Green Hydrothermal Titanium Dioxide Nanoparticle Synthesis and Characterization with Cayratia Pedata Leaves for Biomedical Applications

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Abstract— **Titanium dioxide (TiO_2) is one of the most revised materials due to their gentle properties, accessibility, and chemical and thermal strength. Titanium dioxide nanoparticles are an incredible class of materials through a broad range of applications owing to the unique properties comprising photo catalytic activity, biocompatibility, and UV-blocking competences. Existing nanoparticle synthesis schemes often involve using unsafe chemicals and high energy consumption, increasing environmental worries and producing toxicity. In contrast, green synthesis methods give workable outcomes through applying bio-based materials such as microorganisms, plants and agricultural waste for NP synthesis. Green synthesis of TiO_2 nanoparticles using Hydrothermal Synthesis is designed in this paper. In this paper, NP synthesis is successfully synthesized with the help of green hydrothermal synthesis scheme using TiO_2 and plant extract of the Cayratia Pedata. The yellow-colored paste acquired is completely dried, gathered and packed for additional analysis. The major aim of this work is to synthesize bio mediated Titanium dioxide and scrutinize its antioxidant property. Furthermore, TiO_2 NPs is further studied through the X-Ray Diffraction, Fourier Transform Infrared Spectroscopy, Scanning Electron Microscopy and Photocatalytic activity, Antibacterial Activity to explore the structural, optical, morphological and antioxidant properties.**

Index Terms— **Green synthesis, Titanium dioxide (TiO_2) nanoparticles, Hydrothermal synthesis, Cayratia Pedata, Scanning Electron Microscopy**

I. INTRODUCTION

Nanotechnology has grown exponentially across disciplines from medicine to engineering. Nanoparticles (NPs) are often created via a diverse of physical and chemical techniques. But these techniques are not appropriate for medicinal and biological applications owing to its risky nature to the environment. Therefore, a green synthesis is designed to prepare nanomaterials, since the technique is effortless, eco-friendly and less expensive. In the past few decades, metal oxide semiconductors such as zinc oxide (ZnO), Magnesium oxide (MgO), Copper oxide (CuO) etc., are broadly applied and prepared via physical, chemical and biological methods. However, Titanium Dioxide Nanoparticles (TiO_2 NPs) are eminent semiconductors through a broad band gap of 3.2eV.

A green synthesis scheme to synthesis the TiO_2 NPs was introduced[1] to examine antibacterial properties. The designed scheme uses the Cymodocea serrulate to synthesis the NPs. Followed by this, the characterization procedure was carried out for biomedical application. A novel green synthesis and characterization of zirconium NP for dental insert fields was studied[2]. The resultant synthesized nanoparticles are fine candidate for a dental implant because of their admirable antimicrobial properties.

Synthesized mesoporous silver-titanium (Ag/TiO_2) via green synthesis scheme by mangosteen pericarp extract was developed[3]. The considered extract was worked as both adsorbent and photocatalyst materials. A Photocatalytic Polyaromatic hydrocarbons (PAH) applying magnetic $CrFe_2O_4$ NP was introduced[4] for water treatment application. By applying co precipitation scheme, NP was productively synthesized.

A pectin aerogel subjected with zinc oxide (ZnO) were generated for promising application in food packaging [5]. The designed model uses the beetroot extract as minimizing, limiting and stabilizing agent. Green synthesis procedure was employed to synthesize the ZnO NPs. Synthesizing TiO_2 NPs via associating the probable of Gram-positive bacteria was investigated[6]. Green creation of zinc oxide NPs was described[7] to contribute biological domains. Here, Monsoon longifolium plant leaves are to be used to get the synthesized NPs. Undoped yttrium, bismuth co-doped TiO_2 was developed[8] for photocatalytic domain. It utilizes the Podophyllum pinnatum leaf extract for synthesis.

Gold-titania NPs mixture was explored[9] to get rid of the viable ciprofloxacin and phytotoxicity estimation on plant growth. These NPs demonstrate great photocatalytic degradation characteristics. A tropical almond was employed as a natural capping operator in the green synthesis to deal with the augment of TiO_2 [10].

II. CONTRIBUTIONS

Major contributions involved in the work are described as follows.

- An efficient green and hydrothermal synthesis method is designed for improving the synthesis of TiO_2 nanoparticles through the aqueous leaf extraction, TiO_2 synthesis and characterization.
- Hydrothermal synthesis of TiO_2 is carried out with lesser time usage by applying Pressurized hot water extraction scheme.
- To obtain TiO_2 nanoparticles, Green hydrothermal synthesis process is performed with the aqueous leaf extract and Titanium tetraisopropoxide.
- Characterizes the synthesized TIO_2 nanoparticles to examine the physical, chemical, and structural properties, which are crucial for their performance and applications in various fields.
- Finally, results of green hydrothermal synthesis are analyzed and their discussion is provided to estimate the performance.

III. RELATED WORKS

Green synthesis of photocatalytic TiO_2/Ag NPs for a competent water remediation was introduced[11]. The results present green and simple method to organize TiO_2/Ag NPs through high photocatalytic efficiency. A TiO_2 and green dyes as photo sensitizers were used to build Dye-sensitized solar cells was described[12].

An anticancer action of TiO_2 through the endophytic fungus Aspergillus sp was investigated[13]. Green model of visible light TiO_2 by way of starch for the reduction of organic toxin was described[14]. Green synthesis and anticancer actions of TiO_2 NPs model through the water extract of Tulbhagia violacea was introduced[15]. The structural, morphological, and optical properties of the TiO_2-NPs were investigated through several characterization methods and evaluated for biological activities.

An antioxidant investigation of TiO_2 NPs was performed[16] to synthesize bio mediated TiO_2 compute its antioxidant property. TiO_2 were synthesized through the selective medicinal plant extracts. Band gap production of TiO_2 NPs was prepared[17] using green route and its visible light driven for environmental remediation. The degradation potential of Ca-TiO_2 NPs was evaluated via the degradation of 4-Nitro phenol.

Antibacterial, antioxidant and photocatalytic properties of TiO_2 was developed[18] using TiO_2. The designed NPs reveal 81.29% DPPH radical scavenging activity at the concentration of 60μg/mL. Proficient eradication of ecological contaminants was carried out[19] with the help of green synthesized metal NPs of Clitoria ternatea. To boost the photocatalytic dye degradation, antibacterial activities, synthesis of TiO_2 nanoparticles were performed[20].

IV. METHODOLOGY

Current research shows the impact of green synthesis of metal oxide nanoparticles where oxides of metals like zinc, gold, copper, silver, nickel, etc., are gaining importance. Nanoparticle synthesis is used in the understanding of optical biosensors, which supports materials with high-quality optical properties like optical absorption, optical emission, photoluminescence, chemiluminescence, etc. Green synthesis method of creating TiO_2 nanoparticles from the plant Cayratia pedate commonly known as 'Birdfoot Grapevine', which belongs to the family Vitaceae. It is a woody climber with a cylindrical stem and grows mostly in tropical forests. Conventionally, the leaf of the plant is applied in the healing of ulcers, inflammation, and scabies. The synthesized nanoparticles are employed for diverse domains such as sensors, lasers, drug discovery, etc. In this work, investigate a green hydrothermal synthesis of TiO_2

nanoparticles from suitable precursor titanium tetraisopropoxide.

Green Hydrothermal Synthesis of Cayratia Pedata Leaves for TiO_2 NP Synthesis

TiO_2 nanoparticles comprise unique magnetic, thermal, optical and electrical properties. Oxide is typically determined in three various forms such as brookite polymorphs, and rutile. Photocatalytic degradation and separation, electrical and electrochromic, sensing instruments, and photovoltaic cells are the most famous applications of TiO_2. Titanium oxide nanoparticles like all other metal NPs had separate morphologies (shape, size, and texture) and surface chemistry. It is used in the manufacturing of papers, meals, colors, cosmetics, and pharmaceuticals. Hazardous compounds in water are ruined through colloidal titanium oxide nanoparticles. Chemical and physical procedures such as chemical precipitation, Chemical deposition, hydrothermal sol-gel, and are commonly applied to build titanium oxide. All of these traditional methods require high pressure, high temperature, and risky chemicals. To manufacture nanoparticles on a better scale with fewer toxicity but, ecologically safe, quick, and efficient technologies are essential.

Green synthesis technique is a simple procedure that has achieved popularity as a low cost and environmentally accountable way to generate nanoparticles. It gives more guarantee for use in biomedical applications on the chemical reduction procedure. It also employs less toxic plant extracts and microorganisms in place of pricy chemical reagents. Green synthesis bestows a more realistic, eco-friendly result. It holds immense potential for the future of nanomaterial production, aligning with the rising demand for environmentally accountable practices in materials science and industrial manufacturing. Green synthesis not only diminishes the environmental and health-associated threats associated with traditional methods. It also suggests a pathway toward scalable, cost-effective, and safer manufacture of nanomaterials. This makes it a competent area of research for the growth of sustainable nanotechnology. TiO_2 has diverse qualities that incorporate non-toxicity in nature, superior resistance in chemical erosion, antimicrobial, antioxidant and anticancer activity against cell lines. As compared to other NPs, TiO_2 provided a unique photocatalytic behavior that examined through antioxidant behaviors of TiO_2 nanoparticles. Therefore, efficient Green hydrothermal scheme is employed for TiO_2 NP synthesis by using Cayratia Pedata leaves. Figure 1 demonstrates the green hydrothermal synthesis method to identify the photocatalytic and antibacterial activity for medical application.

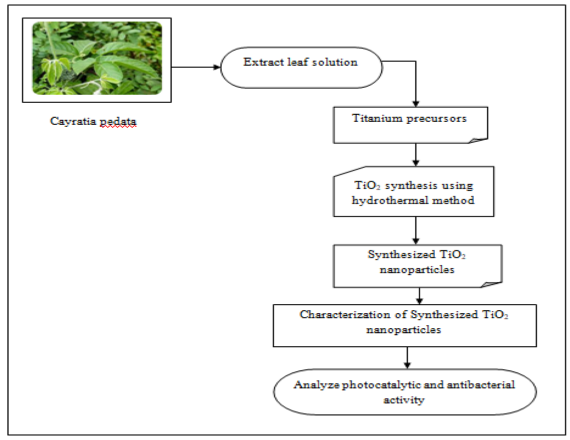

Figure 1 Process of green hydrothermal synthesis method

Figure 1 depicts the procedure involved in the green hydrothermal synthesis method to obtain the TiO_2 nanoparticles via examining the photocatalytic and antibacterial activity. The above-mentioned process includes diverse steps such as aqueous leaf extraction, TiO_2 synthesis, and characterization. A biological resource referred as Cayratia pedate plant leaves are considered as input in the designed method. From the input plant leaves, the aqueous leaf extraction is acquired. Then, the Titanium precursors is added to synthesis the TiO_2 nanoparticles and analyze their characterization. From this, photocatalytic and antibacterial activity of TiO_2 nanoparticles is determined.

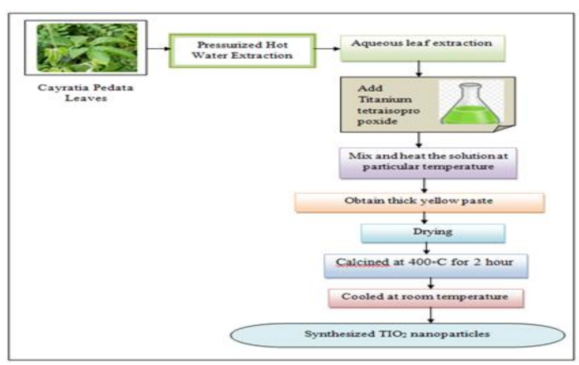

Figure 2 Synthesis of TIO_2 nanoparticles with Cayratia Pedata Plant Leaves

Figure 2 shows the synthesis of TIO_2 nanoparticles with Cayratia Pedata Plant Leaves. Initially, the input collection of plants is performed in the synthesis method. From the Trivandrum district, new Cayratia pedate plant is acquired for this study. This plant is determined through a Biologist, the Department of Botany, University of Kerala. The Titanium tetraisopropoxide is recommended for investigation and bought from Merck.

Followed by the input collection, aqueous extraction is performed from Cayratia pedate plant. The usual levels of partitioning bioactive compounds from plant materials are sample preparation, extraction, and purification. Extraction is also crucial to acquire the maximum amount of the desired compounds using the minimum amount of Cayratia Pedata. It is noteworthy to choose the proper extraction method since higher than 60% of the whole time is applied for the sample preparation stage. In addition, the right choice of extraction method progresses the extracts quality and avoid loss of the target compounds. Several extraction methods have been designed for obtaining the bioactive compounds from plant. However, the existing solvent extraction methods associated with higher extraction time, substantial amounts of solvents, and at times many extraction steps. Therefore, efficient extraction technique called Pressurized hot water extraction is used for getting bioactive compounds from Cayratia Pedata plant leaves for TiO_2.

Fresh plant leaves are harvested and completely washed to eradicate the soil, dust, and other contaminants. Then, the leaves are preserved (usually at room temperature). This process minimizes the moisture content and makes handling simpler. After that, the leaves are slice into smaller parts for increasing the surface area. With this, the efficient solvent penetration is achieved in the extraction. In Pressurized Hot Water Extraction, water used as solvent, and it is employed under high pressure and greater temperature to boost its extraction efficiency. Also, the distilled or purified water is usually suggested to evade contamination that influences the extraction.

The prepared plant input (typically 5–50 grams based on the scale of extraction) is filled into the extraction tub or the extraction chamber. According to the plant's characteristics and compounds considered for extraction, plant material is typically mixed with water in a particular ratio. The water is heated to temperatures between100°C and 250°C, and pressure is given to preserve the water in a liquid form. The pressure frequently varied from 1 to 20 MPa (megapascals). At these temperatures, the water demonstrates enhanced solvent properties and obtains a wider range of bioactive elements than at ambient temperatures. The heat aids break down the plant cell walls, enhancing the discharge of bioactive compounds into the water.

The extraction vessel is usually circulated to develop the association between the heated water and plant material for enhancing the efficiency of compound discharge. The plant material is subjected to the hot water for a particular time (i.e., 15–60 minutes) based on the type of compounds being extracted. Upon passing the desired extraction time, the plant material is isolated from the extracted solution (liquid extract). This is usually carried out via filtration to avoid plant residues. The extract is subjected through a filter where the Whatman No. 1 filter paper is employed to eliminate the any solid particles or superior plant fragments. The extracted compound is cooled to room temperature.

To perform synthesis, Titanium Tetra Isopropoxide is considered as precursor solution. Gradually add the leaf extract to the titanium precursor solution. After that, the mixture is blended at 65˚C for 20 minutes. The samples are collected and permitted to heat overnight at matching temperature. This process is continued until getting a thick yellow paste. This paste is then dried scrupulously and calcined at 400◦C for 2 hours prior to gather and pack individually for further characterization. The Calcination ignores the impurities in the sample and gets purified form of the nanoparticles. Later, the nanoparticles are characterized using techniques such as UV visible spectrometer, XRD, SEM, and FTIR. From the assessment, it is verified as TiO_2 nanoparticles. This procedure is continued for 55˚C and 75˚C, temperatures above and below the operating temperature. The reaction between Titanium tetra isopropoxide and plant extract does not occur when tested with 55˚C. By using 75˚C as the working temperature, it created ashes as the temperature is too elevated. At 65˚C, a yellow color change is obtained without any side effects. In addition, the sample is verified to be TiO_2 nanoparticles when performing characterization.

Characterization of Synthesized TIO_2 Nanoparticles

Upon completing the TIO_2 nanoparticles synthesis using Cayratia Pedata plant, the characterization process is carried out to recognize the structural and other important properties of the Titanium dioxide nanoparticles. The diverse techniques such as X-ray diffraction (XRD), Fourier transforms infrared (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and UV-Visible spectroscopy (UV-Vis).

XRD of explored TIO_2 nanoparticles is noted through the PAN analytical XPERT PRO Diffractometer. FTIR spectrum is noted via Perkin Elmer spectrophotometer noted from 400 to 4000 cm^{-1}. The Surface morphology of TiO_2 nanoparticles is displayed via SEM. SEM spectrum is noted through using Quanta FEG-250. UV–Visible Diffuse Reflectance Spectrophotometer (DRS) spectrum is scrutinized using a Shimadzu 2700 spectrophotometer. The reflectance spectrum is observed in the range of 200–800 nm. The antibacterial activity of TiO_2 NPs is analyzed for both gram-positive and gram-negative bacteria through the disk diffusion method.

UV-Visible Spectroscopy

UV-Visible Spectroscopy is a valuable scheme to scrutinize the optical properties of titanium nanoparticles. This scheme is applied to scrutinize the optical properties of TiO_2 nanoparticles namely light absorption characteristics. TiO_2 nanoparticles often exhibit strong UV absorption, but their bandgap is inferred through examining the absorption spectra. The sample of TiO_2 nanoparticles are subjected to UV-Visible light, and the reflected light is computed. Owing to their bandgap, TiO_2 nanoparticles typically demonstrate a characteristic absorption edge in the UV region. The onset of absorption offers insight into the band gap energy of the material which is frequently around 3.0 to 3.2 eV for TiO_2. TiO2 nanoparticles typically show a good absorption in the UV region that is associated to their high energy band gap. The shift in absorption towards visible light is a outcome of variations in nanoparticle size, morphology, or doping. Furthermore, it aids to find any surface plasmon resonance for doped or adapted nanoparticles. It gives an indication of the degree of particle dispersion via observing the peak shape and intensity. The band gap of the TiO_2 nanoparticles is estimated from UV-Visible spectra through the Tauc plot.

Scanning Electron Microscopy (SEM)

SEM is a great technique applied to scrutinize the surface morphology and size distribution of TiO_2 nanoparticles at elevated magnification. In TiO_2 nanoparticles, SEM exposes diverse morphological features namely shape (spherical, rod-like, or irregular), particle aggregation, and surface texture.

TiO_2 nanoparticles normally reveal sizes ranging from 10 to 100 nm, and SEM facilitate for the clear-cut measurement of these dimensions. The morphology of TiO_2 nanoparticles is strongly associated to their synthesis conditions, comprising the precursor concentration, temperature, and reaction time, and SEM imaging assist correlate these factors with nanoparticle formation. In addition, SEM recognizes the extent of aggregation or agglomeration of nanoparticles, which is critical for understanding their dispersion properties in solution or composite materials. SEM gives helpful insight into the microstructural features of TiO_2 nanoparticles that directly manage their performance in applications such as photocatalysis, solar cells, and sensors.

SEM worked by scanning the sample TiO_2 nanoparticles surface via concentrated beam of electrons. It generates secondary electrons that build high-resolution images. The resulting images present detailed information regarding the shape, size, and aggregation state of the nanoparticles. SEM also makes known structural features such as uniformity and surface roughness which are significant factors in diverse applications comprising catalysis and drug delivery. In addition, SEM is functional for observing the extent of nanoparticle agglomeration which influences the material's stability and performance.

X-Ray Diffraction (XRD)

XRD is applied to find out the crystalline structure, stage purity, and crystallite size of TiO_2 nanoparticles. XRD operates through directing X-rays onto the sample TiO_2 nanoparticles, where they work together through the crystalline planes of the material. The X-rays are diffracted at particular angles, and the resulting diffraction model gives complete information regarding the crystal structure, phase, and size of the nanoparticles. Through the diffraction peaks, positions and intensities are employed to learn the phase and crystallite size.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR determine the functional sets and chemical bonding existed on the surface of TiO_2 nanoparticles. FTIR operates via estimating the absorption of infrared radiation, which outcomes the vibrations in molecular bonds. The resulting spectrum exhibits peaks respective to diverse functional sets or bonds, allows for identification of surface chemistry. In TiO_2 nanoparticles, FTIR distinguish the existence of Ti–O bonds normally emerging around 400–800 cm^{-1} and surface hydroxyl groups (–OH). FTIR also expose the existence of organic molecules or stabilizing agents added to the nanoparticle surface, which control their reactivity and stability in diverse applications.

Transmission Electron Microscopy (TEM)

It gives high-resolution images and comprehensive structural examination of TiO_2 nanoparticles. TEM enables detailed examination of their size, shape, and morphology for TiO_2 nanoparticles. TiO_2 nanoparticles usually varied in size from 10 nm to 100 nm. TEM gives correct measurement of these dimensions with elevated accuracy. The images acquired by TEM expose dissimilar particle shapes such as spherical, rod-like, or even complex structures based on the synthesis method. Moreover, it is capable of distinguishing between different structural forms of TiO_2 namely anatase, rutile, and brookite through giving insights into the interior lattice structure and crystallographic features.

Photocatalytic Activity of TIO_2 NPs

The action of photocatalytic in synthesized TiO_2 NPs is scrutinized through the methylene blue dye in UV light irradiation (wavelength = 300 nm). In the catalytic activity, 10 ppm dye solution is employed. The 100 mL MB dye compound is combined by means of 10 mg nanocatalyst of TiO_2 NPs. The merged catalyst solution is stimulated under dark state via magnetic stirrer. The dark states assist to attain the adsorption-desorption balance position. The combined solutions are reserved in a UV compartment and light irradiated for 120 minutes. For the periodic intervals (30 minutes), the irradiated solution is considered out for 5 mL to calculate the degradation efficiency. The gathered dye solutions are centrifuged at 10000 rpm for 5 minutes to eradicate the superfluous elements and nanocatalyst. The centrifuged samples are computed for UV-Visible spectroscopy. The same working process is followed by reusability analysis. The p-benzoquinone is applied to find out the superoxide ion activity, isopropyl alcohol (IPA) is employed to discover the hydroxyl activity, and triethanolamine (TEOA) is employed to scrutinize the hole activity in the catalytic action. These compounds confine the free radicals, holes, and superoxides, and their concentration is 1 mmol/L.

Antibacterial activity of TiO_2 NPs

The synthesized TiO_2 nanoparticle bacterial actions are estimated through applying gram-positive Staphylococcus aureus (ATCC 6538) and gram-negative Escherichia coli (ATCC 8739) over the fine diffusion scheme. The nutrient broth is protected through bacterial culture (106CFU/mL), and inoculated suspensions are developed for 24 hours. The incubated suspensions are washed over the Mueller-Hinton-mediated petri plates. The gel stab is applied to create the fine about 5 mm on agar mediated petri plates and the similar concentrations (20 µg/ ml) are put it over the well. The given petri plates are incubated for overnight at 37° C. Later, the incubated samples exhibited the zones around the well. The bacterial inactivity is examined by zone of inhibitions in the range of millimeter scale. The bacterial inactivity of TiO_2 nanoparticles is compared with the same concentration of Cayratia Pedata plant extract and antibiotic drug (gentamycin-SD195 Gentamicin, 120 micrograms).

Synthesis of TiO_2 by hydrothermal method

To perform synthesis of TiO_2 NPs, 0.1 N of TTIP is mixed in 20 ml of ethanol solution through the constant stirring for 30 min. Then, gradually include a few drops of distilled water to create the dispersion state. The creation is positioned on the ultrasonic bath for 20 min. Followed by the sonication; the solution is shift into an autoclave at 150 °C for 3 h. Then the product is cool to room temperature, and washed and centrifuged with deionized water to take away the dust particles. Afterward, it is filtered through Whatman No. 1 Filter paper. The filtered product is dried oven at 110 °C for 5 h, and annealed at 500 °C for 2 h. The product TiO_2 NPs is gathered and processed with additional characterization.

V. RESULTS AND DISCUSSION

The performance of the TIO_2 nanoparticles synthesis is examined using Green and Hydrothermal Synthesis method. The outcomes of synthesis method are evaluated in terms of various TIO_2 characterization such as X-ray diffraction (XRD), Fourier transform infrared (FTIR), scanning electron microscopy (SEM), Photocatalytic activity of TiO_2 Antibacterial activity of TiO_2 NPs.

XRD Analysis

The structure of TiO_2 nanoparticles and their crystallinity are verified through the XRD. In the XRD spectrum, each diffraction peaks are associated to the characteristic peaks of TiO_2 in the anatase phase. The diffraction peaks absolutely emerged at 2θ is finely positioned to the crystal planes (101), (004), (200), (105), (211), and (204) are illustrated in Figure 3.

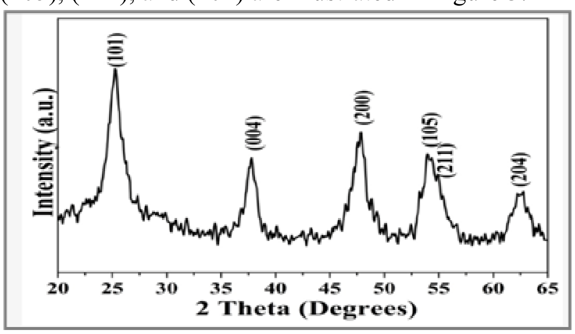

Figure 3 X-ray diffraction pattern of TiO2 NPs

Figure 3 depicts the result of X-ray diffraction patterns using Cayratia Pedata plnat for TiO_2 nanoparticles synthesis. As given in the above graph, the horizontal axis takes the diffraction angle in degrees such as 20, 25, 30, 35, …65 whereas the vertical axis represents intensity in absorption unit (au). The size of the NPs is computed with the help of Debye-Scherrer equation. From this, the average crystalline size of the nanoparticles formed is predicted and found to be 52.24 nm. Thus, the green synthesis of titanium nanoparticles with the Cayratia Pedata leaves through X-ray diffraction patterns applied for treatment in the medical field.

Fourier Transform Infrared Spectroscopy Analysis (FTIR)

FTIR offers creation of efficient sets of synthesized TiO_2 nanoparticles. It recommends that the formation of TiO_2 nanoparticles owing to the association of the phenolic compounds, alkynes, terpenoids, and flavonoids. It finds intensity over a range of wavelengths at a time.

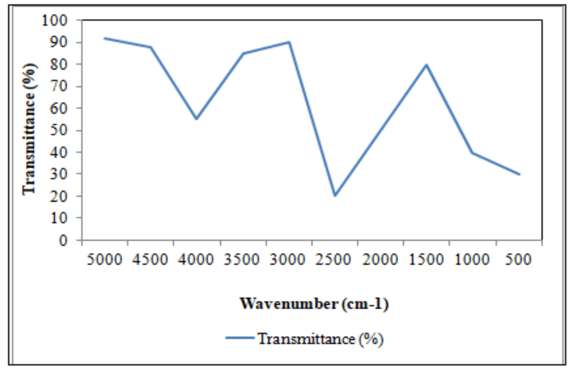

Figure 4 FTIR Analysis

Figure 4 reveals the FTIR of the synthesized TiO_2 nanoparticles in the range 500–5000cm 1. The functional groups are accountable to minimize the titanium ions to TiO_2, and it is observed as bands. Each of the bands corresponds to varied stretching modes. The broad band measured at 3250 cm^{-1} respective to O–H stretching of phenolic composites. The alkene group's attendance is attributed to 1550 cm^{-1} and the band at 1250 cm^{-1} respective to C–N stretching bonds of the amines. The C–O extended the esters and carboxylic useful sets and the band among 950 and 1150. The numerous sharp bands at 491 cm^{-1} and 435 cm^{-1} are attributed to the occurrence of TiO_2 extended bands.

Scanning Electron Microscopic Images

The SEM assessment of bacterial bio-films is carried out to examine the impact of TiO_2 on the biofilm structural design of test bacteria. Hence, the SEM analysis represents the TiO_2 NPs reduced the bio-film growth and modified the bio-film construction of test bacteria.

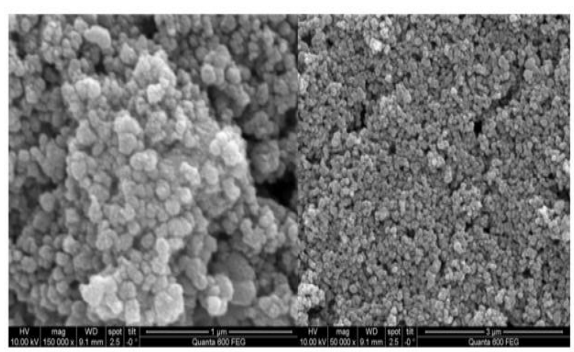

Figure 5 SEM images of prepared using TiO_2 NPs

Figure 5 illustrates the SEM images of prepared TiO_2 NPs. The surface morphology of the TiO_2 NPs is examined with the help of SEM. The SEM images demonstrate that the NP firm leads a boost in the TiO_2 NP size12 and that the TiO_2 NPs had a consistent spherical morphology. The mean particle size of a spherical formed TiO_2 NPs is determined in the range of 32–48 nm. The Particle size acquired from SEM outcomes is fine associated through the mean crystalline size from XRD. In general, diminish in particle size is indirectly associate to the surface point of the material. As a result, the lower particle size material rapidly enters the toxic components with the bacterial surface that led the procedure of decomposition.

Photocatalytic activity of TiO_2

The photocatalytic actions of TiO_2 are examined through the competence to corrupt organic pollutants such as dyes or other impurities under UV or visible light exposure. The Photocatalytic activity of TiO_2 NP Green synthesis method is shown in figure 6.

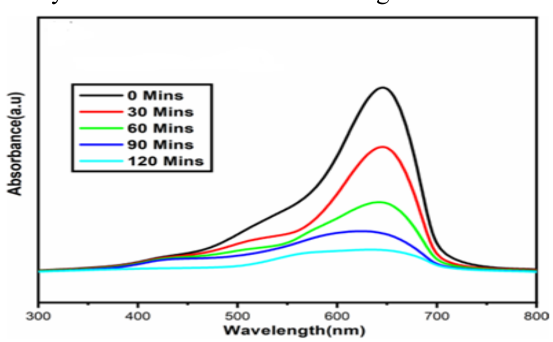

Figure 6 Photocatalytic activity of TiO_2 NPs

Figure 6 depicts the performance of the photodegradation of methylene blue. Methylene blue dye is taken as a pollutant in this analysis. The UV absorption spectra of methylene blue at 665 nm matches to π–π^* transition. Absorption peak intensity minimization outcomes point out the degradation of methylene blue. The green synthesized TiO_2 NPs attained superior degradation effectiveness. Bio mediated TiO_2 NPs outcomes in the greatest degradation of 90% under 110 min of irradiation.

Antibacterial activity of TiO_2 NPs

The antibacterial study of TiO_2 nanoparticles is investigated through the gram-positive and gram-negative bacteria. The bacterial activity of TiO_2 nanoparticles is illustrated in Figure 7.

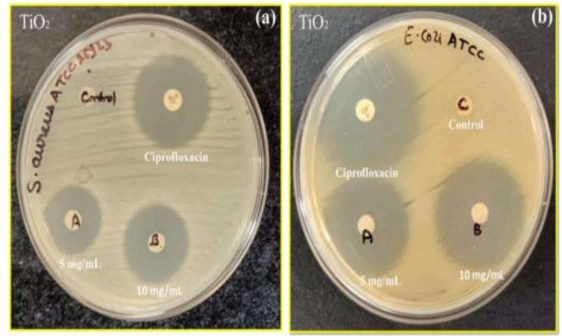

Figure 7 Antibacterial activity of TiO_2 NPs (a) S. aureus (Gram-positive bacteria), and (b) E. coli (Gram-negative bacteria)

The zone inhibition layer of the TiO_2 NPs is evaluated Escherichia coli (E. coli), and Staphylococcus aureus (S. aureus) are computed in mm scale as shown in Figure 8.

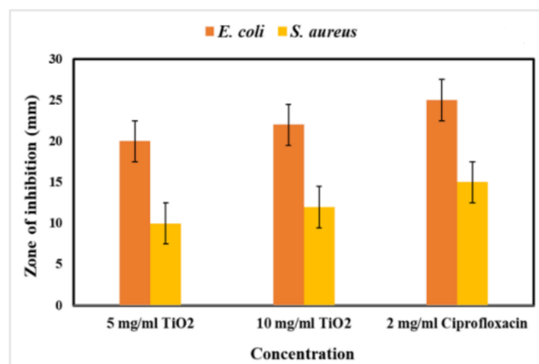

Figure 8 Graph shows the zone of inhibition (mm) against diverse concentrations (mg ml-1) of TiO_2

Figure 8 shows the zone of inhibition in terms of various concentrations. The maximum zone inhibition layer is examined in green synthesized TiO_2 NPs. The zone inhibition layer of pathogenic bacteria E. coli has strong outcomes relative to S. aureus. Thin partitions of gram-negative bacteria are rapidly busted by a positive ion of TiO_2 NPs. From the analysis, the gram-negative bacteria are extremely powerful when compared with gram-positive bacteria. The variation in diameter of zone of inhibition is owing to the variation in vulnerability of bacteria, the morphology of nanoparticles, phase creation particle size, shape, and synthesis method. The zone of inhibition of formed TiO_2 NPs confirms a tremendous antibacterial activity. Thus, the formed TiO_2 NPs are extremely pertinent to biomedical applications.

VI. CONCLUSION

In this article, Green Hydrothermal Synthesis method of titanium oxide nanoparticles and their characterization is designed for diverse biomedical applications. A Pressurized hot water extraction scheme is used to carry out aqueous leaf extraction. With this extracted solution, green hydrothermal synthesis procedure is carried out with Titanium tetraisopropoxide. From this, the synthesized TiO_2 particles are acquired in a cost-effective manner. In addition, various characterizations are examined to determine the structure of the TiO_2 nanoparticles. On the contrary to existing methods produce the nanoparticles, green synthesis method is determined to be ecofriendly and can be achieved by few usages of chemicals. The outcomes confirmed the Green Hydrothermal Synthesis method performs better in synthesizing the TiO_2 particles for biomedical applications.

REFERENCES

[1] Mohankumar N., Srinivasan S., Gnanasekaran C., Ramachandran G., Chelliah C.K., Rajiv Gandhi G., Maruthupandy M., Quero F., Li W.-J., Hayder G., Khaled J.M., Arunachalam A., Manoharan N. Synthesis and characterization of marine seagrass (*Cymodocea serrulate*) mediated titanium dioxide nanoparticles for antibacterial, antibiofilm and antioxidant properties. *Microbial Pathogenesis*. pp 1-11, 2024 Apr;189:106595.

[2] Chowdhury M.A., Hossain N., Mustaf M.G., Mia M.R., Tushar M., Rana M.M., Hossain M.H. Green synthesis and characterization of zirconium nanoparticles for dental implant applications. *Heliyon*. Pp 1-19, 2023 Jan;9(1): e12756.

[3] Yuwono A.H., Septennium F., Dhaneswara D., Sofyan N., Suwartha N., Lalasari L.H., Maulana F.A., Nurhidayah E., Barep A.L. Investigation of methylene blue dye degradation using green synthesized mesoporous silver-titanium. *South African Journal of Chemical Engineering*. 2024 Oct; 50:415-426.

[4] Al-Hunaiti A., Ghazzy A.M., Mahmoud N.T. Photocatalytic polyaromatic hydrocarbons (PAH) utilizing magnetic $CrFe_2O_4$ nanoparticle: green synthesis, characterization, *ab initio* studies, electronic, magnetic features and water treatment application. *Chemical Engineering Journal Advances*. pp 1-12, 2024 Aug 15;19:100631.

[5] Mottola S., Viscusi G., Oliva G., Vigliotta G., Cardea S., Gorrasi G., De Marco I. Pectin/alginate aerogel containing ZnO produced from beetroot extract mediated green synthesis for potential applications in food packaging. *Journal of CO_2 Utilization*. pp 1-11, 2025 Jan;91:103003.

[6] Rathore C., Yadav V.K., Amari A., Meena A., Egbosiuba T.C., Verma R.K., Mahdhi N., Choudhary N., Sahoo D.K., Chundawat R.S., Patel A. Synthesis and characterization of titanium dioxide nanoparticles from *Bacillus subtilis* MTCC 8322 and its application for the removal of methylene blue and orange G dyes under UV light and visible light. *Frontiers in Bioengineering and Biotechnology*. pp 1-23, 2024 Jan; 11:1323249. doi:10.3389/fbioe.2023.1323249. eCollection 2023.

[7] Bekele S.G., Ganta D.D., Endashaw M. Green synthesis and characterization of zinc oxide nanoparticles using *Monoon longifolium* leaf extract for biological applications. *Discover Chemistry*. pp 1-17, 2024 Jul;1:7. doi:10.1007/s44371-024-00007-9.

[8] Parvez M.S., Rahman A., Habib A.K.M.A., Rokon S.M.N. Green synthesis of undoped and yttrium, bismuth co-doped titanium dioxide nanoparticles using *Bryophyllum pinnatum* for photocatalytic application. *Results in Materials*. 2024 Dec; 24:100629.

[9] Balarabe B.Y. Green synthesis of gold–titania nanoparticles for sustainable ciprofloxacin removal and phytotoxicity evaluation on aquatic plant growth. *Hybrid Advances*. 2023 Dec; 4:100107.

[10] Nofrijon Sofyan, Alry Mochtar Jamil, Aga Ridhova, Akhmad Herman Yuwono, Donanta Dhaneswara, Jeffrey W. Fergus, "Graphene oxide doping in tropical almond (terminalia catappa L.) fruits extract mediated green

synthesis of TiO_2 nanoparticles for improved DSSC power conversion efficiency", Heliyon, volume, 10, issue 8, April 2024, Pages 1-19. Doi: 10.1016/j.heliyon. 2024.e29370.

[11] Cantarella M., Mangano M., Zimbone M., Sfuncia G., Nicotra G., Scalisi E.M., Brundo M.V., Pellegrino A.L., Giuffrida F., Privitera V., Impellizzeri G. Green synthesis of photocatalytic TiO_2/Ag nanoparticles for an efficient water remediation. *Journal of Photochemistry and Photobiology A: Chemistry*. 2023 Sep 1; 443:114838.

[12] Hendi A.A., Alanazi M.M., Alharbi W., Ali T., Awad M.A., Ortashi K.M., Aldosari H., Alfaifi F.S., Qindeel R., Naz G., Alsheddi T.H. Dye-sensitized solar cells constructed using titanium oxide nanoparticles and green dyes as photosensitizers. *Journal of King Saud University – Science*. 2023 Apr;35(3):102538.

[13] Alhadrami H.A., Hassan H.M., Alhadrami A.H., Rateb M.E., Hamed A.A. Green synthesis and anticancer activity of titanium dioxide nanoparticles using the endophytic fungus *Aspergillus* sp. *Journal of Radiation Research and Applied Sciences*. 2025 Mar;18(1):1-10.

[14] Abu Hasim N.N., Mohamad Idris N.H., Hamzah H.H., Rustier G., Schrodec B., Lee H.L. Green technique of visible light active titanium dioxide nanoparticles using starch for the degradation of organic pollutant. *Desalination and Water Treatment*. 2023 Mar; 287:46-58. doi:10.5004/dwt.2023.29346.

[15] Mbenga Y., Adeyemi J.O., Mthiyane D.M.N., Singh M., Onwudiwe D.C. Green synthesis, antioxidant and anticancer activities of TiO_2 nanoparticles using aqueous extract of *Tulbhagia violacea*. *Results in Chemistry*. 2023 Dec; 6:101012.

[16] [16] Blissymbol B., Yasotha P., Kalaiselvi V., Gopi S. An antioxidant study of titanium dioxide (TiO_2) nanoparticles against mace of *Myristica fragrans* Houtt, rhizomes of *Curcuma longa* Linn and *Kaempferia galanga* extracts. *Results in Chemistry*. 2024 Jan; 7:101187.

[17] Tasisa Y.E., Sarma T.K., Krishnaraz R., Sarma S. Band gap engineering of titanium dioxide (TiO_2) nanoparticles prepared via green route and its visible light driven application for environmental remediation. *Results in Chemistry*. 2024 Oct; 11:101234.

[18] Ahmad W., Kumar S., Ahmed S. Microwave-assisted one-step biosynthesis of titanium dioxide nanoparticles: Antibacterial, antioxidant, and photocatalytic properties. *Kuwait Journal of Science*. 2024 Apr;51(2):1-8.

[19] Sa N., Alkhayer K., Behera A. Efficient removal of environmental pollutants by green synthesized metal nanoparticles of *Clitoria ternatea*. *Heliyon*. 2024 Sep;10(9): e32015.

[20] Rathi V.H., Jeice A.R., Jayakumar K. Green synthesis of Ag/CuO and Ag/TiO_2 nanoparticles for enhanced photocatalytic dye degradation, antibacterial, and antifungal properties. *Applied Surface Science Advances*. 2023; 13:101522.