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Madhuca longifolia leaf extract mediated synthesis of ZnO nanoparticles and their Antibacterial, Antioxidant, and Photocatalytic activity

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ABSTRACT

The biogenic synthesis of ZnO NPs is a promising substitute for the standard method of NP synthesis. In the current study, ZnO nanoparticles were produced biologically. Leaf extract from the *Madhuca longifolia* (M-ZnO NPs) plant was used to create ZnO NPs, which were then examined using UV-vis, XRD, FTIR, SEM, and TEM. SEM and TEM examination and XRD validated the size and crystalline nature of Zinc oxide nanoparticles, respectively. Functional groups involved in the production of ZnO NPs were visible in the FTIR spectra. By scavenging DPPH free radicals at various concentrations, the antioxidant activity of green ZnO NPs was determined. By using the agar well diffusion method, ZnO NPs were tested for their bactericidal potential against Gram-negative bacteria *E. coli* and Gram-positive bacteria *Staphylococcus aureus* bacterial strains. A 96% photodegradation of MB dye and 91% degradation of textile wastewater was observed in green-produced ZnO NPs when exposed to sunshine. The recent work proved that ZnO NPs have substantial antioxidant, antibacterial, and photocatalytic activity. Therefore, the study offers a straightforward, practical, economical, and ecologically secure green synthesis technique for the biofabrication of multifunctional ZnO nanoparticles.

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INTRODUCTION

Nanotechnology is a rapidly growing and widely accepted research field in modern material science with the purpose of synthesis of new materials at the nanoscale level. Many researchers reported that nanomaterials have various functions and huge efficiency that are not noticed in their bulk phase and nano-dimension particles are extensively studied due to having electronic, magnetic, catalytic, optical, antimicrobial, wound healing, and anti-inflammatory properties [1].

Zinc oxide is a well-known semiconductor (n-type) with low-coat, nontoxic, and photocatalytic properties. ZnO has a wide bandgap semiconductor with 3.2 eV and photocatalytic activity is a commonly investigated function of semiconductors [1]. Zinc oxide nanoparticles are well known for their wide range of applications in diverse fields. Due to the extremely low toxicity of ZnO nanoparticles, recent research works have paid high attention to their function and employment, especially in the biomedical field property. The demand and production of green ZnO NPs increased gradually because a better eco-friendly alternative is required to avoid the use of high-energy inputs and toxic chemicals.

Looking at the harmful and toxic effects of nanomaterials on the environment, current research focused on a low-toxic, cost-effective, and eco-friendly green approach. The biological process of NPs synthesis using plant extracts, microorganisms, algae, and enzymes is considered a healthier substitute for existing processes of NPs synthesis because of their hazards to the environment [2, 3].

Nanotechnology has offered a ray of hope in the biomedical field as an antimicrobial and drug-delivery agent for various diseases. However, the mechanisms of the bactericidal potential of ZnO NPs are still unknown, but Rajani *et al.*(2022) [4]; explain some mechanisms such as membrane disruption, generation of reactive oxygen species (ROS), disruption of the cell wall, leakage of nuclear and cytoplasmic material by the action of nanomaterials.

Textile wastewater contains a huge amount of non-biodegradable hazardous dyes which causes some serious health and environmental issues. It is an immediate need to introduce a promising technology for dye removal with high efficiency and low toxicity. Nanotechnology has emerged as one of the prime technology with great potential for the degradation of textile dyes from wastewater more efficiently and effectively than previous methods. Current methods involve in the treatment of wastewater and water purification, are disgraced due to their insufficiency in water purification and high-cost demands. Nanotechnology can help to conquer this issue by removing organic dyes, heavy metal pollutants, pesticides, and other chemical pollutants from water that are related to a serious hazard to the ecosystem because of their toxicity to water inhabitants and every living organism, including humans [5].

ZnO NPs are semiconductors that have photocatalytic properties so ZnO NPs can photodegrade textile dyes efficiently. ZnO NPs are widely employed as photocatalysts in the photodegradation of organic pollutants in air and water [6]. Vasantharaj *et al.* (2021) [7]; was successfully reported the degradation of synthetic textile dyes by green synthesize ZnO NPs using *Ruellia tuberosa* plant extract.

In this study, *Madhuca longifolia* plant leaf extract was applied for the facile green synthesis of ZnO nanoparticles. The phytochemistry study of *M. longifolia* plant shows that it is rich in protein, sugar, alkaloids, vitamin, phenolic compounds, sapogenins, triterpenoids, steroids, saponins, flavonoids, glycosides, etc. [8, 9]. ZnO nanoparticle synthesis using *M. longifolia* leaf extract is an environmentally benign synthetic method. *M. longifolia* has higher constituents of phenolic compounds which are amenable to the ZnO NPs synthesis. These bio-compounds have reducing properties and acted as capping agents in the nanoparticle synthesis process.

The bio-synthesized *M*-ZnO NPs were investigated for their antioxidant, antibacterial, and photocatalytic activities. The photodegradation performances of methylene blue (MB) dye by the green synthesized ZnO nanoparticles under sunlight irradiation were investigated.

EXPERIMENTS

Materials and Methods

Leaves of *Madhuca longifolia* were taken from the local garden of Jaipur, India. Plant leaves were cleaned with distilled water and dry to grind to make their powder. 5 g of *M. longifolia* plant leaves powder boiled in 100 ml distilled water for 20 minutes. After cooling, leaf extract was filtered using Whatman's filter paper and stored in the refrigerator for further use. Zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) was used for ZnO NPs synthesis. MB dye was purchased from Thermo Fisher Scientific company, India. Textile dye samples were collected from the textile industry area, Sanganer, Jaipur.

Biosynthesis of ZnO NPs

8 gm zinc nitrate was added into 25 ml distilled water and heated at 60^{o}C. 50 ml *M. longifolia* plant leaf extract was added drop by drop into the heated zinc nitrate solution under the continuous stirrer. The solution was continuously stirred until a pale yellow colour paste was obtained. The paste was dried in the furnace at 400^{o}C for 2 hours. The ZnO NPs were collected and stored for further characterization and applications.

Characterization

The bio-synthesized ZnO NPs were sonicated before characterization and every application for uniform dispersion and obtaining higher efficiency. The ZnO NPs synthesis was confirmed by the UV-Vis spectroscopy of the compounds. The UV-Vis absorption peak of ZnO NPs centred at 376 nm. The optical analysis was carried out by the UV-Vis spectrophotometer (GENESIS 180). The morphology and size of ZnO NPs were analyzed by SEM images (Model-ZESIS), at a working distance of 10 mm and a voltage of 20 kV. Transmission electron microscopy was performed using a TALOS HR-TEM apparatus operating at 80 kV. The crystalline structure and grain size were confirmed by XRD using Rigaku (Model-SMART LAB) diffractometer. The functional groups

and chemical composition of ZnO NPs were studied by using an FTIR spectrometer (Bruker ALPHA).

Antioxidant activity

The free radical scavenging capacity of green synthesized ZnO NPs was evaluated against DPPH radical. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) provides an easy method of determining antioxidant activity. DPPH was offered as a free radical source and ZnO NPs were applied as a radical scavenger. In the presence of ZnO NPs, the DPPH solution's deep violet colour gradually turns pale yellow. The absorbance at 517 nm gradually decreases as ZnO NP concentration increases. The ZnO nanoparticles' ability to scavenge free radicals is assessed by the reduced absorbance in the DPPH solution. M-ZnO NPs were treated in ten different doses to test the antioxidant potential of ZnO nanoparticles.

Antibacterial assessment

M-ZnO NPs were tested for their bactericidal activity against *S. aureus* and *E. coli* bacterial strains using an agar well diffusion assay. It is a relatively quick and effective test to determine antimicrobial activity. Different concentrations of *M*-ZnO NPs were pipetted into the well of agar plates. After that, the plates were incubated at 37 ^{0}C in the bacterial incubator for 24 hours. The bactericidal activity of *M*-ZnO NPs was in the form of a diameter of the Inhibition Zone (IZ). After 24 hours, the inhibition zones were observed.

Photocatalytic activity of ZnO NPs

The prepared green ZnO NPs examined for the photocatalytic degradation of textile dyes. The photocatalytic activities of *M*-ZnO NPs was assessed for dye methylene blue and dye-containing textile wastewater. The degradation of dye was observed in three different conditions-1.) Dye degradation in direct sunlight with *M*-ZnO NPs, 2.) Dye degradation in sunlight without NPs, 3.) Dye degradation in dark (without sunlight) with *M*-ZnO NPs.

After certain time intervals, degradation of pollutants was observed and absorbance of dye pollutants at their respective wavelength was used to measure the residual dye amount measured using a UV-vis spectrophotometer.

RESULT AND DISCUSSION

Characterization of ZnO nanoparticles

UV visible studies

UV-vis absorption spectra are used to evaluate the optical properties of nanoparticles. UV- vis absorption spectra of green ZnO NPs shown in Figure 1. UV-vis absorption spectra reveal the mono dispersion of zinc oxide nanoparticles. The wavelength of absorption peaks of ZnO nanoparticles at 376 nm can be allied with ZnO intrinsic band-gap absorption. This happened because of the bounce of e^{-} from the valence to the conduction band [10].

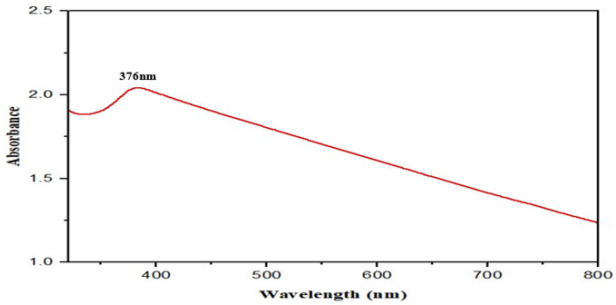

Figure 1: Uv-vis absorption spectra of M-ZnO NPs

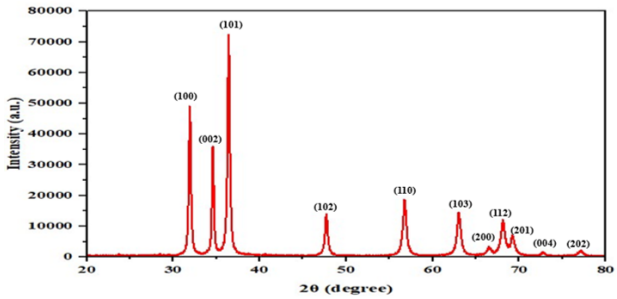

Figure 2: XRD pattern of M-ZnO nanoparticles

XRD analysis

X-ray diffraction is used to determine the phase and crystallographic structure of a material. The green synthesized ZnO nanoparticles were characterized using powder XRD to confirm the nanoparticles as zinc and to examine the structural information and crystalline behaviour. The XRD profile of the optimized green ZnO NPs is reported in Figure 2. The prominent XRD peaks were obtained at 2θ values of 31.79°, 34.43°, 36.28°, 47.53°, 56.5°, 62.79°, 67.83°, and 68.67° Bragg peaks corresponding entirely to (100), (002), (101), (102), (110), (103), (112) and (201) indicating crystalline wurtzite structure of ZnO NPs and this data matched with JCPDS No. 36-1451 [11, 12].

SEM analysis

SEM images are used to predict the structural morphology of particles. SEM image of ZnO NPs (Figure 3) observed that the shape of most of the particles is spherical and the average size is between 70-120 nm.

The SEM analysis of ZnO NPs coupled with the EDX spectrum confirms the zinc oxide particles and the presence of other elements.

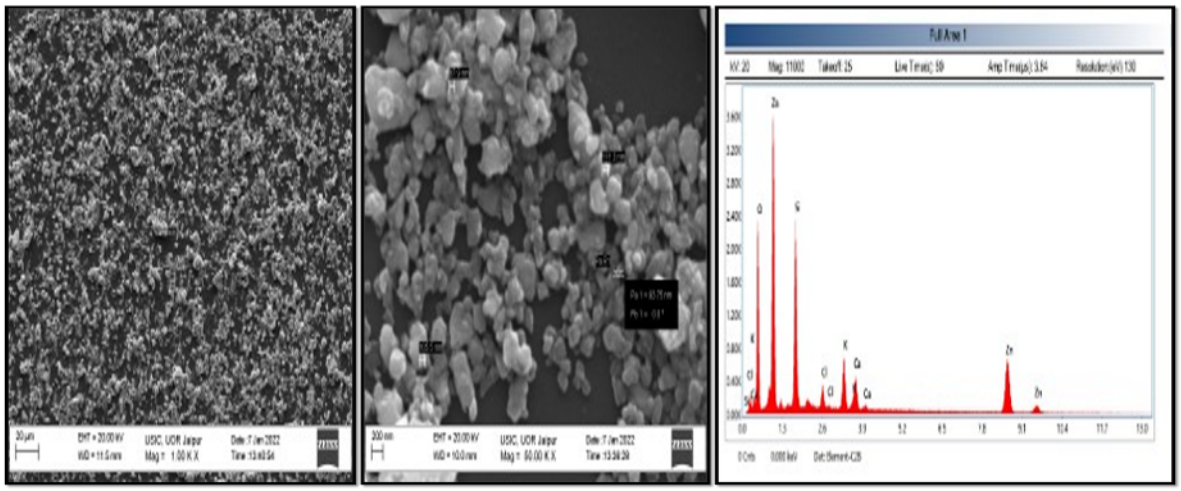

Figure 3: SEM image of M-ZnO nanoparticles at different magnifications

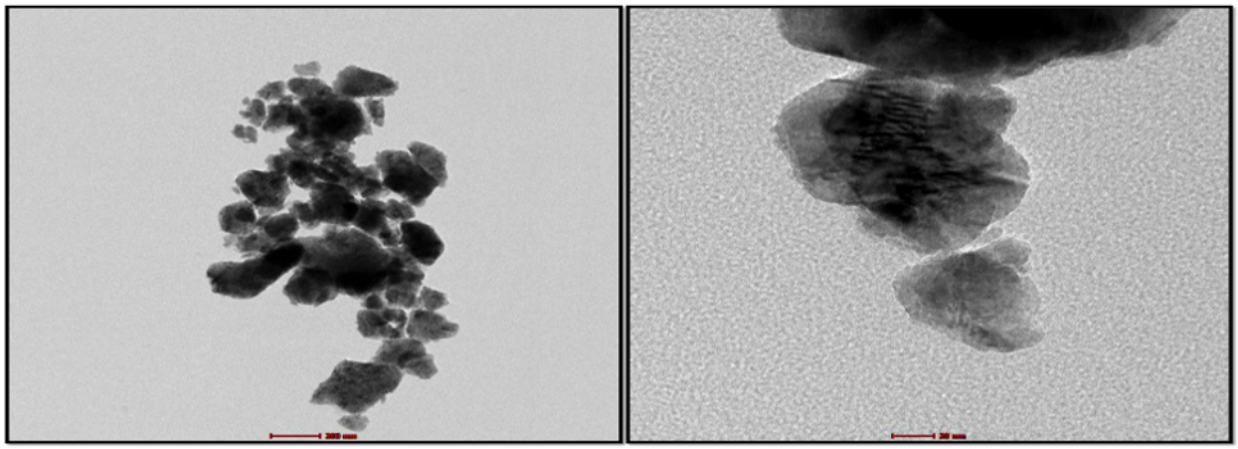

Figure 4: HR-TEM micrograph of M-ZnO NPs at different magnifications

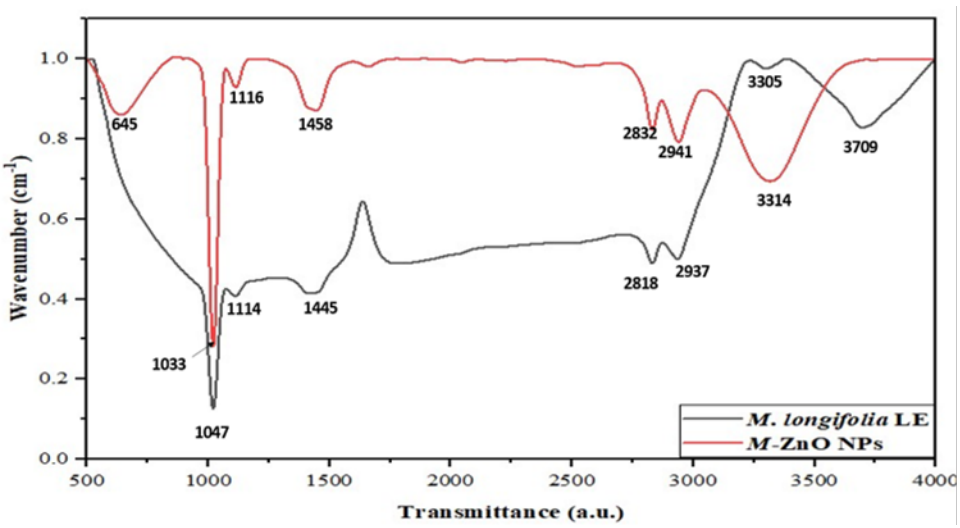

Figure 5: FTIR spectra of *M. longifolia* leaf extract and M-ZnO NPs

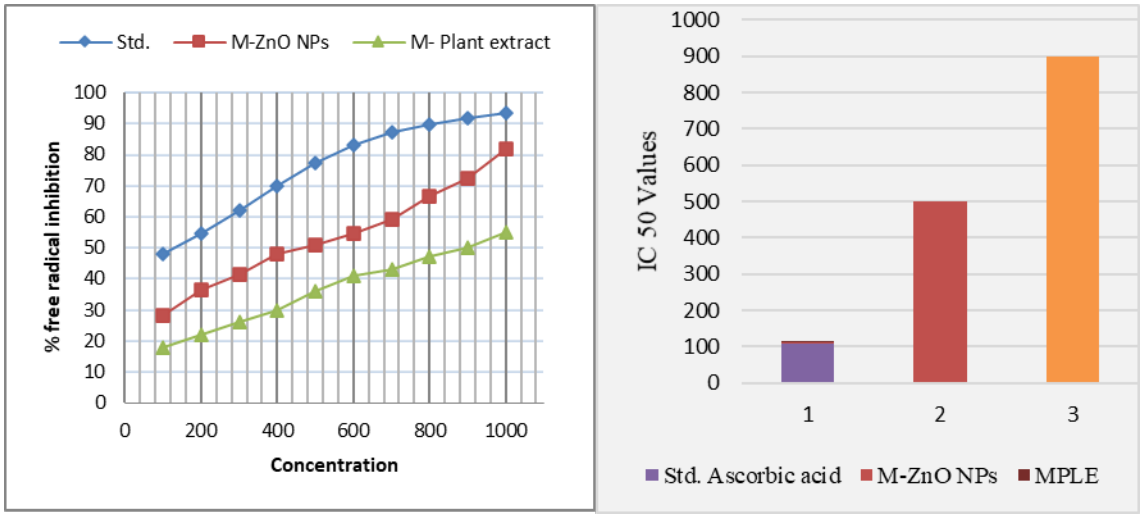

Figure 6: Free radicals inhibition percentage of *M. longifolia* leaf extract and M-ZnONPs and their IC_{50} values

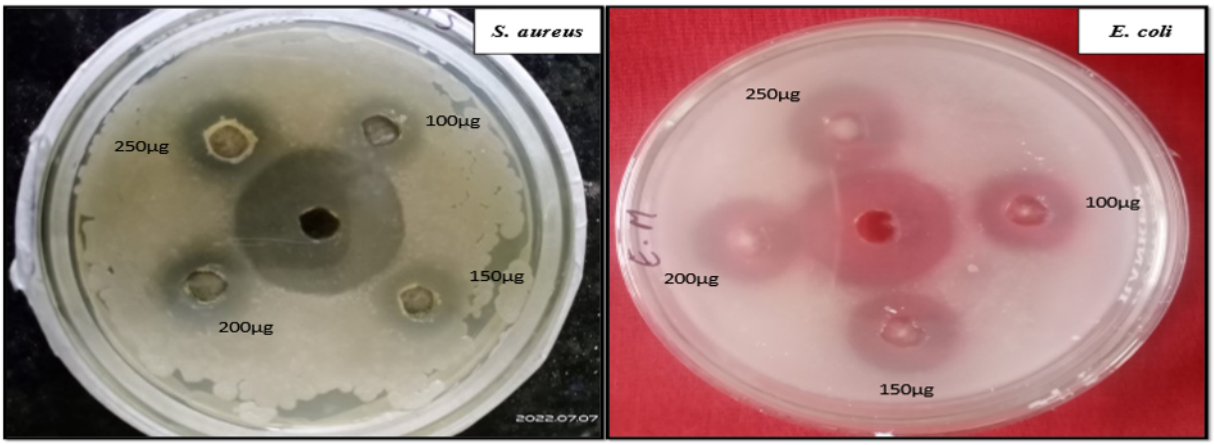

Figure 7: Inhibition zone of *M*-ZnO NPs against *S. aureus* and *E. coli* bacteria at different concentrations

Table 1: Antibacterial activity of *M. longifolia* leaf extract mediated synthesized ZnO nanoparticles

Bacteria	Inhibition Zone (mm)				
	Standard	100μg/ml	150μg/ml	200μg/ml	250μg/ml
S. aureus	32	16	17	19	19
Escherichia coli	31	19	20	22	23

The elemental inspection by the EDX spectrum shows that zinc is the major element that is present in the highest amount. Oxygen is also observed in the EDX spectrum which is related to the formation of ZnO NPs. Other elements observed in the EDX profile, are correlated with plant extracts that were used for ZnO NPs synthesis. Silica impurities were observed due to the glass slide used for thin film formation.

TEM analysis

High resolution of the TEM was used to analyze the shape, size, and density of nanoparticles. The TEM image at different magifications (20 and 200 nm) are hown in Figure 4. ZnO are quite monodisperse and nearly spherical in shape. However, some larger aggregates were also observed in the sample image because the aggregation is the result of the high surface energy of ZnO nanoparticles.

FTIR analysis

The functional groups found in plant extracts that were utilised in the production of ZnO NPs were confirmed by FTIR spectroscopic analysis. For bio-reduced ZnO NPs, phytochemicals function as capping agents. The bio-reduction of ZnO NPs is carried

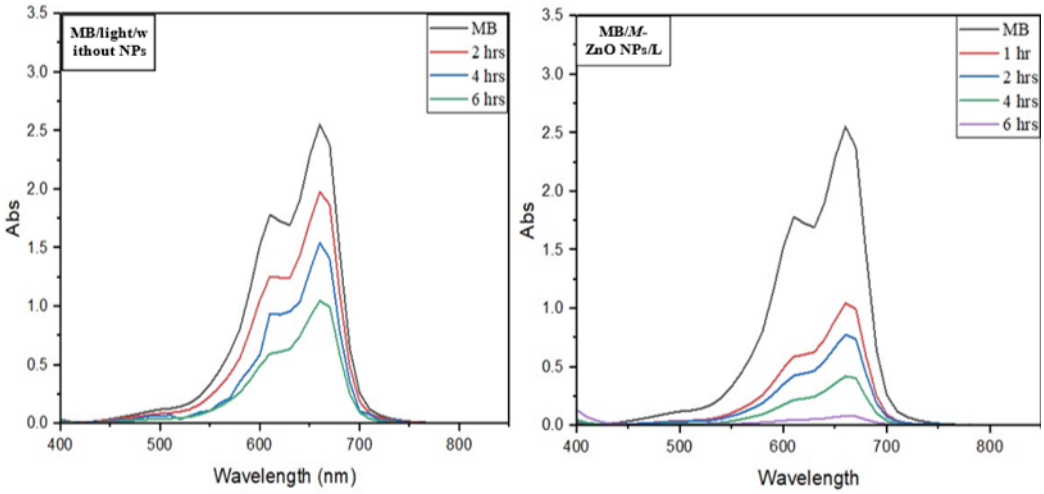

Figure 8: MB dye degradation in the absence and presence of *M*-ZnO NPs under sunlight illumination

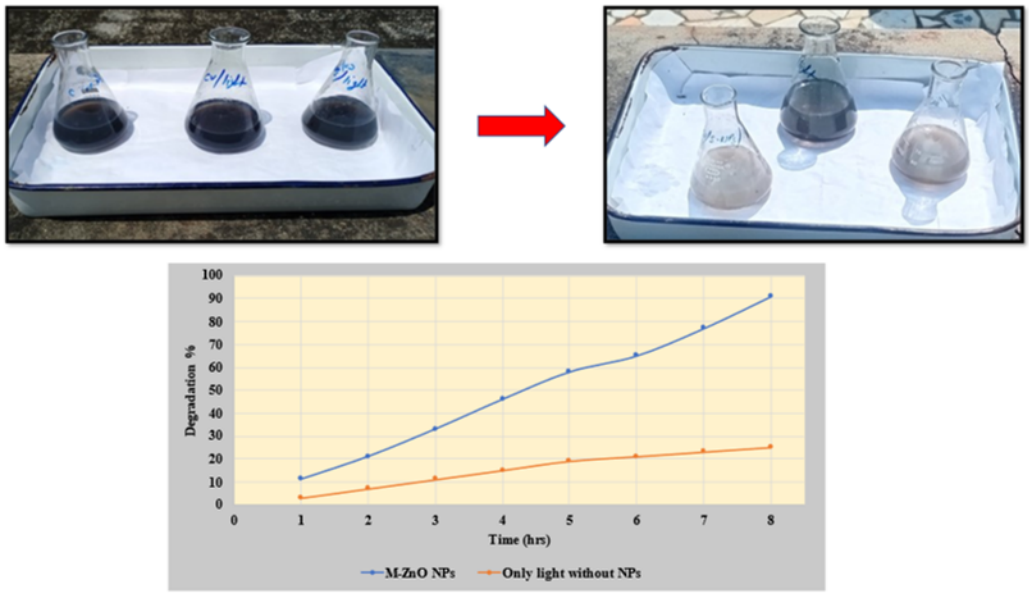

Figure 9: Dye degradation percentage of textile wastewater in the presence and absence of ZnO NPs

out using predetermined wave numbers to identify specific bond vibration peaks in the FTIR method. Figure 5 displays the FT-IR spectra of M. longifolia leaf extract and M-ZnO NPs. Because ZnO NPs span between 400 and 800 cm-1, the band at 645 is a sign of their presence. The absorption bands at 1047, 1114, 1445, 2818, 2937, 3305, and 3709 cm^{-1} in *M. longifolia* leaf extract wound be shifted to 645, 1033, 1116, 1458, 2832, 2941, 3314 cm^{-1} in synthesized ZnO NPs. The absorption between 3200-3400 (peak band at 3314, 3305) indicates the O-H stretch of the phenol group [13]. The peak at 2818, 2937 for *M. longifolia* leaf extract and 2832, 2941 for *M*-ZnO NPs is because of C-H stretching vibrations in the aromatic compound. Further, the peak at 1033 and 1047 indicate the starching between C-O of polyphenol. The absorption region at 942–1714 cm^{-1} is due to C=N, C=O, NH, and C=C stretching vibrations of aromatic compounds [14].

Antioxidant activity of green ZnO nanoparticles

The antioxidant potential of green synthesized ZnO NPs was evaluated against DPPH radical. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) provides an easy method of determining antioxidant activity. DPPH was used as the free radical source and ZnO NPs were used as a radical scavenger. In the presence of ZnO NPs, the deep violet colour of the DPPH solution gradually changed into pale yellow colour. With the increase of ZnO NPs concentration, absorbance at 517 nm gradually decreases. The

decrease in absorbance gives evidence of the radical scavenging ability of ZnO NPs.

DPPH solution was prepared by dissolving 0.004gm DPPH in 100 mL of 80% methanol. In the presence of a free radical scavenger (ZnO NPs) that can change in absorbance at 517 nm which was determined spectrophotometrically. 80% of methanol was prepared by dilution of methanol with distilled water. In brief, a series of with 10 various concentrations values from 100μl/ml to 1000μl/ml of ZnO NPs solution was added and mixed with 3ml DPPH solution. ZnO NPs solution was made by dispersing 1mg NPs in 10ml distilled water. The mixture was left in dark for 30 minutes. The absorbance against a blank (80% methanol), and a control sample (DPPH+ methanol) without the addition of a test sample and a series of different concentrations of the test samples (ZnO NPs) was recorded at 517 nm. The absorbance difference between control and test samples is considered as actual absorbance.

$$Antioxidant\ \ activity\ \ Index = \frac{IC_{50}\ of\ the\ standard\ Ascorbic\ acid\ (\mu l/ml)}{IC_{50}\ of\ Sample\ (\mu l/ml)}$$

The IC_{50} value for *M. longifolia* leaf extract is 900 μl/ml and green ZnO NPs are 500 μg/ml (Figure 6). results indicate that the antioxidant activity of *M. longifolia* leaf extract-mediated ZnO NPs is much higher than *M. longifolia* leaf extract.

Bactericidal activity of green ZnO nanoparticles

Green synthesized ZnO nanoparticles were tested for their bactericidal activity against *S. aureus* and *E. coli* bacteria by agar well diffusion assay. Inhibition zone values were determined for the *M*-ZnO NPs at different concentrations (100 μg/ml, 150 μg/ml, 200 μg/ml, and 250 μg/ml). ZnO NPs showed an excellent inhibitory effect against both *S. aureus* and *E. coli* bacteria due to their surface activity. In Figure 7, the diameter (mm) of inhibition zones of ZnO NPs solution around each well is shown. The result shows that inhibition zone diameter increase with the increasing concentration of ZnO NPs. *M. longifolia* leaf extract-mediated synthesized ZnO NPs are found the highest bactericidal activity against *E. coli* (23 mm) at 250 μg concentration (Table 1). But Pachaiappan *et al*., 2021 [15]; reported that green synthesized ZnO NPs using *Justicia adhatoda* leaves extract showed higher bactericidal activity against *S. aureus* bacteria than *E. coli.* The result indicates that the bactericidal activity of green ZnO NPs depends on the concentration of NPs. Asha *et al*. (2022) [16]; reported that the antimicrobial potential of zinc oxide nanoparticles depends upon the size, smaller size NPs has better bactericidal effect than a larger one.

Photodegradation of Textile dyes

In the sunlight irradiation but absence of *M*-ZnO, little degradation of MB dye was observed. The effect of both ZnO NPs on the photocatalytic degradation of MB was investigated and shown in Uv-vis spectroscopy graphs. However, no photocatalytic degradation of MB dye was observed in dark conditions. The absorbance of dye-polluted water decreases with the increasing illumination time of sunlight irradiation. It is clearly seen in Uv-visible spectroscopy data that the relative concentration of dye reduced with sunlight illumination time. In the photocatalytic degradation process, the photogeneration of electron-hole pairs is generally responsible for the elimination of dye pollutants [17]. The degradation percentage of MB dye in the presence of ZnO NPs was calculated using the equation [18].

$$Degradation\ \% = [A_o - \frac{A_t}{A_o}] \times 100$$

where A_0 is the absorbance value at the initial stage and the absorbance at time "*t*" is A_t. After 6 hours, 96.08% degradation of MB dye was observed in the presence of *M*-ZnO NPs (Figure 8). Only 48% degradation was observed in the absence of nanoparticles. Anbuvannan *et al*. 2015 [1]; also reported the efficient photocatalytic property of green ZnO NPs against MB dye in sunlight illumination.

As we can see in Figure 9, textile industry-contaminated water becomes transparent in the presence of *M*-ZnO NPs after 8 hours of sunlight illumination. After treatment of *M*-ZnO NPs, 91% of dye degradation was observed while only 25% degradation was observed in the absence of nanoparticles.

ZnO NPs have been shown to exhibit inhibitory and antibacterial properties, antioxidant properties and photocatalytic activity. It was also proven in previous findings that green synthesis of nanoparticles improves their properties and reduced their hazards [19–21].

CONCLUSION

In this study, Zinc oxide nanoparticles were synthesized by a simple, cost-effective, and eco-friendly approach using *Madhuca longifolia* plant leaves extract. The phytochemicals of *M. longifolia* leaf extract effectively work as reducing agents in ZnO NPs synthesis. The biogenic ZnO NPs were found to have 376 nm absorption spectra with a hexagonal wurtzite structure. Powdered XRD results confirm the crystalline structure of NPs. SEM and TEM images validated the formation of nanoparticles with 90 nm of average size. FTIR results

showed the presence and role of phytochemicals in the synthesis of ZnO NPs. Results revealed that ZnO NPs showed significant antioxidant and antimicrobial activity. ZnO nanoparticles effectively photodegrade methylene blue dye under sunlight illumination. The objective of this study is to investigate the properties of bio-synthesized ZnO NPs which can help to reduce the hazards of chemically synthesized nanoparticles and enhance the efficiency of ZnO NPs. This study successfully facile green synthesized multifunctional ZnO nanoparticles using plant parts.

Abbreviations

ZnO- zinc oxide, NPs- nanoparticles, *M*-ZnO NPs- *Madhuca longifolia* leaf extract mediated zinc oxide nanoparticles, XRD- X-ray Diffraction, SEM- Scanning Electron Microscope, EDX- Energy Dispersive X-ray spectroscopy, TEM- Transmission Electron Microscope, FTIR- Fourier Transform Infrared spectroscopy, Uv-vis spectroscopy- Uv-visible spectroscopy.

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Authors Contributions

Rajani: conceptualization, formal analysis, investigation, writing-original draft;

Rishi Kesh Meena: investigations, writing-review, and supervision;

Preeti Mishra: conceptualization, writing-review, and editing.

Declarations of Competing Interest

The authors declare that they have no competing interests or personal relationships that could have appeared to influence the work reported in this paper.

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