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# Assessment of Antioxidant, Anti inflammatory and Anti microbial efficacy of Piper longum and Piper betle mediated titanium dioxide nanoparticles

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**Abstract**

Background: Titanium nanoparticles (TiO2 NPs) hold promise for various applications due to their unique properties. This study presents a novel green synthesis method utilizing Piper longum and Piper betle extracts for the first time. The investigation further explores the potential antimicrobial, antioxidant, and anti-inflammatory activities of the synthesized nanoparticles.

Methods: The synthesized TiO2 NPs were characterized using various techniques (UV-Vis, FT-IR, SEM, XRD) to confirm their formation and properties. Subsequently, their antibacterial efficacy against common pathogens (Streptococcus mutans, E. faecalis, Staphylococcus aureus, Candida albicans) was evaluated. The nanoparticles were also assessed for their antioxidant activity using the H2O2 assay and anti-inflammatory potential through the egg albumin denaturation assay.

Results: The synthesized nanoparticles exhibited a zone of inhibition of 9 ± 0.5 mm against bacterial strains, comparable to established standards. Notably, they demonstrated a maximum anti-inflammatory inhibition of 80.83% and displayed promising antioxidant activity with a maximum inhibition of 86.23% at a concentration of 50 µg/ml.

Conclusion: This study successfully established a green synthesis approach for TiO2 NPs using P. longum and P. betle. The resulting nanoparticles demonstrated remarkable antibacterial, antioxidant, and anti-inflammatory properties, suggesting their potential for future applications in therapeutic interventions and immunomodulation.

Categories: Nano biotechnology, Health care.

**Keywords:** Titanium-nanoparticles, Green-synthesis, Piper longum, Piper betle, Antibacterial, Anti Inflammatory, Anti Oxidant.

## I. INTRODUCTION

Plants have been a cornerstone of traditional medicine for centuries, offering a natural treasure trove of compounds with potential therapeutic benefits [1]. Piper species, particularly Piper longum and Piper betle, are noteworthy for their diverse biological activities, including analgesic, antioxidant, and anti-inflammatory properties widely recognized in India and Southeast Asia [2, 3, 4]. Studies by Sunila et al. [5] further suggest the presence of anti-cancer properties within Piper longum extracts, specifically piperine.

The emergence of nanotechnology has revolutionized various scientific fields, offering promising solutions for a sustainable future [6]. Nanoparticles, due to their unique size-dependent properties like enhanced optics, improved catalytic activity, and high surface-to-volume ratio, have garnered significant scientific interest [7]. Their applications range from consumer products to healthcare interventions, including combating diseases, addressing energy storage needs, and facilitating drug/gene delivery systems [7].

Green synthesis presents an innovative and efficient approach for nanoparticle production compared to conventional methods relying on hazardous chemicals. This eco-friendly approach utilizes natural resources like plant extracts, bacteria, and fungi for the synthesis process [8, 9, 10]. Green synthesis offers several advantages, including cost-effectiveness, reduced environmental impact, and the potential for scale-up for mass production.

Among various metal oxide nanoparticles, titanium dioxide (TiO2) stands out due to its remarkable properties like stability, high efficiency in light utilization, exceptional surface area, potent photocatalytic activity, and favorable electronic band structure [11]. These unique characteristics contribute to its extensive applications in diverse fields such as solar cells, antimicrobial coatings, environmental remediation, development of electrochemical devices, and even the cosmetics industry [11].

This study proposes a novel and environmentally sustainable method for synthesizing titanium nanoparticles. We aim to utilize **polyphenols**

**and alkaloids** extracted from Piper longum as a green alternative to conventional hazardous chemicals in the synthesis process.

## II. MATERIALS AND METHODS:

The research was conducted at the Gold Lab of Saveetha Dental Colleges. All chemicals necessary for the synthesis of the nanoparticles were commercially procured.

### 2.1 Plant Extract Preparation:

Fresh leaves of Piper longum and Piper betle were used for the experiment. Two leaves of similar size from each plant species, weighing approximately 3.2 grams each, were selected for Saveetha Dental College's herbal garden. To ensure consistency, the leaves were meticulously washed with deionized water to remove any surface contaminants. Subsequently, they were crushed into a fine powder using a mortar and pestle. The resulting powder (a total of 6.4 grams) was then mixed with 100 milliliters of deionized water in a flask. This mixture was subjected to boiling for 20 minutes to facilitate the extraction of desired bioactive compounds from the plant material[11]. Finally, the solution was filtered using Whatman No. 7 filter paper to obtain the concentrated plant extract.

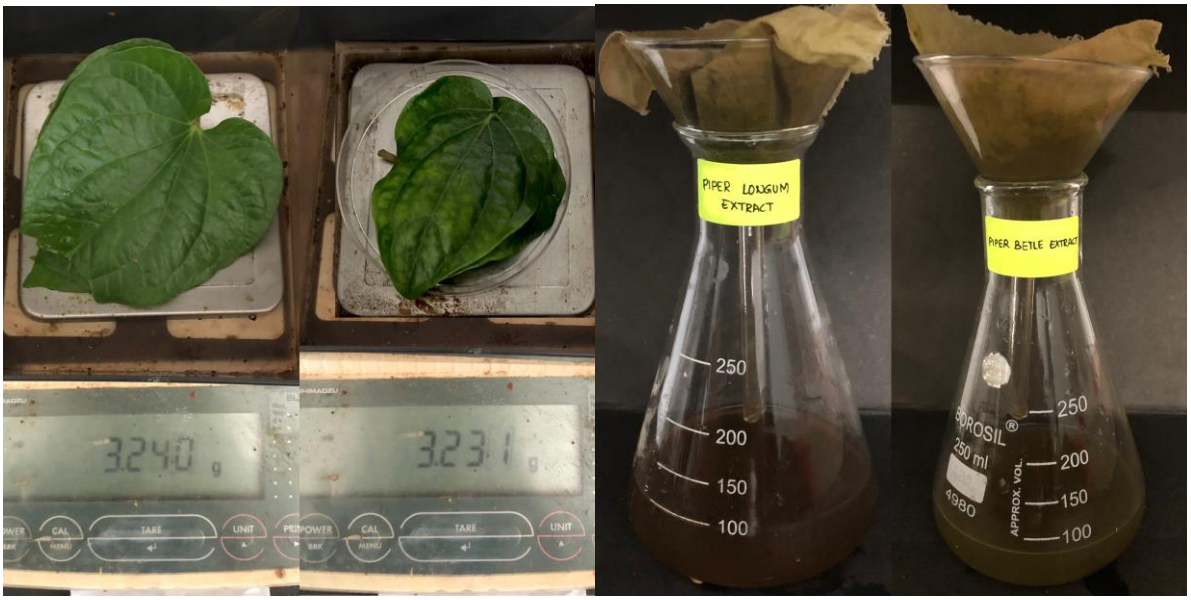

***Figure 1** showing the weight of Piper Longum and Piper Betle Leaves taken and ground into fine paste and diluted and filtered.*

### 2.2 Synthesis of Titanium Dioxide Nanoparticles (TiO2):

Aqueous extracts from Piper longum and Piper betle were combined to form a unified plant-based solution crucial for the subsequent nanoparticle synthesis. In parallel, a precursor solution was prepared by dissolving a precisely weighed amount (0.398 g) of commercially obtained titanium dioxide ($TiO_2$) powder in 50 mL of deionized water.

Equal volumes (50 mL each) of the plant-based solution and the precursor solution were then carefully mixed in a reaction vessel equipped with a magnetic stirrer. This mixture underwent continuous magnetic stirring at a controlled speed of 600-700 rpm for a prolonged period of 72 hours.

The progress of the reaction was monitored throughout this period using two complementary techniques. Visual observation revealed a gradual change in the color of the solution, serving as a preliminary indication of nanoparticle formation. Additionally, confirmatory measurements were conducted at regular intervals (every 24 hours) using a UV-Vis spectrophotometer. This analytical technique provided valuable insights into the light absorption properties of the reaction mixture, further solidifying the evidence for nanoparticle synthesis [12].
Following the 72-hour stirring period, a significant color change was observed, suggesting the reduction of metal ions ($Ti^{4+}$) within the mixture. Subsequently, the reaction mixture was centrifuged at 8000 rpm for 10 minutes to separate the solid components (nanoparticles) from the liquid components (supernatant) (Figure 2).

**Figure: 2** **Showing the Plant Extract Mixture, and Titanium Oxide Precursor Solution**

### 2.3 Characterization of Synthesized TiO2 Nanoparticles:

The synthesized titanium dioxide (TiO2) nanoparticles underwent extensive characterization to determine their structural and morphological properties. This involved employing established analytical techniques:

- **UV-Vis spectrophotometry:** An ELICO SL 210 instrument was used to analyze the light absorption properties of the nanoparticles within the 250-650 nm wavelength range. To assess potential changes during synthesis, absorption spectra were recorded at various time points (1, 12, 24, 36, and 48 hours).
- **Fourier Transform Infrared (FT-IR) spectroscopy:** A Bruker Alpha II instrument facilitated the identification of functional groups present within the nanoparticles. Each sample was analyzed using 64 scans with a resolution of 4 cm-1 across the 4000-550 cm-1 wavenumber range to obtain a comprehensive spectral fingerprint.
- **Scanning Electron Microscopy (SEM):** The morphology and surface features of the nanoparticles were investigated using a JEOL FE-SEM IT 800 instrument. Samples were prepared by mounting them on a stub and coating them with a thin layer of platinum to enhance conductivity. High-resolution images were captured at various magnifications.

### 2.3.4 X-ray Diffraction (XRD) Analysis:

X-ray diffraction (XRD) analysis was conducted using a Bruker D8 Advance diffractometer. This technique elucidates the crystal structure and phase composition of the synthesized nanoparticles. The instrument utilizes CuKα radiation with a wavelength of 1.5406 Å and operates at 40 kV and 40 mA. The 2θ/θ scanning mode was employed for data collection within the angular range of 5 to 90 degrees, with a step size of 0.029649 degrees.

### 2.4 Antimicrobial Activity Assessment:

The antibacterial efficacy of the green-synthesized titanium dioxide nanoparticles was evaluated using the agar well diffusion method. This method involves:

- Media Preparation: Mueller Hinton agar plates were sterilized through autoclaving at 121°C for 15-20 minutes. The sterilized medium was then poured into sterile Petri dishes and allowed to solidify at room temperature.
- Inoculation: Bacterial and fungal suspensions (e.g., E. coli, Staphylococcus aureus, Candida albicans, Streptococcus mutans) were evenly spread onto the agar surface using sterile swabs.
- Well Creation and Sample Loading: Wells with a diameter of 9 mm were created in the agar using a sterile tip. These wells were subsequently filled with varying concentrations (25 µg, 50 µg, and 100 µg) of the synthesized nanoparticles.
- Control Groups: Standard antibiotics like Amoxicillin (bacteria) and Fluconazole (fungi) were used as positive controls for comparison.
- Incubation and Zone Measurement: The plates were incubated at 37°C for 24 hours (bacteria) and 48 hours (fungi). The extent of the inhibition zone surrounding each well, indicative of antibacterial activity, was measured using a caliper in millimeters (mm).

### 2.5 Antioxidant Activity Assay - H2O2 Scavenging:

The ability of the synthesized nanoparticles to scavenge free radicals was assessed using the H2O2 assay method described by Ruch et al. [13]. Briefly, the following steps were involved:

- Reaction Mixture Preparation: H2O2 solution (0.6 mL) was combined with varying concentrations (25-400 µg/mL) of the nanoparticle extract (0.1 mL). The mixture was then adjusted to a final volume of 0.4 mL using a 50 mM phosphate buffer solution (pH 7.4).
- Spectrophotometric Analysis: After thorough mixing, the absorbance of the reaction mixture was measured at 230 nm using a spectrophotometer. Ascorbic acid served as a positive control in this experiment.

$$H2O2activity(\%)=\frac{Abs(control)-Abs(sample)}{Abs\ (control\ )}x100$$

Where Abs(control) represents the absorbance of the control, and Abs(test) denotes the absorbance of the extracts or standards.

### 2.6 Anti-inflammatory Activity Assessment - Egg Albumin Denaturation Assay:

To evaluate the potential anti-inflammatory properties of the synthesized nanoparticles, the egg albumin denaturation assay was employed. This assay investigates the ability of a substance to inhibit protein denaturation, a crucial step in the inflammatory cascade.

This involved the preparation of a reaction mixture containing 2.8 milliliters of phosphate buffer (pH 6.3) and 0.2 milliliters of freshly obtained egg albumin. Subsequently, varying concentrations (10-50 µg/mL) of the titanium dioxide nanoparticles synthesized using Piper longum and Piper betle extracts were introduced into the mixture.

Following the addition of the nanoparticles, the reaction mixture was incubated for two distinct periods:

- Initial incubation: Ten minutes at room temperature.
- Secondary incubation: Thirty minutes at an elevated temperature of 55°C within a water bath.

Dimethyl sulfoxide (DMSO) served as the control group, representing the baseline level of protein denaturation in the absence of any treatment. Diclofenac sodium, a known anti-inflammatory drug, was included as the standard group for comparison.

After the incubation stages, the reaction mixtures were subjected to spectrophotometric analysis at a wavelength of 660 nm. The resulting absorbance values were then utilized to calculate the percentage of protein denaturation using a standard formula [14].

$$\%\ inhibition = \frac{Absorbance\ of\ control\ -\ Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

## III. RESULTS AND DISCUSSION:

### 3.1 Characterization of Synthesized TiO2 Nanoparticles:

### 3.1.1 Colorimetric Analysis:

A noteworthy characteristic of nanoparticles is their unique interaction with light, often resulting in the manifestation of vivid colors. This phenomenon played a crucial role in monitoring the progress of the titanium dioxide (TiO2) nanoparticle synthesis process. The observed color alterations served as a preliminary indication of the transformation occurring within the reaction mixture.

During the synthesis, the precursor, titanium oxide, exhibits a white color. As the reaction progresses and the phytochemicals present in the plant extract mediate the formation of titanium nanoparticles, a gradual shift in color is observed. This initial transformation typically involves a change from white to a creamy white hue, as illustrated in Figure 3.

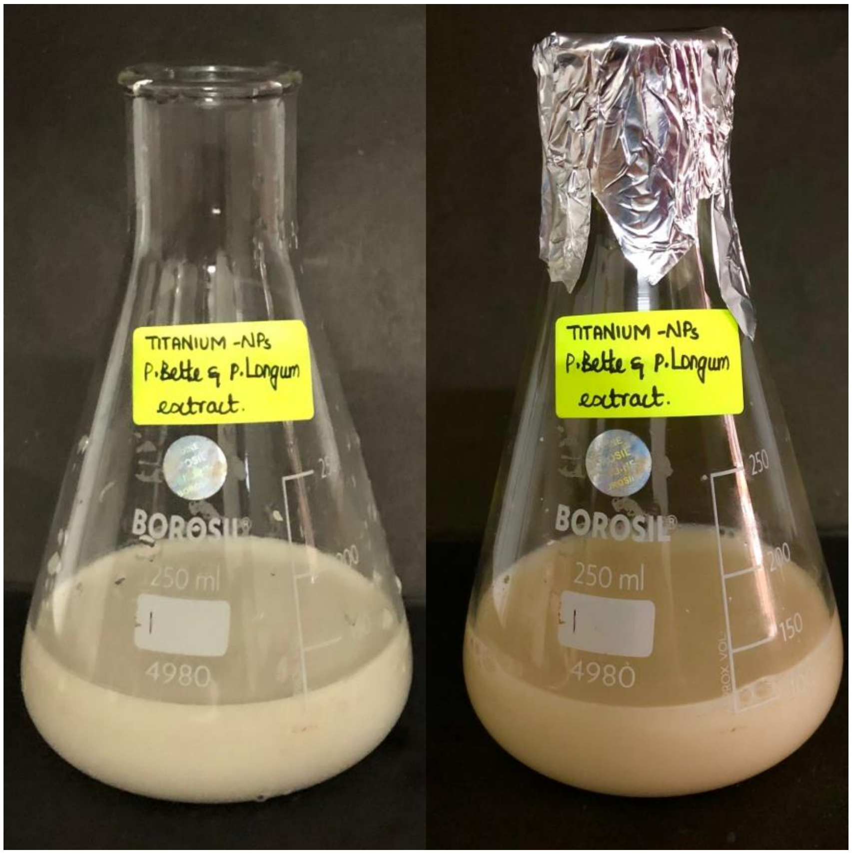

**Figure: 3** *shows a colour shift from White to Creamy White, demonstrating the formation of Titanium nanoparticles.*

### 3.1.2 UV-Visible Spectroscopic Analysis:

Ultraviolet-visible (UV-Vis) spectroscopy served as another valuable tool for characterizing the synthesized titanium dioxide (TiO2) nanoparticles. This technique analyzes the interaction of light with the sample at various wavelengths, providing insights into the material's composition and properties. UV-Vis spectroscopy analysis of the green-synthesized TiO2 nanoparticles revealed a characteristic peak from 270-350 nm with a high absorbance value of 3.0 (Figure 4). This peak aligns well with the typical absorption spectra of TiO2 nanoparticles, strongly suggesting the successful formation of TiO2-NPs in the synthesis process.

Ultraviolet-visible (UV-Vis) spectroscopy served as another valuable tool for characterizing the synthesized titanium dioxide (TiO2) nanoparticles. This technique analyzes the interaction of light with the sample at various wavelengths, providing insights into the material's composition and properties.

The precursor solution, containing a combination of Piper longum and Piper betle extracts, exhibited a characteristic absorption peak at 350 nanometers (nm), as depicted in Figure 4. This peak is attributed to the presence of specific biomolecules within the plant extracts.

Following the synthesis process, the UV-Vis spectrum revealed a critical observation: the emergence of a pronounced peak corresponding to Surface Plasmon Resonance (SPR). This phenomenon arises due to the collective oscillation of electrons on the nanoparticle surface when exposed to light. The presence of a well-defined SPR peak signifies the successful formation of nanosized particles, potentially exhibiting some degree of size distribution (polydispersity)..

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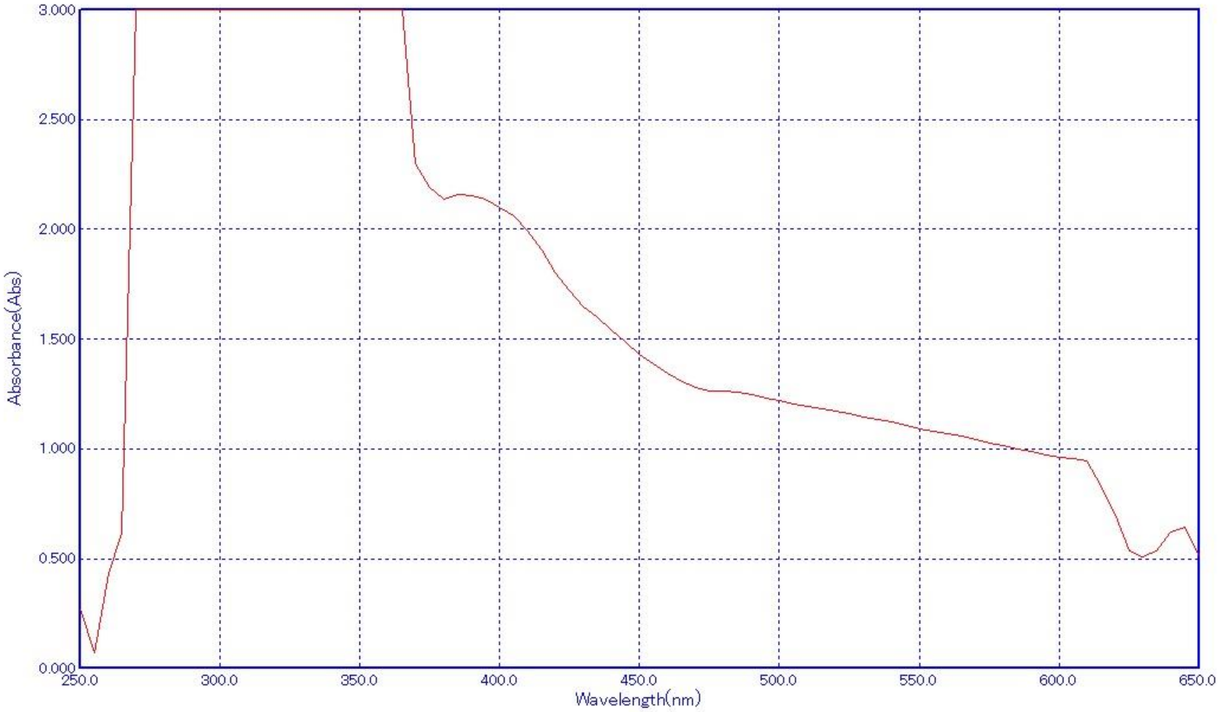

**Figure:4** **UV-Vis spectrophotometer analysis of synthesized TiO2 nanoparticles.**

### 3.1.3 Scanning Electron Microscopy (SEM) Analysis:

Scanning electron microscopy (SEM) was employed to visualize the morphology and size distribution of the synthesized titanium dioxide (TiO2) nanoparticles. The SEM images were captured at varying magnifications (6,000x and 30,000x) to provide a comprehensive perspective, enabling a detailed analysis of the particles' shape, size, and surface features (Figure 5). The micrographs reveal the predominantly spherical morphology of the TiO2 nanoparticles, with their diameters ranging from 20 to 30 nanometers (nm). Notably, some individual particles exhibit a tendency to aggregate, forming structures resembling flower-like clusters. Additionally, the presence of spherical flakes is also observed within the sample.

It's noteworthy that similar morphologies of TiO2 nanostructures have been documented in previous research conducted by Kirthi et al. (2011) [19]. This alignment with established findings strengthens the evidence for the successful synthesis of TiO2 nanoparticles using the green synthesis approach.

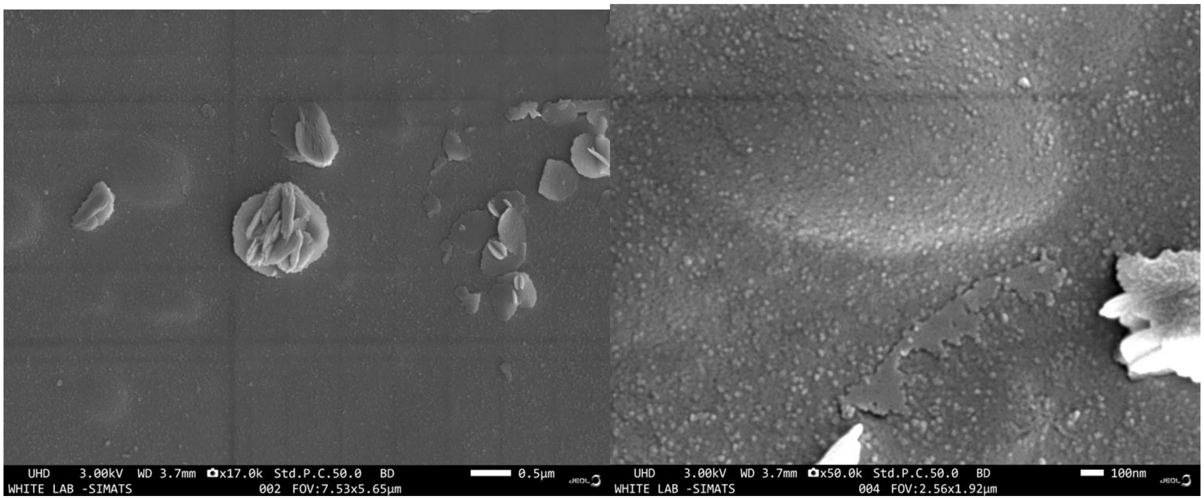

**Figure 5: In Figure A and B, small spherical-shaped nanoparticles are observed. In Figure A, both nanoparticle flakes with a spherical shape and integrated particles resembling flower-like structures are present.**

### 3.1.4 Fourier Transform Infrared (FTIR) Spectroscopy:

Fourier Transform Infrared (FTIR) spectroscopy was employed to elucidate the potential role of biomolecules in the process. The FTIR spectrum of the dried, green-synthesized TiO2 nanoparticles, reveals the presence of several key peaks(Figure 6). These peaks correspond to specific functional groups potentially involved in the following aspects:

- Metal Ion Reduction: Peaks observed around 642 cm-1 can be attributed to Ti-O stretching vibrations, indicating the possible involvement of these groups in the reduction of titanium ions during nanoparticle formation.
- Nanoparticle Surface Interactions: Peaks at 1075 cm-1, 1393 cm-1, and 1621 cm-1 suggest the presence of functional groups like C-O (amino acids), phenol (OH stretching), C=C (vinylidene), and N-H (secondary amines) potentially interacting with the surface of the synthesized nanoparticles.

The presence of these functional groups, particularly those associated with proteins, aligns with the established notion that protein molecules exhibit a high affinity for binding to metal surfaces. This binding interaction can play a crucial role in preventing the nanoparticles from aggregating and stabilizing them within the surrounding medium.

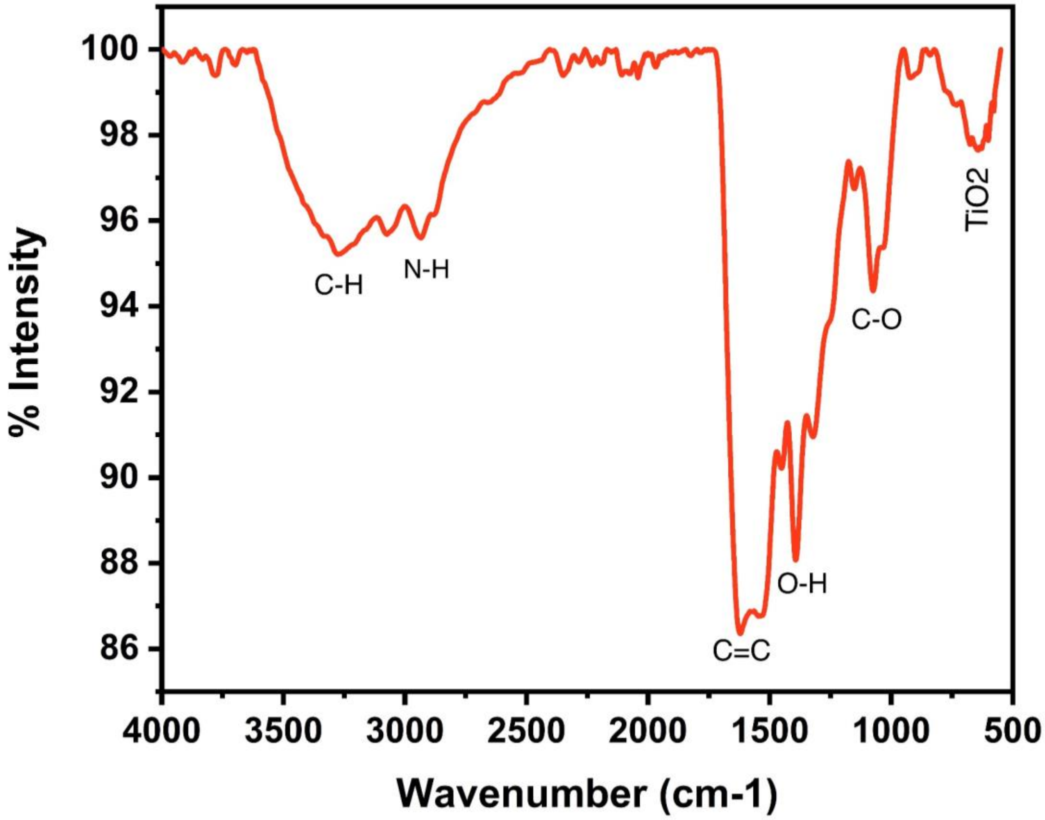

**Figure: 6** **FT-IR spectrum of synthesized TiO2NPs.**

**3.1.5 XRD:** X-ray diffraction (XRD) analysis served as a definitive tool to ascertain the crystalline nature of the synthesized titanium dioxide (TiO2) nanoparticles. The obtained XRD pattern for the synthesized nanoparticles reveals the presence of several distinct peaks across the 2θ range (20° to 80°)(Figure 7). These peaks hold significance as they correspond to specific crystal planes within the TiO2 crystal structure.

The characteristic crystalline nature of TiO2 is unequivocally confirmed by the presence of prominent peaks at 25.259°, 36.893°, 68.647°, and 80.619°. These peaks can be attributed to the (101), (103), (116), and (008) crystal planes, respectively. Additionally, the presence of minor peaks at 38.499° and 47.944° corresponding to the (112) and (200) planes further substantiates the existence of TiO2 within the sample.

It is noteworthy that the reference XRD spectra for pure crystalline TiO2 structures are available through the Joint Committee on Powder Diffraction Standards (file no. 84-1285). The close alignment of the obtained pattern with this reference data reinforces the successful synthesis of crystalline TiO2 nanoparticles.

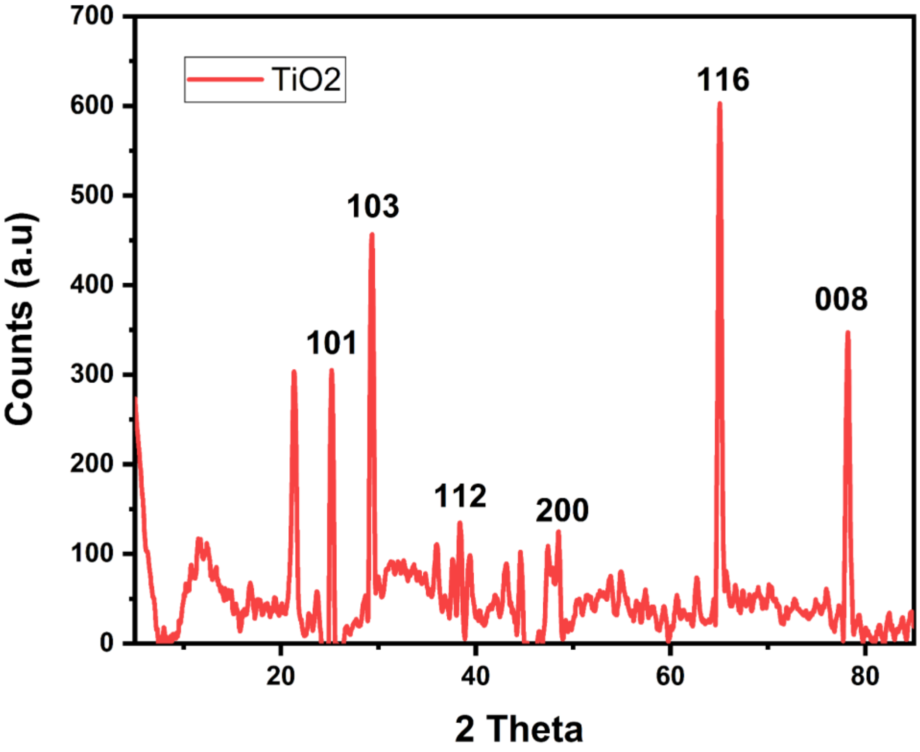

**Figure: 7** **XRD spectrum of synthesized TiO2NPs.**

3.2 Antibacterial Activity of TiO2 Nanoparticles against Oral Pathogens: Titanium dioxide (TiO2) possesses remarkable potential for combating various oral bacterial pathogens, with its effectiveness further amplified when present in its nanoform [15]. A widely accepted notion suggests that metal oxides, including TiO2 nanoparticles, hold a positive surface charge, whereas microorganisms exhibit a negative charge. This inherent polarity fosters an electrostatic attraction between the negatively charged bacterial cell wall and the positively charged nanoparticles. Upon contact with the bacterial surface, the close proximity triggers an oxidative stress response within the microorganism. This phenomenon disrupts the cell membrane integrity, leading to the inactivation of crucial enzymes and ultimately causing bacterial cell death.

Our investigation revealed that the synthesized TiO2 nanoparticles mediated by Piper Longum and Piper Betle extracts demonstrate significant antibacterial, AND ANTIFUNGAL activity at 25 µL, 50 µL, and 100 µL corresponding to a ZOI of 9±0.5mm, respectively matching the controls efficacy. These measurements were made linearly.

**Figure: 8** **Antimicrobial activity of Titanium nanoparticles synthesized from Piper longum and Piper betle formulation against S.Aureus, S. Mutans, C. Albicans and E.Faecalis.**

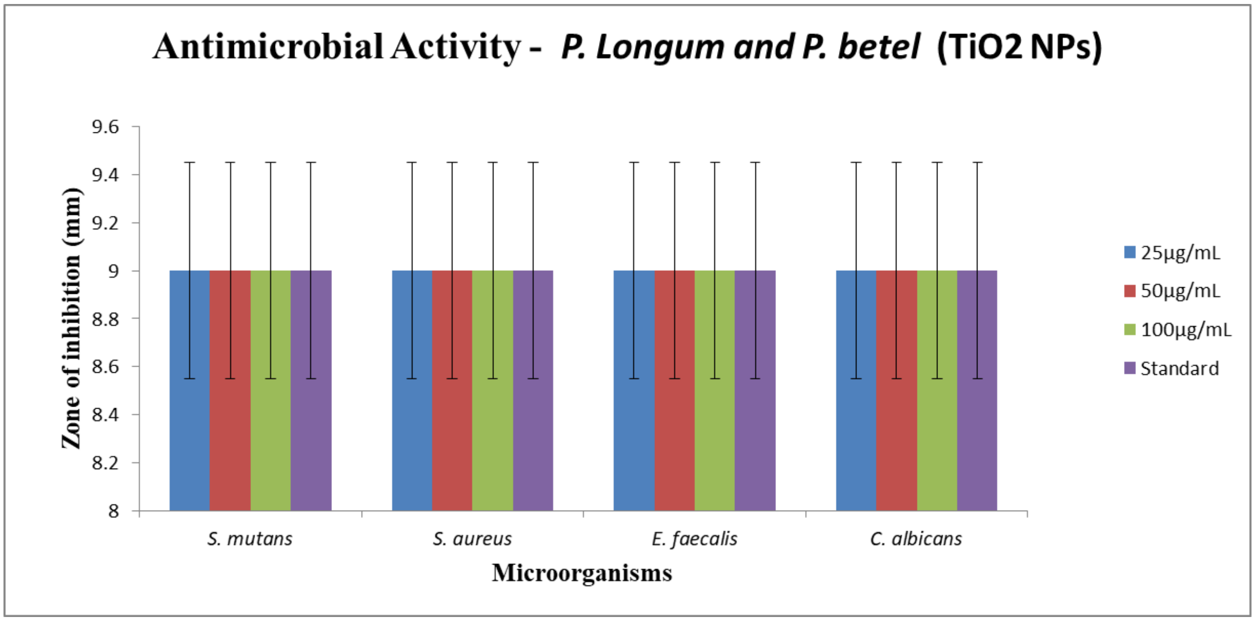

**Figure 9: The antibacterial activity of Titanium nanoparticles synthesized from the Piper Longum and Piper Betle formulation against S. Aureus, S. Mutans, C. Albicans, and E. Faecalis is virtually equivalent to that of the control antibiotic disc.**

### 3.3 Antioxidant Activity of Synthesized TiO2 Nanoparticles:

Our findings reveal that the synthesized TiO2 nanoparticles possess exceptionally high antioxidant activity at lower concentrations, gradually approaching the efficacy of established standards at higher concentrations(Figure 10). The nanoparticles exhibited a maximum inhibition activity of 86.23% at 50 µg/ml, with a minimum inhibition of 50.62% observed at the lowest tested dose of 10 µg/ml. The determined IC50 value for these nanoparticles was 9.61 µg/ml, signifying their ability to scavenge 50% of the hydrogen peroxide (H2O2) radicals.

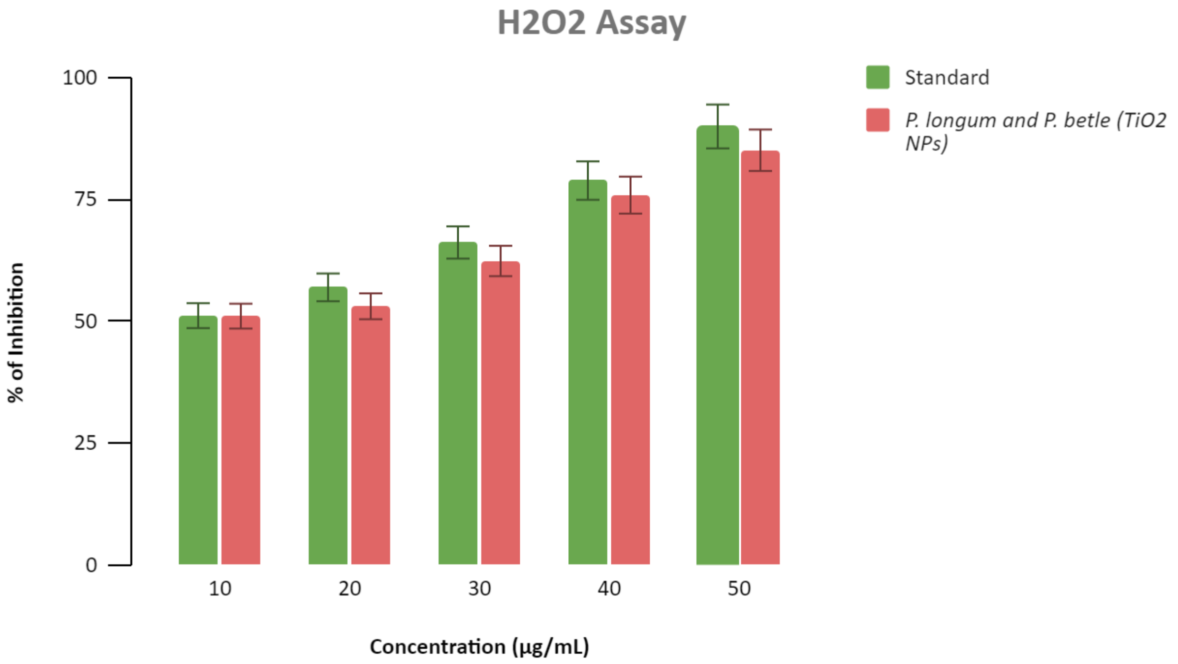

**Figure: 10 Antioxidant activity of Titanium nanoparticles synthesized from Piper Longum and Piper Betle formulation with antioxidant activity nearly equivalent to the standard, Ascorbic Acid.**

### 3.4 Anti-inflammatory Activity Assessment:

In-vitro bioassay results explore the potential anti-inflammatory properties of the synthesized Green Synthesized-TiO2 nanoparticles (NPs) by evaluating their ability to inhibit heat-induced denaturation of egg albumin (Figure 11). The observed results reveal a concentration-dependent decrease in egg albumin denaturation across all tested dosages of the TiO2 nanoparticles. The highest measured concentration (50 µg/mL) demonstrated the most significant inhibition, reaching 80.83%. Notably, the reference anti-inflammatory medication, Diclofenac, exhibited a slightly higher inhibition percentage of 84.17% at the same concentration.

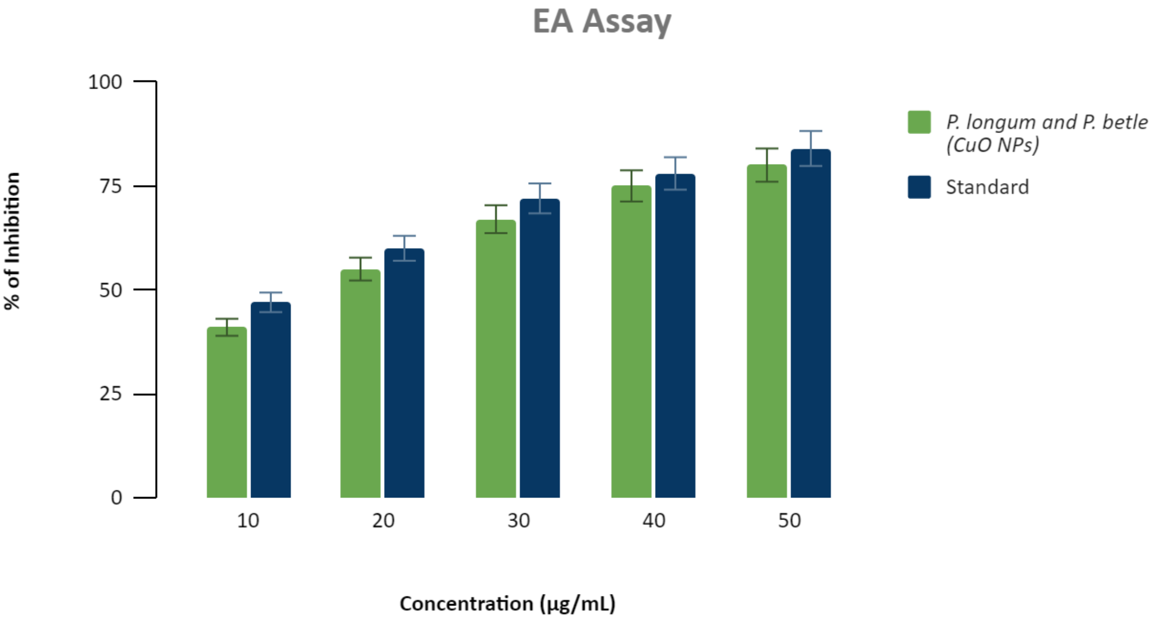

**Figure: 11 Anti-Inflammatory: An illustration of the Titanium nanoparticle's anti-inflammatory properties, which are equivalent to those of the conventional anti-inflammatory drug, Diclofenac Sodium.**

## IV. DISCUSSION

The Piperaceae family Boasts over 10 genera and 1500 species, have a long history of medicinal use in Asia [3-5]. They contain valuable bioactive compounds like phenolics, flavonoids, and terpenoids that could potentially be used to develop new drugs [9,10]. This method is different from traditional techniques and could be a more sustainable approach for producing TiO2-NPs. As antibiotic resistance becomes a growing threat, researchers are looking for safer ways to fight infections. This study focused on creating titanium dioxide nanoparticles (TiO2-NPs) using extracts from Piper betle (PB) and Piper longum (PL) to see if they could kill bacteria. The urgency of finding alternatives is highlighted by the concerning number of deaths caused by antibiotic-resistant bacteria [11].

Fourier-transform infrared spectroscopy (FTIR) showed that the Piper plant extracts contained helpful molecules that stuck to the titanium dioxide nanoparticles (TiO2-NPs) and made them smaller and more stable [15]. Other tests conducted by Kirthi et al., confirmed that the nanoparticles were between 10 and 50 nanometers in size [17, 19]. Finally, microscopic images revealed that the nanoparticles were round or flower-shaped and all similar in size, similar to the studies conducted by Khan et al., indicating a successful synthesis process [18,20].

The antibacterial activity of the synthesized TiO2-NPs was investigated against various concentrations. The agar disc diffusion method measured the zone of inhibition (ZOI) in millimeters. The NPs demonstrated antimicrobial activity comparable to the control antibiotics, cefixime and clotrimazole. The antimicrobial properties of the Piper extracts are likely due to their acidic nature and phytochemicals like terpenes and phenolics, which can damage bacterial cell walls [21]. This disrupts membrane permeability, leading to cell death. Similar antibacterial properties of TiO2-NPs have been observed against various bacteria [22]. Studies by Hatano, T. et al., showed that these NPs have potential applications in topical formulations (creams and ointments) to combat S. aureus on the skin and in oral hygiene products like toothpaste and mouthwash to fight S. aureus colonization in the mouth [23, 24]. The promising antibacterial activity of these green TiO2-NPs opens new avenues for combating bacterial diseases.

Green synthesis of TiO2-NPs using Piper extracts offers an eco-friendly alternative to chemical techniques. The NPs' ability to generate reactive oxygen species (ROS) can induce oxidative stress in bacterial cells, inhibiting protein synthesis and DNA replication [24]. Plants are rich sources of bioactive compounds, particularly phenols with free radical scavenging properties due to hydroxyl groups [25, 26].

Interestingly, the synthesized TiO2-NPs exhibited remarkable free radical scavenging activity comparable to ascorbic acid, a well-known standard. Previous research by Kumar, A. et al. and Kähkönen, M.P. et al., has documented the anti-inflammatory and antioxidant properties of Piper betle and Piper longum extracts [27, 28]. These plants are known to contain significant amounts of phenols, polyphenols, and tannins, which likely contribute to the observed antioxidant effects, aligning with prior studies [29-31].

Flavonoids and saponins present in Piper extracts are known to modulate inflammatory pathways by suppressing enzymes involved in inflammatory mediator production. This translates to their anti-inflammatory and pain-relieving properties. Prior research by Koblyakov, V.A. et al and Kumar, S. et al, confirms the presence of high levels of flavonoids in Piper extracts, potentially explaining the observed anti-inflammatory effects of the synthesized nanoparticles [32, 33]. These findings suggest the potential anti-inflammatory activity of the NPs, possibly due to the bioactive compounds from the Piper extracts used in the green synthesis process.

This study's in-vitro nature limits its generalizability to real-world clinical scenarios. Further research is needed to explore the effect of synthesis temperature on the size and shape of the NPs [34]. More tests are required to assess antimicrobial activity against a broader range of bacteria, including those causing localized and systemic infections. Cytotoxicity testing is also crucial for future product development [35]. Continuous efforts are needed to develop eco-friendly and commercially viable methods that harness the potential of natural agents for nanoparticle synthesis

## V. CONCLUSION:

This study presents a novel and cost-effective approach for synthesizing titanium dioxide nanoparticles (TiO2-NPs) utilizing co-extracts of Piper Longum and Piper Betle. These readily available plant extracts act synergistically as both reducing and stabilizing agents, facilitating a rapid and efficient nanoparticle production process. Notably, the synthesized TiO2-NPs exhibit promising properties, including potent antimicrobial activity against a broad spectrum of pathogens, reduction of inflammatory responses in tissues, and the ability to combat oxidative stress. These characteristics open exciting possibilities for their application in various therapeutic areas. However, extensive research is still required before these nanoparticles can be safely integrated into patient care. Rigorous in vitro studies will elucidate the nanoparticles' interaction with human cells and potential cytotoxicity. Subsequent in vivo studies in animal models will be crucial to assess their efficacy and potential for adverse effects. Finally, well-designed clinical trials are essential to definitively determine the safety and effectiveness of TiO2-NPs, particularly when considering their incorporation into oral hygiene products. This innovative synthesis method, coupled with the promising properties of the resulting nanoparticles, warrants further investigation to unlock their full potential in the realm of medical advancements.

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