 12nm to 21nm. While in Figure 3.5b, the particles are spherical in shape with little bigger in size as well as good yield is achieved with 1:1ratio of the jamun extract to TTIP solution. The average size of the particle is found to be 20nm in size. In Figure 3.5c, particles of bigger size and more agglomeration are observed. The average particle size is found to be 23nm. Here the volume of leaf extract is less compared to volume of the metal precursor. So the enlargement in size of the particles happened due to the coagulation of the particle that is generated from the high concentration of TiO_2 nuclei at high TTIP concentration (Kim et al., 2005, Wang et al., 2005). The composition analysis of the synthesized TiO_2 NPs is determined by the EDAX analysis and results are shown in the inset of Figure 3.5(a-c) along with the HRSEM images. It is clearly visible that Ti and O are the major elements found. This also confirms the presence of TiO_2.

3.3.3 TEM analysis of TiO_2 NPs

Figure 3.6(a-c) give the TEM images and SAED (selected area diffraction) pattern of TiO_2 nanoparticles synthesized from the *Syzygium cumini* (jamun leaf) extract by green route. The average size of the particle in the Figure 3.6(a),(b)&(c) is found to be 10nm,11nm and 13nm respectively with spherical shape which is very close to the crystal size calculated by Debye Scherrer's equation in the XRD result. In the SAED image, the diffraction rings with bright circular spots match with the XRD pattern and confirmed the anatase phase of the TiO_2 nanoparticles in the Figure 3.6(a),(b)&(c) (Saranya et al., 2018). As the volume of the extract increased in the reaction mixture a spherical, less agglomerated and small size particle was formed because of the biomolecules present in the extract act as capping/stabilizing agent.

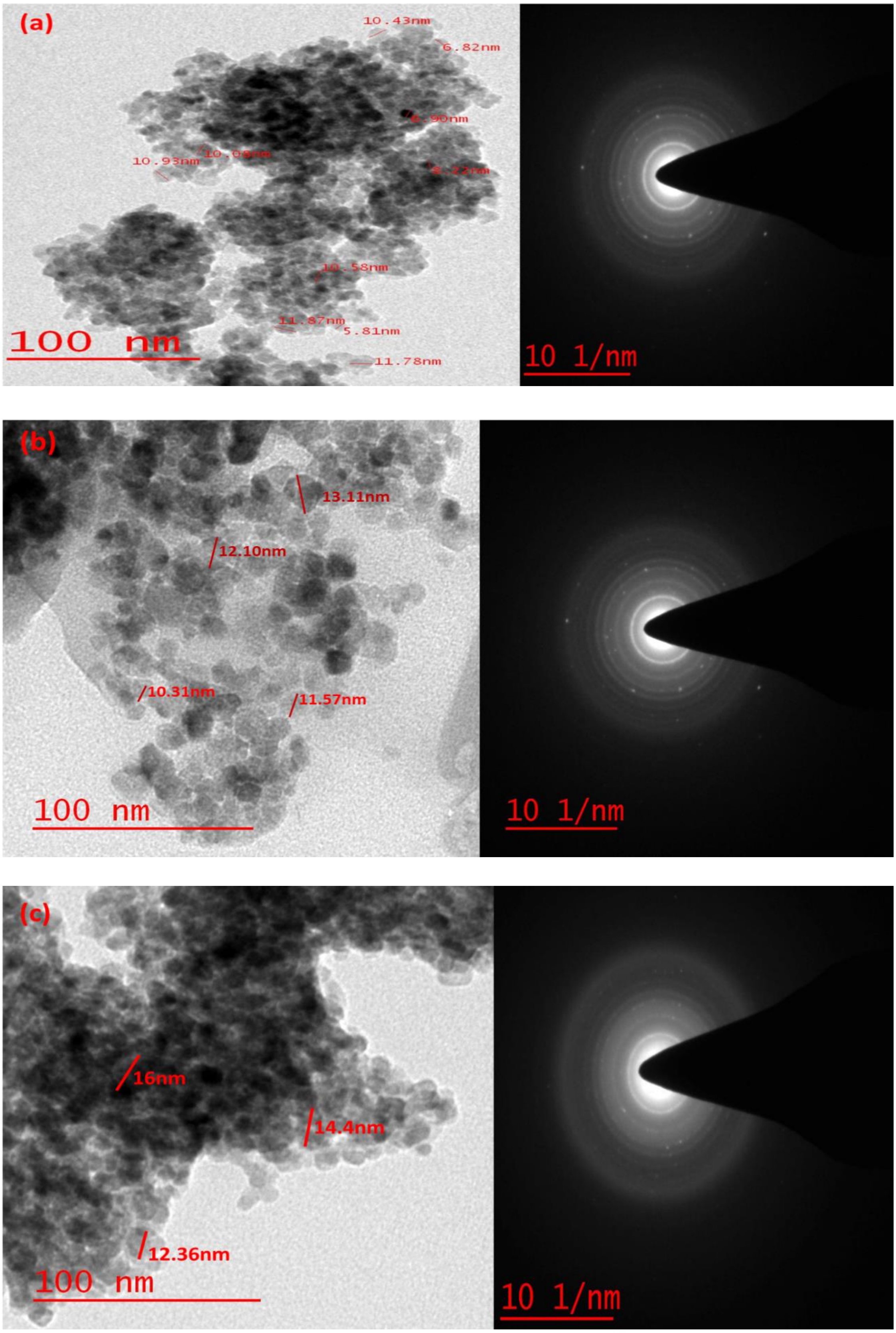


Figure 3.6 TEM analysis and SAED pattern of synthesized TiO_2 NPs at (a) 2:1 (b) 1:1 and (c) 1:2 ratio of leaf extract to metal precursor

3.3.4 Dynamic light scattering (DLS) of TiO_2 NPs

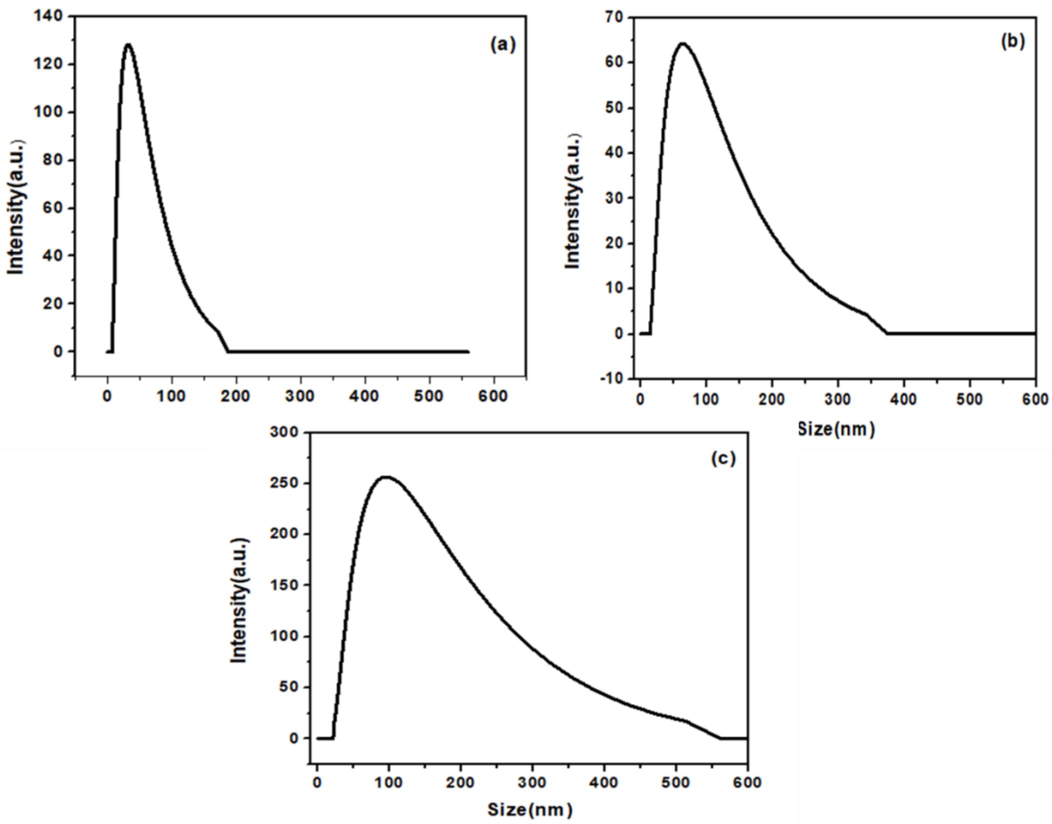


Figure 3.7 Particle size distribution of synthesized TiO_2 NPs at (a) 2:1 (b) 1:1 and (c) 1:2 ratio of leaf extract to metal precursor

To analyze the particle size distribution, green synthesized TiO_2 NPs are subjected to dynamic light scattering (DLS) analysis. Here the material is first dispersed uniformly in distilled water using ultra-sonicator for DLS analysis. The DLS spectra of particles are shown in the Figure 3.7(a), (b) &(c). The average particle size at maximum intensity was observed at 55nm, 72nm and 90nm for 2:1, 1:1 and 1:2 ratio of leaf extract to metal precursor respectively. Herein also the increase in value of average particle size was observed with increase in TTIP concentration in the reaction mixture for the synthesis which further justifies results as obtained from XRD, HRSEM and TEM results. However, the higher particle size value obtained from DLS than from XRD, HRSEM and TEM at given condition is attributed to the presence of water film on the surface of the particle and time taken by the analyzer for analysis responsible for the agglomeration of particle with bigger particle size (Canesi et a., 2010, Cho et al., 2004, Nadtochenko et al., 2005).

3.3.5 N_2 adsorption and desorption analysis

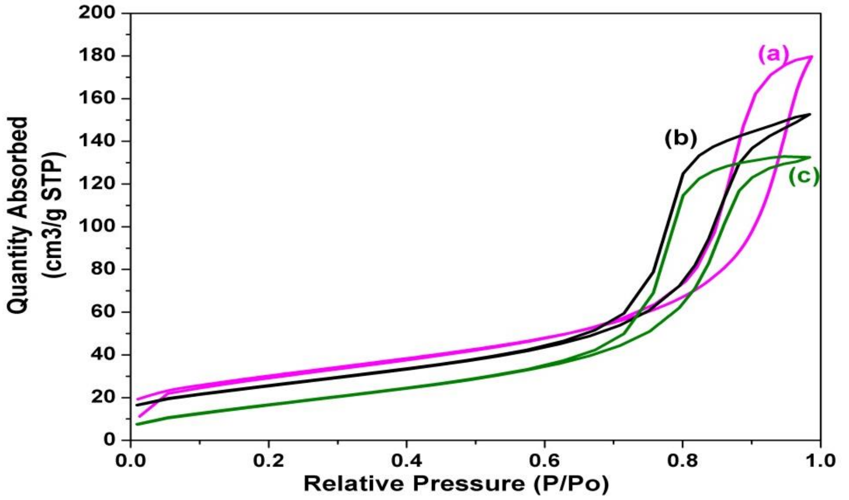


Figure 3.8 N_2 adsorption–desorption isotherms of synthesized TiO_2 NPs at (a) 2:1 (b) 1:1 and (c) 1:2 ratio of leaf extract to metal precursor

The N_2 adsorption–desorption isotherms of the synthesized TiO_2 NPs is determined and shown in the Figure 3.8 (a-c) at different ratios (2:1,1:1,1:2) of jamun leaf extract to TTIP metal precursor. It is observed that, as the volume of extract decreased in the reaction mixture for the synthesis of nanoparticle, the surface area decreased. The highest surface area obtained by taking 2:1 ratio is $105m^2g^{-1}$. The surface area varied from $105m^2g^{-1}$ to $55m^2g^{-1}$, with decreased in fraction of the leaves extract is attributed to the increased the particle size of the synthesized TiO_2 NPs (Kim et al., 2009).

3.3.6 UV-Vis spectroscopy of TiO_2 NPs

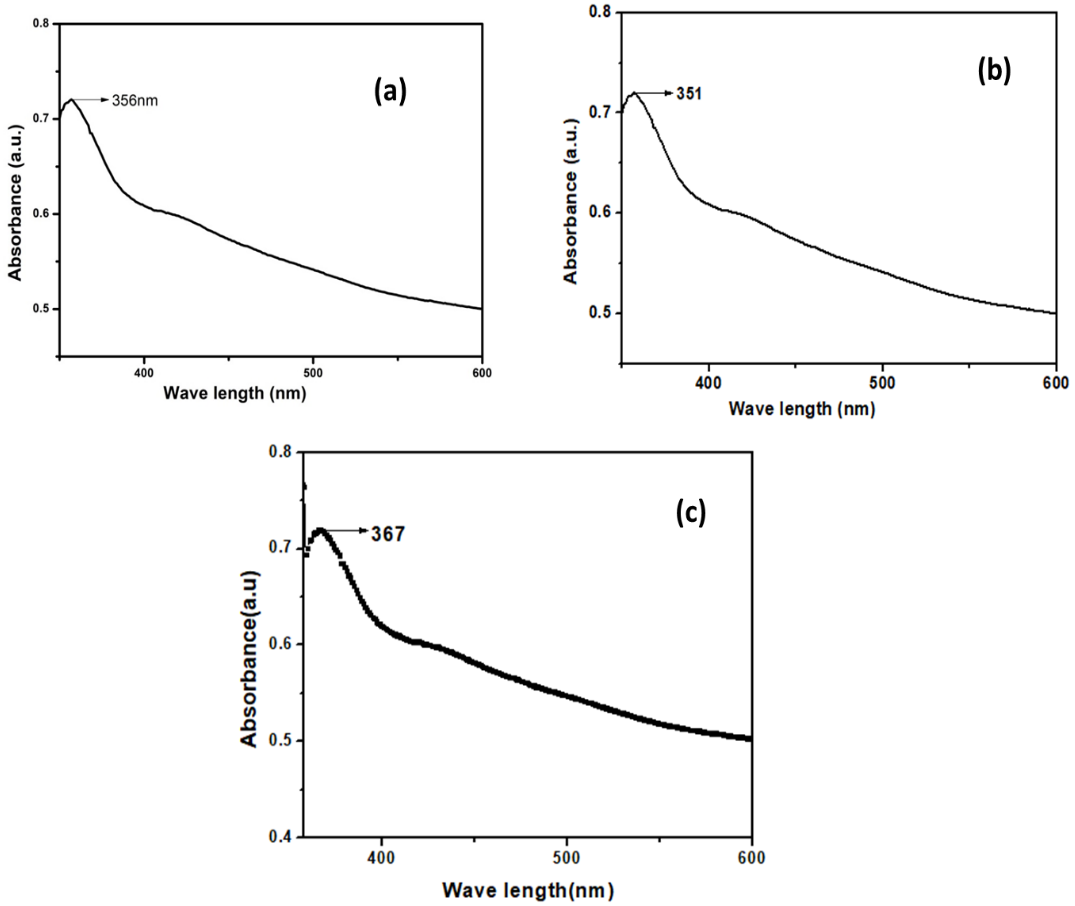


Figure 3.9: UV- Visible spectra of synthesized TiO_2 NPs (a) 2:1 (b) 1:1 and (c) 1:2 ratio of leaf extract to metal precursor

UV- Visible spectrophotometer is used to study the absorption of TiO_2 nanoparticles and absorption spectra of the synthesized TiO_2 using different ratios of *Syzygium Cumini* extract to TTIP metal precursor are shown in the Figure 3.9 (a-c). Results obtained as shown in the Figure 3.9 showcases a prominent peak at 356 nm, 351nm and 367nm with absorbance less than 1. Energy Band gap of synthesized TiO_2 nanoparticles by green route can be estimated using equation 3.5 (Vijayalakshmi et al., 2012)

$$Eg = \frac{hc}{\lambda} \qquad (3.5)$$

where Eg: Band gap energy (eV), h: Planck's constant, c: light velocity (m/s), and λ: wavelength at maximum absorbance (nm). From equation (3.5), the band gap energy (Eg) of the synthesized particles is calculated and results are shown in the Table 3.3. Band gap energy of TiO_2 particle as given by the literature is 3.0eV≤ Eg ≤ 3.7eV **(**Lo´pez and Go´mez, 2012**).** Calculated value of band gap energy for the synthesized TiO_2 nanoparticles also lies in the same range which further confirms the successful formation of TiO_2 NPs in all three cases and the particle has the ability to be used as photo catalyst for various applications (Jwo et al., 2005).

Table 3.3 UV-Visible absorption spectra of TiO_2 nanoparticles synthesized using different ratios of *Syzygium cumini* (jamun) leaf aqueous extracts

Leave extract to metal precursor	**Wave length(nm)**	**Band gap energy(Eg) in eV**
1:1	356	3.48
2:1	351	3.53
1:2	367	3.38

3.3.7 FTIR & Surface Charge Analysis

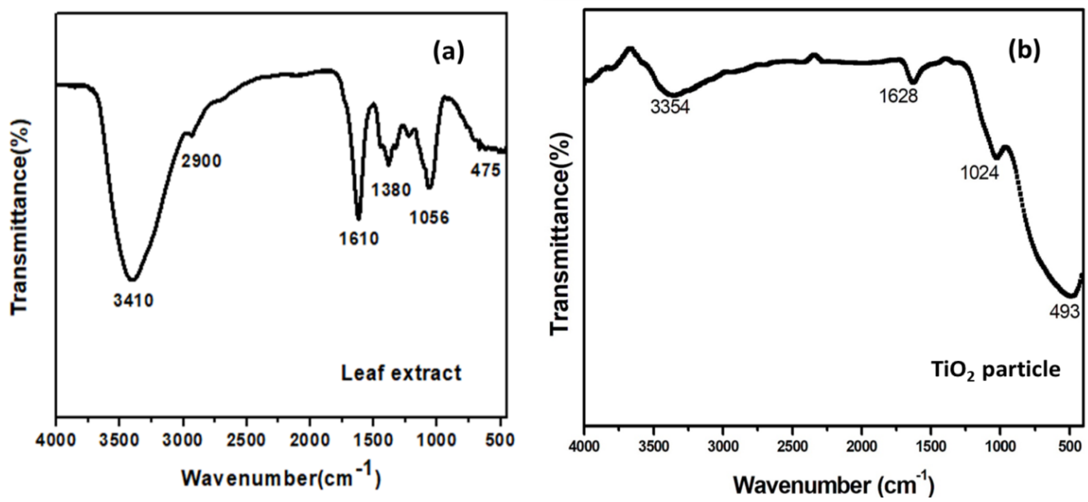


Figure 3.10 FTIR plot of (a) *Syzygium cumini* (jamun) extract; (b) Synthesized TiO_2 NPs

To identify the biomolecules present in the *Syzygium cumini* (jamun) leaf extract responsible for the capping/stabilization of the metallic ions (Ti^{+4}) of the metal precursor TTIP, FTIR spectroscopy is employed. FTIR results of *Syzygium cumini* (jamun) extract show in the Figure3.10(a) reveal that the peak at 3410 cm^{-1} is due to intramolecular bonded alcohols present in leaf extract (Kumar et al., 2013). Another broad peak at 2900 cm^{-1} is due to presence of carboxylic group whereas the peak at 1610 cm^{-1} indicates the presence of secondary amines, amides, quinones or conjugated ketones. Another peak at 475 cm^{-1} is for S stretch and indicates the presence of polysulphides (Kumar et al., 2013). Aromatic group is ($AryleOeCH_2$) represented by peaks 1056 cm^{-1}. The presence of above mentioned peaks in leaf extract indicates that, the extract added to the TTIP solution is acting as stabilizing/capping media in order to control the particle size formation from agglomeration.

The FTIR spectra of green synthesized TiO_2 NPs is shown in Figure 3.10(b). It reveals that, green synthesized TiO_2 NPs has a peak at 3354 cm^{-1} and a small peak at 1628 cm^{-1} due to hydroxyl group and surface adsorbed water respectively (Ghaly et al., 2011). Peaks at 1024 and 493 cm^{-1} are due to Ti-O stretching and Ti-O-Ti bridging stretching mode (Peiro et al., 2011; Yu et al., 2006). Removal of all the organic compounds during calcination at 570 °C for 3 h is confirmed by no peak recorded at 2900 cm^{-1}(Martra et al., 2000).

Further the impact of biomolecules from leave extract on the synthesis of TiO_2 can be predicted by Zeta potential analysis of the TTIP solution and the TTIP solution leaf extract. Biomolecules present in the leaves extract act as capping/stabilizing agent studied by Akhtar et al., 2013. The influence of the extract on the particle synthesis in terms of zeta potential is shown in Figure 3.11. TTIP undergoes hydrolysis to produce

TiO_2 particles when comes in contact with water. But proper shape and size can be provided with help of capping/stabilizing agent. As shown in Figure 3.11, the zeta potential value of TTIP solution was positive value but when it was mixed with the jamun leaves extract it went to negative value i.e. -19mV which is due to the adsorption of capping /stabilizing agents biomolecules on the surface of the TiO_2 nanoparticle particles and change the surface charge negative (Akhtar et al., 2013). If the zeta potential value is more negative, the particle will be more colloidal in nature and with less agglomerated (Vadlapudi and Amanchy, 2017).

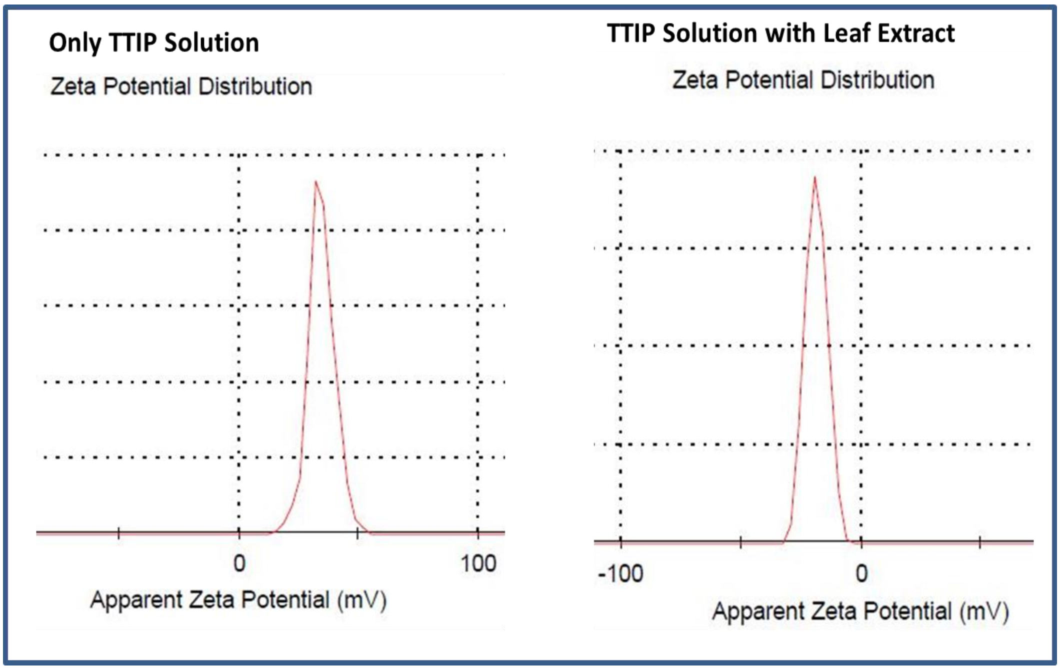


Figure 3.11 Zeta potential analysis of TTIP solution and TTIP with *Syzygium cumini* (jamun) extract

3.3.8 Antibacterial property of the synthesized TiO_2 NPs

It is beneficial for the green synthesized TiO_2 particles to have antifouling property for its successful application in various fields. Pure PDMS (poly-dimethyl siloxane) polymer without TiO_2 and particles synthesized using different ratios of jamun leaf extract to metal precursor are shown in the Figure 3.12. Here PDMS polymer is used to immobilize the particles in it for antibacterial test against gram negative (*E. coli*) and gram positive (*B.cereus*). Figure 3.12(b), (c) & (d) showed the inhibition zone formation around the polymer incorporated with TiO_2 particles after 24 h for gram negative (*E. coli*). But in case of Figure 3.12(a), the overgrowth of bacteria is observed without any zone formation. The inhibition zone represents a clear area around the polymeric matrix without any bacterial growth. So, the TiO_2 particles synthesized using different ratios of jamun leaf extract showcased antibacterial property and encouraged its usage for various biomedical applications. Similar results were observed in gram positive (*B.cereus*) bacteria as shown in Figure 3.12 (e-h).

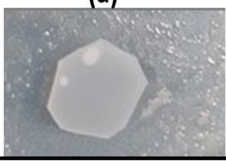
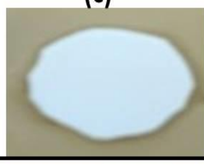
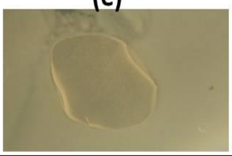
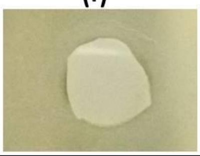
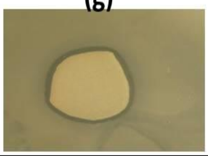
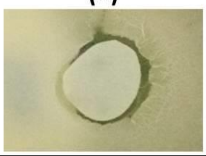
Type	Without TiO2	TiO2 NPs (1:2)	TiO2 NPs (1:1)	TiO2 NPs (2:1)
Gram Negative	(a) 	(b) 	(c) 	(d) 
Gram Positive	(e) 	(f) 	(g) 	(h) 

Figure 3.12 Antibacterial test of the synthesized TiO_2 NPs by Inhibition zone formation

3.4 Conclusion

Green synthesis technique is adopted for the synthesis of TiO_2 nanoparticles by using different ratios of jamun leaf extract to metal precursor (TTIP). The synthesized spherical TiO_2 particles are in the nano meter scale and increase in the particle size with increase in volume of the precursor in the reaction mixture is observed. TiO_2 nanoparticles with non-agglomerated spherical shaped and moderate grain size are obtained for 1:1 ratio of aqueous leave extract. Crystalline nature and anatase phase of the synthesized particle is confirmed from the XRD analysis. Other characterization techniques are adopted to know the size, shape and surface area of the green synthesized TiO_2 nanoparticles. Results also revealed the influence of leaf extract ratios on the morphology of the particles. From the FTIR analysis, the functional groups which act are responsible for capping /stabilizing action of the extract is identified. Antibacterial study of the synthesized TiO_2 has shown good results against gram negative (*E. coli*) and gram positive (*B.cereus*). Work done in this chapter is a quick and environmental friendly way for the preparation of TiO_2 NPs with good photocatalytic property.