American Journal of Psychiatric ehabilitation

Expert Opinion Article

Harnessing Costus Pictus D. For Sustainable Tio_2 Nanoparticles: Antimicrobial And Larvicidal Efficacy Unveiled

Shalini KR[1], Udayakumar R[2*] And Udhayan S[3]

[1,2*]Department of Physics, Annamalai University, Annamalai Nagar, Tamil Nadu, India
[3]Adhi College of Engineering and Technology, Department of Physics, Sankarapuram, Kanchipuram-631605, Tamil Nadu, India

***Corresponding Author:** e-mail: drru.krs24@gmail.com

Abstract
The present study explores the green synthesis, physicochemical characterization, and biomedical applications of biosynthesized TiO_2 NPs using leaf extract of *Costus pictus* D. The synthesized TiO_2 NPs were characterized through several techniques, including UV- Visible spectroscopy, photoluminescence (PL) analysis, HR-TEM, and DLS. UV-Vis spectroscopy revealed a distinct absorbance maximum at 313 nm, with an estimated energy bandgap of 3.24 eV, which was further supported by PL spectral analysis. Furthermore, Photoluminescence analysis indicated the occurrence of structural imperfections, including oxygen vacancies and self-trapped excitons, which influence the photonic behavior of the nanoparticles. HR-TEM revealed a uniform spherical morphology with an average particle size of 21 nm. In contrast, DLS measurements indicated a significantly larger hydrodynamic diameter of 657 nm, attributed to the inclusion of bio-capping agents. The moderate zeta potential of -6.47 mV suggests a reasonable level of colloidal stability. The antibacterial efficacy of the TiO_2 nanoparticles was evaluated on both Gram-positive and Gram-negative bacteria, with *Bacillus pumilus* and *Bacillus subtilis* showing the greatest sensitivity. Notably, antifungal activity was determined to be particularly effective against *Aspergillus niger*. At the same time, larvicidal assays demonstrated significant mortality rates in *Aedes aegypti* and *Culex quinquefasciatus* larvae, increasing proportionally with the concentration of the treatment. These findings underscore the multifunctional capabilities of *Costus pictus*- mediated TiO_2 nanoparticles as eco-friendly antimicrobial and larvicidal agents, emphasizing their promising applications in biomedicine and environmental health.

Keywords: Green synthesis, Titanium dioxide nanoparticles, *Costus pictus*, Antibacterial activity, Antifungal potential, Larvicidal efficacy

1. Introduction

Nanotechnology represents a cutting-edge interdisciplinary domain operating within a scale of 1 to 100 nanometers. It has garnered significant interest among researchers across various scientific fields due to the materials' unique physical, chemical, and biological properties at this nanoscale [1]. With transformative applications spanning healthcare,pharmacology, and environmental science, nanomedicine has emerged as an up-and-coming area, facilitating advancements in disease diagnosis, targeted therapy, and regenerative medicine. Among the myriad types of nanomaterials, metallic and metal oxide nanoparticles have gained prominence for their exceptional catalytic, antibacterial, and biomedical capabilities [2].

Traditional physical and chemical nanoparticle synthesis methods often raise concerns about cost, toxicity, and environmental sustainability. To address these challenges, contemporary research has begun to favor green synthesis methodologies, especially those that utilize plant extracts as reducing and stabilizing agents. These biologically mediated techniques offer enhanced environmental compatibility and improved biocompatibility of the resulting nanoparticles, thereby establishing them as viable candidates for eco-conscious biomedical applications [3,4].

Among the various botanical resources employed for nanoparticle synthesis, *Costus pictus D*., commonly called the "insulin plant," distinguishes itself due to its ethno pharmacological significance. This species, native to Central America and widely cultivated in southern India, has attracted attention for its antidiabetic, anti-inflammatory, and antioxidant properties, particularly in Ayurvedic medicine [5]. Its phytochemical constituents have been demonstrated to serve as effective agents in reducing and stabilizing metal ions, thereby providing a promising platform for nanoparticle biosynthesis.

Specifically, titanium dioxide (TiO_2) nanoparticles are well-regarded for their photocatalytic, antibacterial, and anticancer properties. When synthesized through green methods, they typically exhibit enhanced biological compatibility relative to those produced via chemical synthesis [6]. In an earlier study [7], TiO_2 nanoparticles were effectively produced with the aid of the aqueous leaf extract of

Costus pictus D. and characterized utilizing techniques such as XRD, SEM, FTIR, GC-MS, and NMR. This study confirmed the crystalline and functional characteristics of the synthesized nanoparticles. It demonstrated their biomedical potential through significant anticoagulant and antiplatelet activities, underscoring their applicability in hematological and therapeutic contexts.

Building on these findings, the present study employs advanced analytical methods, including UV-Vis spectroscopy, photoluminescence spectroscopy, HRTEM, and zeta potential analysis to investigate further the optical, morphological, and colloidal stability of the TiO_2 NPs. This follow-up study also seeks to transition from exclusive physicochemical assessment to the evaluation of biological efficacy, exploring the biosynthesized nanoparticles' antimicrobial, antifungal, and larvicidal activities.

2. Materials and Methods

2.1 Materials

Titanium tetrafluoride (TiF_4), an analytical-grade precursor obtained from M/s Sigma Aldrich, Mumbai, served as the titanium source for the synthesis of nanoparticles. All glassware utilized during the experimental procedures underwent thorough cleaning and sterilization in a hot-air oven to eliminate potential contaminants. High-purity deionized water was consistently used throughout the study to uphold experimental integrity and ensure the reproducibility of results.

2.1.1 Collection of Plant Material

In the current study, fresh leaves of *Costus pictus* D. were meticulously obtained from the Karaikudi region (10.07°N, 78.78°E), Tamil Nadu, India. The plant's identification was verified with the assistance of taxonomists from the Department of Botany at Annamalai University (11.3837°N, 79.7248°E), Annamalai Nagar, Chidambaram, Tamil Nadu, India. A reference specimen of the plant was carefully preserved in the greenhouse of the Department of Botany at Annamalai University in August 2022. The leaves underwent a thorough washing process with running water, followed by rinsing with fresh distilled water to remove impurities and contaminants, ensuring the purity of the plant material.

2.1.2 Preparation of Leaf Extract

The collected leaves were dried naturally and then grinded into a fine powder. A 5 -gram of the powdered material was combined with 100 mL of deionized water and heated to 80 °C for one hour under precisely controlled conditions. The resulting solution was filtered through Whatman No. 1 filter paper and kept at 4 °C for later use.

2.1.3 Synthesis of TiO_2 Nanoparticles

For the biosynthesis of TiO_2 nanoparticles, 20 mL of the freshly prepared *Costus pictus D*. leaf extract was combined with 3.4 g of TiF_4. The mixture was stirred continuously under controlled heating conditions until the formation of a visible precipitate was observed. The resulting precipitate was collected, dried at 100 °C for six hours, and subsequently calcined at 500 °C for one hour to yield *Costus pictus*-mediated TiO_2 nanoparticles.

2.2 Characterization Techniques

2.2.1 Instrumentation Optical Studies

The optical absorption characteristics of the synthesized TiO_2 nanoparticles were examined utilizing a SHIMADZU UV-1800 UV–Visible spectrophotometer. The nanoparticles were uniformly dispersed in an appropriate solvent, and the absorbance spectrum was recorded across a suitable wavelength range. This analysis aimed to elucidate the optical properties, specifically the absorption edge, indicative of the material's electronic transitions and band gap energy.

Photoluminescence (PL) Studies

Photoluminescence (PL) analysis was employed to explore the synthesized nanoparticle's electronic structure and emission behaviour. The PL spectra provided vital insights into the defect states, recombination processes, and optical quality of the TiO_2 nanomaterial, thereby offering complementary information to the UV–visible findings.

Morphological Analysis

The morphological properties of the synthesized nanoparticles were analyzed using HR-TEM. Imaging was performed with a JEOL JEM-2100F microscope. The nanoparticles were dispersed in an appropriate solvent and subjected to ultrasonication for sample preparation to ensure a homogeneous suspension. This suspension was placed onto a carbon-coated copper grid and allowed to air dry at room temperature. The HR-TEM images provided a detailed examination of particle shape, size distribution, and lattice structure, offering a comprehensive understanding of the nanoparticles' morphology.

Zeta Potential Analysis

Dynamic Light Scattering (DLS) and zeta potential measurements were performed to evaluate the hydrodynamic size distribution and colloidal stability of the TiO_2 nanoparticles. These measurements were conducted using a Malvern Zetasizer under standard conditions. Before analysis, the nanoparticle suspension underwent ultrasonication to prevent agglomeration and ensure uniform dispersion. Zeta potential values were used to evaluate the electrostatic stability of the particles, with higher absolute values indicating improved dispersion in aqueous media.

2.3 Biological Activity Assessments

2.3.1 Antibacterial Activity

The antibacterial efficacy of the synthesized titanium dioxide (TiO_2) nanoparticles was assessed using the standard disc diffusion technique. Mueller-Hinton Agar (MHA) was prepared and dispensed into sterile Petri dishes, solidifying under aseptic conditions. Fresh bacterial cultures were grown and evenly spread across the agar surface using sterile swabs to ensure uniform inoculation.

Sterilized filter paper discs, measuring 6 mm in diameter, were impregnated with the synthesized TiO_2 nanoparticles and meticulously placed onto the inoculated agar plates. Ampicillin (20 µL/disc) served as the positive control, while DMSO-impregnated discs functioned as negative controls, thereby confirming that the observed antimicrobial activity resulted solely from the nanoparticles. All plates were incubated at 37 °C for 24 hours to facilitate microbial growth and nanoparticle diffusion.

The antibacterial activity was quantified by measuring the diameter of the inhibition zones surrounding each disc in millimetres. All experiments were performed in triplicate to verify the reliability and consistency of the results.

2.3.2 Antifungal Activity

The antifungal activity of the TiO_2 nanoparticles was evaluated using the agar disc assay. Sabouraud Dextrose Agar (SDA) was prepared, sterilized, and then poured into Petri plates, which solidified under aseptic conditions. Fresh fungal cultures were uniformly swabbed across the agar surface using sterile cotton.

Sterile 6 mm filter paper discs impregnated with the synthesized TiO_2 nanoparticles were accurately positioned on the inoculated plates. Amphotericin B was used as a positive control, while DMSO-soaked discs served as the negative controls. The plates were kept at 37 °C for 24 hours to facilitate fungal growth and enable nanoparticle diffusion. Antifungal activity was evaluated post-incubation by measuring the inhibition zone diameter around each disc. All experiments were performed in triplicate to verify the reliability and consistency of the findings.

2.3.3 Larvicidal Activity

The larvicidal potential of the synthesized TiO_2 nanoparticles was evaluated by the adult emergence inhibition method as outlined by World Health Organization (WHO) guidelines. A stock solution of TiO_2 nanoparticles (1 mg/mL) was prepared and serially diluted to achieve test concentrations between 10 and 100 µg/mL.

Larvae of *Aedes aegypti* and *Culex quinquefasciatus* were collected and introduced into the nanoparticle solutions, with ten larvae per test concentration. A control group maintained in distilled water was established for comparative analysis. The tests were conducted under controlled laboratory conditions, with a temperature maintained at 30 ± 2 °C and relative humidity set at 75 ± 5%.

Larval mortality and inhibition of adult emergence were recorded after the specified exposure period. Each experiment was replicated three times to ensure statistical reliability and reproducibility of the results.

The mortality percentage was determined using the appropriate formula.

% of mortality = (No. of dead larvae / No. of larvae Tested) x 100

3. Result and Discussion

3.1 UV-Vis Spectrophotometric Analysis

UV-Visible spectroscopy, a powerful and widely employed analytical technique, served as a crucial tool in confirming the reduction of titanium tetrafluoride (TiF4) and the subsequent formation of titanium dioxide nanoparticles (TiO2 NPs). This method enabled detailed insight into the optical properties of the biosynthesized TiO2 NPs and provided essential confirmation of nanoparticle formation. Distinct color changes were observed in the aqueous leaf extract of Costus pictus D. following the introduction of TiO2, indicating nanoparticle synthesis. These changes are attributed to the presence of phytochemicals, which play a pivotal role in both reducing and stabilizing metal precursors, as previously reported by (Dobrucka et al. 2017)[8].

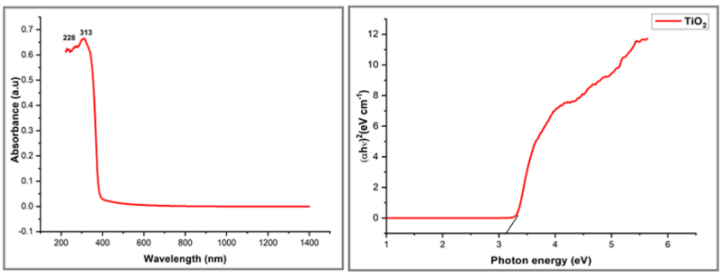

Fig. 1 (a) UV - Vis and (b) Tauc Plot pattern of C. p @ TiO_2 NPs

The UV-Vis absorption spectrum of the *Costus pictus*-mediated TiO_2 NPs was recorded in the range of 200–800 nm (Fig. 1a). Notably, absorption peak was observed at 313 nm, corresponding to polyphenolic compounds and reduced TiO_2 nanoparticles, respectively, as supported by (Rajkumari et al. 2019) [9]. (Sankar et al. 2015) similarly documented the optical excitation of TiO_2 nanoparticles within the 270 - 320 nm range [10]. Additionally, characteristic absorption peaks at 356 nm (Sethy et al., 2020) [11] and 360 nm (Nasrollahzadeh and Sajadi, 2015) [12] have been reported for TiO_2 NPs synthesized using various plant extracts, including *Syzygium cumini* and *Euphorbia heteradena jaub*, highlighting the influence of phytochemical constituents on nanoparticle formation and absorption behavior.

To determine the band gap energy of the synthesized TiO_2 NPs, a Tauc plot was constructed (Fig. 1b) based on the Tauc relation:

$(\alpha h\nu) = A\,(h\nu - E_g)\, n$,

where **$\alpha h\nu$** represents the absorption coefficient, A denotes a constant, hν signifies the photon energy, and E_g represents the band gap energy. The Tauc plot, formulated as $(\alpha h\nu)^2$ versus photon energy (hν), was extrapolated to estimate a direct band gap of 3.24 eV for the synthesized TiO_2 nanoparticles.

Comparable results have been reported by (Ngoepe et al. 2020) [13], who recorded a band gap energy of 3.53 eV for TiO_2 nanoparticles synthesized using an aqueous extract of *Monsonia burkeana*, further corroborating the effectiveness of plant-mediated green synthesis

1.1 Photoluminescence Analysis

The photoluminescence (PL) spectrum of titanium dioxide (TiO_2) nanoparticles synthesized via a green synthesis approach was recorded at room temperature to evaluate their electronic and defect-related properties. The PL spectrum, obtained under excitation at 313 nm, is illustrated in Fig. 2. Similar emission characteristics have been previously reported for TiO_2 nanoparticles synthesized using cinnamon powder extract [14] highlighting the consistency of phytochemical-mediated synthesis routes in modulating optical responses.

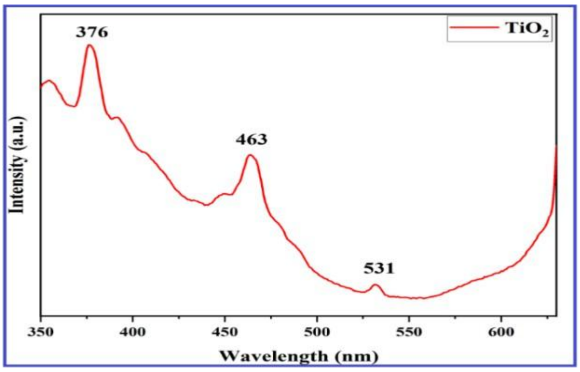

Fig. 2 Photoluminescence spectrum of C. p @ TiO_2 NPs

The PL emission behavior of anatase-phase TiO_2 nanoparticles is primarily influenced by three key physical mechanisms: oxygen vacancies [15,16], self-trapped excitons [15,17], and surface state defects [18]. These surface states are commonly associated with Ti^{4+} ions in close proximity to oxygen-deficient sites. Such intrinsic defects arise from the non- equilibrium growth dynamics during nanoparticle formation and significantly affect the luminescent properties of TiO_2, as emphasized by Zeng et al., 2010 [19].

The recorded PL spectrum reveals a prominent ultraviolet emission peak at 376 nm, along with visible peaks at 463 nm and 531 nm. The ultraviolet emission is attributed to band-edge recombination, offering a direct probe of the material's band gap. The corresponding band gap energy, calculated from the UV emission peak, is approximately 3.24 eV - closely matching the value derived from the Tauc plot analysis. The emission peak at 463 nm is ascribed to self-trapped excitons within TiO_2 octahedral structures, while the blue emission at 531 nm is indicative of oxygen vacancies.

Because of their large surface area relative to volume, TiO_2 nanoparticles inherently possess a significant number of oxygen vacancies, a characteristic supported by the findings of (Siddhapara and Shah 2012) [20]. These defects enhance the nanoparticles' optical activity and broaden their functional versatility.

Furthermore, the ultraviolet-visible (UV-Vis) spectrum analysis corroborates a band gap of 3.24 eV, underscoring the strong ultraviolet absorption capacity of the synthesized TiO_2 nanoparticles. This property is crucial in promoting the production of reactive oxygen species (ROS), thereby enhancing the nanoparticles' antimicrobial and insecticidal activity. Such multifunctional attributes underscore the potential of green-synthesized TiO_2 nanoparticles in diverse applications, ranging from biomedical treatments to environmental disinfection strategies.

1.2 HRTEM Analysis

High-Resolution Transmission Electron Microscopy (HRTEM) was used to examine the morphology, particle size, and crystallinity of the green-synthesized titanium dioxide (TiO_2) nanoparticles (NPs). The micrographs revealed well- dispersed, nanocrystalline particles with predominantly spherical morphology (Fig. 3a).

The particle size distribution ranged from 19 to 24 nm, with an average diameter of approximately 21 nm. These values are consistent with those estimated using Scherrer's formula, based on the previously obtained X-ray diffraction (XRD) data for *Costus pictus* D.-mediated TiO_2 NPs [7]. The observed particle dimensions and uniformity align with earlier studies on biosynthesized TiO_2 NPs derived from other plant sources such as *Azadirachta indica* [21] and *Aloe barbadensis mill* [9], supporting the reliability and reproducibility of phytochemical-mediated synthesis.

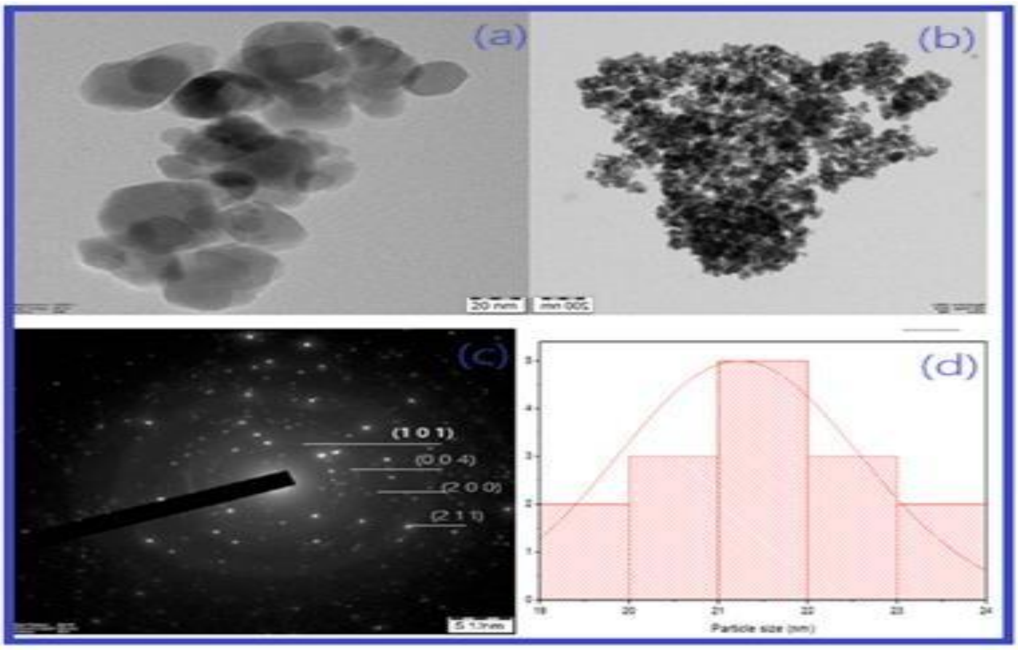

Fig. 3 *Costus pictus* induced TiO_2 NPs (a) HR-TEM image (b) d- spacing image.

The SAED pattern (Fig. 3b) displayed a series of concentric rings composed of discrete bright spots, confirming the polycrystalline nature of the sample. The diffraction rings corresponded to the (101), (004), (200), and (211) lattice planes of the anatase phase of TiO_2, further validating the crystalline identity inferred from XRD data. The interplanar spacing (d-spacing) observed in the SAED pattern was measured at 3.27 nm, which closely matches the 3.54 nm value previously recorded via XRD [7].

In parallel, the photoluminescence (PL) spectrum provided additional insight into the surface and structural defects of the synthesized TiO_2 NPs, specifically highlighting the presence of oxygen vacancies and self-trapped excitons. These intrinsic defects, as also supported by the SAED patterns and HR-TEM images, significantly influence the optical and surface characteristics of the nanoparticles, which in turn affect their interactions with biological and environmental systems.

To complement the morphological analysis, SEM was employed to examine the surface topography of annealed TiO_2 NPs, while EDS was used to verify their chemical composition [7]. The EDS spectrum confirmed the presence of titanium (Ti) and oxygen (O) with atomic weight percentages of 76% and 24%, respectively, indicative of a high concentration of oxygen vacancies - a key feature enhancing photo catalytic and antimicrobial efficacy.

Together, the HRTEM, SAED, PL, SEM, and EDS analyses provide a comprehensive understanding of the structural integrity, elemental composition, and defect states of the biosynthesized TiO_2 NPs. These findings underscore their suitability for potential applications in antimicrobial formulations and environmental remediation technologies.

1.3 Dynamic Light Scattering and Zeta Potential Analysis

Dynamic Light Scattering (DLS) is a fundamental technique in nanomaterials research, playing a pivotal role in determining the hydrodynamic diameter and size distribution of nanoparticles in colloidal suspensions. This method relies on analyzing variations in the scattered light intensity resulting from the Brownian motion of particles. Renowned for its sensitivity, speed, and accuracy, DLS is widely used to characterize various nanoscale entities, including metallic nanoparticles and biological markers such as cancer biomarkers [22].

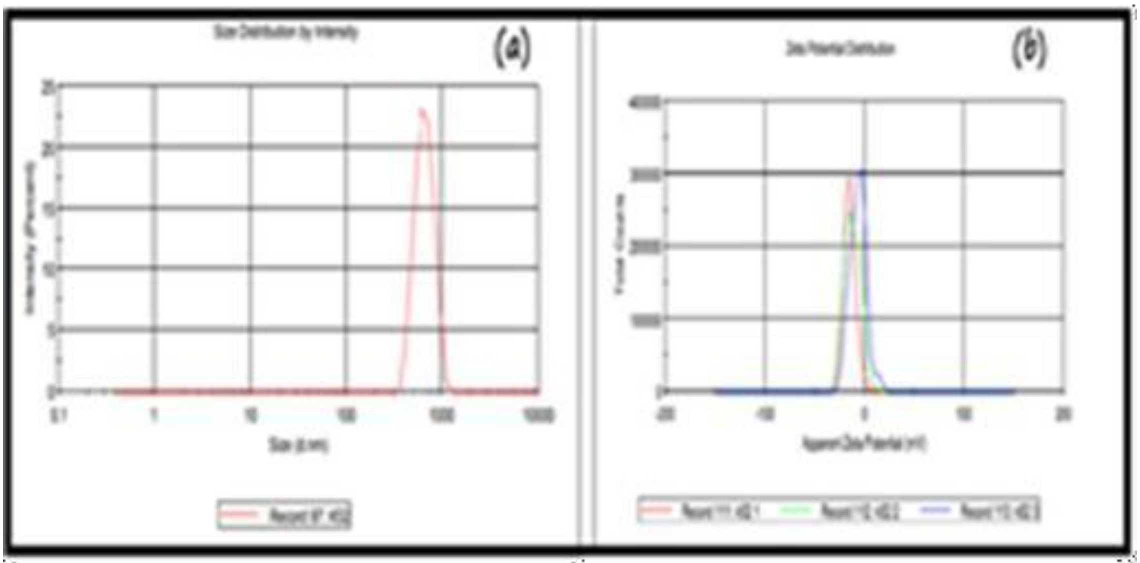

Fig. 4 (a) Dynamic light scattering (DLS) for C. p @ TiO_2 NPs and (b) Zeta potential measurements for C. p @ TiO_2 NPs

Fig. 4(a) illustrates the size distribution profile of *Costus pictus* D.- mediated TiO_2 nanoparticles as determined by DLS. The analysis revealed an average hydrodynamic diameter of 657 nm. It is important to note that DLS typically yields larger size values compared to methods like TEM, SEM, or XRD. This discrepancy is attributed to the presence of phytochemical stabilizing agents from the leaf extract, which adsorb onto the nanoparticle surface and form a hydrated shell. While TEM and SEM capture the dry core dimensions of nanoparticles, DLS accounts for this surrounding solvation layer, thereby reflecting the full hydrodynamic volume.

Furthermore, variations in particle size estimates via DLS may arise due to non-uniform dispersion within the suspension. Larger particles tend to dominate the scattering signal, potentially skewing the size distribution and leading to overestimation, whereas smaller particles may contribute minimally to the signal [23].

In addition to size characterization, zeta potential analysis was conducted to assess the electrokinetic potential and colloidal stability of the prepared TiO_2 NPs. The zeta potential reflects the magnitude of the repulsive forces between similarly charged particles in suspension and is a critical indicator of nanoparticle stability. In the present study, the TiO_2 nanoparticles exhibited a zeta potential of - 6.47 mV (Fig. 4b), suggesting moderate colloidal stability.

The observed negative surface charge is likely a result of the bioactive capping molecules present in the *Costus pictus* D. extract, which facilitate electrostatic repulsion among particles and prevent agglomeration. This stabilizing effect is consistent with previous findings, where plant-derived biomolecules were reported to contribute to nanoparticle surface modification and stability [24,25].

For comparative context, TiO_2 nanoparticles synthesized using the aqueous extract of *Syzygium cumini* exhibited a zeta potential of -18.7 mV [11], while those produced using *Withania somnifera* root extract and propolis displayed zeta potentials of - 24 mV and - 32.4 mV, respectively [26,27]. These values indicate that while the *Costus pictus*-mediated TiO_2 NPs possess moderate stability, other phytogenic sources may offer stronger electrostatic stabilization.

In summary, DLS and zeta potential analyses collectively underscore the influence of phytochemical capping on particle size and stability, highlighting the nuanced interplay between synthesis method and nanomaterial behavior in colloidal systems.

1.4 Antibacterial Activity

The antibacterial efficacy of biosynthesized titanium dioxide nanoparticles was assessed against a broad spectrum of bacterial strains, encompassing both Gram-positive (*Staphylococcus aureus, Enterococcus faecalis, Bacillus pumilus,* and *Bacillus subtilis*) and Gram-negative species (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Salmonella* sp.). The activity was evaluated at concentrations of 500, 750, and 1000 µg/mL. The synthesized TiO_2 nanopowder demonstrated significant antibacterial activity against all tested strains, as illustrated in Fig. 5.

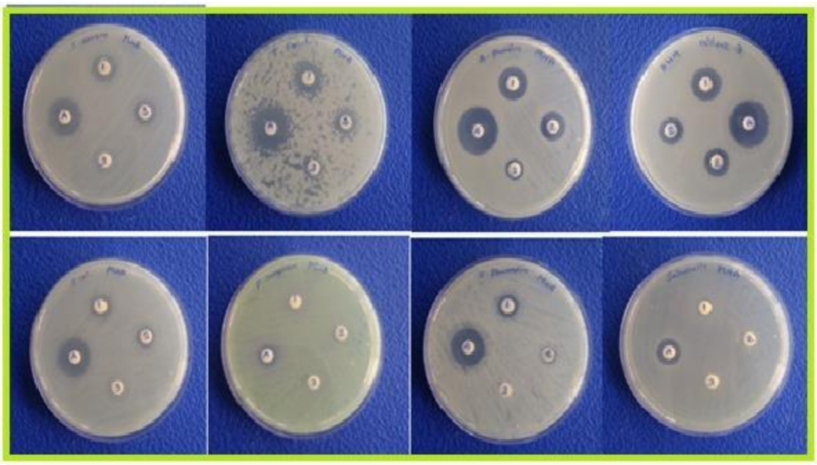

Fig. 5 Antibacterial activity of CP @ TiO_2 NPs against Gram- positive and Gram negative bacteriae. a) ***Staphylococcus aureus b) Enterococcus faecalis c) Bacillus pumilus d) Bacillus subtilis e) Escherichia coli, f) Pseudomonas aeruginosa g) Klebsiella pneumoniae h) Salmonella sp.***

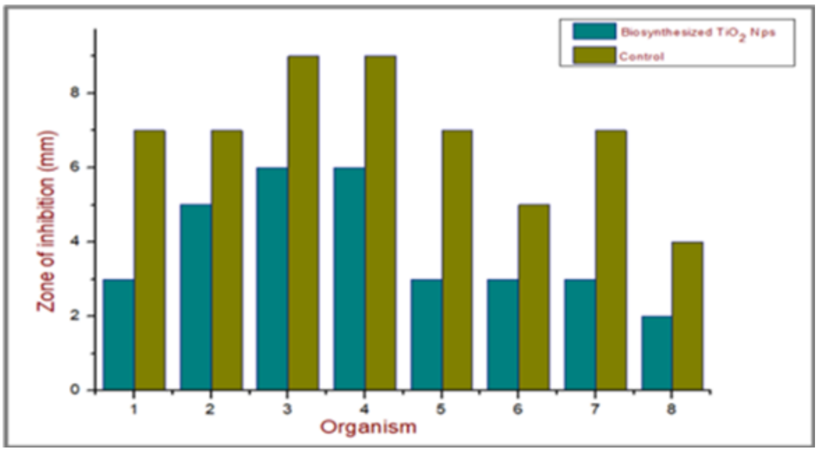

Fig. 6 Antibacterial screening activity of CP @ TiO_2 NPs a) ***Staphylococcus aureus b) Enterococcus faecalis c) Bacillus pumilus d) Bacillus subtilis e) Escherichia coli, f) Pseudomonas aeruginosa g) Klebsiella pneumoniae h) Salmonella sp.***

Table – 1 Effects of C. p @ TiO_2 NPs against the growth of different bacterial pathogens

S.NO	Organism	500 µg/ml	750 µg/ml	1000 µg/ml	Control
Gram - Positive bacteria					
1	Staphylococcus aureus	2 mm	2 mm	3 mm	7 mm
2	Enterococcus faecalis	2 mm	4 mm	5 mm	7 mm
3	Bacillus pumilus	2 mm	4 mm	6 mm	9 mm
4	Bacillus subtilis	4 mm	5 mm	6 mm	9 mm
Gram - negative bacteria					
1	Escherichia coli	1 mm	2 mm	3 mm	7 mm
2	Pseudomonas aeruginosa	2 mm	2 mm	3 mm	5 mm
3	Klebsiella pneumoniae	1 mm	1 mm	3 mm	7 mm
4	Salmonella sp.	-	-	2 mm	4 mm

Zone of inhibition measurements (Table - 1 and Fig. 6) revealed a concentration-dependent antibacterial response, with higher TiO_2 concentrations yielding more pronounced zones of inhibition. Among the Gram-positive strains, *Bacillus pumilus* and *Bacillus subtilis* exhibited the greatest susceptibility, with inhibition zones reaching up to 6 mm. In contrast, *Salmonella* sp. was the least affected, showing a zone of inhibition of only 2 mm. Notably, the Gram-negative bacteria exhibited consistently smaller zones of inhibition than their Gram-positive counterparts. This disparity may be attributed to the structural differences in bacterial cell walls, specifically the presence of a double-layered outer membrane in Gram-negative bacteria that imparts increased resistance [28].

Compared to the standard antibiotic control (ampicillin), the biosynthesized TiO_2 nanoparticles showed notable antibacterial activity even at lower concentrations. Similar antibacterial effects of TiO_2 nanoparticles synthesized through different green and chemical routes have been documented (Jayaseelan et al., 2013) [29].

The proposed mechanism underlying the bactericidal action of TiO_2 nanoparticles involves the production of highly reactive oxygen intermediates, especially hydroxyl radicals (•OH), which destabilize bacterial membranes by initiating phospholipid peroxidation, ultimately resulting in bacterial eradication [30,31]. Additionally, the nanoparticles' capacity for direct interaction with bacterial surfaces contributes to their bactericidal action [32]. When bacterial membrane integrity is compromised, cells may attempt repair; however, excessive damage often leads to the release of intracellular contents and eventual cellular demise [33]. Such interactions predominantly occur at the cell membrane interface, although some nanoparticles are capable of membrane penetration [34].

In the case of TiO_2 nanoparticles synthesized using *Costus pictus* D., the measured zeta potential was -6.8 mV, which is more positive than that of various Gram-positive bacteria, including *Enterococcus faecalis*, *Bacillus pumilus* and *Bacillus subtilis*. Literature indicates that metal oxide nanoparticles generally carry a positive surface charge, while bacterial cells typically possess a negatively charged envelope. This electrostatic attraction facilitates strong interactions, leading to oxidative stress and bacterial cell death.

Table – 2 MIC and MBC values for the C. p @ TiO_2 NPs against chosen bacterial pathogens

S. No.	Organism	MIC (μg/ml)	MBC (μg/ml)
1.	*Staphylococcus aureus*	31.2	125
2.	*Enterococcus faecalis*	7.8	31.2
3.	*Bacillus pumilus*	7.8	31.2
4.	*Bacillus subtilis*	7.8	31.2
5.	*Escherichia coli*	31.2	125
6.	*Pseudomonas aeruginosa*	15.6	62.5
7.	*Klebsiella pneumoniae*	62.5	250
8.	*Salmonella sp.*	125	500

Further, the MIC and MBC values for the bio-mediated TiO_2 nanoparticles were assessed and are summarized in Table - 2. The most sensitive strains, *Enterococcus faecalis*, *Bacillus pumilus*, and *Bacillus subtilis*, exhibited the lowest MIC of 7.8 μg/mL and MBC of 31.2 μg/mL. Conversely, *Klebsiella pneumoniae* and *Salmonella* sp. displayed higher resistance, exhibiting MIC values of 62.5 μg/mL and 125 μg/mL, respectively.

These results underscore the enhanced susceptibility of Gram-positive bacteria, suggesting that the antimicrobial efficacy of TiO_2 nanoparticles could be further optimized via surface modifications. The potent antibacterial activity observed is likely due to the nanoparticles' reduced dimensions, uniform spherical morphology, and enhanced surface area, which provide numerous reactive sites for microbial interaction [35]. These features contribute to the disruption of bacterialmembranes and the leakage of essential cellular components, such as minerals, proteins, and nucleic acids, ultimately resulting in bacterial cell death.

In conclusion, the findings highlight the promising potential of biosynthesized TiO_2 nanoparticles as eco- friendly and effective antibacterial agents. Their remarkable efficacy, especially against Gram-positive bacteria, offers exciting prospects for applications across biomedical, pharmaceutical, and environmental domains.

1.5 Antifungal Activity

The incidence of opportunistic fungal infections has risen markedly among immunocompromised individuals, with candidiasis emerging as the most prevalent invasive fungal disease [36]. Given the limitations associated with conventional antifungal therapies— including adverse side effects, drug resistance, and therapeutic failure— attention is shifting toward the development of novel antifungal agents, which offer both efficacy and biocompatibility [37].

Titanium dioxide nanoparticles (TiO_2 NPs) have demonstrated significant antifungal activity against various pathogenic fungal strains. For instance, prior studies have demonstrated their effectiveness against *Ustilago tritici*, the causative agent of wheat rust, using both sol-gel and green synthesis methods [38].

In this study, TiO_2 nanoparticles synthesized using Costus pictus D. leaf extract was assessed for their antifungal efficacy against *Aspergillus niger* and *Candida albicans* through the measurement of the inhibition zone. As shown in Table - 3 and Fig. 6, the nanoparticles exhibited notable antifungal activity. *Aspergillus niger* was particularly susceptible, with a maximum inhibition zone of 9 mm, while *Candida albicans* displayed a smaller inhibition zone of 4 mm. These results suggest a higher sensitivity of *Aspergillus niger* to the biosynthesized TiO_2 nanoparticles, underscoring their potential as effective antifungal agents.

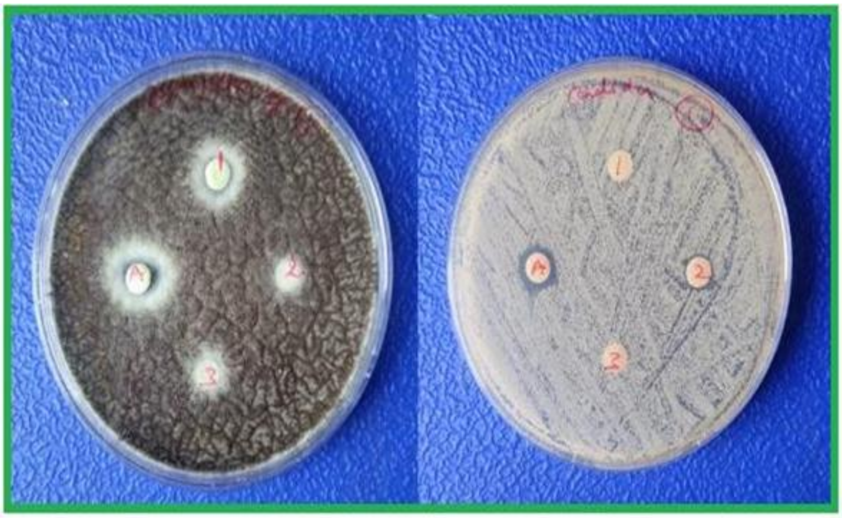

Fig. 6 Antifungal activity of C. p @ TiO_2 NPs against

a) *Aspergillus niger* b) *Candida albicans*

Table – 3 Effect of C. p @ TiO_2 NPs on the growth of tested fungal pathogens

Sample	**Zone of Inhibition in diameter (mm)**	
	Aspergillus niger	***Candida albicans***
Sample (TiO_2 NPs)	9	4
Standard (Amphotericin -B)	11	8

The results align with previous reports on the antifungal efficacy of TiO_2 nanoparticles, further confirming their potential as eco-friendly alternatives to conventional antifungal compounds [39]. TiO_2 NPs are particularly appealing because of their low toxicity, enhanced biocompatibility, and environmentally benign synthesis process.

To quantify their antifungal potency, the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the biosynthesized TiO_2 nanoparticles were determined. MIC refers to the lowest concentration of an antimicrobial agent that prevents observable fungal growth. As detailed in Table - 4, the MIC values were found to be 62.5 µg/mL for *Aspergillus niger* and 125 µg/mL for *Candida albicans*. Corresponding MFC values were 250 µg/mL and 500 µg/mL, respectively. These findings confirm the strong fungicidal activity of the biosynthesized TiO_2 nanoparticles, particularly against *Aspergillus niger*.

Table – 4 MIC and MFC values of C. p @ TiO_2 `NPs on the growth of tested fungal pathogens

S No.	**Organism**	**MIC (µg/mL)**	**MFC (µg/mL)**
1	*Aspergillus Niger*	62.5	250
2	*Candida albicans*	125	500

Although the antifungal activity of TiO_2 nanoparticles is somewhat lower than that of standard antifungal drugs such as amphotericin B, they still offer a compelling alternative due to their green synthesis, low environmental impact, and potential for further functionalization [40]. However, a deeper understanding of their antifungal mechanisms is essential. It is hypothesized that their bioactivity arises from their nanoscale size, high surface exposure, and the production of reactive oxygen species (ROS), which can damage fungal cell membranes and internal structures.

In particular, in vivo research is needed to confirm the safety, effectiveness, and potential therapeutic applications of these nanoparticles in living organisms. Nevertheless, the results emphasize the growing relevance of bioinspired nanomaterials in managing fungal infections. The demonstrated antifungal efficacy of *Costus pictus*- mediated TiO_2 nanoparticles holds significant promise for future uses in the healthcare and pharmaceutical fields [41].

Larvicidal Activity

In the present study, titanium dioxide (TiO_2) nanoparticles synthesized using Costus pictus D. leaf extract were assessed for their effectiveness in controlling larvae of *Aedes aegypti and Culex quinquefasciatus* - two well-known vectors responsible for transmitting arboviral diseases such as dengue, Zika, chikungunya, and malaria. The larvicidal potential was assessed using a dose-dependent approach, with TiO_2 nanoparticle concentrations varying from 10 µg/mL to 100 µg/mL. As shown in Fig. 7, a progressive increase in larval mortality was observed with increasing nanoparticle concentration. For *Aedes aegypti*, mortality rates at 10 µg/mL and 25 µg/mL were 30%, reaching a peak of 70% at 100 µg/mL. In contrast, *Culex quinquefasciatus* exhibited a lower sensitivity, with 10% mortality at the two lowest concentrations and a maximum of 60% mortality at 100 µg/mL. These results suggest that *Aedes aegypti* larvae are more susceptible to green-synthesized TiO_2 nanoparticles than *Culex* species.

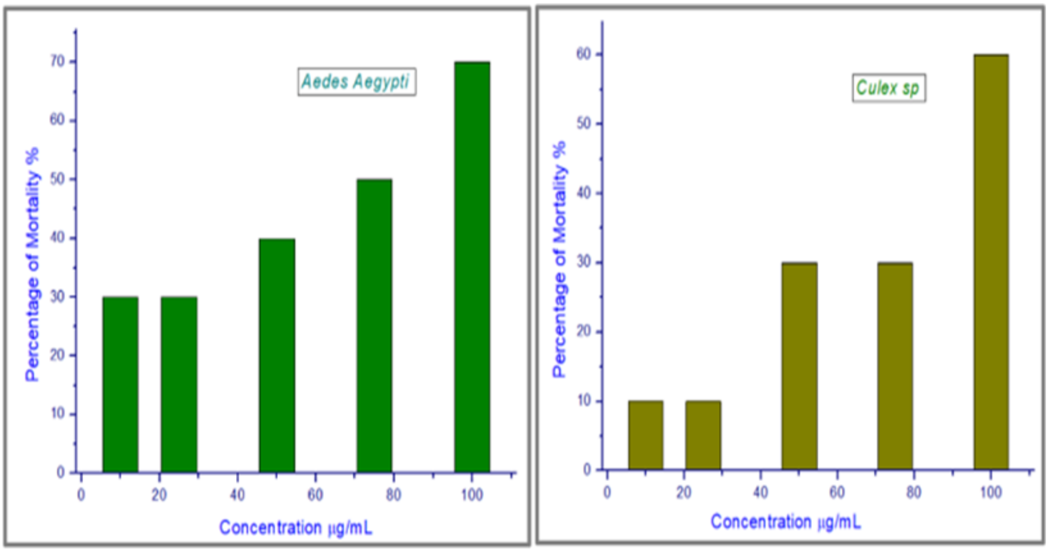

Fig. 7. Larvicidal activity of C. p @ TiO_2 NPs on (a) *Aedes aegypti* (b) *Culex quinquefasciatus*

These results align with previous research that has demonstrated the larvicidal efficacy of metal oxide nanoparticles synthesized through plant- mediated green chemistry. For instance, TiO_2 and ZnO nanoparticles synthesized from *Cuscuta reflexa* stem extract have been reported to cause substantial mortality in *Anopheles stephensi* larvae across all developmental instars, achieving complete mortality after 48 hours at concentrations ranging from 25 to 250 ppm [42]. Similarly, (Murugan et al. 2015) reported LC_{50} values for TiO_2 nanoparticles against *Aedes aegypti* ranging from 4.02 ppm (1st instar) to 7.527 ppm (pupal stage), reinforcing their potent bioactivity [43]. Additional studies have confirmed the effectiveness of TiO_2 nanoparticles against *Anopheles subpictus* and lice, while chromium-doped TiO_2 nanoparticles have also shown high larvicidal toxicity [44-46].

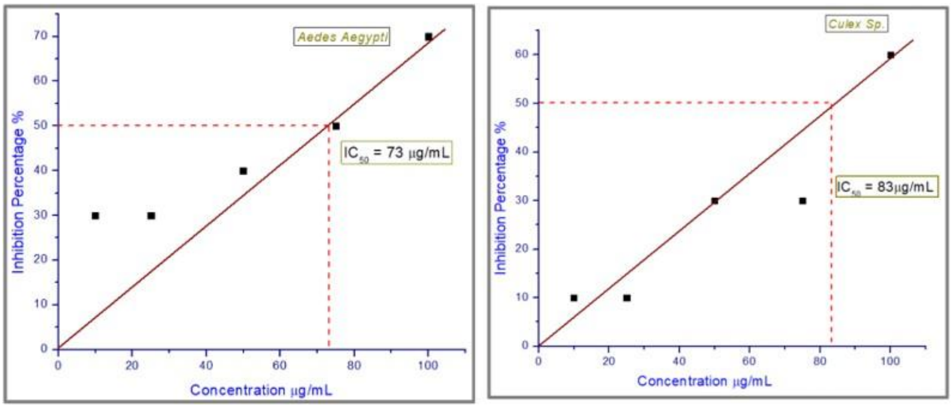

Fig. 8 IC_{50} value for Larvicidal activity

Fig. 8 presents the IC_{50} values for larvicidal activity, providing quantitative validation of the concentration-dependent toxicity of the synthesized TiO_2 nanoparticles.

The results of this investigation highlight the potential of green-synthesized TiO_2 nanoparticles as an eco-friendly and sustainable alternative to synthetic larvicides. Although their efficacy may be lower compared to conventional chemical agents, their biodegradable nature, reduced environmental footprint, and potential for selective toxicity position them as promising options for integrated vector control programs [47].

Nevertheless, further studies are needed to better understand their specific mechanisms of action, evaluate their environmental safety, and create optimized formulations suitable for field application.

4 Conclusions

In conclusion, this study successfully demonstrates the biosynthesis of TiO_2 NPs using the aqueous leaf extract of *Costus pictus D*., emphasizing a sustainable and environmentally friendly approach to nanoparticle production. Comprehensive physicochemical characterizations including UV-vis spectroscopy, photoluminescence (PL) analysis, HR-TEM, and dynamic light scattering (DLS) confirm the formation of spherical TiO_2 nanoparticles that exhibit favourable optical properties, with a direct band gap of 3.24 eV and moderate colloidal stability. The presence of surface defects, such as oxygen vacancies and self- trapped excitons, enhances the photonic and reactive characteristics of the nanoparticles, thereby providing a robust foundation for future research efforts. Functionally, the biosynthesized TiO_2 nanoparticles demonstrated significant antimicrobial activity, exhibiting strong antibacterial effects, particularly against Gram-positive strains, as well as effective antifungal properties against *Aspergillus niger* and *Candida albicans*. Furthermore, larvicidal assessments indicated a concentration-dependent efficacy targeting *Aedes aegypti* and *Culex quinquefasciatus*, suggesting their potential applicability in vector control strategies.

These results highlight the multifunctional capabilities of *Costus pictus*-mediated TiO_2 nanoparticles for biomedical, environmental, and pest management applications. Nevertheless, additional in vivo research and mechanistic studies are essential to validate their safety and optimize their practical implementation. This study contributes to the expanding body of research in green nanotechnology and paves the way for developing biocompatible nanomaterials that offer wide-ranging societal benefits.

Acknowledgements

The authors would like to thank the Annamalai University for providing the laboratory and library facilities.

Declarations Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Conflict of Interest

The authors declare no competing interests.

Ethical approval

This article does not contain any studies with human volunteers or animals being involved by any of the authors.

Informed Consent

None.

References

1. Fakhari, Shabnam, Mina Jamzad, and Hassan Kabiri Fard. "Green synthesis of zinc oxide nanoparticles: a comparison." Green chemistry letters and reviews 12, no.1 (2019): 19-24.
2. Ahmed, Shakeel, Mudasir Ahmad, Babu Lal Swami, and Saiqa Ikram. "A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a

green expertise." Journal of advanced research 7, no. 1 (2016): 17-28.
3. Rai, Mahendra, Avinash P. Ingle, Sonal Birla, Alka Yadav, and Carolina Alves Dos Santos. "Strategic role of selected noble metal nanoparticles in medicine." Critical reviews in microbiology 42, no. 5 (2016): 696-719.
4. Kim, Dokyoon, Kwangsoo Shin, Soon Gu Kwon, and Taeghwan Hyeon. "Synthesis and biomedical applications of multifunctional nanoparticles." Advanced Materials 30, no. 49 (2018): 1802309.
5. Kaur, Manbir, and Ashi Mannan. "In vitro Antidiabetic Activity of Chamaecostus cuspidatus." Pharmacognosy Research 14, no. 1 (2021).
6. Boujday, Souhir, Frank Wünsch, Patrick Portes, Jean-François Bocquet, and Christophe Colbeau-Justin. "Photocatalytic and electronic properties of TiO2 powders elaborated by sol–gel route and supercritical drying." Solar Energy Materials and Solar Cells 83, no. 4 (2004): 421-433.
7. Shalini, Udayakumar R and Udhayan. "Leaf extract from Costus pictus D.: Unveiling the power of synthesized TiO2 nanoparticles through phytochemical analysis and exciting Antiplatelet and Anticoagulant" Journal of Neonatal Surgery 14(32s) (2025).
8. Dobrucka, Renata. "Synthesis of titanium dioxide nanoparticles using Echinacea purpurea herba." Iranian journal of pharmaceutical research: IJPR 16, no. 2 (2017): 756.
9. Rajkumari, J., C. Maria Magdalane, B. Siddhardha, J. Madhavan, G. Ramalingam, Naif Abdullah Al-Dhabi, Mariadhas Valan Arasu, A. K. M. Ghilan, V. Duraipandiayan, and K. Kaviyarasu. "Synthesis of titanium oxide nanoparticles using Aloe barbadensis mill and evaluation of its antibiofilm potential against Pseudomonas aeruginosa PAO1." Journal of Photochemistry and Photobiology B: Biology 201 (2019): 111667.
10. Sankar, Renu, Kadarmohideen Rizwana, Kanchi Subramanian Shivashangari, and Vilwanathan Ravikumar. "Ultra-rapid photocatalytic activity of Azadirachta indica engineered colloidal titanium dioxide nanoparticles." Applied Nanoscience 5 (2015): 731-736.
11. Sethy, Naresh Kumar, Zeenat Arif, Pradeep Kumar Mishra, and Pradeep Kumar. "Green synthesis of TiO2 nanoparticles from Syzygium cumini extract for photo-catalytic removal of lead (Pb) in explosive industrial wastewater." Green Processing and Synthesis 9, no. 1 (2020): 171-181.
12. Nasrollahzadeh, Mahmoud, and S. Mohammad Sajadi. "Synthesis and characterization of titanium dioxide nanoparticles using Euphorbia heteradena Jaub root extract and evaluation of their stability.Ceramics International 41, no. 10 (2015):14435-14439.
13. Ngoepe, Nkgaetsi Marius, Morongwa Mary Mathipa, and Nomso Charmaine Hintsho- Mbita. "Biosynthesis of titanium dioxide nanoparticles for the photodegradation of dyes and removal of bacteria." Optik 224 (2020): 165728.
14. Nabi, Ghulam, Waseem Raza, and M. B. Tahir. "Green synthesis of TiO2 nanoparticle using cinnamon powder extract and the study of optical properties." Journal of Inorganic and Organometallic Polymers and Materials 30, no. 4 (2020): 1425-1429.
15. Saraf, C. S., O. P. Rupela, and D. M. Hegde. "Biological nitrogen fixation and residual effects of winter grain legumes in rice and wheat cropping systems of the Indo-Gangetic plain." (1998): 14-30.
16. Serpone, Lawless, and Rr Khairutdinov. "Size effects on the photo physical properties of colloidal anatase TiO2 particles: size quantization versus direct transitions in this indirect semiconductor." The Journal of Physical Chemistry 99, no. 45 (1995): 16646- 16654.
17. Tang, Aimin, Masayuki Amagai, Lawrence G. Granger, John R. Stanley, and Mark C. Uddy. "Adhesion of epidermal Langerhans cells to keratinocytes mediated by E- cadherin." Nature 361, no. 6407 (1993): 82-85.
18. Forss, L., and M. Schubnell. "Temperature dependence of the luminescence of TiO 2 powder." Applied Physics B 56 (1993): 363-366.
19. Zeng, Haibo, Guotao Duan, Yue Li, Shikuan Yang, Xiaoxia Xu, and Weiping Cai. "Blue Luminescence of ZnO nanoparticles based on non-equilibrium processes: defect origins and emission controls." Advanced functional materials 20, no. 4 (2010): 561- 572.
20. Siddhapara, Kirit, and Dimple Shah. "Characterization of nanocrystalline cobalt doped TiO2 sol–gel material." Journal of crystal growth 352, no. 1 (2012): 224-228.
21. Krishnasamy, A., Sundaresan, M., Velan, P., 2015. Rapid phytosynthesis of nano-sized titanium using leaf extract of Azadirachta indica. Int. J. ChemTech Res. 8, 2047–2052.
22. Ghotekar, Suresh, Trupti Pagar, Shreyas Pansambal, and Rajeshwari Oza. "A review on green synthesis of sulfur nanoparticles via plant extract, characterization and its applications."Advanced Journal of Chemistry-Section B 2, no. 3 (2020): 128-143.
23. Tomaszewska, Emilia, Katarzyna Soliwoda, Kinga Kadziola, Beata Tkacz-Szczesna, Grzegorz Celichowski, Michal Cichomski, Witold Szmaja, and Jaroslaw Grobelny. "Detection limits of Dls and Uv-Vis spectroscopy in characterization of polydisperse nanoparticles colloids." Journal of Nanomaterials 2013, no. 1 (2013): 313081.

24. Dhanjal, Soniya, and Swaranjit Singh Cameotra. "Aerobic biogenesis of selenium nanospheres by Bacillus cereus isolated from coalmine soil." Microbial cell factories 9 (2010): 1-11.
25. Romanello, Marina Belen, and Maria M. Fidalgo de Cortalezzi. "An experimental study on the aggregation of TiO2 nanoparticles under environmentally relevant conditions." Water research 47, no. 12 (2013): 3887-3898.
26. Al-Shabib, Nasser A., Fohad Mabood Husain, Faizan Abul Qais, Naushad Ahmad, Altaf Khan, Abdullah A. Alyousef, Mohammed Arshad et al. "Phyto-mediated synthesis of porous titanium dioxide nanoparticles from Withania somnifera root extract: broad- spectrum attenuation of biofilm and cytotoxic properties against HepG2 cell lines." Frontiers in Microbiology 11 (2020): 1680.
27. Hamed, Moaaz T., Basant A. Bakr, Yahya H. Shahin, Bassma H. Elwakil, Marwa M. Abu-Serie, Faizah S. Aljohani, and Adnan A. Bekhit. "Novel synthesis of titanium oxide nanoparticles: Biological activity and acute toxicity study." Bioinorganic Chemistry and Applications 2021, no. 1 (2021): 8171786.
28. Bekele, Eneyew Tilahun, Enyew Amare Zereffa, Noto Susanto Gultom, Dong-Hau Kuo, Bedasa Abdisa Gonfa, and Fedlu Kedir Sabir. "Research Article Biotemplated Synthesis of Titanium Oxide Nanoparticles in the Presence of Root Extract of Kniphofia schemperi and Its Application for Dye Sensitized Solar Cells." (2021).
29. Jayaseelan, Chidambaram, Abdul Abdul Rahuman, Selvaraj Mohana Roopan, Arivarasan Vishnu Kirthi, Jayachandran Venkatesan, Se-Kwon Kim, Moorthy Iyappan, and Chinnadurai Siva. "Biological approach to synthesize TiO2 nanoparticles using Aeromonas hydrophila and its antibacterial activity." Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 107 (2013): 82-89.
30. Cho, Min, Hyenmi Chung, Wonyong Choi, and Jeyong Yoon. "Different inactivation behaviors of MS-2 phage and Escherichia coli in TiO2 photocatalytic disinfection." Applied and environmental microbiology 71, no. 1 (2005): 270-275.
31. Nadtochenko, V., N. Denisov, O. Sarkisov, D. Gumy, C. Pulgarin, and J. Kiwi. "Laser kinetic spectroscopy of the interfacial charge transfer between membrane cell walls of E. coli and TiO2." Journal of Photochemistry and Photobiology A: Chemistry 181, no. 2-3 (2006): 401-407.
32. Jeng, Hueiwang Anna, and James Swanson. "Toxicity of metal oxide nanoparticles in mammalian cells." Journal of Environmental Science and Health Part A 41, no. 12 (2006): 2699-2711.
33. Li, Qilin, Shaily Mahendra, Delina Y. Lyon, Lena Brunet, Michael V. Liga, Dong Li, and Pedro JJ Alvarez."Antimicrobial nanomaterials for water disinfection and microbialcontrol: potential applications and implications." Water research 42, no. 18 (2008): 4591-4602.
34. Jang, Heeun, Lindsay E. Pell, Brian A. Korgel, and Douglas S. English. "Photoluminescence quenching of silicon nanoparticles in phospholipid vesicle bilayers." Journal of Photochemistry and Photobiology A: Chemistry 158, no. 2-3 (2003): 111-117.
35. Zhang, Huanjun, and Guohua Chen. "Potent antibacterial activities of Ag/TiO2 nanocomposite powders synthesized by a one-pot sol– gel method." Environmental science & technology 43, no. 8 (2009): 2905-2910.
36. Salari, S., N. Sadat Seddighi, and P. Ghasemi Nejad Almani. "Evaluation of biofilm formation ability in different Candida strains and anti-biofilm effects of Fe3O4-NPs compared with Fluconazole: an in vitro study." Journal de mycologie medicale 28, no. 1 (2018): 23-28.
37. Arastefar, Amir, and Parisa Badiee. "A Review of Antifungals and their Mono-and Combination-therapy in the Treatment of Invasive Fungal Infections." Journal of Kerman University of Medical Sciences 23, no. 6 (2016): 829-842.
38. Irshad, Muhammad Atif, Rab Nawaz, Muhammad Zia Ur Rehman, Muhammad Adrees, Muhammad Rizwan, Shafaqat Ali, Sajjad Ahmad, and Sehar Tasleem. "Synthesis, characterization and advanced sustainable applications of titanium dioxide nanoparticles: A review." Ecotoxicology and environmental safety 212 (2021): 111978.
39. Ahmadpour Kermani, Shima, Samira Salari, and Pooya Ghasemi Nejad Almani. "Comparison of antifungal and cytotoxicity activities of titanium dioxide and zinc oxide nanoparticles with amphotericin B against different Candida species: In vitro evaluation." Journal of clinical laboratory analysis 35, no. 1 (2021): e23577.
40. Rajamehala, M., Renugaa Su, Muthu Kumara A. Pandian, B. Gopalakrishnan, and Vijay Pradhap M. Singh. "Green synthesis of titanium dioxide and its application on anti- fungal activity." Research Journal of Pharmacy and Technology 15, no. 2 (2022): 695-700.
41. Sagadevan, Suresh, Shahla Imteyaz, Baranya Murugan, Jayasingh Anita Lett, Nanthini Sridewi, Getu Kassegn Weldegebrieal, Is Fatimah, and Won-Chun Oh. "A comprehensive review on green synthesis of titanium dioxide nanoparticles and their diverse biomedical applications." Green Processing and Synthesis 11, no. 1 (2022): 44-63.
42. Soni, Namita, and Ramesh C. Dhiman. "Larvicidal activity of Zinc oxide and titanium dioxide

nanoparticles Synthesis using Cuscuta reflexa extract against malaria vector (Anopheles stephensi)." Egyptian Journal of Basic and Applied Sciences 7, no. 1 (2020): 342- 352.
43. Murugan, Kadarkarai, Devakumar Dinesh, Krishnamoorthy Kavithaa, Manickam paulpandi, Thondhi Ponraj, Mohamad Saleh Alsalhi, Sandhanasamy Devanesan et al. "Hydrothermal synthesis of titanium dioxide nanoparticles: mosquitocidal potential and anticancer activity on human breast cancer cells (MCF-7)." Parasitology research 115 (2016): 1085-1096.
44. Rajakumar, Govindasamy, Abdul Abdul Rahuman, Chidambaram Jayaseelan, Thirunavukkarasu Santhoshkumar, Sampath Marimuthu, Chinnaperumal Kamaraj, Asokan Bagavan et al. "Solanum trilobatum extract-mediated synthesis of titanium dioxide nanoparticles to control Pediculus humanus capitis, Hyalomma anatolicum anatolicum and Anopheles subpictus." Parasitology Research 113 (2014): 469-479.
45. Gandhi, P. Rajiv, C. Jayaseelan, E. Vimalkumar, and R. Regina Mary. "Larvicidal and pediculicidal activity of synthesized TiO2 nanoparticles using Vitex negundo leaf extract against blood feeding parasites." Journal of Asia- Pacific Entomology 19, no. 4 (2016): 1089-1094.
46. .Rekha, Ravichandran, Mani Divya, Marimuthu Govindarajan, Naiyf S. Alharbi, Shine Kadaikunnan, Jamal M. Khaled, Mohammed N. Al- Anbr, Roman Pavela, and Baskaralingam Vaseeharan. "Synthesis and characterization of crustin capped titanium dioxide nanoparticles: Photocatalytic, antibacterial, antifungal and insecticidal activities." Journal of Photochemistryand Photobiology B: Biology 199 (2019): 111620.
47. Murugan, Kadarkarai, Palanimuthu Aruna, Chellasamy Panneerselvam, Pari Madhiyazhagan, Manickam Paulpandi, Jayapal Subramaniam, Rajapandian Rajaganesh et al. "Fighting arboviral diseases: low toxicity on mammalian cells, dengue growth inhibition (in vitro), and mosquitocidal activity of Centroceras clavulatum- synthesized silver anoparticles." Parasitology research 115 (2016): 651-662.