



Green Synthesis and Multifunctional Applications of *Moringa Oleifera*-enhanced TiO₂ Nanocomposites

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ABSTRACT

This study investigates the green synthesis and detailed characterization of TiO₂ nanoparticles (TiO₂-NPs) doped with *Moringa oleifera* leaf (MOL) extract, aiming to explore their multifunctional applications. MOL extract acted as both a reducing and stabilizing agent during nanoparticle synthesis, thereby enhancing the physicochemical properties of the resulting composites. The photocatalytic activity of the synthesized TiO₂-MOL composites was evaluated through the degradation of methylene blue dye under solar irradiation, with optimal performance achieved at 4 wt.% MOL loading. Key parameters, including pH, catalyst dosage, dye concentration, and catalyst reusability, were systematically optimized to determine the most effective conditions. The optimum photocatalytic performance was observed at neutral pH, 150 mg catalyst loading, and a dye concentration of 1×10^{-4} M. Characterization techniques such as Scanning electron microscopy (SEM), High-resolution transmission electron microscopy (HR-TEM), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), light absorption capacity, and interactions between MOL bioactive compounds and TiO₂. Furthermore, the TiO₂-MOL composites exhibited notable antimicrobial, antibacterial, and anti-inflammatory properties, attributed to the synergistic effects between the photocatalyst and MOL's bioactive constituents. These findings suggest that MOL-doped TiO₂ nanocomposites hold significant promise for sustainable environmental remediation and biomedical applications.

Keywords: *Moringa Oleifera* leaves; TiO₂ nanoparticles; Methylene blue; Antimicrobial activity; Antibacterial activity; Anti-inflammatory activity.

1. INTRODUCTION

The environmental consequences of dye pollution have emerged as a critical area of study due to its far-reaching ecological and health impacts. Industrial sectors such as textile processing, leather tanning, paper manufacturing, polymer production, and pharmaceutical formulation are primary sources of dye-contaminated effluents. These effluents, characterized by their recalcitrant and toxic nature, contribute to severe water pollution challenges, disrupting aquatic ecosystems and posing significant risks to human health and environmental sustainability (Suba *et al.* 2024). Methylene blue has evolved into a cornerstone of clinical and scientific innovation. (Natrayan *et al.* 2024). Methylene blue is a multifaceted synthetic dye and drug widely employed in biology, chemistry, and pharmacy. Renowned for its vibrant blue hue and distinct chemical properties, it is essential for staining, diagnostics, and chemical analyses. Modern research thrives on exploring nanoscale materials, defining the essence of

nanotechnology. This field focuses on manipulating matter at atomic and molecular levels, unlocking unprecedented applications.

Nanotechnology bridges disciplines, driving innovations across science and industry. (Panhwar *et al.* 2021; Mani *et al.* 2023; Murugaiyan *et al.* 2024; Mani *et al.* 2024). *Moringa oleifera*, known as the "Miracle Tree" or "Tree of Life," is acclaimed for its potent health benefits. Its nutritional profile is unmatched, offering a wide range of essential compounds. Additionally, it plays a vital role in enhancing environmental sustainability (Kashyap *et al.* 2022). *Moringa oleifera* leaf extract, packed with bioactive compounds like phenolics, flavonoids, and alkaloids, acts as a reducing and stabilizing agent in nanoparticle synthesis. These compounds control nanoparticle morphology, prevent aggregation, and enhance stability. As a result, *Moringa* extract is widely used in the green synthesis of metal and

metal oxide nanoparticles (e.g., TiO_2), with applications in antimicrobial activity and dye degradation (Kaur *et al.* 2025). *Moringa oleifera* Leaf is widely used in traditional medicine across the globe. Recent studies highlight its therapeutic potential, showcasing anti-diabetic, anti-cancer, anti-hypertensive, and antimicrobial properties. These bioactivities position *Moringa* as a valuable plant in modern medicine. Its diverse medicinal applications continue to expand, drawing significant scientific interest (Abdel *et al.* 2023). The botanical classification of *Moringa oleifera* Leaf is shown in table 1. The biological activity of leaf extracts is contingent on the solvent used, which selectively isolates specific bioactive compounds. This variability leads to distinct pharmacological properties. Thus, solvent choice plays a crucial role in determining the therapeutic potential of the extract (Tiwari *et al.* 2020). Titanium dioxide (TiO_2) is an inorganic material characterized by its superior chemical stability, high electrochemical coupling efficiency, broad absorption spectrum, and outstanding photostability (Adewi *et al.* 2024). TiO_2 nanoparticles (TiO_2 -NPs) are widely employed owing to their low production costs, exceptional chemical stability, high refractive index, and robust oxidative capabilities. Additionally, the presence of oxygen vacancies within their crystalline lattice enhances their functional versatility. These nanostructures exhibit remarkable electrical and ionic properties, which can be precisely tuned for specific applications. Such modifications enable TiO_2 -NPs to serve as advanced materials in sensor technology and electronic device fabrication. Their unique physicochemical properties further expand their potential in cutting-edge technological fields (Ghareeb *et al.* 2024).

The green synthesis of TiO_2 nanoparticles (TiO_2 -NPs) using biological extracts has been extensively explored, accompanied by comprehensive characterization analyses. Detailed investigations into the morphological and structural properties of the synthesized nanoparticles are presented, providing valuable insights. The study emphasizes the eco-friendly and sustainable approach of green synthesis, which minimizes environmental impact. Furthermore, the potential applications of green-synthesized TiO_2 -NPs in photocatalysis and antimicrobial activities are critically evaluated. This approach offers significant advantages in enhancing catalytic efficiency and biocompatibility (Verma *et al.* 2022). *Moringa oleifera* leaf (MOL) extract has long been acknowledged for its potential in antimicrobial and medicinal applications. MOL/ TiO_2 nanocomposites demonstrate robust antimicrobial activity against diverse microorganisms. TiO_2 , in both micro- and nanoscale forms, is recognized as a cost-effective and efficient alternative to silver-based antimicrobial agents. Incorporating small amounts of MOL with affordable TiO_2 nanoparticles presents a synergistic strategy to enhance photocatalytic efficiency and antimicrobial performance (Ghosh *et al.* 2022). In

this study, MOL/ TiO_2 nanocomposites were synthesized using an aqueous extract of *Moringa oleifera* leaves, serving as both a reducing and stabilizing agent. The photocatalytic performance of the synthesized composites was evaluated for Methylene Blue dye degradation under light irradiation. The results confirmed their high efficiency as photocatalysts, showcasing their potential in environmental remediation. This approach emphasizes the eco-friendly nature of photocatalytic degradation, offering a sustainable solution for pollutant elimination. Unlike conventional methods, this technique utilizes light-activated photocatalysts to degrade contaminants without resorting to harsh chemicals. Such advancements underline the significance of MOL/ TiO_2 composites in green technology applications. This study aimed to develop unique MOL/ TiO_2 hybrid photocatalysts by incorporating varying weights of *Moringa oleifera* leaf (MOL) extract. The photocatalytic activity of the synthesized MOL/ TiO_2 nanocomposites was assessed using Methylene Blue as a model pollutant under solar light irradiation. The composites exhibited significantly enhanced photodegradation performance compared to pristine TiO_2 and MOL alone. Furthermore, the MOL/ TiO_2 nanocomposites demonstrated notable antimicrobial, anti-inflammatory, and antibacterial properties, highlighting their multifunctional potential.

The antibacterial activity arises from the synergistic effects of TiO_2 -induced reactive oxygen species (ROS) generation and bioactive phytochemicals in MOL, which lead to bacterial membrane disruption and oxidative stress-mediated inhibition. This dual mechanism reduces bacterial resistance compared to conventional antibiotics and exhibits anti-inflammatory potential. These findings underscore the effectiveness of the hybrid system for environmental and biomedical applications (Sivagami *et al.* 2022). This study focused on evaluating the antimicrobial, antibacterial, and anti-inflammatory activities of TiO_2 nanoparticles synthesized via a green synthesis route, utilizing *Moringa oleifera* leaf extract as a bioreducing and stabilizing agent. Additionally, the photocatalytic performance of the synthesized nanoparticles was systematically investigated for the degradation of organic dye pollutants, highlighting their potential in environmental and biomedical applications (Narayanan *et al.* 2023). MOL/ TiO_2 composites serve as a natural source of bioactive compounds with antioxidant and anti-inflammatory properties, positioning them for potential applications in functional foods and medicinal formulations. Their inherent therapeutic characteristics suggest their promising role in promoting health benefits. The incorporation of *Moringa oleifera* leaf extract into TiO_2 enhances these properties, making the composite suitable for use in nutraceuticals and pharmaceutical products. This synergy offers an innovative approach to developing sustainable and biofunctional materials for future health-oriented applications (Xu *et al.* 2019). Scaling up involves optimizing the green synthesis of

TiO₂ nanoparticles using MOL extract for sustainable industrial applications. Key challenges include cost-effective large-scale production, batch-to-batch variability, and ensuring consistent physicochemical properties. Process standardization, resource sustainability, and yield optimization are crucial for industrial feasibility.

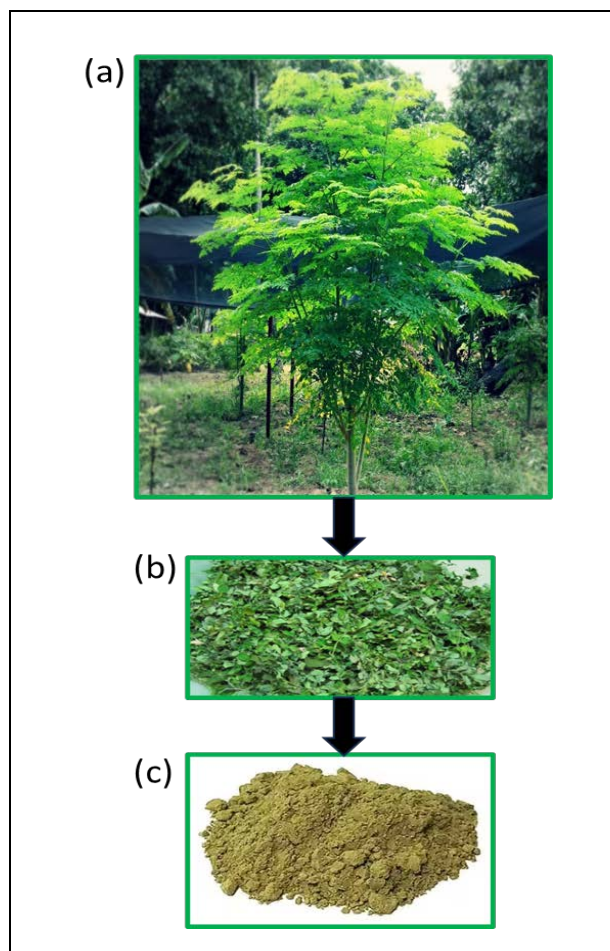


Fig. 1 (a-c): *Moringa Oleifera* leaf powder extraction

Table 1. Botanical classification of *Moringa Oleifera* Leaves

Kingdom	Plantae
Subkingdom	Tracheopionta
Division	Magnoliophyta
Class	Magnalipsida
Subclass	Dilleniidae
Superorder	Violanae
Order	Moringales
Family	Moringaceae
Genus	Moringa
Tamil Name	Murungai
English Name	Moringa

2. MATERIALS AND METHODS

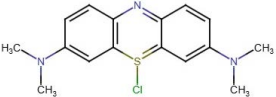
2.1 Preparation of MOL Extract

Fresh *Moringa oleifera* leaves (1 kg) were procured from Thiruvavur District, Tamil Nadu, India, and thoroughly washed with distilled water to remove impurities. The leaves were shade-dried at ambient temperature (25–30°C) for 7–10 days to minimize the degradation of bioactive compounds. Once dried, the leaves were ground into a fine powder using a laboratory-grade grinder (Fig. 1). A hydroalcoholic solution, comprising distilled water, ethanol (99.9%), and methanol (99.8%) in an 80:10:10 ratio, was prepared as the extraction solvent. The powdered leaves were immersed in 8 L of the solvent mixture and incubated at 30°C for 72 hours in an orbital shaker to enhance the extraction of bioactive constituents. Following incubation, the mixture was centrifuged at 4000 rpm for 10 minutes to separate the solid residues from the liquid phase. The obtained supernatant was further filtered through Whatman No.1 filter paper to remove any remaining particulates. The filtered extract was concentrated by solvent evaporation at 50°C using a rotary evaporator. The resultant hydroalcoholic extract was stored in amber glass bottles at 4°C to preserve its stability and bioactivity for subsequent photocatalytic degradation studies (Pedraza *et al.* 2021).

2.2 Biosynthesis of TiO₂ NPs

The biosynthesis of TiO₂ nanoparticles began with the preparation of a plant extract by boiling 20–50 g of fresh, washed leaves in 100–200 mL of distilled water at 80°C for 30 minutes, followed by filtration to obtain a clear solution. Separately, a titanium precursor solution was prepared by dissolving 0.2 M TiOSO₄ in 50 mL of distilled water under constant stirring. The plant extract (20–30 mL) was added dropwise to the titanium precursor solution at room temperature with continuous stirring. The pH of the mixture was adjusted to 7 using 1–2 M NaOH, and the reaction was maintained at 60°C for 2–4 hours, resulting in the formation of a gel-like precipitate. The precipitate was filtered and thoroughly washed with distilled water to remove impurities. The filtered solid was dried at 80°C for 12 hours, followed by calcination at 500°C for 3 hours in a ceramic crucible to achieve crystallization of the TiO₂ nanoparticles. Short-term stability is ensured by maintaining extract storage at 4°C and enhancing TiO₂ crystallinity through 500°C calcination. However, long-term structural integrity, photocatalytic recyclability, and degradation resistance under continuous operational conditions require further investigation. The calcined product was ground into a fine powder using a mortar and pestle and stored in an airtight container for subsequent applications. (Verma *et al.* 2022) This green synthesis method utilizes plant extracts as sustainable reducing and capping agents.

Table 2. Physicochemical properties and chemical structure of methylene blue

Physicochemical properties	Description
Molecular formula	C ₁₆ H ₁₈ N ₃ ClS
Molecular weight	319.85 g/MOL
Solubility	43.6 g/L in water at 25°C
Color	Deep blue solution in water or alcohol
Chemical Structure	
Melting point	100–110°C

2.3 Photodegradation with Solar Light

Fig. 2 demonstrates the photocatalytic degradation of dye using TiO₂ doped with *Moringa oleifera* leaves (MOL) under solar light, examining various doping percentages. Minimal dye degradation is observed under dark conditions (Dye/Dark, Dye/Catalyst/Dark), emphasizing the critical role of light in activating photocatalysis. Solar light alone (Dye/Solar) results in limited degradation, while the presence of the catalyst under solar light significantly accelerates the process (Dye/Catalyst/Solar). Among the doping levels studied, 4 wt.% achieves the highest degradation (99%), attributed to enhanced light absorption, suppressed electron-hole recombination, and an optimal distribution of active sites. Lower doping levels (e.g., 2 wt.%) show moderate degradation due to insufficient active sites. Conversely, higher doping at 8 wt.% leads to a decline in degradation efficiency, likely caused by particle agglomeration and reduced surface area, which impair catalytic activity. This behavior underscores the need to optimize doping concentrations to achieve a balance between light absorption and active site availability. The findings highlight that TiO₂-MOL serves as an effective photocatalyst under solar light, with 4 wt.% doping providing the highest efficiency for dye degradation, demonstrating its potential for sustainable wastewater treatment applications. MOL-TiO₂ composites offer a sustainable approach for wastewater treatment and biomedical applications, minimizing synthetic chemical dependency. However, a detailed life-cycle assessment, cost-benefit analysis, and potential ecotoxicological effects of prolonged use need further evaluation.

2.4 Characterization of *Moringa oleifera* Leaves Nanocomposites

Morphological and elemental assessments of *Moringa oleifera* leaves /TiO₂ nanoparticles were analyzed using a field emission scanning electron microscope (FEI Quanta FEG200- FESEM). The Jeol 2100 HR-TEM analyzed nanocomposite morphology at

200 kV, 0.2 nm resolution, with samples prepared by ultrasonic dispersion in ethanol on carbon-coated copper grids. Using a Benchtop X-Ray Diffractometer with Cu-K at 0.15,405 Å in the Bragg's angle range 2θ (10–80°). To learn more about their composition and how it affects their performance, scientists turned to FTIR spectroscopy (ATR diamond accessory of IR tracer 100 AH / Japan). Using a UV-visible spectrophotometer, the optical properties of the sample synthesis were examined at (UV 2100, Shimadzu). The anti-inflammatory activity of MOL/TiO₂ catalysts was assessed via the albumin denaturation method, showing their potential to inhibit protein denaturation.

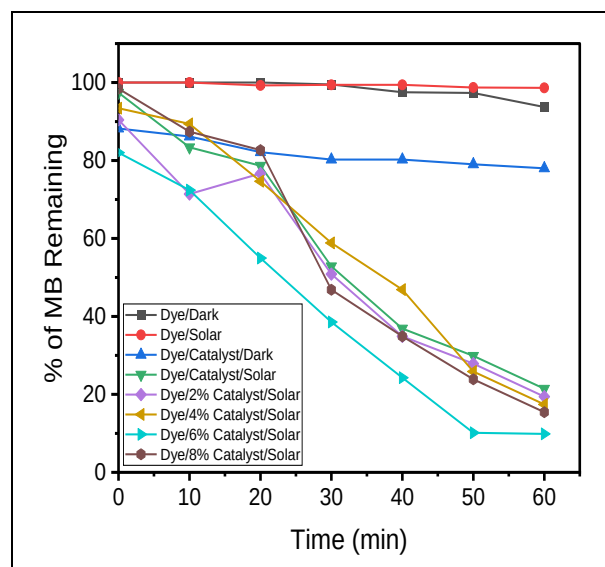


Fig. 2: Primary analysis of MOL/TiO₂ catalyst with Methylene Blue dye under solar light ([MB] = 4×10^{-4} M, 4 wt.% MOL/TiO₂ = 2 g L⁻¹, airflow rate = 8.1 mL s⁻¹, pH = 7.0, ISolar = 1.381×10^{-6} einstein L⁻¹ s⁻¹)

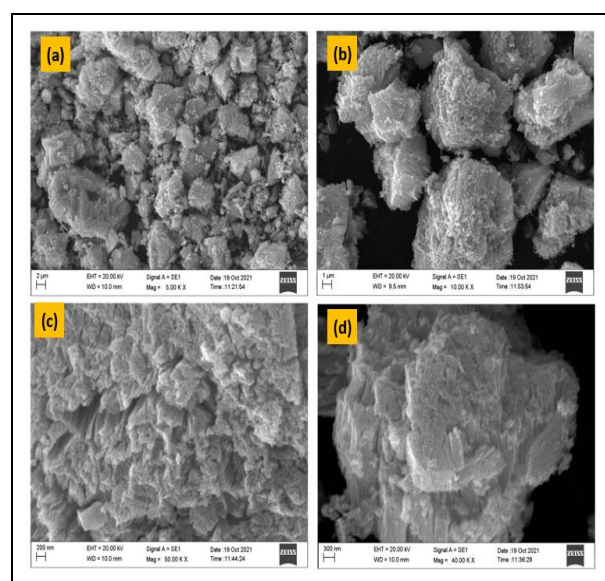


Fig. 3: SEM images of 4 wt.% MOL/TiO₂ nanocomposites (a) 2 μm, (b) 1 μm, (c) 200 nm and (d) 300 nm

3. RESULTS AND DISCUSSION

3.1 SEM

SEM images of the 4 wt.% *Moringa oleifera* leaves/TiO₂ composite reveal key morphological features across different magnifications. At the 2 μ m scale (fig. 3a), the composite shows a heterogeneous distribution of particles with rough, uneven surfaces, indicating the successful incorporation of *Moringa oleifera* leaves into the TiO₂ matrix. This roughness suggests an increased surface area, essential for enhanced photocatalytic performance. The 1 μ m image (fig. 3b) provides a closer view of these particles, showing irregular shapes and rough textures, further emphasizing the integration of organic components with the inorganic TiO₂. At the 200 nm scale (fig. 3c), finer surface textures and porosity become more apparent, highlighting the presence of a high surface area, which is beneficial for improved light absorption and catalytic efficiency. The 300 nm (fig. 3d) shows well-defined, flaky, and layered nanostructures, confirming the uniform dispersion of *Moringa oleifera* leaves within the TiO₂ matrix (Inkson *et al.* 2016). The consistent rough and porous morphology across all magnifications suggests a strong interaction between the organic and inorganic components, enhancing the composite's structural and functional properties. These observations confirm the successful doping process, resulting in a composite with potentially superior photocatalytic activity due to its increased surface area and improved light interaction.

3.2 HR-TEM

HR-TEM images of the 4 wt.% *Moringa oleifera* leaves/TiO₂ nanocomposites provide detailed structural and morphological insights. At 100 nm magnification (fig. 4a), the agglomerated nanoparticles suggest effective incorporation of *Moringa oleifera* leaves-derived components within the TiO₂ matrix, confirming the successful doping process. The 50 nm magnification (fig. 4b) further substantiates the uniform dispersion and integration of organic material, which is essential for optimizing the composite's functional properties. At a 20 nm magnification (fig. 4c), the nanoparticles exhibit distinct and well-defined shapes, with sizes ranging from 10 to 20 nm. This level of size control is critical for enhancing the surface area, which in turn improves the photocatalytic efficiency of the composite (Perumalsamy *et al.* 2024). The 2 nm magnification Fig. 4d shows clear lattice fringes with a spacing of 0.27 nm, corresponding to the (101) plane of anatase TiO₂. This confirms the retention of TiO₂'s crystalline structure post-doping, which is essential for preserving its photocatalytic activity. Overall, the HR-TEM analysis provides conclusive evidence of the successful doping of *Moringa oleifera* leaf components into TiO₂, maintaining the crystalline integrity of TiO₂

and enhancing the composite's potential for photocatalytic applications.

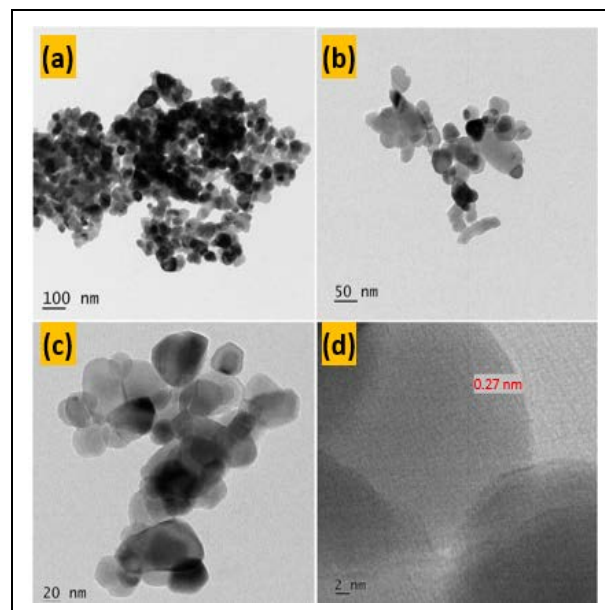


Fig. 4: HR-TEM images of 4 wt.% MOL /TiO₂ nanocomposites at (a) 100 nm, (b) 50 nm (c) 20 nm and (d) 2 nm resolutions

3.3 X-Ray Diffraction Analysis of Green Synthesized TiO₂ NPs

XRD analysis of the materials shows key structural features that confirm the successful doping of *Moringa oleifera* leaves into TiO₂. The TiO₂ (anatase phase) peaks, including (101) at 25.3°, (004) at 37.8°, (200) at 48.0°, (105) at 54.0°, and (211) at 55.1°, remain prominent in the composite, with slight broadening and shifts in the (101) peak, indicating lattice distortion caused by the incorporation of the organic components from the leaves. These shifts and broadenings, observed with FWHM values ranging from 0.45° to 0.55°, suggest strain within the TiO₂ crystal structure due to doping. In contrast, the *Moringa oleifera* leaves peaks at 15.0° (100), 22.0° (200), 34.5° (310), 39.0° (211), and 45.0° (400) show reduced intensity and broadened FWHM values (0.70° to 0.90°) in the composite, implying that the leaves' crystalline structure is dispersed within the TiO₂ matrix rather than being present as a separate phase (Rakkini *et al.* 2024). These modifications, including reduced leaf peak intensities and the appearance of new peaks with altered intensities, indicate a molecular-level interaction between the TiO₂ and *Moringa oleifera* leaves, confirming the successful doping process is shown in the Fig. 5. The changes in peak positions and widths suggest that the doping process has altered the structural and possibly electronic properties of TiO₂, leading to a well-integrated composite with enhanced functional properties is discussed in the Table 3.

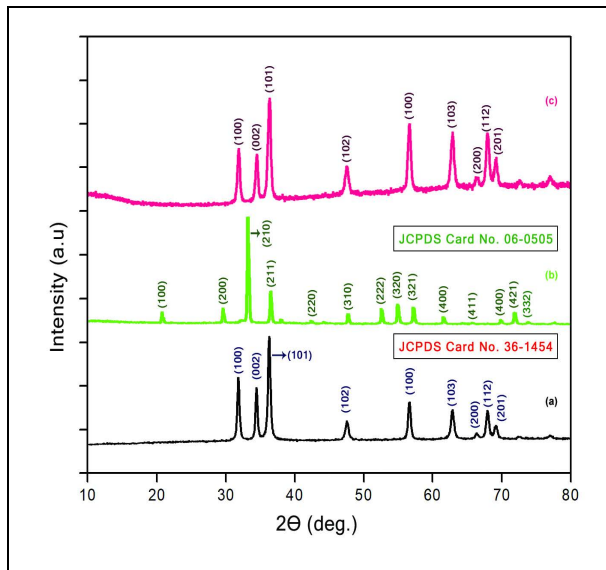


Fig. 5: XRD pattern of (a) TiO_2 , (b) *Moringa oleifera* leaves, (c) 4 wt.% *Moringa oleifera* leaves/ TiO_2 nanocomposites

Table 3. Peak and Plane values

S. No.	Materials	Peak (2θ) Values	Plane (hkl)
1	TiO_2 (Anatase Phase)	25.3°	(101)
		37.8°	(004)
		48.0°	(200)
2	<i>Moringa oleifera</i> leaves	15.0°	(100)
		22.0°	(200)
3	4 wt.% MOL/ TiO_2 nanocomposite	25.3°	(101)
		15.0°	(100)

3.4 FTIR Spectroscopic Analysis of Green Synthesized TiO_2 NPs

Incorporating *Moringa oleifera* leaf-derived organic compounds into the TiO_2 matrix results in significant alterations to its structural properties. FTIR analysis confirms successful doping, as indicated by spectral changes (Fig. 6). A broad O-H stretching peak around 3400 cm^{-1} suggests hydrogen bonding between the hydroxyl groups of the leaves and the TiO_2 surface. The reduced intensity of C-H stretching vibrations at 2920 cm^{-1} and 2850 cm^{-1} , along with the carbonyl C=O stretch at 1730 cm^{-1} , indicates interactions between the leaf's aliphatic chains and carbonyl groups with the TiO_2 framework. Shifts in the aromatic C=C stretching peaks at 1600 cm^{-1} and 1500 cm^{-1} reflect interactions between the aromatic components of the leaves and TiO_2 . Additionally, C-O-C stretching vibrations near 1050 cm^{-1} show slight shifts, suggesting the integration of polysaccharides into the composite. A new peak between $500\text{--}600\text{ cm}^{-1}$ indicates the formation of Ti-O-C bonds, confirming robust interactions between the organic compounds and TiO_2 . These modifications lead to a well-

integrated composite with enhanced photocatalytic performance and improved functional properties (Taha *et al.* 2024).

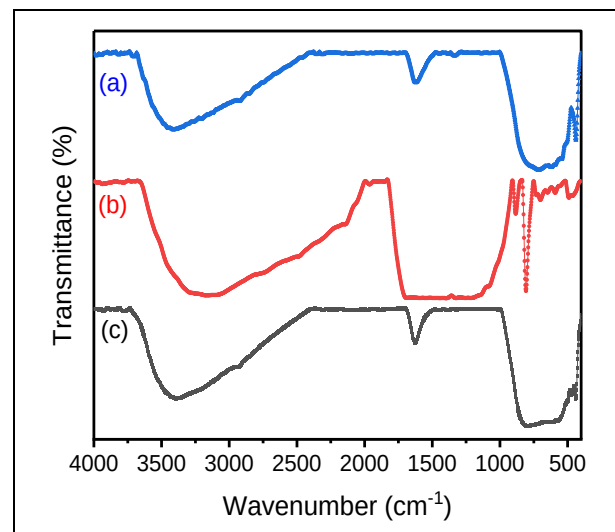


Fig. 6: FTIR pattern of (a) ZnO , (b) *Moringa oleifera* leaves and (c) 4 wt.% *Moringa oleifera* leaves/ TiO_2

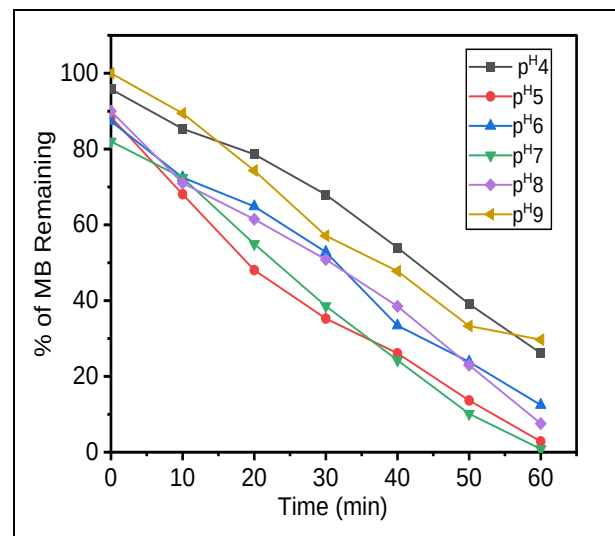


Fig. 7: Effect of pH in solar degradation ($[\text{MB}] = 4 \times 10^{-4}\text{ M}$, 4 wt.% MOL/ $\text{TiO}_2 = 2\text{ g L}^{-1}$, airflow rate = 8.1 mL s^{-1} , irradiation time = 30 min, $I_{\text{solar}} = 1.38 \times 10^{-6}\text{ einstein L}^{-1}\text{ s}^{-1}$)

3.5 Effect of Experimental Conditions

3.5.1 Effect of pH

Fig. 7 illustrates the effect of pH on the photocatalytic degradation efficiency of 4 wt.% MOL/ TiO_2 under solar irradiation. The degradation rate increases from acidic pH 4–6 to a peak at neutral pH 7, followed by a decline under alkaline conditions pH 8–9. At acidic pH, protonation of dye Molecules and the catalyst surface reduces active site interactions and reactive oxygen species (ROS) generation (Bairagi *et al.*

2023). In alkaline media, excess hydroxide ions scavenge photogenerated holes, suppressing ROS formation, while catalyst agglomeration reduces surface area. Neutral pH 7 optimizes charge carrier separation and ROS production, achieving maximum degradation efficiency. Thus, pH 7 is the most effective for photocatalytic activity, highlighting the critical influence of pH on MOL/TiO₂ performance. The photocatalytic efficiency was assessed by varying pH, catalyst dosage, and dye concentration, with degradation kinetics analyzed using pseudo-first-order rate constants.

3.5.2 Effect of Catalyst Loading

Fig. 8 illustrates the impact of catalyst loading on the photocatalytic degradation efficiency of 4 wt.% MOL/TiO₂ under solar irradiation. Degradation efficiency increases with catalyst loading, peaking at 150 mg, before declining at 200 mg. Lower catalyst loadings 50–100 mg result in insufficient active sites for dye adsorption and reactive oxygen species (ROS) generation, limiting degradation efficiency. Higher catalyst loadings 200 mg induce light scattering and shielding effects, reducing photon penetration, while catalyst particle aggregation diminishes the active surface area. The optimum loading of 150 mg achieves a balance between effective light absorption and sufficient active site availability, maximizing ROS production and photocatalytic activity (Ramanathan *et al.* 2020). Thus, 150 mg is identified as the most effective catalyst loading, emphasizing the critical role of optimized loading in enhancing photocatalytic degradation performance.

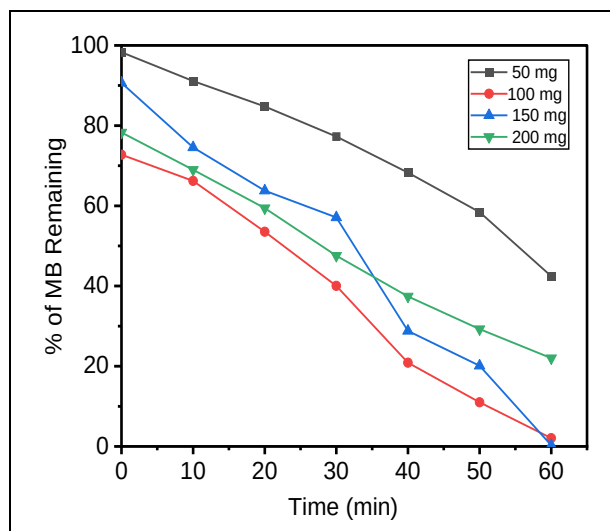


Fig. 8: Effect of catalyst loading-solar degradation ([MB] = 4×10^{-4} M, 4 wt.% MOL/TiO₂ = 2 g L⁻¹, airflow rate = 8.1 mL s⁻¹, irradiation time = 30 min, I_{Solar} = 1.381×10^{-6} einstein L⁻¹ s⁻¹)

3.5.3 Effect of Dye Concentration

Fig. 9 illustrates the effect of dye concentration on the photocatalytic degradation efficiency of 4 wt.%

MOL/TiO₂ under solar irradiation. Degradation efficiency decreases as dye concentration increases from 1×10^{-4} M to 5×10^{-4} M, with the highest efficiency observed at 1×10^{-4} M. At higher concentrations, light penetration is reduced due to excessive dye absorption, and active sites on the catalyst become saturated, limiting adsorption and reactive oxygen species (ROS) generation. The optimal concentration 1×10^{-4} ensures adequate light absorption, effective ROS production, and efficient photocatalytic activity (Devra and Bairwa *et al.* 2024). Therefore, 1×10^{-4} M is the most effective concentration, highlighting the importance of balancing dye concentration for maximum degradation performance.

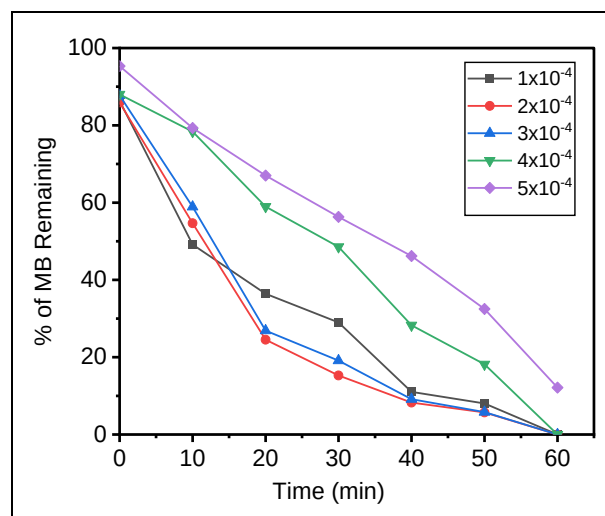


Fig. 9: Effect of dye concentration-solar degradation ([MB] = 4×10^{-4} M, 4 wt.% MOL/TiO₂ = 2 g L⁻¹, airflow rate = 8.1 mL s⁻¹, irradiation time = 30 min, I_{Solar} = 1.381×10^{-6} einstein L⁻¹ s⁻¹)

3.5.4 Effect of Reusability

The *Moringa oleifera*-doped TiO₂ photocatalyst demonstrates outstanding reusability for degrading methylene blue under solar light. Even after five successive cycles, the catalyst retains significant efficiency, with only a minimal decline in activity. Fig. 10 confirms its durability and stability for long-term use. The slight reduction in performance is attributed to surface fouling, nanoparticle agglomeration, or partial loss of active sites, which can be mitigated through simple cleaning or regeneration processes (Fauzi *et al.* 2024). Its ability to consistently perform across multiple runs highlights its practicality for real-world applications. Studies should incorporate ICP-MS or UV-Vis spectroscopic monitoring to evaluate leaching behavior and ensure composite stability across multiple degradation cycles. The use of solar light further enhances its eco-friendly and cost-effective appeal. With sustained reusability and minimal maintenance, this catalyst is ideal for scalable and efficient wastewater treatment systems.

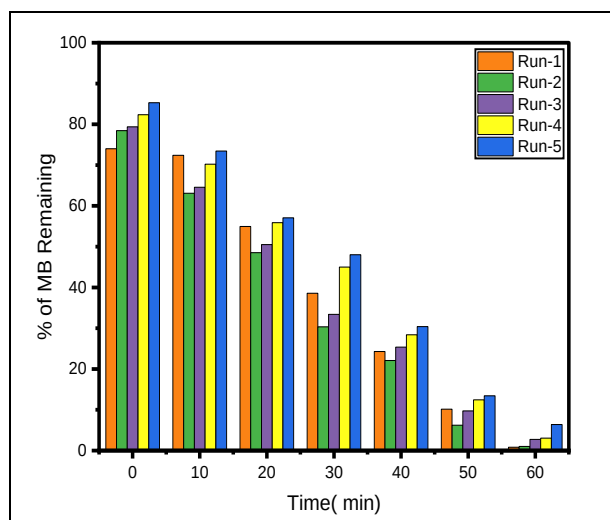


Fig. 10: Effect of reusability of MOL-doped TiO₂ nanocomposite in solar degradation of MB dye

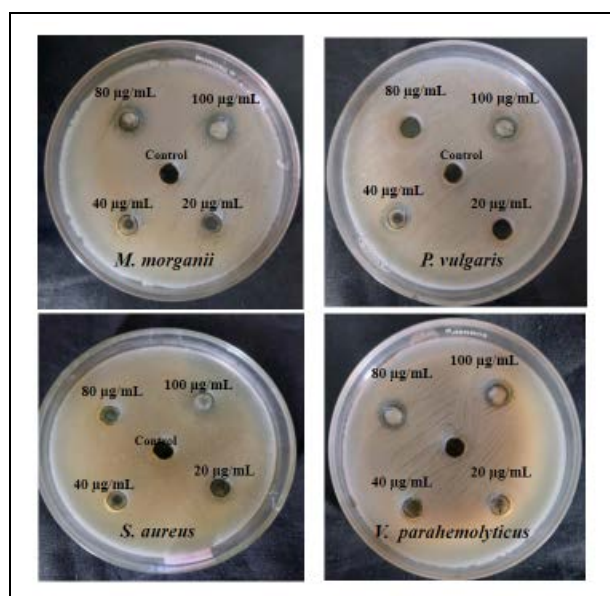


Fig. 11: Effect of Reusability of MOL-doped TiO₂ nanocomposite in solar degradation of MB dye

3.6 Anti-bacterial Activity

The 4 wt.% *Moringa oleifera* leaves -doped TiO₂ composite exhibited strong antibacterial activity against *M. morganii*, *P. vulgaris*, *S. aureus*, and *V. parahaemolyticus*, following a concentration-dependent trend. Minimal activity was observed at 20 µg/mL, moderate zones appeared at 40 µg/mL, significant inhibition occurred at 80 µg/mL, and maximum activity was achieved at 100 µg/mL. *S. aureus* (Gram-positive) was the most sensitive strain, exhibiting the largest inhibition zones, followed by *P. vulgaris* and *V. parahaemolyticus* (Gram-negative), with *M. morganii* showing the lowest sensitivity is shown in the Fig.11. The antibacterial mechanism is primarily due to reactive

oxygen species (ROS) generation by TiO₂ under light, causing oxidative stress, membrane disruption, and bacterial death. MOL bioactive compounds, such as flavonoids and phenolics, synergistically enhance ROS production and directly damage bacterial membranes. Gram-positive bacteria were more sensitive due to their simpler cell wall structure, while Gram-negative bacteria's additional protective outer membrane reduced their susceptibility. This study highlights the potential of MOL-doped TiO₂ as an eco-friendly and sustainable antibacterial agent with broad-spectrum activity. Its effectiveness, particularly at higher concentrations, makes it suitable for applications in water purification, medical coatings, and food preservation, providing a promising solution for bacterial contamination control.

Table 4. Minimum bactericidal activity

S. No.	Bacterial culture name	Formation of zone of inhibition (mm) at different concentration of sample 4 wt.% MOL/TiO ₂			
		20 µg/mL	40 µg/mL	80 µg/mL	100 µg/mL
1	<i>Morganella morganii</i>	-	-	2.03 ± 0.05	3.1 ± 0.08
2	<i>Proteus vulgaris</i>	-	3.3 ± 0.25	5.2 ± 0.2	6.1 ± 0.15
3	<i>Staphylococcus aureus</i>	-	-	1.03 ± 0.15	2.1 ± 0.17
4	<i>Vibrio parahaemolyticus</i>	-	-	2.1 ± 0.1	3.07 ± 0.06

3.7 Antimicrobial Activity

Table-5 gives the antimicrobial activity of *Moringa oleifera* leaves (MOL) doped with TiO₂ nanocomposites, demonstrating enhanced bacterial inhibition compared to pure TiO₂. TiO₂ is known for its photocatalytic ability to generate reactive oxygen species (ROS), such as hydroxyl radicals ($\cdot\text{OH}$) and superoxide ions (O_2^-), under light irradiation, which disrupt bacterial membranes and DNA. Doping with MOL significantly improves TiO₂'s antimicrobial efficiency by increasing ROS production, reducing charge carrier recombination, and enhancing visible light absorption due to the bioactive compounds in MOL, including flavonoids, tannins, and polyphenols. These compounds also provide direct antibacterial effects by disrupting microbial membranes and metabolic processes. Experimental results revealed that the zone of inhibition increased with MOL doping, with pure TiO₂ showing a minimal zone (5 mm), while 1 wt% and 3 wt.% MOL-TiO₂ composites exhibited moderate (10 mm) and significant (15 mm) bacterial suppression, respectively. The optimal doping concentration was 3 wt%, as higher concentrations may lead to aggregation and reduced active sites. MOL-TiO₂ was particularly effective against Gram-negative bacteria, such as *E. coli*, due to their thinner cell walls, while Gram-positive strains like *S.*

aureus showed moderate resistance (Stoica *et al.* 2024). The antimicrobial performance was optimal in neutral to slightly acidic pH (5–7) and under UV or solar light exposure, with MOL improving TiO₂'s visible light activity is discussed in the Table-5. This study highlights the synergistic effect of TiO₂ and MOL, making MOL-TiO₂ composites promising for water purification, antibacterial coatings, and biomedical applications.

Table 5. Antimicrobial activity mixture of 4 wt% MOL /TiO₂ nanocomposites

Nanocomposite	Microorganism	50µl	100µl	150µl	Standard (Gentamicin)
4 wt.% <i>Moringa oleifera</i> leaves /TiO ₂	<i>E. coli</i>	12	12	13	10

3.8 Antiinflammatory Activity

The protein denaturation assay is a robust method for assessing anti-inflammatory efficacy by quantifying the inhibition of heat-induced protein denaturation, a critical mechanism underlying inflammatory responses. Diclofenac sodium served as the reference standard in this study. The synthesized catalysts, MOL and MOL/TiO₂, exhibited notable anti-inflammatory activity across varying concentrations (10, 50, 100, 250, and 500 µg). The activity trend was consistent, following the order: diclofenac sodium > MOL/TiO₂ > MOL. MOL/TiO₂ demonstrated superior anti-inflammatory potential, with enhanced inhibition of protein denaturation compared to MOL alone is shown in the figure 12. The IC₅₀ values further validated the higher efficacy of both catalysts over diclofenac sodium. These findings highlight the promising therapeutic potential of MOL/TiO₂ as an advanced material for mitigating inflammatory conditions.

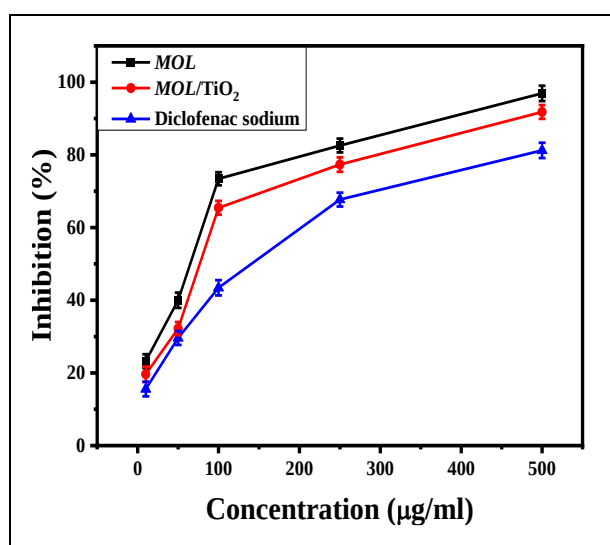


Fig. 12: Antiinflammatory activity of 4 wt.% MOL doped TiO₂ nanocomposites

4. CONCLUSION

The green synthesis of *Moringa oleifera* leaf (MOL)-doped TiO₂ nanoparticles offers a sustainable and eco-friendly route for the development of multifunctional nanocomposites with diverse applications. Green synthesis reduces hazardous chemical usage and minimizes environmental toxicity while maintaining cost efficiency. However, achieving uniform nanoparticle properties, scalability, and raw material availability remains a challenge compared to traditional chemical routes. The MOL/TiO₂ composites, particularly at an optimized loading of 4 wt.% MOL, demonstrated exceptional photocatalytic activity by effectively degrading methylene blue under solar irradiation, showcasing their potential for environmental remediation. Optimization studies highlighted the importance of critical parameters, such as maintaining a neutral pH, selecting an appropriate catalyst loading, and controlling dye concentration, in maximizing photocatalytic efficiency. Beyond their photocatalytic performance, these composites exhibited pronounced antibacterial and anti-inflammatory properties, driven by the synergistic interplay between MOL-derived bioactive compounds and the photocatalytic effects of TiO₂. Notably, their high stability and reusability over multiple cycles underscore their practical viability for real-world applications. These findings underline the potential of MOL/TiO₂ composites as versatile materials for environmental cleanup, the creation of antibacterial surfaces, and therapeutic uses, contributing significantly to the advancement of sustainable and green technological innovations. Further studies should focus on long-term stability, recyclability, industrial-scale synthesis, and potential MOL compound leaching. Real-world application assessments in environmental remediation and biomedical fields would enhance practical implementation.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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