

Original Research

Paranitrophenol and Organic Azo Dye Degradation: Catalytic Power of *Terminalia* Leaf Silver Nanoparticles

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Abstract

Terminalia species is a widely available plant that can be used to synthesise silver nanoparticles (AgNPs) and harness their catalytic capacity. This experimental design will explore the potential of *Terminalia* AgNPs in contributing to the degradation process of toxic chemicals generated by industries such as textiles, food, and pharmaceuticals. This research uses four leaf varieties of *Terminalia* species—*T. arjuna*, *T. bellirica*, *T. chebula*, and *T. catappa*—to synthesise AgNPs, evaluate their optimal synthesis conditions, morphological characteristics, methylene blue, methyl orange, para-para-nitrophenol degradation, and cytotoxicity. Water extracts of *T. arjuna*, *T. bellirica*, and *T. catappa* successfully synthesised AgNPs at an optimal condition of 90°C and 45 minutes with AgNP peaks between 420 nm and 480 nm. *T. catappa* showed the presence of spherical AgNPs of 40 nm - 60 nm under scanning electron microscopy. The catalytic potential of *Terminalia* AgNPs was observed at different volumes (20 µL and 50 µL). The highest rate of 0.4013 min⁻¹ catalytic function was obtained with 50 µL of *T.bel*_AgNP, and the lowest rate of 0.1561 min⁻¹ was obtained with 20 µL of



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T.cat_AgNP. *Terminalia* AgNP demonstrated successful degradation of methylene blue and methyl orange with and without the addition of a catalyst (sodium borohydride). In methylene blue degradation, the highest rate was obtained with 266.67 ppm *T.arj_AgNP* and sodium borohydride at 0.0647 min^{-1} . In methyl orange degradation, the highest rate was obtained at 266.67 ppm *T.bel_AgNP* and sodium borohydride with a rate of 0.0762 min^{-1} . These findings highlight a higher degradation efficiency of methyl orange when sodium borohydride is added. The cytotoxicity study using *Artemia salina* exposure to 800 ppm and 200 ppm AgNPs showed 100% viability. Overall, these findings highlight *Terminalia* leaf AgNPs as potential, non-toxic substitutes for environmental remediation.

Keywords

Terminalia; silver nanoparticles; para-nitro phenol; photocatalysis; methylene blue; methyl orange; cytotoxicity

1. Introduction

Nanotechnology is the science of designing, manipulating, and controlling matter at the atomic level, on the scale of 1 to 100 nm. This field has offered modern solutions to various scientific challenges across the petroleum, agricultural, food production, and healthcare industries [1]. Among these industries, the widely utilized nanoparticle applications are in food packaging, water treatment, energy storage, medicine delivery, diagnostics, and cosmetics [2]. Nanoparticles can be formed from different metals depending on the application required and efficiency; for example, nanoparticles are classified as carbon-based, metal, or semiconductor. This research focuses on metal nanoparticles due to their localized surface plasmon resonance (LSPR), which endows them with excellent optical and electrical properties. Commonly utilized industrial metal nanoparticles included gold, silver, copper, zinc, aluminum, and iron [3].

Among these various metal nanoparticles, silver nanoparticles (AgNPs) garnered research attention due to their unique properties, such as higher surface area, non-toxicity to animal cells, optical properties, biocompatibility, eco-friendliness, chemical stability, and cost-effectiveness. They have been extensively studied and utilized as solutions for multiple industrial applications, such as antioxidants or medicines, and environmental remediation of organic pollutants [4]. Additionally, AgNPs exhibit size-dependent catalytic activity and have been shown not to penetrate human skin. These findings highlight the potential of AgNP for safe human use at industrial levels [5]. These AgNPs are used in various applications, such as the health care, food, textile, and cosmetic industries [6].

The synthesis of AgNPs can be achieved using two fundamental methods: the top-down approach, in which nanoparticles are produced from bulk materials, and the bottom-up approach, in which molecules or atoms assemble (Figure 1). The top-down approach requires the breakdown of bulk silver into nanoscale particles, which can be performed by physical methods such as electro spraying, laser ablation, lithography, and evaporation-condensation, which can generally produce 10 to 100 nm particles [7]. However, the major drawbacks are the extensive use of force and the absence of stabilizing agents, which can prevent agglomeration during synthesis. The bottom-up

approach can be achieved by chemical or biological means. Chemical methods include chemical vapor deposition, co-precipitation, irradiation, electrochemical, and photo reduction with the involvement of reducing agents, and biological methods using plants and microbes. Chemical methods also use chemicals as reducing or stabilizing agents, which could be hazardous and cannot be used for human purposes. Generally, these conventional methods require expensive procedures, toxic chemical usage, and high energy, which reduces their eco-friendliness. Instead, biological methods offer more environmentally friendly synthesis, are cost-effective, highly scalable, simple, and encourage biocompatibility [3, 8].

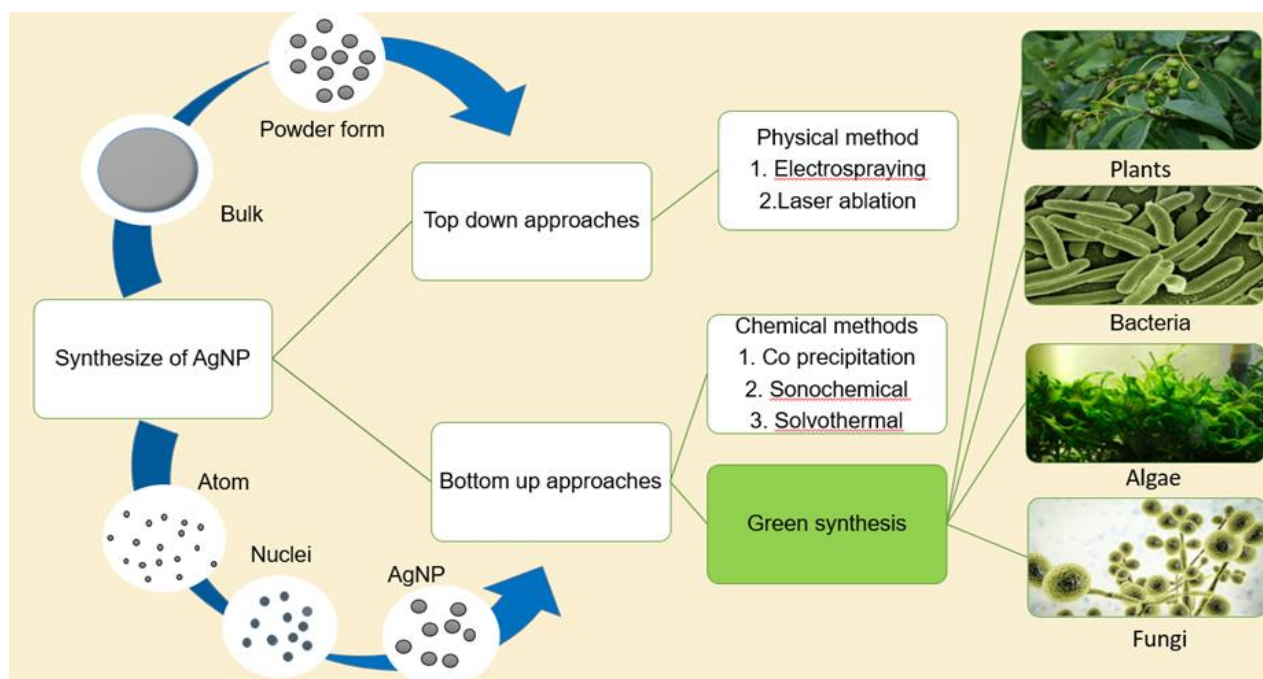


Figure 1 AgNP synthesis from top-down approaches using physical methods and bottom-up approaches using chemical and biological processes (adapted from [9]).

Plant-based synthesis is primarily selected for research, as microbial synthesis is less feasible due to lengthy processing times, susceptibility to contamination, and difficulty scaling up industrially [10]. AgNP synthesis by plants, particularly medicinal plants, has attracted significant attention due to their diverse phytochemical profiles, which have positively influenced the properties of the synthesized nanoparticles, such as antibacterial and anticancer [11].

The primary focus of this research is to explore the potential of using plant-based nanoparticles for catalytic applications derived from *Terminalia* leaves. The *Terminalia* genus is widely distributed, especially in South Asia, South Africa, and Australia, and belongs to the Combretaceae family [12, 13]. This genus comprises more than 100 tropical species. It includes prominent *Terminalia* species like *T. arjuna*, *T. bellirica*, *T. chebula*, and *T. catappa*, which are known for their rich phytochemical content, including terpenes, flavonoids, and phenols. Due to their antimicrobial, antioxidant, antimalarial, and anticancer properties, these species have been used in traditional medicine to treat several diseases, including cancer, heart problems, gastrointestinal disorders, and malaria [14, 15]. *Terminalia*-derived AgNPs have been found helpful in industries such as medicine, environmental remediation, agriculture, and cosmetics [16]. Therefore, the active compounds

present in *Terminalia* sp. can be incorporated into nanoparticle synthesis and used in industrial applications.

In recent years, advances in nanotechnology have driven demand to address water contamination from industrial pollutants. Toxic organic dyes are a significant source of contamination, as they have been used in the manufacture of paper, food, textiles, and drugs. These industries discharge large amounts of industrial waste containing dyes into water bodies, posing a significant environmental hazard [17]. This practice of contaminating water by industrial dyes has caused substantial changes in the soil, fauna, and flora, ultimately leading to their death. In terms of human health, these contaminated drinking water sources led to lung, kidney, and skin problems [18].

Among water pollutants, phenolic contaminants are commonly found in pesticides, herbicides, and explosives. One of the most toxic phenolic compounds is para-nitrophenol (PNP), which is considered a hazardous nitro-aromatic substance. The approved LNP level is 0.22 $\mu\text{mol/l}$; however, these levels have been exceeded and require remediation [19, 20]. Experiments incorporating reducing agents such as sodium borohydride (NaBH_4), which converts PNP to the harmless Para-Aminophenol (PAP), have been studied. This approach is found to be kinetically challenging, calling for much more feasible approaches [21]. Many industries produce synthetic dyes or pigments containing one or more benzene rings and $-\text{N}=\text{N}-$ functional groups in their molecular structure. Azo dyes are among the most common artificial dyes used in manufacturing processes, leading to high effluent levels in water bodies [22, 23]. Methylene blue (MB) and methyl orange (MO) are synthetic dyes that are not biodegradable and are discharged into the environment from industries such as food, paper, and textiles [24]. Conventional removal methods include adsorption, photocatalysis, coagulation, oxidation, and electrochemistry. However, these methods are costly, ineffective, and energy-intensive. Due to their enhanced catalytic activities, plant-based AgNPs can act as excellent biological catalysts, offering a more economical and environmentally friendly alternative [25, 26].

Cytotoxicity assessment is essential before AgNPs are used for human applications, as they are widely used in water treatment, food packaging, and cancer treatment. Conventional cytotoxicity testing techniques include cell culture tests, MTT assay, and LDH-release assay. These assays are highly accurate but expensive, unethical, time-consuming, and labor-intensive, and require specialized equipment [27]. A rapid, easy, and affordable substitute for cytotoxicity testing is the use of brine shrimp as a model organism, which are sensitive to toxins, ethically accepted, and have a short life cycle; aseptic conditions are not required [28].

This research aims to synthesize AgNPs using four *Terminalia* leaf species, obtain optimum conditions for AgNPs synthesis, assess the PNP catalytic activity of AgNPs, study and determine the photocatalytic activity of AgNPs using MB and MO, and evaluate the cytotoxic activity of AgNPs using *Artemia salina*. Additionally, morphological characterization of AgNP using Scanning Electron Microscopy (SEM) is performed. The study emphasizes the many uses of AgNPs, highlighting their potential for environmental remediation as an affordable and sustainable alternative to catalysts.

2. Materials and Methods

2.1 Sample Collection and Preparation

Four *Terminalia* varieties were collected from Thirukkivil, Sri Lanka, and shade-dried for 10 days (Figure 2).

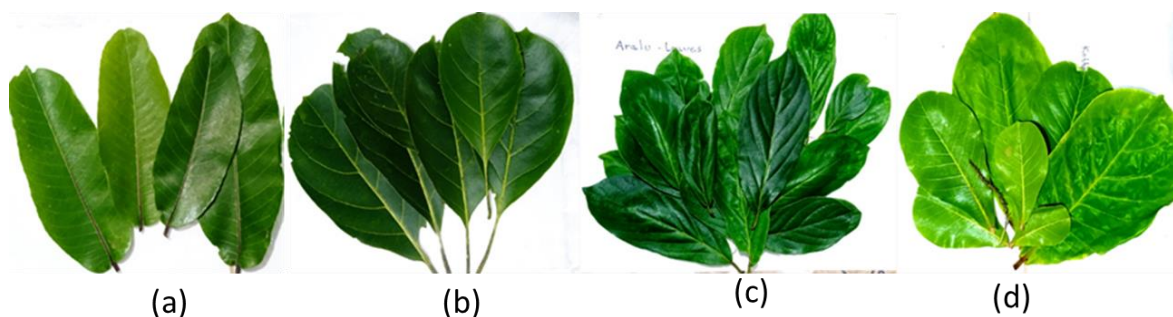


Figure 2 Four varieties of *Terminalia* leaves and their code names; (a): *T. arjuna* (*T.arj*), (b): *T. bellirica* (*T.bel*), (c): *T. chebula* (*T.cheb*) and (d): *T. catappa* (*T.cat*) (Own source).

2.2 Water Extraction

The selected *Terminalia* leaves were ground into a fine powder using a mortar and a pestle. Each powdered sample was weighed on the analytical balance to obtain 2 g, and 50 mL of distilled water (DW) was added to each sample. These mixtures were placed in a hot-air oven at 100°C for 30 minutes. After incubation, the solutions were filtered through Whatman filter paper (No. 1) to obtain the respective water extracts (WEs) [29], which were stored at 4°C for future use.

2.3 Silver Nanoparticle Synthesis

The green synthesis of the *Terminalia* leaves was conducted as follows. First, 1 mL of each WE was mixed with 9 mL of 1 mM silver nitrate (AgNO_3) and heated at 60°C and 90°C for 15, 30, 45, and 60 minutes, and at room temperature (RT) for 24 hours. Following synthesis, the absorbance was recorded from 320 nm to 520 nm using DW as the blank [30]. The synthesized products were stored at 4°C for future use.

2.4 SEM Analysis

*T.cat*_AgNP (4000 ppm) was centrifuged at 4000 rpm for 4 minutes using a High-speed mini centrifuge, and the formed supernatant was discarded. This step was repeated until a prominent pellet was obtained [31]. The pellet was dried at 40°C for 24 hours and sent to the Sri Lanka Institute of Nanotechnology for analysis using a Field-Emission SEM with a secondary electron detector. The dried pellet was gold-coated, and the images were captured at the nanoscale using AZtec software at different magnifications.

2.5 PNP-Catalysis

Terminalia leaf AgNPs were used to catalyze the reduction of PNP. The UV-visible spectra of a 0.01 mM PNP solution were obtained. Then 2 mL of PNP was mixed with 1 mL of 0.1M of freshly prepared NaBH₄ solution, and the spectra were recorded every 5 minutes for 30 minutes. For the AgNP catalytic reduction, the previous step was repeated with the addition of 20 µL and 50 µL of AgNPs, and the spectra were recorded [32]. All absorbance was recorded using a UV–visible spectrometer between 280 and 540 nm with DW as the blank.

2.6 Photocatalysis of AgNP on MB and MO

The UV-visible spectra of a 0.1 mM MB dye solution were obtained. The first experiment was conducted by adding 0.5 mL of 4000 ppm/266.67 ppm AgNPs to 50 mL of MB solution and leaving it under sunlight. In the second experiment, the solution mixture was mixed with 1 mL of 0.2 M NaBH₄ and exposed to sunlight. The absorbance of the mixtures was monitored using a UV–visible spectrophotometer between 380 nm and 780 nm at regular time intervals with DW as the blank. The degradation was also monitored using a MO dye solution over the wavelength range of 340 nm to 620 nm [16].

2.7 Cytotoxicity

Brine shrimp eggs were hatched in the seawater under a yellow LED for 24 hours. Cytotoxicity was analyzed at 800 ppm and 200 ppm AgNP concentrations, with seawater as the control. After hatching for 24 hours, into a 96-well plate, two hatched shrimps and their respective AgNP solutions were added into wells in triplicate. This system was left for 24 hours, and afterwards the percentage (%) viability was calculated using equation (1) [33].

$$\text{Viability \%} = \frac{\text{Total no of viable shrimps} - \text{Total no of non-viable shrimps}}{\text{Total no of viable shrimps}} \times 100 \quad (1)$$

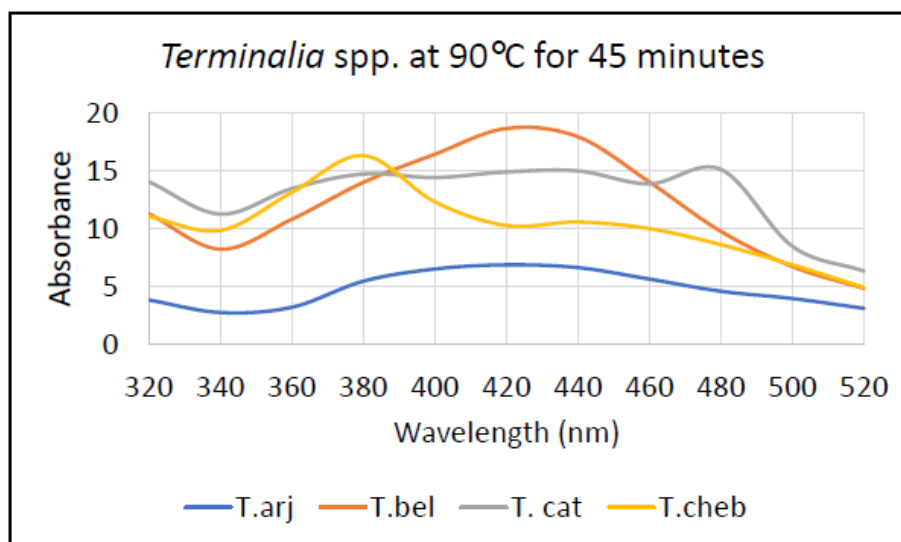
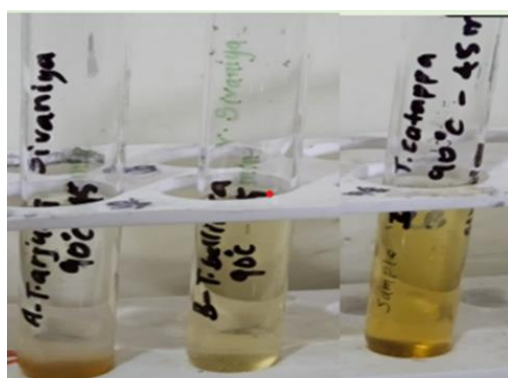
3. Results

3.1 Green Synthesis of *Terminalia*_AgNPs

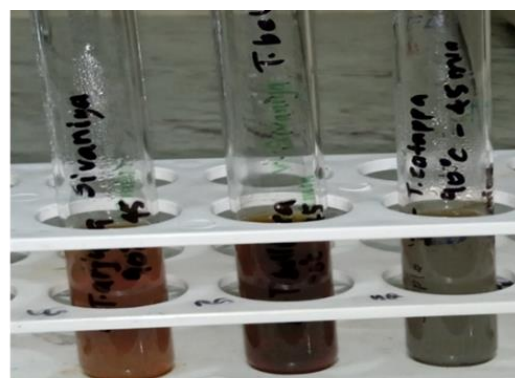
The optimum condition for *Terminalia*_AgNP synthesis was confirmed as 90°C for 45 minutes for *T.arj*, *T.bel*, and *T.cat*, while *T.cheb* did not show the presence of AgNP under all tested conditions (Table 1). *T.arj*, *T.bel*, and *T.cat* showed characteristic surface plasmon resonance peaks between 420–480 nm (Figure 3) and a visible color change to greyish-brown after incubation (Figure 4), confirming AgNP synthesis. SEM analysis revealed spherical *T.cat*_AgNPs with particle sizes ranging from 40 nm to 60 nm (Figure 5).

Table 1 Optimization of the AgNPs; (✓) shows the presence, (×) absence of the AgNPs.

Sample	90°C				60°C				RT
	15 min	30 min	45 min	60 min	15 min	30 min	45 min	60 min	24 hrs
<i>T.arj</i>	×	✓	✓	✓	×	✓	✓	✓	×
<i>T.bel</i>	×	✓	✓	✓	✓	✓	✓	✓	×
<i>T.cheb</i>	×	×	×	×	×	×	×	×	×
<i>T.cat</i>	×	✓	✓	✓	×	✓	✓	✓	×

**Figure 3** UV-visible spectrum of AgNPs synthesized using four different varieties of *Terminalia* leaf at 90°C for 45 minutes.

(a)



(b)

Figure 4 A characteristic colour change from pale yellow (a) to dark greyish-brown (b).

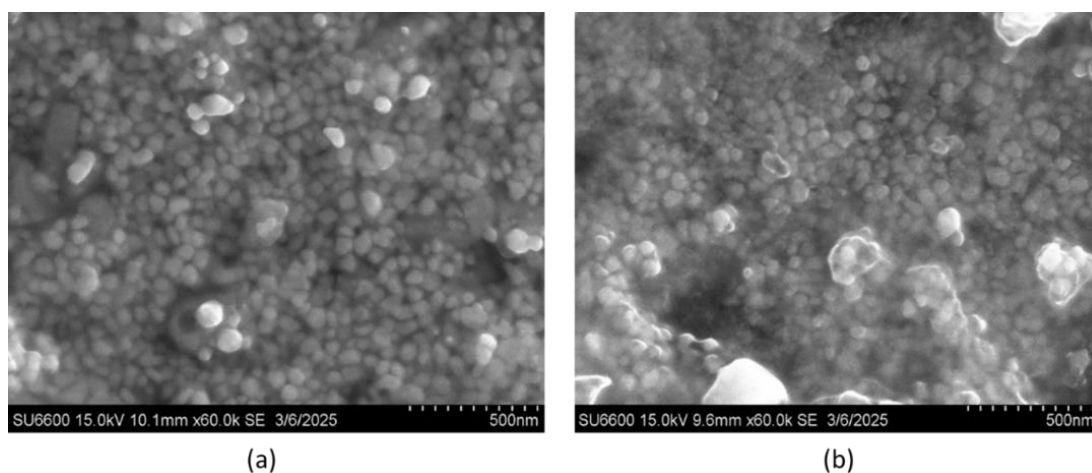


Figure 5 Images of SEM analysis of *T.cat*_AgNP at (a) 15.0 kV, 10.1 mm × 60.0 k. 500 nm (b) 15.0 kV 9.6 mm × 60.0 k. 500 nm (Images from Hitachi SU6600 SEM at SLINTEC).

3.2 PNP Degradation *Terminalia*_AgNPs

Absorption spectra showing the catalytic degradation of PNP by AgNPs. The shift of the PNP peak at 300 nm (Figure 6a) to 400 nm with the addition of NaBH₄ was observed, in the absence of PNP degradation (Figure 6b). The introduction of 20 μL AgNPs resulted in successful degradation, indicated by a decrease in the 400 nm peak and the formation of PAP (Figure 7). A faster degradation was observed with 50 μL AgNPs, completing within 10 minutes (Figure 8).

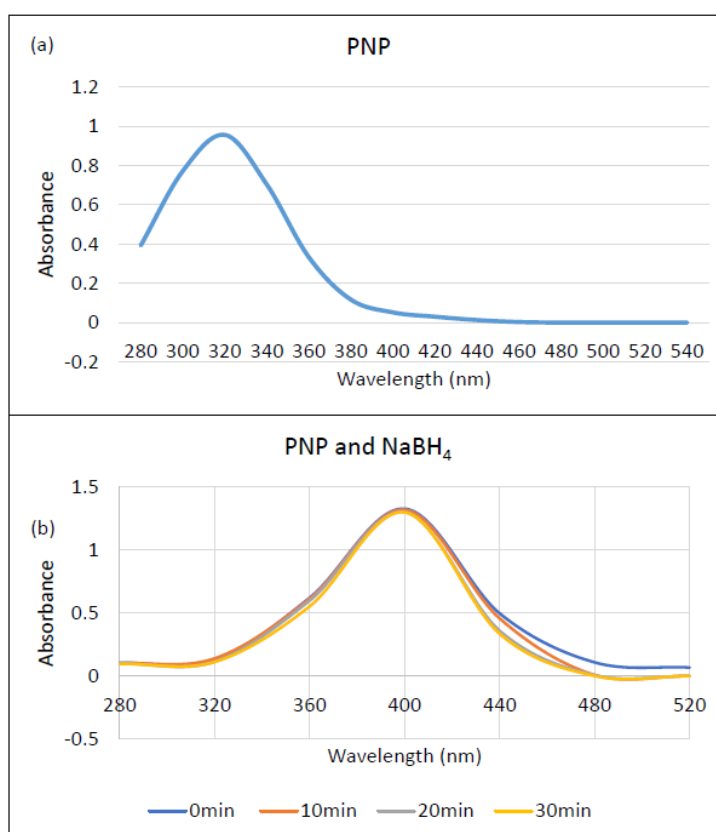


Figure 6 UV-visible absorption spectra for (a) 0.01 mM PNP and (b) 2 mL of 0.01 mM PNP and 1 mL of 0.1 M NaBH₄.

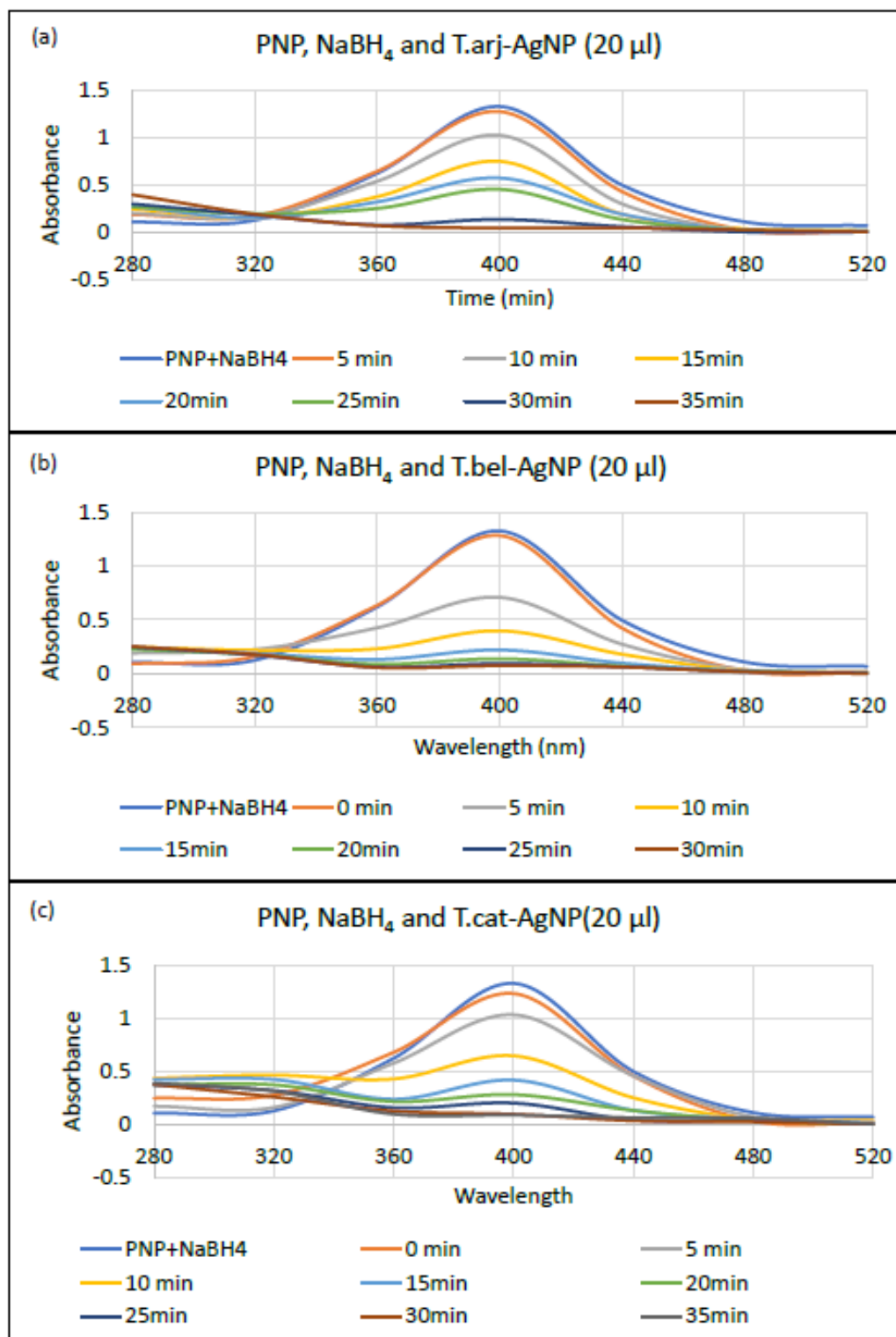


Figure 7 UV-visible absorption spectra for PNP degradation with 20 µL AgNP and 0.1 M NaBH₄: (a) *T.arj*_AgNP, (b) *T.bel*_AgNP, and (c) *T.cat*_AgNP.

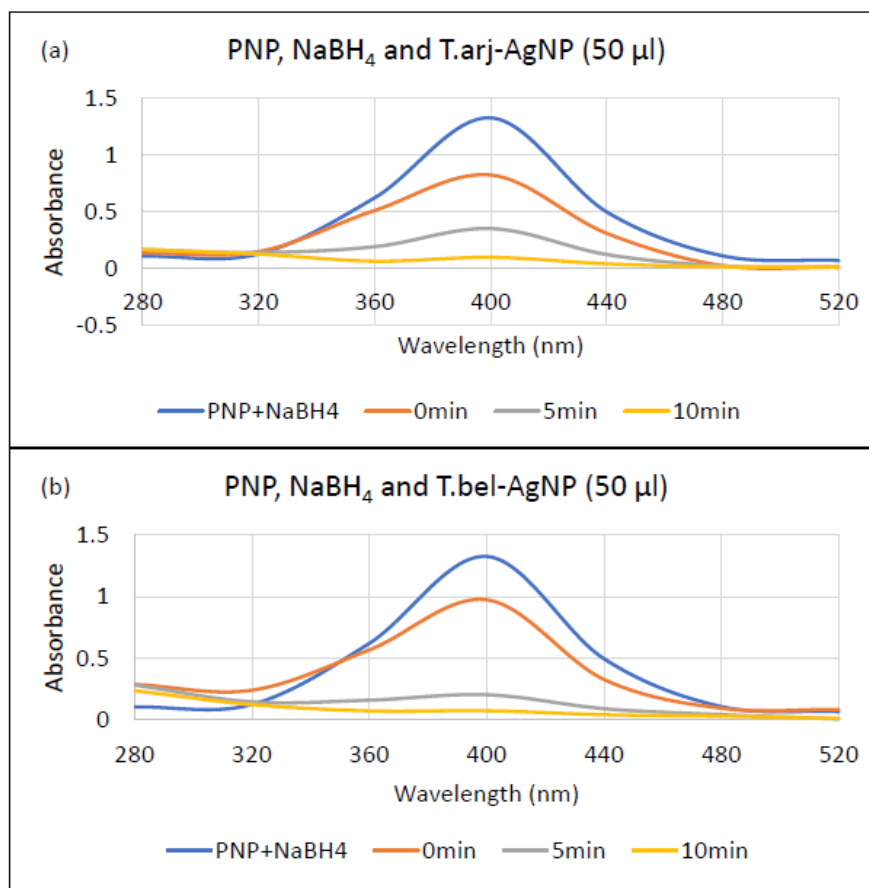


Figure 8 UV-visible absorption spectra for PNP degradation with 50 µL AgNP and 0.1 M NaBH₄: (a) *T.arj*_AgNP and (b) *T.bel*_AgNP.

3.3 Photocatalytic Activity Against MB and MO Using *Terminalia*_AgNPs

No degradation was observed in the experiments without NaBH₄. However, a significant decrease in the MB peak (Figure 9a) intensity was observed with the addition of both 4000 ppm and 266.67 ppm AgNPs in the presence of NaBH₄ (Figure 10, Figure 11, Figure 12), with enhanced degradation observed with 4000 ppm.

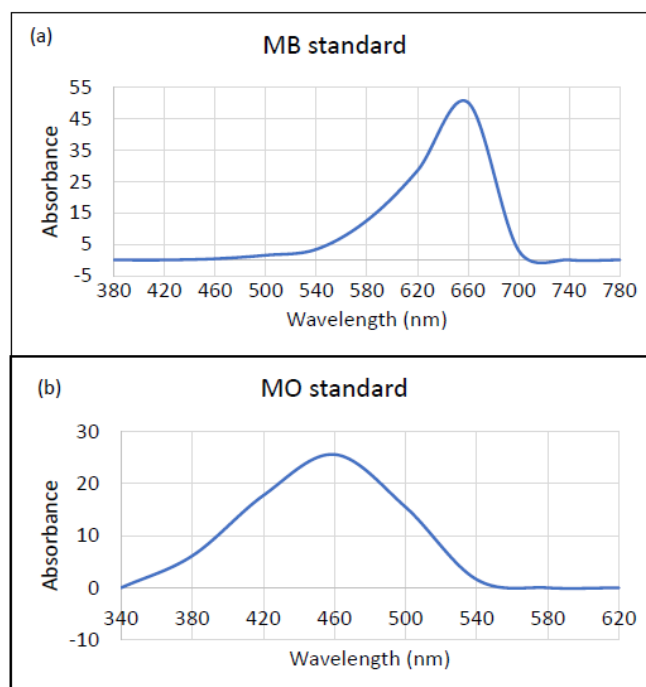


Figure 9 Standard curve for dye: (a) 0.1 mM MB and (b) 0.1 mM MO.

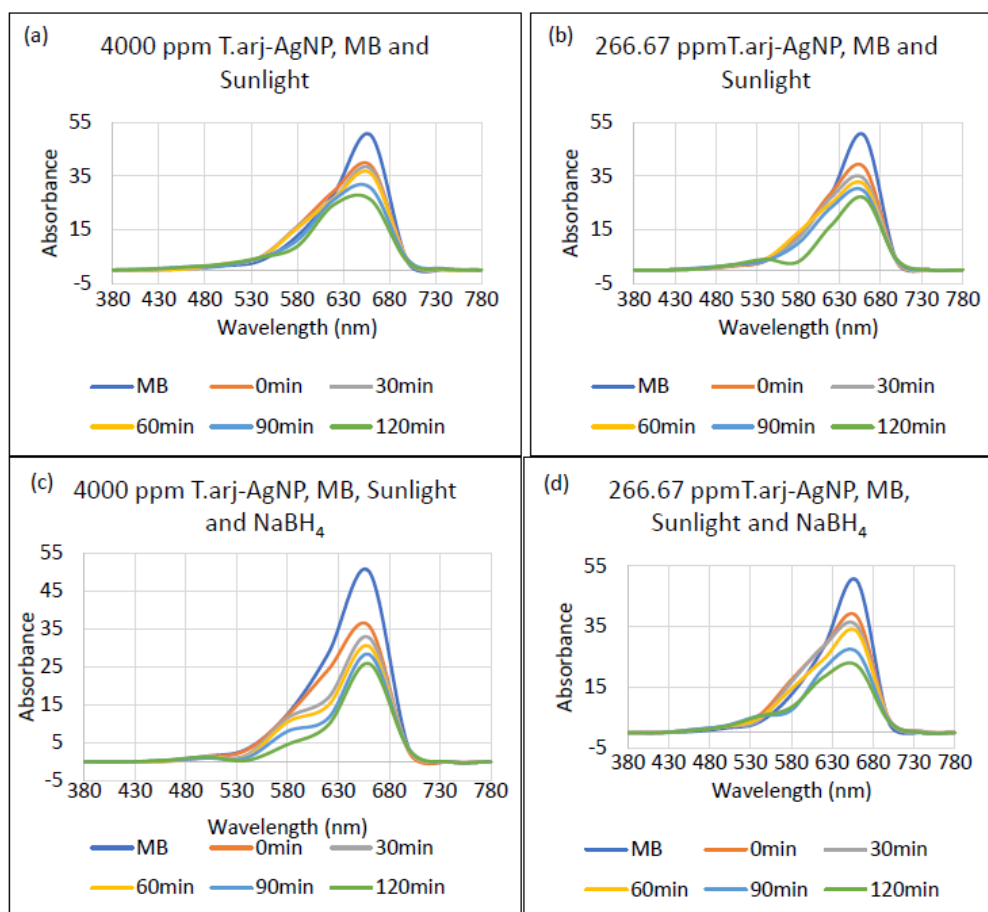


Figure 10 Degradation of 50 mL of 0.1 mM MB dye using 0.5 mL of *T.arj*_AgNP under various conditions: (a) 4000 ppm under sunlight, (b) 266.67 ppm under sunlight, (c) 4000 ppm under sunlight and 0.2 M NaBH₄, (d) 266.67 ppm under sunlight and 0.2 M NaBH₄.

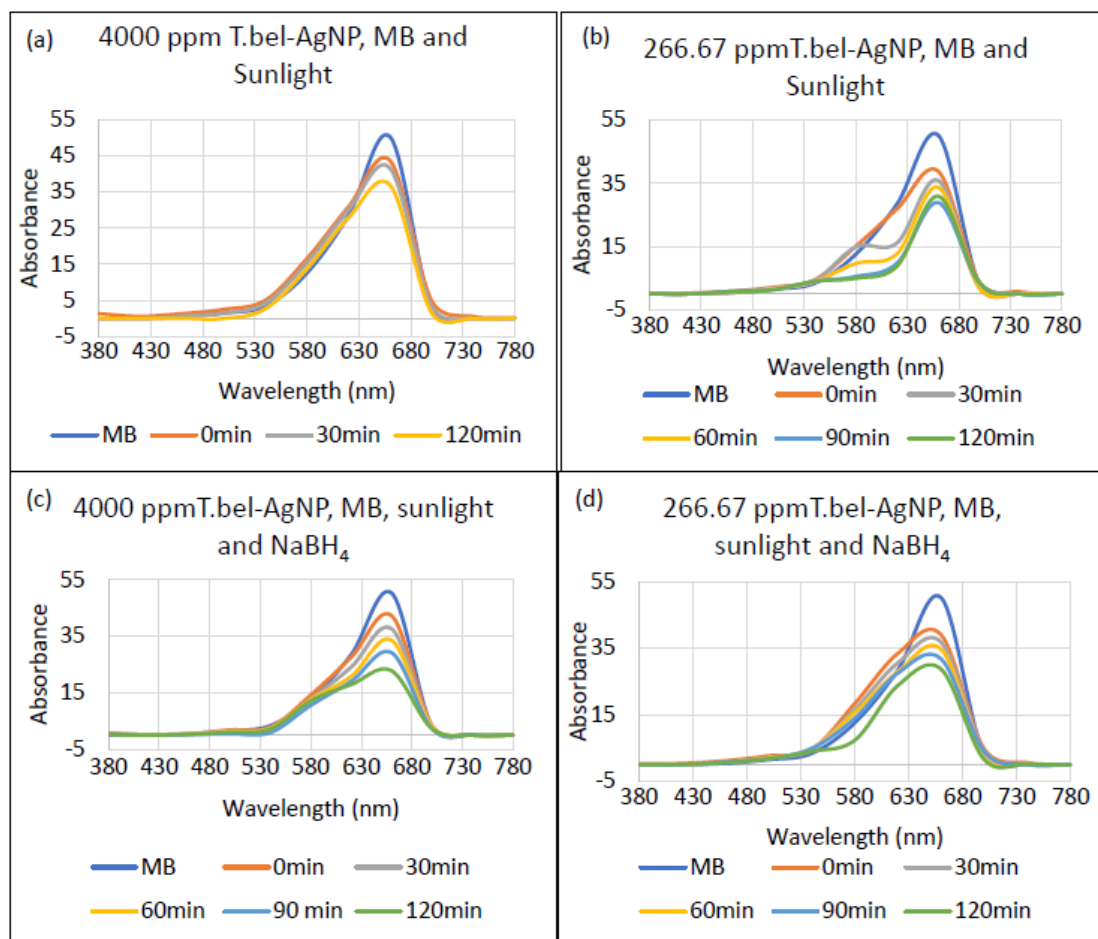


Figure 11 Degradation of 50 mL of 0.1 mM MB dye using 0.5 mL of *T.bel*_AgNP under various conditions: (a) 4000 ppm under sunlight, (b) 266.67 ppm under sunlight, (c) 4000 ppm under sunlight and 0.2 M NaBH₄, (d) 266.67 ppm under sunlight and 0.2 M NaBH₄.

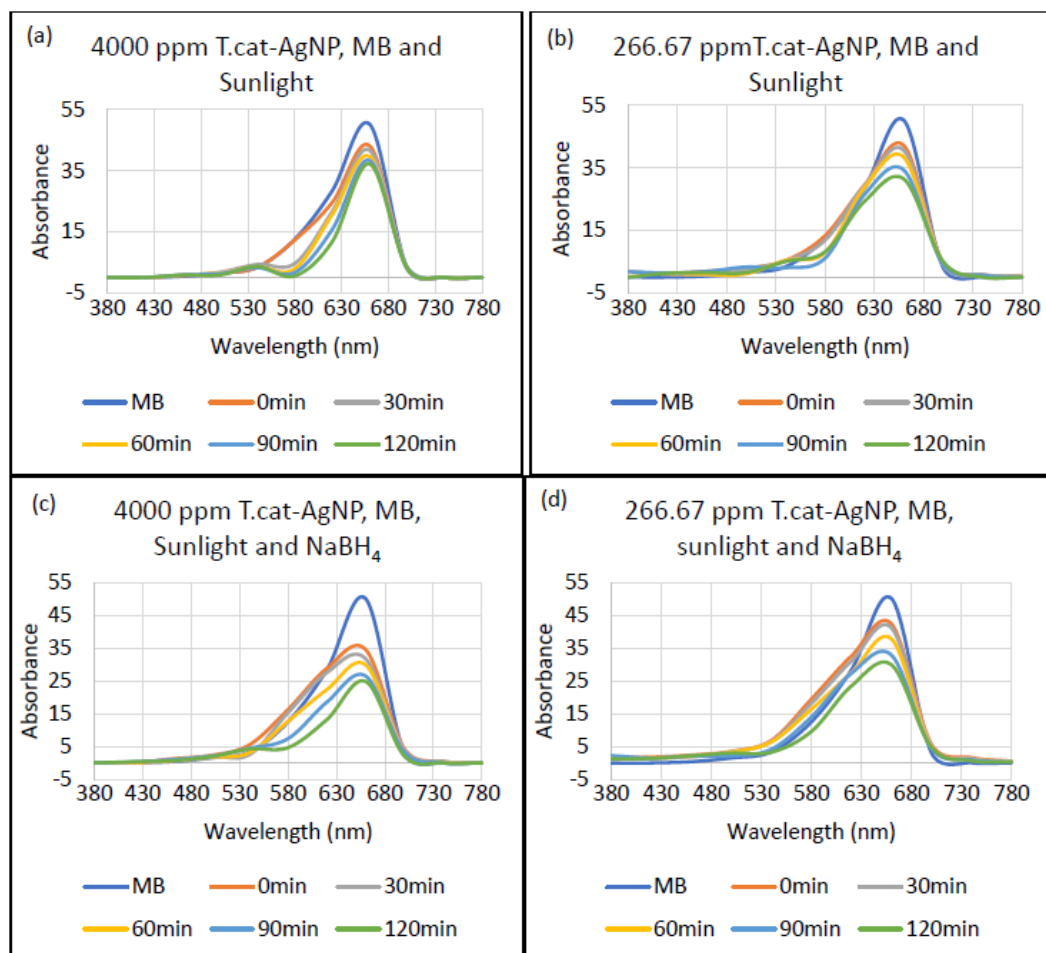


Figure 12 Degradation of 50 mL of 0.1 mM MB dye using 0.5 mL of *T.cat_AgNP* under various conditions: (a) 4000 ppm under sunlight, (b) 266.67 ppm under sunlight, (c) 4000 ppm under sunlight and 0.2 M NaBH_4 , (d) 266.67 ppm under sunlight and 0.2 M NaBH_4 .

No degradation was observed in the experiments without NaBH_4 . In contrast, the MO peak (Figure 9b) intensity decreased significantly with 4000 ppm and 266.67 ppm AgNPs in the presence of NaBH_4 (Figure 13, Figure 14, Figure 15), with greater degradation observed at 4000 ppm. Overall, MO exhibited higher degradation efficiency than MB.

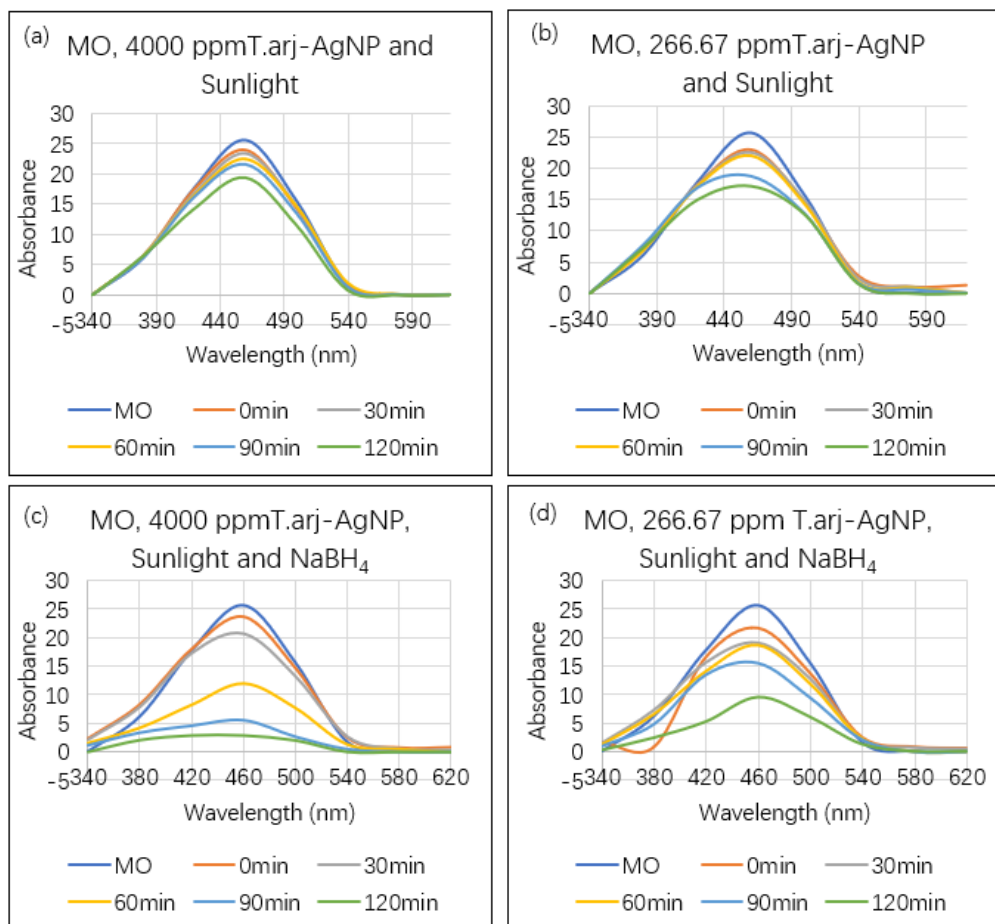


Figure 13 Degradation of 50 mL of 0.1 mM MO dye using 0.5 mL of *T.arj*-AgNP under various conditions: (a) 4000 ppm under sunlight, (b) 266.67 ppm under sunlight, (c) 4000 ppm under sunlight and 0.2 M NaBH_4 , (d) 266.67 ppm under sunlight and 0.2 M NaBH_4 .

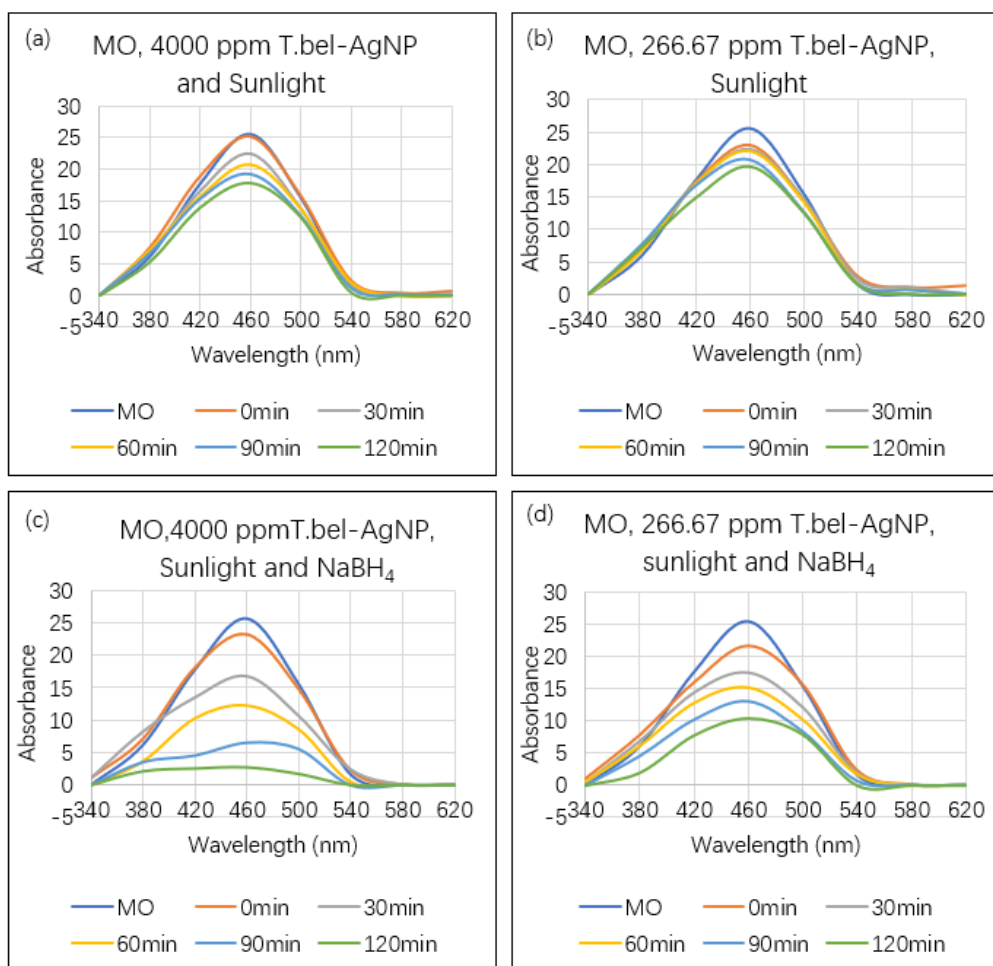


Figure 14 Degradation of 50 mL of 0.1 mM MO dye using 0.5 mL of *T.bel*_AgNP under various conditions: (a) 4000 ppm under sunlight, (b) 266.67 ppm under sunlight, (c) 4000 ppm under sunlight and 0.2 M NaBH₄, (d) 266.67 ppm under sunlight and 0.2 M NaBH₄.

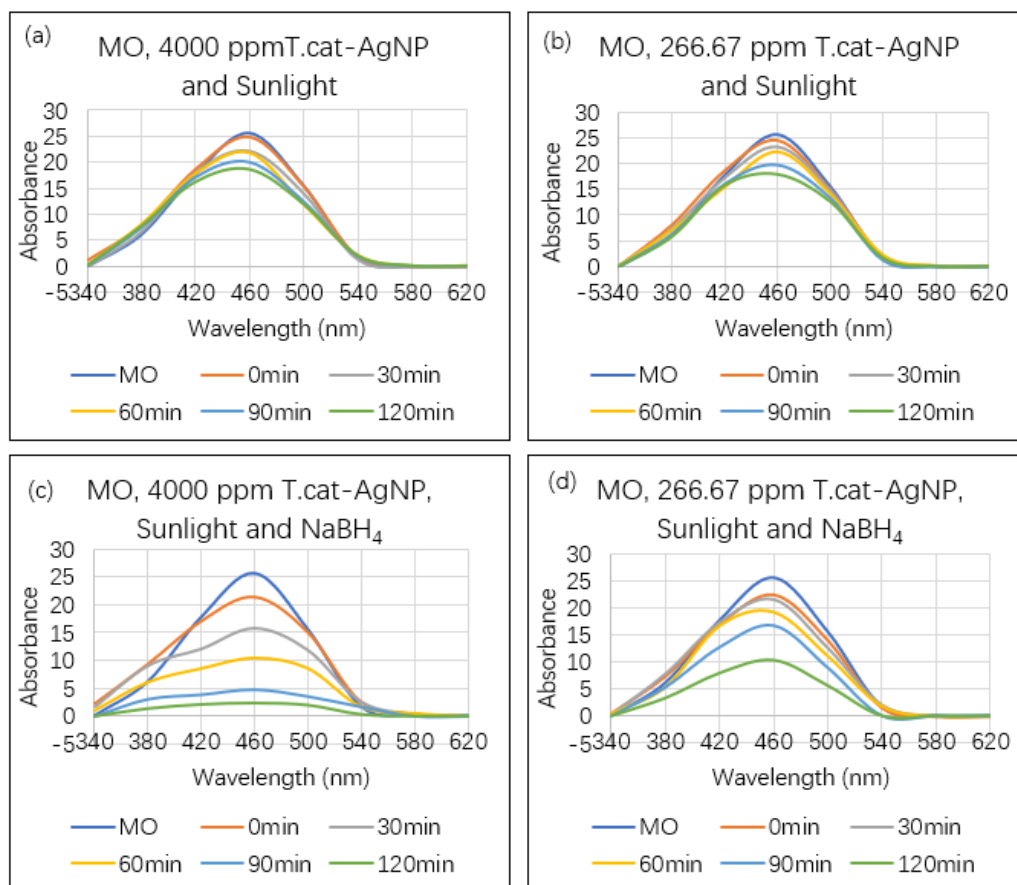


Figure 15 Degradation of 50 mL of 0.1 mM MO dye using 0.5 mL of *T.bel*_AgNP under various conditions: (a) 4000 ppm under sunlight, (b) 266.67 ppm under sunlight, (c) 4000 ppm under sunlight and 0.2 M NaBH₄, (d) 266.67 ppm under sunlight and 0.2 M NaBH₄.

3.4 Cytotoxicity Activity *Terminalia*_AgNPs

The cytotoxicity assay results indicated 100 % viability at 800 ppm and 200 ppm of AgNPs (Figure 16).

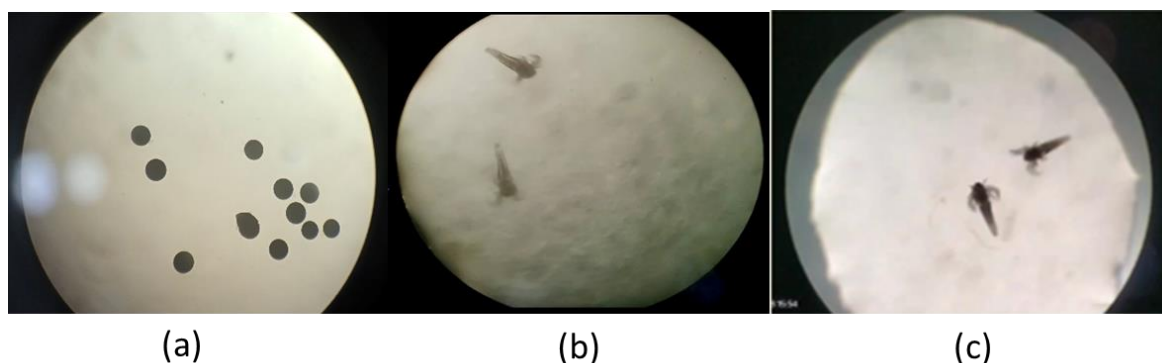


Figure 16 Different developmental stages of *Artemia salina* morphology: (a) eggs, (b) hatchlings before *Terminalia*_AgNP incubation, and (c) hatchlings following AgNP incubation.

4. Discussion

AgNPs contain unique properties that make them valuable in medicine, catalysis, and environmental protection. Conventional methods of AgNPs synthesis are costly, energy-intensive, and environmentally harmful. However, green synthesis using plant extracts offers a sustainable alternative, which utilizes phytochemicals as natural reducing and stabilizing agents [34]. In this study, AgNPs were synthesized using WEs from leaves of four different *Terminalia* species. The research sample was selected because, unlike edible plants, harvesting leaves is a sustainable and ethical option that does not disrupt the food chain [35]. This investigation's primary focus is to evaluate the catalytic properties of synthesized AgNPs and contrast them with past findings in the literature.

Before extraction, an essential step is the shade-drying process, which preserves heat-sensitive bioactive molecules and maintains antioxidant and antimicrobial properties [36, 37]. For extraction, various solvents can be used, including ethanol, methanol, and DW. For this research, which focuses on green synthesis methods, DW is used for extraction as it is also cost-effective, eco-friendly, pure, and safe for human-targeted products such as drugs and food. The polarity feature of DW is another added advantage for efficient extraction of phytochemicals [16, 38]. Previous research has utilized methanol as the extracting solvent to improve phenolic extraction [29, 39]; however, due to its toxicity and additional purification steps, it is not a suitable solvent for use in medicines and food.

During AgNP synthesis, bioactive compounds from plant extracts function as reducing agents and convert Ag^+ to Ag^0 . The subsequent aggregation and stabilization form the final nanoparticle structure, giving them their specific shape and size. Biomolecules also serve as capping agents, preventing further aggregation [40].

In AgNP synthesis, the LSPR effect occurs when incident light excites the free electrons at the nanoparticles' surfaces. This leads to a collective oscillation that produces a distinctive absorption peak in UV-Vis spectra between 420 nm and 480 nm and a color change depending on the LSPR wavelength [41]. AgNP synthesis depends on various conditions, and temperature and time duration were the parameters examined in this research. Among the synthesis conditions, 90°C for 45 minutes (Table 1) was selected as the optimal condition, confirmed by maximum absorbance in the UV-Vis spectroscopy; SPR peaks at 420 nm for *T.arj*_AgNP and *T.bel*_AgNP, and 440 nm for *T.cat*_AgNP (Figure 3).

Previous research has documented successful AgNP synthesis for these species: India for *T.arj*_AgNP at 413 nm [16], for *T.bel*_AgNP at 427 nm [14] for *T.cat*_AgNP at 420 nm [42] in Indonesia and at 434 nm [43] in Saudi Arabia. *T.cheb* did not exhibit AgNP synthesis, however, studies from different countries, such as India [44] and Nepal [45] showed AgNP formation, which can be due to environmental, geographical, or phytochemical variables influencing its stabilizing and reducing properties. However, certain conditions did not produce AgNPs, demonstrating the impact of temperature and reaction time on synthesis efficiency. Further, a darker color change from a lighter color (Figure 4) after incubation indicated AgNP formation, as evidenced by light scattering.

SEM analysis was used to examine the surface shape, size, and distribution of AgNPs [46]. The SEM results revealed that the AgNPs had a spherical and uniform structure and were 40–60nm in size (Figure 5). The consistency of AgNP formation in *T.cat*_AgNP is supported by previous studies by [14, 16] on *T.bel*_AgNP and *T.arj*_AgNP, which reported similar morphologies.

Band gap energy (E) is the minimum energy required for electrons to move from the valence to the conduction band [47]. This study confirmed the semiconductor nature of AgNPs; E = 2.95 eV for *T.arj*_AgNP and *T.bel*_AgNP, E = 2.82 eV for *T.cat*_AgNP, which requires less than 3 eV for excitation [48], determined using the Tauc relation equation (2) [49]. A previous study also documented a band gap energy of 2.8 eV (semiconductor). SPR-induced electron oscillations suggest potential applications in electronics and catalysis [50].

$$E = \frac{h (\text{plank's constant})(6.626 \times 10^{-34} \text{ Js}) \times c (\text{Speed of light})(3 \times 10^8 \text{ ms}^{-1})}{\lambda (\text{Wavelength of AgNP})(10^{-9})} \quad (2)$$

The catalytic activity of AgNPs using PNP-catalysis and photocatalysis plays a crucial role [16]. PNP has an absorption peak at 320 nm in an acidic or neutral solution (Figure 6a). The peak shifts to 400 nm as a result of increased electron delocalization caused by the deprotonation of its hydroxyl group upon the addition of NaBH₄ (Figure 6b). In the absence of a catalyst, the reduction reaction is kinetically slow. Through the adsorption of both para-nitrophenolate and BH₄⁻ ions, biological catalysts such as AgNPs accelerate the response by promoting electron transfer from BH₄⁻ to the nitro group [51]. The color change from yellow to colorless occurs when the 400 nm peak decreases and a 290 nm peak appears, indicating the formation of PAP [52, 53].

In this study, the evaluation of PNP degradation potential was conducted using 20 µL and 50 µL of AgNP. Due to the low volume of *T.cat*_AgNP, an experiment using 50 µL was not performed. After successful PNP degradation at different volumes of *Terminalia* AgNPs (Figure 7, Figure 8), the reaction kinetics were evaluated to determine the reaction rate. This was assessed according to equation (3) following pseudo-first-order reaction kinetics.

$$\ln \frac{C_t}{C_0} = kt \quad (3)$$

where k is the rate constant, and C_t and C₀ represent the concentration of PNP at time interval 't' and at zero, respectively [54].

The rate constant, k, was determined using the linear regression slope, which showed that in the presence of 20 µL AgNPs, the rates followed the order of *T.bel*_AgNP > *T.arj*_AgNP > *T.cat*_AgNP within 35 minutes with a color change from yellow to colourless. However, 50 µL AgNPs degraded PNP in 10 minutes, with *T.bel*_AgNP showing the highest rate. This suggests that degradation efficiency is in the order of *T.bel*_AgNP > *T.arj*_AgNP > *T.cat*_AgNP (Table 2). The rate constant indicates a correlation in which higher AgNP volumes lead to faster degradation and greater efficiency, due to higher levels of natural reducing agents. Compared to a previous study by [16], the current study achieved a higher rate constant (0.4013 min⁻¹), despite a 40× higher PNP volume and a 500× lower concentration. Variations in volume and concentration of PNP and NaBH₄ caused the faster degradation.

Table 2 Rate constants for the kinetic study of PNP degradation using 20 μL and 50 μL of *Terminalia*_AgNPs.

Sample	20 μL of AgNP (min^{-1})	50 μL of AgNP (min^{-1})
<i>T.arj</i> _AgNP	0.1856	0.338
<i>T.bel</i> _AgNP	0.1889	0.4013
<i>T.cat</i> _AgNP	0.1561	-

AgNPs degrade azo dyes such as MB and MO through photocatalysis under sunlight. Exposure to light leads to the SPR effect and interband transitions, enabling electron excitation from the valence band to the conduction band upon photon absorption. This ultimately leads to the generation of radicals, hydroxyl, and oxygen, which break the azo bonds in the dye molecule. Additionally, AgNPs act as a relay surface, facilitating successful electron transfer between the dye and NaBH_4 and further enhancing the reduction by donating electrons and hydrogen atoms [55, 56]. The successful photocatalytic degradation of MB is initially observed as a colour change from deep blue to light blue, and finally to colourless, upon exposure to AgNPs. Further confirmation of degradation is provided by the absorption spectrum, which shows that the absorption peak at 660 nm of MB (Figure 9a) gradually decreases with increasing exposure time to AgNPs, and complete degradation occurs when the absorption peak reaches the baseline [57]. Similarly, in MO, the colour changes from orange to colourless, and the decrease in the absorption peak at 460 nm (Figure 9b) indicates successful MO degradation upon exposure to AgNPs [58].

Enhanced degradation is observed in the addition of NaBH_4 with 4000 ppm and 266.67 ppm AgNP in MB degradation (Figure 10, Figure 11, Figure 12) and MO degradation (Figure 13, Figure 14, Figure 15). The reaction kinetics were also evaluated using equation 3, following pseudo-1st-order kinetics.

In this study, based on the linear regression slope, the MB degradation rate was higher with 4000 ppm AgNPs in the order of *T.arj*_AgNP > *T.bel*_AgNP > *T.cat*_AgNP. The overall degradation rate increased with the addition of NaBH_4 for both 4000 ppm and 266.67 ppm AgNPs. However, the highest rate with the addition of NaBH_4 is observed with 266.67 ppm of *T.arj*_AgNP ($K = 0.0647 \text{ min}^{-1}$), and the lowest rate with the addition of NaBH_4 is observed with *T.cat*_AgNP ($K = 0.0426 \text{ min}^{-1}$) (Table 3). These behaviors are likely due to improved dispersion and increased active site availability, which enhance electron transfer.

Table 3 Rate Constant for the Kinetic study of MB Degradation at different conditions.

Sample	4000 ppm AgNP		266.67 ppm AgNP	
	Sunlight (min^{-1})	Sunlight and NaBH_4 (min^{-1})	Sunlight (min^{-1})	Sunlight and NaBH_4 (min^{-1})
<i>T.arj</i> _AgNP	0.0508	0.0501	0.0494	0.0647
<i>T.bel</i> _AgNP	0.0425	0.0600	0.0419	0.0430
<i>T.cat</i> _AgNP	0.0236	0.0541	0.0375	0.0426

In the MO degradation study, the overall degradation rates were higher with 266.67 ppm AgNPs under sunlight, and they increased further with the addition of NaBH_4 . Under sunlight, the highest rate of degradation is observed with *T.bel*_AgNP ($K = 0.0338 \text{ min}^{-1}$). With the addition of NaBH_4 ,

4000 ppm AgNPs showed higher rates compared to 266.67 ppm AgNPs in the order of *T.cat*_AgNP > *T.arj*_AgNP > *T.bel*_AgNP (Table 4).

Table 4 Rate Constant for the Kinetic study of MO Degradation at different conditions.

Sample	4000 ppm AgNP		266.67 ppm AgNP	
	Sunlight (min ⁻¹)	Sunlight and NaBH ₄ (min ⁻¹)	Sunlight (min ⁻¹)	Sunlight and NaBH ₄ (min ⁻¹)
<i>T.arj</i> _AgNP	0.0213	0.1962	0.0329	0.0738
<i>T.bel</i> _AgNP	0.0338	0.1917	0.0201	0.0762
<i>T.cat</i> _AgNP	0.0274	0.2120	0.0304	0.0676

A similar study conducted previously [16] demonstrated rapid degradation within 14 minutes with a lower rate constant (MO: 0.166 min⁻¹; MB: 0.138 min⁻¹), which may be due to differences in AgNPs concentration, dye concentration, or reaction conditions. Overall, MO exhibited higher kinetic degradation than MB in both studies, indicating a stronger catalytic interaction with AgNPs. The structural differences between MO and MB can also lead to different reaction rates; MO contains two aromatic rings, whereas MB contains three adjacent aromatic rings, which increases MB's stability [59].

AgNPs are widely recognized to cause cytotoxicity by producing reactive oxygen species that impair cellular processes, causing oxidative stress, membrane damage, and apoptosis [60, 61]. Research has documented that the interaction of silver atoms in AgNPs with the chitin layer of *A. salina* leads to cellular and cuticle damage [62]. This research showed 100% viability for brine shrimp (Figure 16), suggesting that there were no harmful effects at 200 ppm and 800 ppm of *Terminalia*_AgNPs. The lack of toxicity suggests that *Terminalia*_AgNPs exhibit biocompatible qualities or release fewer reactive oxygen species. A similar study conducted [3] on zebrafish showed comparable results, but using brine shrimp as a model organism is novel for this plant species. These findings suggest that AgNPs may have potential without significant cytotoxic effects.

5. Conclusions

This study explores a green synthesis approach to synthesize AgNPs using *Terminalia* leaf extract. The findings of this research highlight success in AgNP synthesis from *T. arjuna*, *T. bellirica*, and *T. catappa*. The optimum synthesis condition was determined to be 90°C for 45 minutes, and SEM analysis showed spherical *T.cat*_AgNPs with a size range of 40 nm to 60 nm. The conductivity study showed semiconductor properties ($E = 2.95$ eV for *T.arj*_AgNP and *T.bel*_AgNP, and $E = 2.82$ eV for *T.cat*_AgNP). *Terminalia* AgNPs exhibited efficient PNP degradation, with a higher AgNP concentration; the highest rate was 0.4013 min⁻¹ with 50 µL of *T.bel*_AgNP. These findings confirm the potential of *Terminalia* leaf AgNPs as catalysts in environmental remediation. Further confirmation of *Terminalia* leaf AgNPs as a potential substitute in environmental remediation was confirmed by MB and MO degradation. It was established that the lowest rate of 0.0236 min⁻¹ was with 4000 ppm *T.cat*_AgNP, and the maximum rate of 0.0647 min⁻¹ was with 266.67 ppm *T.arj*_AgNP and NaBH₄ for MB degradation. In MO degradation, 4000 ppm *T.cat*_AgNP and NaBH₄ showed the highest rate of 0.2120 min⁻¹. The current study also demonstrated 100% viability of *A. salina* after 24 hours of exposure to 800 ppm and 200 ppm AgNPs, confirming the safety profile of *Terminalia*

leaf AgNPs. However, to improve the effectiveness of *Terminalia* leaf AgNPs in real-world applications, further research is needed on their safety profile in animal models. These significant findings on PNP degradation, photocatalytic degradation, and cytotoxicity studies emphasize the potential of *Terminalia* leaf AgNPs as catalysts.

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Author Contributions

Dean and senior supervisor Mathivathani Kandiah carried out the planning and design of the research and analyzed with revision of manuscript. Experiments were executed and recorded by Sivaniya Vinayagamoorthy. Beneli Gunaratne and Ominda Perera have overlooked the experiment work.

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Competing Interests

The authors have declared that no competing interests exist.

Data Availability Statement

Data relevant to this research can be requested from the authors at mathi@bms.ac.lk.

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