Green Synthesis and Characterization of TiO_2 Nanoparticles using Piper Betel leaf Extract for Antimicrobial Activity

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***Abstract*—The synthetic techniques for metal oxide nanoparticles often involve toxic solvents, high pressure, high energy, and high temperatures. While these methods can be effective, they are not environmentally friendly. In contrast, plant-based synthesis of metal oxide nanoparticles offers several advantages, including cost-effectiveness, simplicity, and compatibility with medical applications. In this study, we utilized Piper betel leaf extract to synthesize titanium dioxide (TiO_2) nanoparticles. TiO_2 possesses several desirable properties, such as a high refractive index, good light absorption, non-toxicity, chemical stability, and relatively low production costs. The characteristics of the synthesized TiO_2 nanoparticles were thoroughly studied using XRD, FTIR, UV-Vis spectroscopy, scanning electron microscopy with detailed results presented throughout the study. We also analyzed the antibacterial effect of TiO_2 nanoparticles on E. coli strains using a well diffusion assay to confirm the inhibition zone of the bacteria. This research highlights the potential of green synthesis methods in producing effective antibacterial agents.**

***Index Terms*—Tio_2 nanoparticles, Piper betel, Morphological characterize, Microscopy analysis, antimicrobial action.**

I. INTRODUCTION

Nanoparticles differ from larger particles of the same composition primarily due to their larger surface area-to-volume ratio. Metal oxide nanoparticles, especially titanium dioxide (TiO_2), have garnered significant attention in medical fields for their antibacterial properties, resistance to microbes, drug delivery capabilities, and applications in immunochromatography, tumor imaging, anticancer activities, and pathogen identification [1-2]. TiO_2, an n-type semiconductor, is notable for its light absorption, surface adsorption, and charge transport properties [3]. It exists in three crystal structures: anatase, rutile, and brookite, with the anatase phase being particularly valued for applications in photovoltaic cells, photocatalysis, and antimicrobial uses. As an inert, nontoxic, and cost-effective material, TiO_2 nanoparticles exhibit a high refractive index and excellent UV light absorption. These attributes make them ideal for enhancing whiteness and opacity in products like sunscreen lotions, paints, plastics, papers, solar cells, medicinal applications, inks, food colorants, and toothpastes. Various synthesis methods are employed to produce metal oxide nanoparticles tailored for specific applications [4]. Metal nanoparticles show good antibacterial properties due to their large specific surface area and volume ratio and have long-term stability and good biocompatibility, making them the most promising antibacterial material. With the enhancement of microbial resistance to antibiotics and the development of drug-resistant strains, metal nanoparticles have aroused increasing interest among researchers [5]. Metal nanoparticles can be used as effective growth inhibitors for a variety of microorganisms, so they are suitable for various medical devices. Compared with microparticles, nanoparticles have the advantages of more controllable, longer-lasting drug release, more specific targeting, and higher surface-to-volume ratio. They can reduce drug load and administration time and are convenient for patients to use [6]. Because of its inherent basic characteristics, titanium dioxide (TiO_2)

has been developed as an excellent photocatalytic material for many different industrial applications [7]. When titanium is used as a nanomaterial, it has antibacterial activity and has broad application prospects in reducing bacterial growth and reducing infection. It has been reported that the antibacterial activity of titanium is closely related to its photocatalytic performance [8]. The photoactivation of TiO_2-NPs significantly improves its antibacterial activity against sensitive Bifidobacterium, Escherichia coli, Salmonella typhimurium, and Staphylococcus aureus [9].

II. PREPARATION OF GREEN EXTRACT

For the preparation of piper betel leave extract the piper betel leaf extract, the thoroughly by distilled water to remove the dust particles. The 50g of washed piper betel leaves were boiled with 100ml of deionized water for 3 hours at 80^0 C in glass beaker using magnetic stirred. Then the extract was cooled filtered using filter paper and stored for further process.

Preparation of the titanium dioxide (TiO_2) nanoparticles

Initially 0.1M of Titanium Nitrate was mixed with 0.2M of NAOH and add 50ml of distilled water. Then the mixture was magnetically stirred 3 hours at 80°C.After stirring for 3 hours, precipitate was obtained. The precipitate was kept in ceramic crucible and dried for 4 hours in hot oven at 120°C.After drying, the white colored powder was obtained. The white powder was crushed using Agate mortar and pestle to get fine powder. Later, the powder was Calcined for 6 hours at 500°C and stored for further process.

Surface Modification of TiO_2

0.5g of prepared titanium Dioxide (TiO_2) were taken into the three conical flask and added 100ml of distilled water separately, then 10ml,20ml,30ml of piper betel leaf extract were taken into the burette and added drop wise into the conical flask respectively. Then the mixture was magnetically stirred for 3 hours. After stirring for 3 hours, the precipitate was obtained. Then the precipitate was kept in ceramic crucible and dried for 12 hours at 80°C.The powder was obtained and the powder was crushed using Agate Mortar and pestle to get fine powder.

X- ray diffraction (XRD)

The X-ray diffraction (XRD) analysis of TiO_2 nanoparticles was conducted using X-rays with a wavelength of 1.54 Å, as illustrated in Figure 5.1. Notable peaks were identified at 25.49°, 48.0°, 53.95°, 62.87°, 70.09°, and 75.19°, corresponding to the crystallographic planes (101), (200), (105), (211), (204), (220), and (215), respectively. These findings align well with JCPDS Card No. 89-4921, confirming the presence of anatase TiO_2. The broadening of the peaks suggests that the crystalline size of the nanoparticles is quite small, indicating a high degree of nanoscale features in the material [10]. The average grain size formed in the biosynthesis was determined using scherrer's formula,

$d = K\lambda/\beta \cos\theta$

where, K is constant (0.9), λ is the wavelength used in XRD (1.5406 Å), θ is the Bragg angle, β is the FWHM (full width half maximum), which shows the average crystalline size of TiO_2 NPs were estiated and listed in table 1.

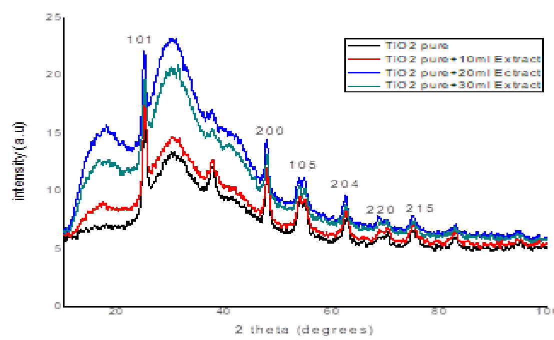

Fig. 1 x-ray diffraction pattern of Tio_2

Table 1 Estimation of crystalline size

Sample	Extract conc. (ml)	Crystalline size (nm)
TiO_2	Pure	17
	10	15
	20	12
	30	10

Fourier Transform-Infrared spectroscopy (FTIR) Analysis

The FTIR spectroscopic technique is commonly employed to assess the purity and composition of synthesized materials, as well as to identify functional groups and types of bonds present in a system. Fig. 2

presents the FTIR spectrum for TiO_2 nanoparticles and TiO_2 doped with leaf extract. In the spectrum for TiO_2 nanoparticles, a peak at 3398.69 cm^{-1} is attributed to the stretching of hydrogen bonds in the O-H (alcohol) group. The peak at 2328.10 cm^{-1} corresponds to the C ≡ C functional group (alkynes), indicative of a variable presence that is not typical in symmetrical alkynes. Additionally, the peak at 1631.37 cm^{-1} is associated with C = C, characterized by medium weak multiple bands. Finally, the peak observed at 747.72 cm^{-1} reflects the stretching and vibration modes of the O–Ti–O bond, confirming the structural features of the TiO_2 nanoparticles [10].

Table. 2 FTIR frequency assignment for Tio_2 nanoparticle

Wave number(cm^{-1})	Assignment
3398.69	H-bond of O-H (Alcohol) group
2328.10	C ≡ C – (alkynes, variable not present in symmetrical alkynes)
1631.37	C = C (medium weak multiple bands)
747.72	stretching and vibration modes of O – Ti – O

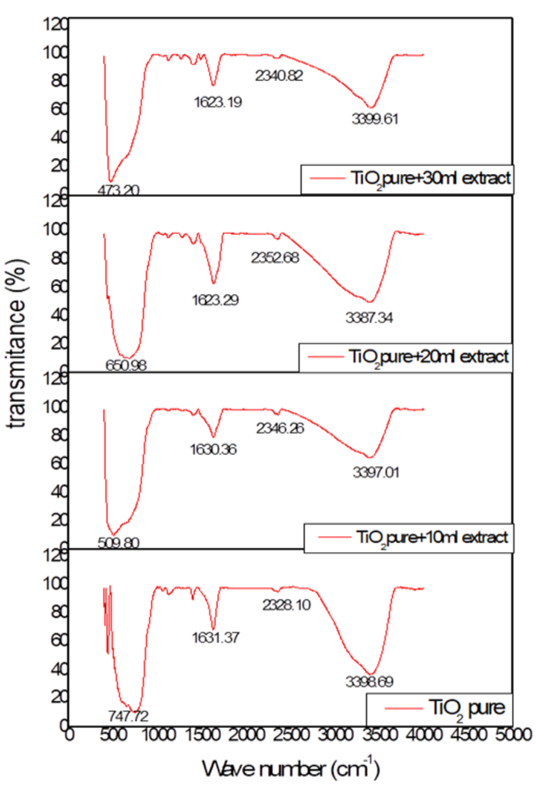

Fig.2 FT-IR spectra of TiO_2 nanoparticles

UV-VIS Spectroscopy

UV-Vis spectroscopy is an effective method for determining the particle size and energy band gap of prepared TiO_2 nanoparticles. The energy band gap (Eg) of the nanoparticles can be calculated using the equation: Eg=hc/ λ where:

- h is Planck's constant (4.135×10^{-15} eVs),
- c is the speed of light (3×10^{8} m/s),
- λ is the wavelength (in nm).

The calculated band gap values for the TiO_2 nanoparticles are summarized in the accompanying table. These values provide insights into the optical properties and potential applications of the nanoparticles in various fields, such as photocatalysis and solar energy conversion [11].

Table 2 Estimation of Energy band gap

Sample	Extract conc. (ml)	Energy band gap (eV)
TiO	Pure	3.82
	10	3.88
	20	3.95
	30	3.97

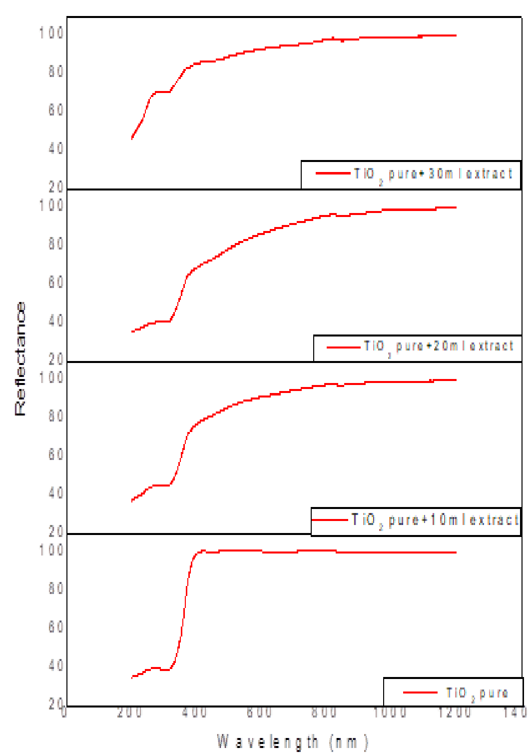

Fig.3 UV-VIS Spectrum of TiO_2 nanoparticles

III. SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS

Scanning electron microscopy (SEM) was employed to analyze the morphology of the synthesized TiO_2 nanoparticles. The SEM images were captured using a Carl Zeiss EVO 18 scanning electron microscope. Fig.4 presents the SEM images of TiO_2 nanoparticles at various magnifications, while Fig. 5 displays the SEM image of TiO_2 doped with leaf extract. The SEM analysis reveals that the TiO_2 nanoparticles exhibit a heterogeneous morphology and appear to be spherical in shape. Additionally, the images indicate that the synthesized TiO_2 sample is porous in nature, which may enhance its surface area and catalytic properties. This morphology is significant for potential applications in areas such as photocatalysis and environmental remediation.

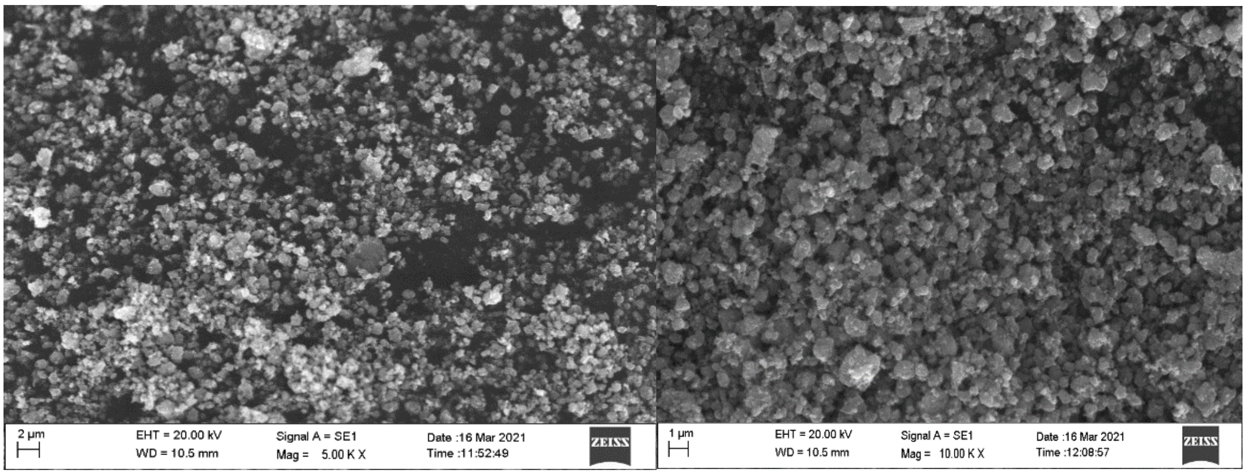

Fig.4 SEM image of TiO_2

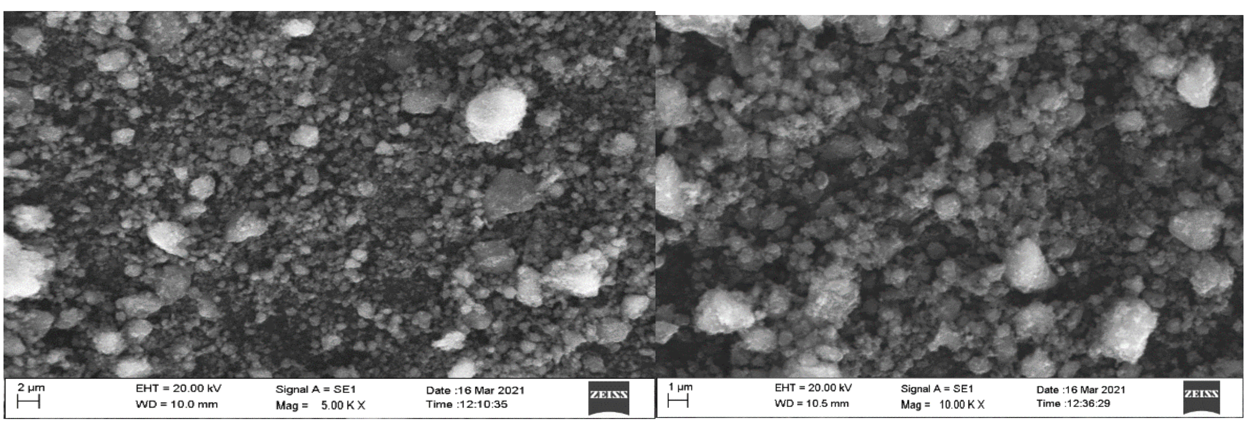

Fig.5 SEM image of TiO_2 doped with Extract

Energy Dispersive X-ray spectroscopy (EDX) Analysis

Energy dispersive X-ray analysis (EDX) is a powerful technique used for the elemental analysis and chemical characterization of samples. The EDX spectrum of TiO_2 nanoparticles is displayed in Fig.6, where the prominent peaks for titanium (Ti) and oxygen (O) confirm the successful formation of TiO_2 nanoparticles. Fig.7 presents the EDX spectrum for TiO_2 doped with piper betel leaf extract. The EDX data for both TiO_2 nanoparticles and the doped sample are summarized in Table 3 and Table 4, respectively. These tables provide the compositions of the prepared samples, presented in both atomic and weight percentages. This elemental analysis is crucial for understanding the material's composition and confirming the incorporation of the leaf extract into the TiO_2 matrix.

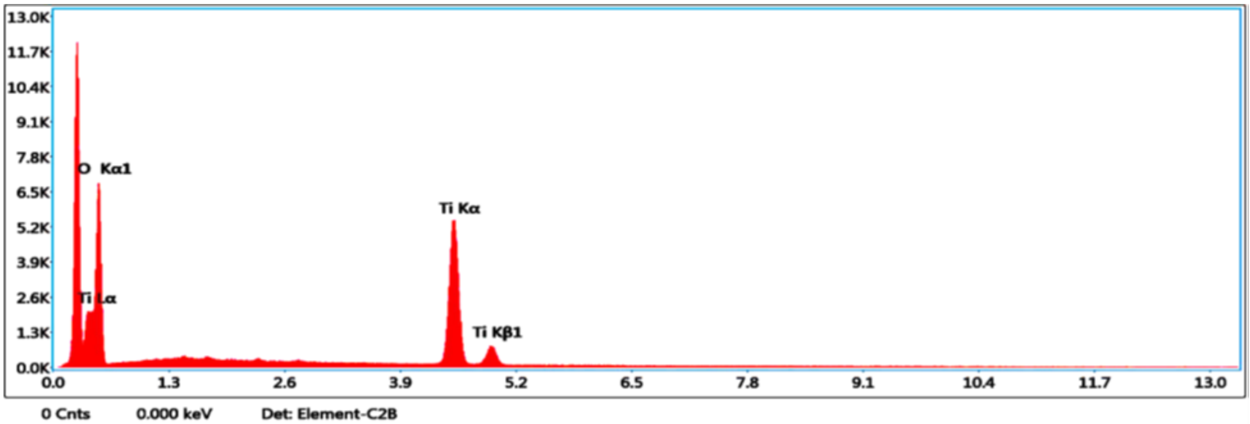

Fig.6 EDX Spectrum of TiO_2

Table 3. Elemental ratio of TiO_2 Nanoparticle

Element	Atomic Percentage (%)	Weight Percentage (%)
O	62.2	83.1
Ti	37.8	16.9

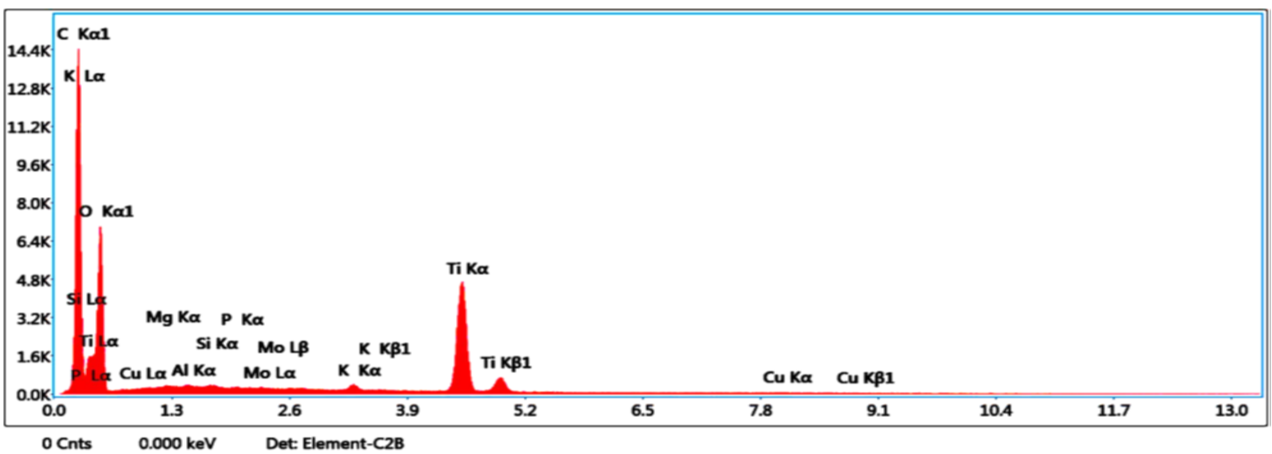

Fig.7 EDX Spectrum of TiO_2 doped with Piper betel leaf Extract

Table 4 Elemental ratio of TiO_2 doped with Piper betel leaf Extract

Element	Atomic Percentage (%)	Weight Percentage (%)
C	41.7	54.8
O	38.8	38.3
Mg	0.9	0.6
Al	0.6	0.3
Si	0.4	0.2
P	0.2	0.1
Mo	0.3	0.0
K	0.6	0.2
Ti	16.2	5.3
Cu	0.5	0.1

Antibacterial activity of titanium dioxide nanoparticles

The antibacterial activity of TiO_2 NPs was evaluated against pathogens by disc diffusion assay such as E. coli, S. aureus. The mean of triplicates of obvious zone of inhibition (in millimetres) around each disc was estimated for each of the pathogens. The capability of the TiO_2 nanoparticles to inhibit bacterial growth has been listed in Table 5. Highest zone of inhibition was observed by the highest concentration of titanium dioxide nanoparticles and the maximum zone of inhibition was observed against E. coli (43 ± 0.11) and S. aureus (40 ± 0.23). Colthup et al., reported that TiO_2 NPs are the mostly examined for their photocatalytic antimicrobial action among different nanoparticles [12]. Wiley et al., have suggested that the potential mechanisms involving the interactions between nanoparticles and biological molecules [13]. The microbes have a negative charge while metal oxide nanoparticles have a positive charge which creates an electromagnetic reaction between the microbes and treated material surface, once the reaction was created, the microbe was oxidized and finally leads to cell death. The interaction of nanoparticles with phosphorus or sulphur containing compounds like DNA and thiol groups of proteins can causes damage of microbe by inhibition of DNA replication and protein inactivation [14]. They are the reason for the pits in bacterial cell walls, which leads to increased cell permeability and cell death [15]. The recent reports were suggested that the metal nanoparticles have a durable electrostatic interaction with the cell wall of the bacteria and causes cell death [16,17,18]. Thus, the antibacterial action of TiO_2 NPs possesses excellent ability to be utilized as antibacterial agents against microbes.

Table 5
Zone of inhibition observed against different bacterial pathogens with different concentrations by disc diffusion method

Pathogens	Zone of inhibition (mm)	
	Tio_2	Tio_2 with leaf Extract
E.coil	40	43
S.aureus	35	40

IV. CONCLUSION

This study presents an environmentally friendly, low-cost, and renewable method for synthesized TiO_2 nanoparticles using Piper betel leaf extract as a reducing agent. The structural properties of the synthesized nanoparticles were thoroughly characterized. XRD analysis indicated that the mean crystalline size of the TiO_2 nanoparticles was calculated using Scherrer's formula. The energy band gap of the synthesized nanoparticles ranged from 3.8 eV to 4.0 eV, which is higher than that of bulk TiO_2, which has a band gap of 3.2 eV. The morphology of the nanoparticles, including their size and shape, was investigated using SEM, revealing their potential as versatile materials. Antimicrobial action of the synthesized titanium dioxide nanoparticles were determined against test pathogens. Disc diffusion assay and MIC were commonly used to assess the antimicrobial impacts. The disc diffusion test had been used to evaluate the effectiveness of TiO_2 NPs against bacterial strains like E. coli and S. aureus and the diameter of the bacterial inhibition zone depends upon the solubility and infusibility of the titanium dioxide nanoparticles. Micro broth dilution assay was used to determine the MIC of TiO_2 nanoparticles against test bacterial strains. The lack of turbidity in cultural broth indicates the lowest concentration of TiO_2 nanoparticles. The biological synthesis of TiO_2 nanoparticles could be a promising process for production of other metal oxide nanoparticles which could have natural, significant, drug and clinical applications.

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