






## Research Article

# Biologically Reduced Zinc Oxide Nanosheets Using *Phyllanthus emblica* Plant Extract for Antibacterial and Dye Degradation Studies

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The nanostructures synthesized using the green chemistry method have recently attracted the attention of scientists due to their significance in many scientific domains. This work provides an overview of the biosynthesis of zinc oxide (ZnO) nanosheets (NSs) using *Phyllanthus emblica* plant (PEP) extract. X-ray diffraction analysis (XRD), X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM), and Fourier transform infrared (FTIR) were used to analyze the synthesized ZnO-NSs. Evaluation of the antibacterial activity of biosynthesized ZnO-NSs was performed. ZnO-NSs exhibit effective antibacterial activity against Gram-positive (*S. pyogenes* and *S. aureus*) and Gram-negative (*S. typhi* and *E. coli*) bacterial strains. *S. typhi* is the most sensitive microbe towards ZnO-NSs and formed a 21 mm zone of inhibition (ZOI). ZnO-NSs are also tested as a photocatalyst in the degradation of methyl orange (MO) and rhodamine B (RB). The degradation rate of MO was 90%, and RB was 96% after being exposed to UV light for 120 min. The as-synthesized ZnO-NSs exhibited selective dye degradation and showed relatively better photocatalytic activity for positively charged (cationic) dyes. This work could lead to the fabrication of high-yield photocatalysts, which have the potential to degrade textile dyes from aqueous solution.

## 1. Introduction

Nanotechnology is considered a developing field in science and technology. It has been playing a crucial role in the development of various nanomaterials in recent years. Numerous advantages of pharmaceutical nanoparticles have grabbed the attention of many researchers for innovations [1]. The prevalence of infections that are resistant to antibiotic treatment has prompted a constant quest for new substitutes [2]. Water-borne bacteria species constitute a serious hazard to public health among drug-resistant pathogens because they cause the spread of illnesses such as diarrhea [3]. A variety of pathogenic bacterial species have shown inorganic nanoparticles to be poisonous [4, 5]. The bactericidal impact of inorganic nanoparticles is poorly understood, even though their broad-spectrum biocidal action is well documented [6, 7]. It has been suggested that when ions are released into a solution, reactive oxygen species are generated that are harmful to bacteria [8]. Other studies indicated that due to their small size, nanoparticles could enter the cell wall of bacteria and damage organelles, which results in cell death [9, 10]. In contrast to their organic counterparts, inorganic antibiotics have multiple targets [11, 12].

Zinc oxide is considered a quite interesting material because of its application in areas such as optical, endodontics, and gas sensing. In addition, zinc oxide has been considered an antifungal agent which has no toxicity and harmful environmental effects [13–15]. Due to the safety of zinc oxide nanoparticles and their compatibility with human skin, it is accepted as an additive for textiles and surfaces that meet human skin [16]. ZnO nanoparticles express high photocatalytic properties, which enhances their antifungal activity [17, 18]. ZnO nanoparticles produce ROS under UV light. The primary uses of zinc oxide in the chemical, cosmetics, and pharmaceutical sectors are for its photocatalytic and antibacterial properties [19]. Zinc oxide nanoparticles' antibacterial capabilities have been widely investigated [20, 21], and the development of oxidative stress linked to the particles' photocatalytic activity is thought to be the main cause of toxicity [22]. ZnO is a potential water purification product due to its antiseptic characteristics [23–27].

The plant *Phyllanthus emblica*, commonly identified as Indian gooseberry, grows in areas of Indonesia, India, China, and the Malay Peninsula that are tropical and subtropical. Emblica is one of the most significant herbs in the conventional Ayurvedic medical system and has excellent antioxidant properties. Other conventional medical systems employ it for its immunomodulatory, hepatoprotective, antiulcer, anti-inflammatory, and anticancer effects. Flavonoids, gallic acid [24], kaempferol, pyrogallol, ellagic acid, elaeocarpusin, nor sesquiterpenoids, geraniin, corilagin, and prodelphinidins B1 and B2 are some of this plant's chemical components. Plants that have been reported to produce ZnO NPs through biosynthesis are *Citrus aurantifolia* [28], *Calotropis gigantea* [29], *Ocimum tenuiflorum* [30], *Tamarindus indica* [31], Maple leaf [32], *Phyllanthus niruri* [23], *Solanum nigrum* [21], and *Anisochilus carnosus* [22]. Green synthesis of nanomaterials has recently been performed

using microbes and plant extract that have been reported to produce ZnO-NSs due to their accessibility, affordability, nontoxic nature, biodegradability, and environmentally friendly qualities.

In this study, ZnO with nanosheets (NSs) like morphology has been synthesized using a green approach. Zinc nitrate and *Phyllanthus emblica* leaves extract were used to prepare ZnO-NSs. XRD, XPS, SEM, FTIR, and UV spectrophotometer were used to analyze the prepared NSs. Studies have also been carried out to analyze the antibacterial and photocatalytic efficiency of ZnO-NSs. Several methods were reported to synthesize ZnO, but no one in the literature claims to synthesize ZnO-NSs using *Phyllanthus emblica* plant leaves extract.

## 2. Material and Methods

**2.1. Preparation of Zinc Oxide Using *Phyllanthus emblica* Leaves Extract.** Freshly collected PE leaves were washed using distilled water to eliminate any dust, and after that, they were dried at room temperature. A mortar and pestle were used to convert the dried leaves into fine powder. 10 grams of fine powdered PE were added to 100 ml of deionized water under stirring. The subsequent mixture was poured into 0.05 M ZnCl<sub>2</sub> solution under continuous stirring on a magnetic hot plate at 90°C for 2 hours. The yellow-colored precipitate was obtained and cooled down to room temperature. To remove the impurity contents, this extract was then centrifuged for 15 minutes at 1000 rpm. The precipitate was continually washed in methanol and distilled water before being dried at 80°C. Finally, at 650°C, the product was calcined for roughly 3 hours in a muffle furnace. The complete synthesis procedure of ZnO using PE extract is shown in Figure 1.

**2.2. Characterization.** The authors used SEM (MAIA3 TESCAN) to study the physical appearance of ZnO, XRD (Bruker D8 (Germany) was employed to study crystallographic structure, FTIR (Nicolet Avatar 370) was used to analyze the attached functional groups, and chemical composition of synthesized material is studied using XPS Kratos Axis Ultra DLD apparatus (Manchester, UK).

**2.3. Antimicrobial Assay.** The efficiency of the prepared nanomaterials to inhibit human pathogens was evaluated against microorganisms using the disc diffusion method [26]. Gram-negative pathogenic strains of *E. coli* (ATCC® 33876), *S. typhimurium* (ATCC® 14028), and Gram-positive pathogenic strains of *S. aureus* (ATCC® 11632) and *S. pyogenes* (ATCC® 19615) were employed. To ensure that the nanoparticles were distributed uniformly, 20 mg of the prepared samples were used to make dilution in 1 ml of deionized water. After adding nutrient agar and allowing it to settle, the sterilized Petri plates were inoculated with Gram-negative and Gram-positive bacteria. The solid agar was covered with discs of Whatman filter paper, size 6 mm. At 37°C, nutritional broth was added to all the strains for 18 to 24 hours. The sterile cotton swabs were used to make

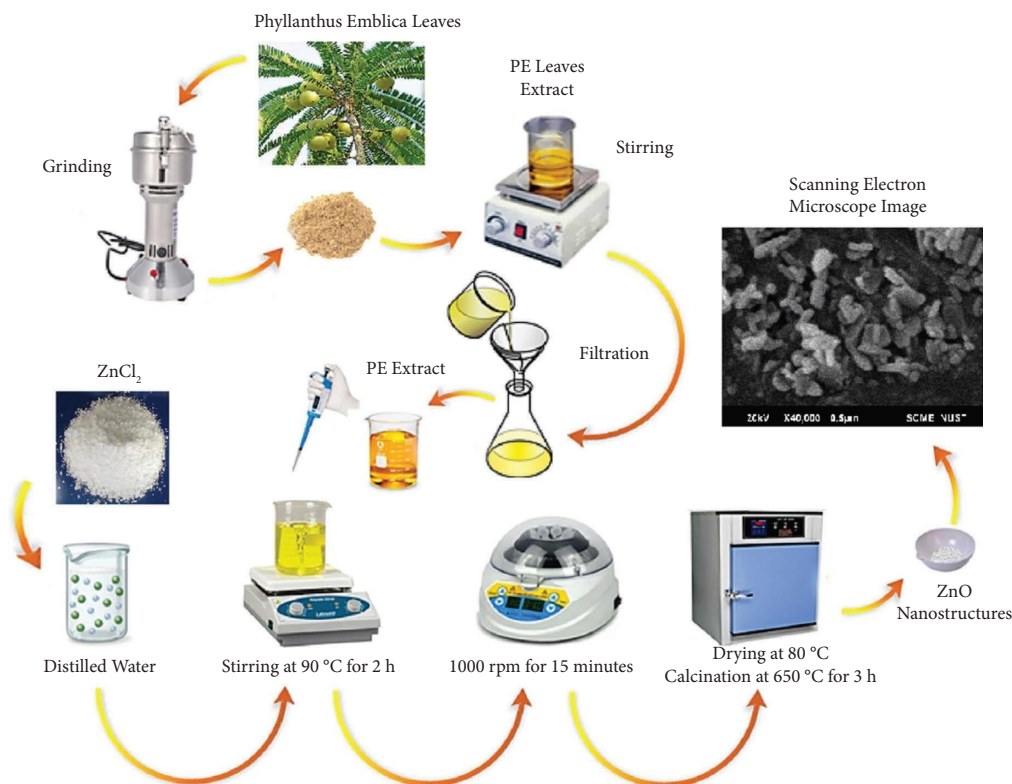


FIGURE 1: The schematic diagram for the complete synthesis procedure of ZnO-NSs.

streaks across the Muller Hinton agar (MHA) surface. The extract (20  $\mu\text{L}$ ) was pipetted onto a sterile paper disc 6 mm in diameter. As a standard reference antibiotic/control, discs containing 40  $\mu\text{L}/\text{mL}$  of ciprofloxacin, were employed. Moreover, the plates were placed in an incubator and subjected to incubation at 37 °C for 24 h after the solvent had evaporated. The development of a clean zone around the discs is proof that the test sample has antibacterial properties. Using an antibiotic zone scale, the diameter of the inhibition zones was assessed. There were three repetitions for each experiment.

**2.4. Photocatalytic Activity Measurement.** ZnO nanoparticles' photocatalytic activity was calculated based on the rate at which methylene orange (MO) and rhodamine B (RB) was oxidized when exposed to UV light. Before illumination, both MO and RB dyes (10 ppm) were mixed for 30 min in the dark with the required amount of catalyst (0.5 g). UV-visible spectrophotometer was used at various time intervals to observe the reaction's progress. The color of reaction mixtures progressively faded until it eventually became colorless. An indication of the successful catalytic activity of ZnO was the absorbance for MO and RB measured with a UV-vis spectrometer.

### 3. Result and Discussion

**3.1. Scanning Electron Microscopy (SEM).** The morphological features of the synthesized material were investigated through SEM. The obtained images of the ZnO sample

showed square-shaped nanosheets with significant particle aggregation, as shown in Figure 2. ZnO-NSs are comparatively homogeneous due to the regular dispersal of Zn cations within a three-dimensional structure. The cluster (agglomeration) in the sample is a result of increased density carried on by the small gap between the particles, while it may also be related to the rapid grain development and nucleation at higher temperatures.

**3.2. Structural Analysis.** Phase and structural analysis of ZnO NPs prepared using *Phyllanthus emblica* is carried out by XRD analysis and shown in Figure 1. All marked diffraction peak positions in Figure 3 are well matched with the standard JCPDS Card: 36-1451. The corresponding X-ray diffraction peaks at observed planes (100), (002), (101), (102), (110), (103), (200), (112), (201), and (004) confirm the formation of hexagonal wurtzite structure of ZnO. The diffraction peaks' observed line broadening is proof that the produced ZnO NPs are in the nanoscale range. Major peaks' increasing full width at half maxima (FWHM) supports the decline in crystallite size. Using the Scherrer formula, the average crystallite size of ZnO-NSs is determined from the X-ray line broadening.

$$D = \frac{k\lambda}{\beta \cos \theta} \quad (1)$$

$D$  and  $\lambda$  represent the crystallite size and radiation's wavelength (1.5406 for Cu  $k\alpha$ ),  $\beta$  is the peak intensity width at half maximum,  $\theta$  is the peak position, and  $k$  is a constant

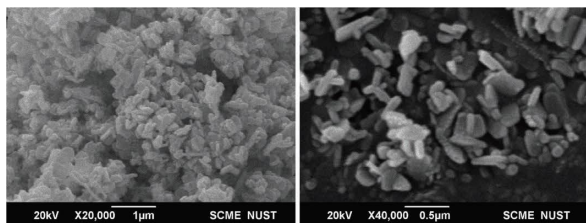


FIGURE 2: SEM micrographs for ZnO nanosheets synthesized using *Phyllanthus emblica* extract.

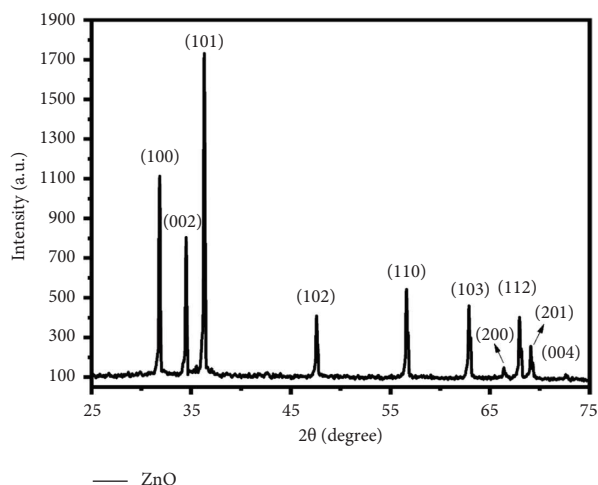


FIGURE 3: Structural analysis of ZnO-Ns using X-ray photoelectron spectroscopy.

(0.94). The synthesized ZnO-Ns have an average crystallite size of 31 nm.

The Williamson–Hall method was used to determine the lattice strain and crystallite size of ZnO, as shown in Figure 4.

$$\beta \cos \theta = k\lambda D + 4\epsilon \sin \theta, \quad (2)$$

where  $\beta$ ,  $D$ , and  $\epsilon$  in the above equation represent full width at half maximum (FWHM), crystallite size, and strain, respectively. The strain is obtained from the linear fit of the data while plotting  $\beta \cos \theta$  against  $4 \sin \theta$ . In comparison to the Williamson–Hall method, which measures crystallite sizes using microstrain, the Scherrer method measures crystallite sizes using the X-rays cohesion length. Any vacancies and defects will make the observed size to be smaller than the actual size.

**3.3. Elemental and Chemical State Analysis.** To identify the constituent elements of the compound synthesized and examine the sample's surface, a surface-sensitive XPS analysis was conducted. Surface scanning was performed to get the survey spectrum, which provides information about the elemental content of the sample surface, as shown in Figure 5(a). Zn and O are recognized with their corresponding distinctive peaks in a low-resolution spectrum (survey analysis). All samples underwent charge shift correction using the adventitious carbon peak binding energy (284.6 eV). High-resolution spectra of the relevant elements

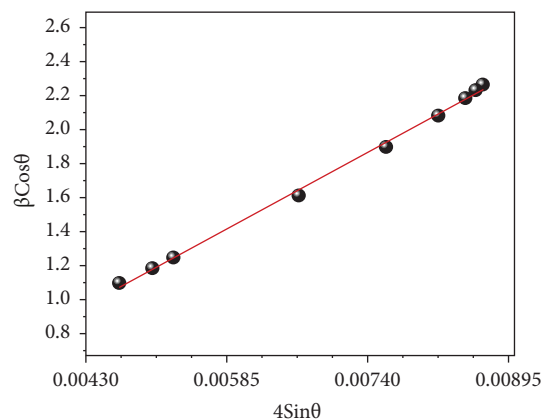


FIGURE 4: Williamson–Hall plot for ZnO-NS.

were examined in the chemical state study. After laser fragmentation, we carried out high-resolution scanning of the sample. Due to spin-orbital coupling, high-resolution spectra of transition metals such as zinc will exhibit a doublet. Zn thus had doublets for the sample examined in this study. These doublets, which are known as  $2p_{3/2}$  and  $2p_{1/2}$ , represent 2p orbitals. Figures 5(b) and 5(c) show the high-resolution spectra of the materials following laser fragmentation, namely the ZnO sample for Zn 2p and O 1s. The binding energies that are displayed are those that match the primary peak, Zn $2p_{3/2}$ . The energy difference between the Zn 2p doublets was 23.1 eV for all samples, which is consistent with previous research [33]. The Zn $^{2+}$  oxidation state is indicated by Zn 2p binding energies in the range of 1022 eV [34]. Pure metallic oxides were chemically represented in the ZnO XPS spectra.

**3.4. Fourier Transform Infrared.** FTIR spectra of ZnO-Ns produced using the green method were captured in the 500–4000  $\text{cm}^{-1}$  range, as shown in Figure 6. The vibrations of the H–O–H bending and O–H stretching were believed to be responsible for the peaks in 1734 and 3418  $\text{cm}^{-1}$ , respectively. This demonstrates that the nanocrystalline ZnO contains a little amount of H $_2$ O. The sample was calcined at 400°C for 3 hours, although not all the adsorbed OH groups were removed. The peak in the range of 1451–1734  $\text{cm}^{-1}$  was linked to the stretching mode of the C=O group, whereas the band at 847  $\text{cm}^{-1}$  corresponds to the vibrations of deformation and elongation of the vibratory Zn–O in ZnO [35].

**3.5. Antibacterial Activity.** According to numerous studies [36, 37], varying particle morphologies have a considerable impact on ZnO's antibacterial efficacy. This morphology-dependent behavior can be addressed considering the percentage of active aspects on the NPs. Nanomaterial studies have been encouraged, to produce specific nanosized ZnO for antibacterial measurements [38]. The antibacterial activity is also significantly influenced by the concentration and particle size. Research findings have shown that the harmful effect of NPs on microorganisms increases with

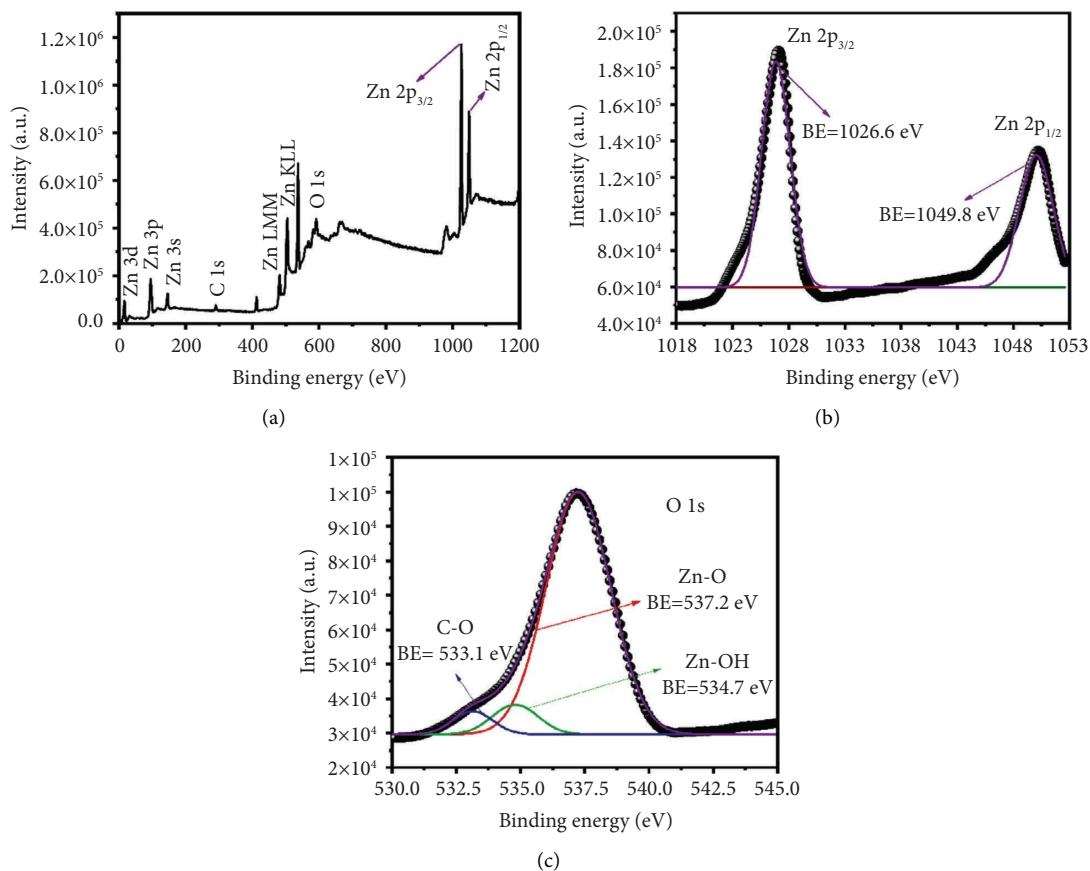


FIGURE 5: X-ray photoelectron spectroscopic micrographs for (a) survey scan and (b, c) high-resolution spectra for Zn 2p and O 1s.

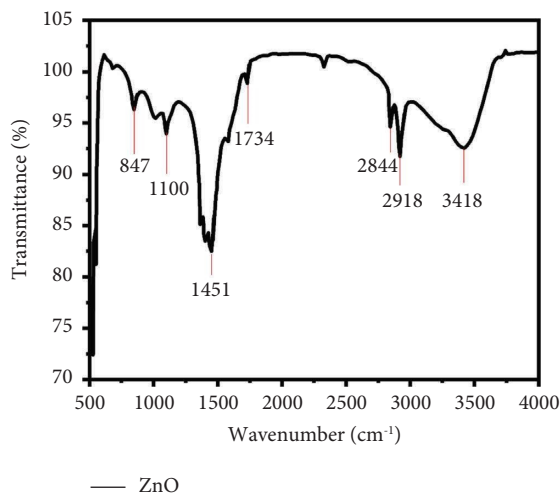


FIGURE 6: FTIR spectra of ZnO-Ns using *Phyllanthus emblica* extract.

decreasing NP size [39, 40]. Smaller NPs are more effective in penetrating bacterial membranes due to their smaller size and larger contact area [41–43]. The ZnO-Ns employed in this study were prepared using a green chemistry approach and shaped like nanosheets with an average length of 97.2 nm.

The microbial sensitivity of ZnO-Ns fluctuates with the microorganisms and the concentrations of the ZnO-Ns. A zone of inhibition is formed for measured values of 30  $\mu\text{g/mL}$ , 50  $\mu\text{g/mL}$ , and 100  $\mu\text{g/mL}$ . The disc diffusion method was used to test the antibacterial activity of ZnO-Ns against diverse microbes, as shown in Figure 7. Antibacterial activity of pure ZnO shows 18 mm, and 21 mm of inhibition zone for *E. coli* (ATCC® 33876) and *S. Typhimurium* (ATCC® 14028) while for *S. aureus* (ATCC® 11632), *S. pyogenes* (ATCC® 19615) ZnO shows 17 mm and 18 mm of inhibition zone as demonstrated in Table 1 and Figure 8. The inhibition zone indicates the sensitivity of the bacteria to toxic substances, resulting in large inhibition diameters for disinfectant-sensitive pathogens and smaller or even no inhibition diameters for resistant pathogens. Our findings demonstrate that ZnO-Ns can only effectively inhibit bacteria at concentrations of 100  $\mu\text{g/mL}$  or above. This validates that greater volume and concentration result in improved antibacterial action.

**3.6. Dye Degradation Study.** The factors that affect the photocatalytic dye degradation efficiency of ZnO-based materials are large surface area, particle size, and the presence of functional groups on the surface [44, 45]. ZnO's surface area and photodegradation abilities are improved when its size is reduced. Figures 9(a) and 9(b) show the degradation of MO and RB over time under UV light

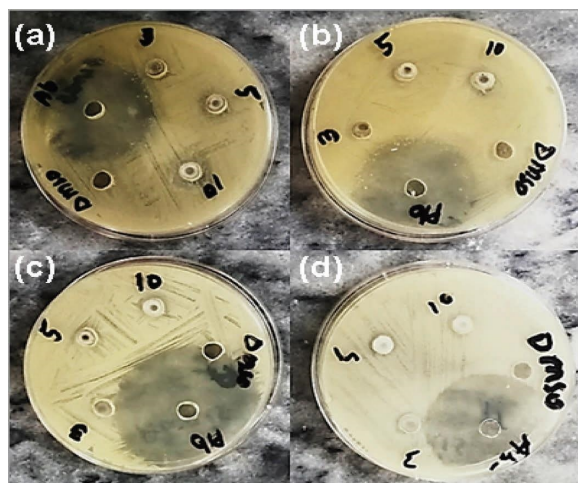


FIGURE 7: Petri plates containing ZnO-NSs employed against microorganisms using disc diffusion method (a) *S. typhi*, (b) *E. coli*, (c) *S. pyogenes*, and (d) *S. aureus*.

TABLE 1: Information zone of inhibition formed against bacterial isolates.

Bacteria	30 $\mu\text{g/mL}$	50 $\mu\text{g/mL}$	100 $\mu\text{g/mL}$
<i>E. coli</i>	$14 \pm 0.28$	$15 \pm 0.36$	$18 \pm 0.34$
<i>S. typhimurium</i>	$15 \pm 0.3$	$16 \pm 0.34$	$21 \pm 0.38$
<i>S. aureus</i>	$13 \pm 0.26$	$14 \pm 0.48$	$17 \pm 0.3$
<i>S. pyogenes</i>	$9 \pm 0.18$	$12 \pm 0.4$	$18 \pm 0.32$

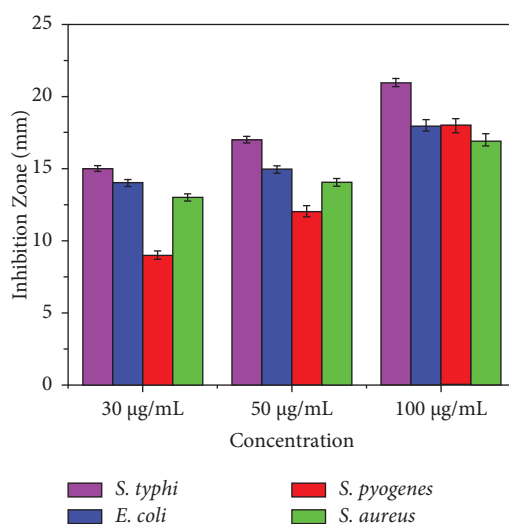


FIGURE 8: Inhibition zone of microorganisms formed using ZnO-NSs synthesized by PE extract.

irradiation in the presence of ZnO-NSs. The relative intensity of UV-visible spectra was used to determine the amount of dye degradation. The MO and RB dyes were kept in the dark for 20 min before exposure to UV light. No change was observed in the absorption behavior of the dyes in the dark before exposure to UV light. The findings showed that the maximum absorbance of MO and RB dye solution occurs at 481 nm and 563 nm, and constantly decreases

when the UV irradiation time is increased. This indicates that ZnO may have accelerated dye degradation with increasing UV exposure time. The degradation % of RB and MO is shown in Figures 10(a) and 10(b); it is observed that the degradation % progressively increased, and about 96% of RB dye and 90% of MO dye degraded within two hours. The kinetics of the photodegradation of organic dyes using ZnO-NSs photocatalyst can be described by several models,

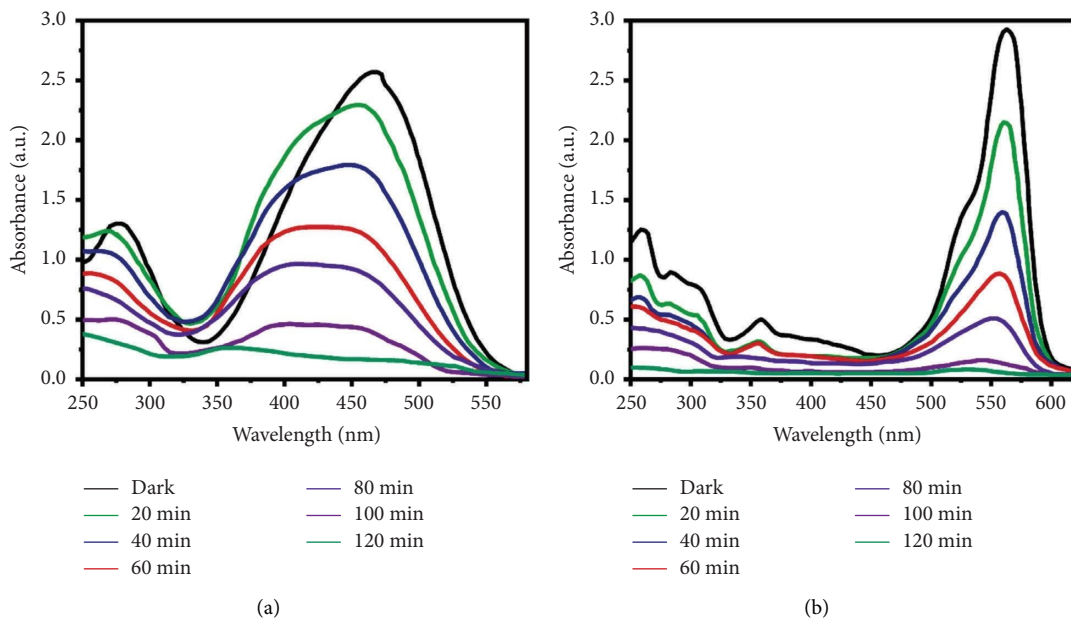


FIGURE 9: The photocatalytic dye degradation efficiency of ZnO-Ns against (a) methyl orange (MO) and (b) rhodamine B (RB) dyes.

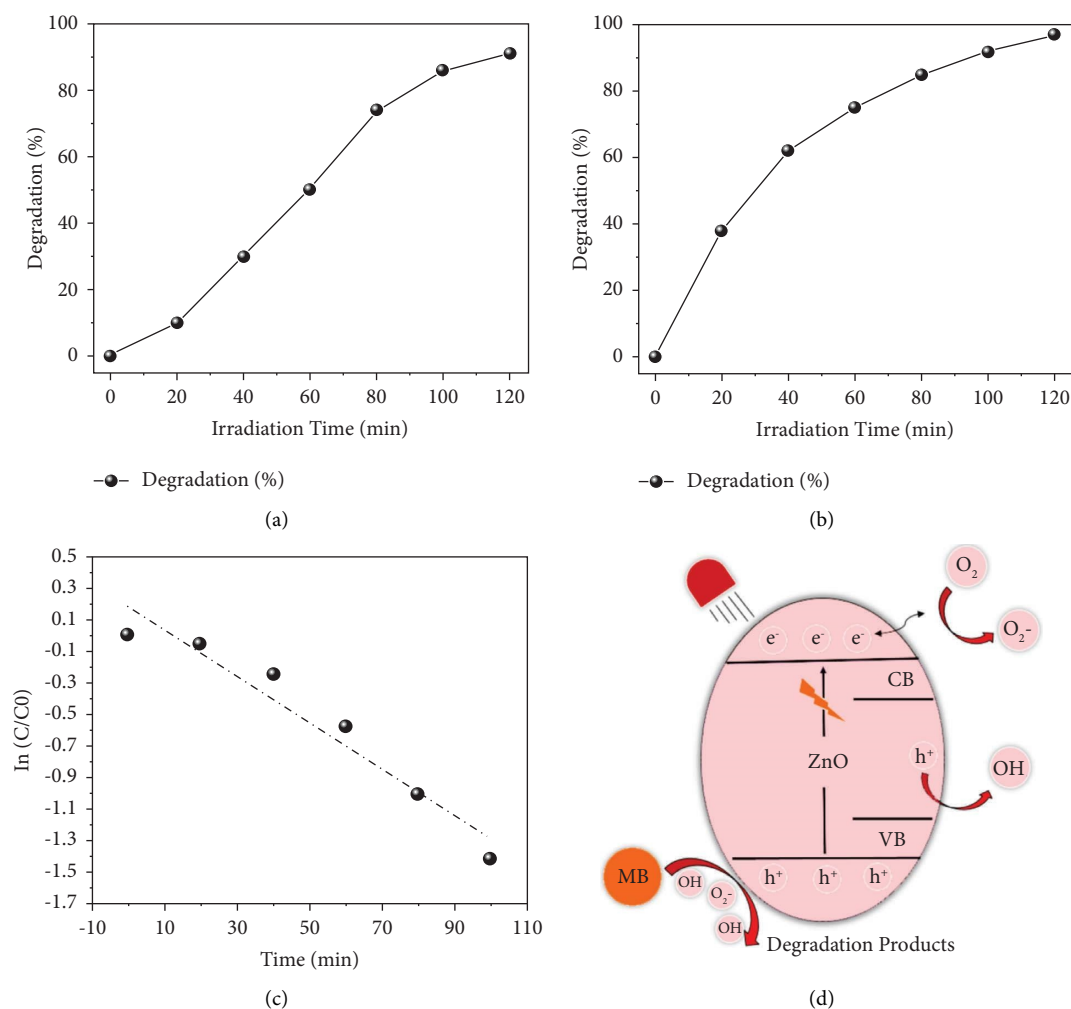


FIGURE 10: Degradation time versus time of exposure graph for (a) MO, (b) RB dyes using ZnO-Ns, (c) pseudo-first-order kinetics, and (d) schematic illustration for charge transportation process of ZnO-Ns.

including the pseudo-first-order kinetics model and the pseudo-second-order kinetics model [46]. These models are based on the observation that the rate of degradation of RB is dependent on the concentration of both RB and ZnO-NSs. By understanding the kinetics of the photo degradation process, it is possible to optimize the conditions for the efficient removal of MB using ZnO photocatalyst. The rate of degradation calculated using the pseudo-first-order kinetics and schematic illustration for the charge transportation process of ZnO-NSs is shown in Figures 10(c) and 10(d).

#### 4. Conclusion

Green leaf extract from the *Phyllanthus emblica* plant was used to successfully prepare ZnO-NSs, which demonstrates its efficiency as an environmentally friendly, nontoxic, and cost-effective technique to synthesize nanomaterials. The PE extracts employed in the production of nanoparticles act as capping and reducing agents. By using the disc diffusion method, the antibacterial activity of produced nanomaterial was analyzed. It was discovered that ZnO-NSs had a larger zone of inhibition for *S. typhi* (21 mm) than all other tested microbes. The growth and survival curves found in this study help us better understand how ZnO NPs work to kill microorganisms over time. Finally, the findings of this study indicate that some of the most severe and prominent food-borne pathogens can be successfully inhibited when ZnO-NSs prepared from PE extract are used as an antibacterial agent in food systems. The comparative dye degradation studies revealed that the catalysts were able to degrade both rhodamine B (cationic) and methyl orange (anionic) dyes. Rhodamine B (RB) and methyl orange (MO) were degraded by ZnO photocatalyst with the highest efficiency of 96% and 90%, respectively, in 120 minutes. Results suggest that many other hazardous organic compounds that are present in both commercial and residential water resources can also be photodegraded using a ZnO photocatalyst.

#### Data Availability

All data used in the findings of this study are included within the manuscript.

#### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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