Supplementary Material

Biosynthesis of ZnO nanoparticles using aqueous extracts of Eclipta prostrata and Piper longum: Characterization and assessment of their antioxidant, antibacterial, and photocatalytic properties

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1. Materials

E. prostrata and *P. longum* was procured from Thu Duc Markets, Ho Chi Minh City, Vietnam. Zinc acetate dihydrate ($Zn(CH_3COO)_2.2H_2O$), sodium hidroxide (NaOH), ethanol (C_2H_5OH) were sourced from Sigma-Aldrich, USA. For antibacterial testing, bacterial strains, including *S.aureus*, *B.cereus*, *E.coli*, and *S.typhimurium* were obtained from the Faculty of Biological Sciences, Nong Lam University.

2. Characterization of ZnONPs

The presence of functional groups in the ZnONPs, as well as the extracts, was determined using an FTIR spectrophotometer (Nicolet Impact 410, Thermo, USA) over a range of 4000 to 400 cm^{-1} with KBr pellet. The crystallinity and phase identification of ZnONPs were analyzed with a scanning rate of 2° min^{-1} using an X–ray diffractometer (Shimadzu XRD–6000, Japan) with a scanning spectrum from 5° to 80°, operating at 40 kV and 100mA. To determine the band gap energy (Eg) of the ZnONPs, Tauc's plot was employed. The morphology of ZnONPs was investigated by Scanning Electron Microscopy (SEM) analysis, utilizing the SU 8010 (Hitachi, Japan), operated at 10 kV. The particle sizes were measures using imageJ. The chemical composition of nanoparticles was investigate using Energy Dispersive X-ray Spectroscopy (EDX) through the SU 8010 (Hitachi, Japan).

3. Antibacterial activity of ZnONPs

Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 11778) and Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14028) were employed to assess the antibacterial activity of the biosynthesized ZnONPs using agar well diffusion method. Initially, these bacterial strains were adjusted to 0.5 McFarland

standard to achieve an approximate concentration (1–2)×10^8 CFU/mL. Wells with a diameter of 5 mm were created on the surface of nutrient agar plates and samples of a concentration of 5 mg mL^{-1} were placed into these wells. Amoxicillin served as the positive control, while plant extracts were used as negative control. Following incubation at 37°C for 18 h, the diameters of inhibition growth zones were measured.

The inhibitory effect of ZnONPs on the growth of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 was investigated using the minimum inhibitory concentration (MIC) assay, as described by Mendes at al., with slight modifications.[1] In brief, 100 µL of bacteria at a density of 10^6 CFU mL^{-1} in Mueller-Hinton Broth (MHB) was inoculated into the wells of 96-well assay plates with varying concentrations of ZnONPs. The highest concentration of ZnONPs used in this evaluation was 40 mg/mL. A two-fold serial dilution was performed until the reaching the lowest concentration of 0.078 mg mL^{-1}. The positive controls (Amoxicillin 100 ppm) and negative control (sterilized distilled water) were included in the wells. The microplates were aerobically incubated at 37°C for 24 h. Subsequently, the wells were incubated with 20 µL of 0.01% resazurin dye and plates were continuously incubated for 1 h. After incubation, the wells were observed for any color change. Following the MIC determination of the ZnONPs, aliquots of 100 µL from five wells with no visible bacterial growth were placed on Mueller-Hinton Agar (MHA) plates and incubated at 37°C for 24 h. The results of this assay were used to plot the correlation between ZnONPs concentration and the inhibition of cell growth.

4. Antioxidant activity of ZnONPs

Antioxidant property of ZnONPs was assessed using DPPH and ABTS tests with modifications following the procedure by Chinnasamy et al.[2] Initially, the synthesized ZnONPs

were dissolved in DMSO. For the DPPH scavenging activity, 1 mL of DPPH solution was added to 2 mL of synthesized ZnONPs at varying concentrations of 250–2500 μg mL^{-1} and incubated in the dark for 30 min at room temperature. The absorbance of the solution was then measured at 517 nm (λ_{max}). For the ABTS activity, a stock of ABTS was prepared by mixing 2.45 mM of potassium persulfate with 7 mM of ABTS at a 1:1 (v/v) ratio, which was kept in dark for 12 h at room temperature. Subsequently, a mixture containing 3 mL of synthesized ZnONPs at various concentrations ranging from 250 μg mL^{-1} to 2500 μg mL^{-1} and 1 mL of the ABTS solution was prepared and incubated in dark for 10 min at room temperature. The absorbance of the mixture was recorded at 734 nm (λ_{max}) using a UV-vis spectrophotometer. Standard gallic acid was used as a control for each of assay. The following formula was employed to calculate the free radical scavenging activity:

Inhibition (%) = (OD of control – OD of sample)/(OD of control)×100

5. Photocatalytic activity of ZnONPs

The photocatalytic properties of ZnO nanoparticles were tested using Crystal Violet dye (CV). A CV dye solution with an initial concentration of 10 mg/L was prepared. Subsequently, 50 mL of the CV solution was then poured into a beaker containing 30 mg of ZnO_EPE, and the suspension was stirred and left in darkness for 60 min to establish adsorption and desorption equilibrium between the CV and the ZnO_EPE catalyst before irradiation. Following this, the suspension was exposed to solar light from 11AM to 2 PM for a period of 120 min. At intervals of 30 min, 3 mL aliquot was taken from the reacted solution and centrifuged at 5000 rpm for 10 min to isolate the catalyst. Subsequently, the resulting solution was analyzed using a UV-vis spectrophometer (UVD-2950, Labomed, USA). A similar procedure was also carried out for ZnO_PLE. The rate of CV degradation was calculated using the fomula below:

Dye degradation (%) = $\frac{(C_o - C_{t)}}{C_o}.100$ (S1)

where, C_o is the initial dye concentration before sunlight irradiation and C_t is the dye concentration after sunlight irradiation for a given time t.

6. Statistical analysis

The analysis of experimental data involved employing one-way analysis of variance (ANOVA) with Origin 8.6 (OriginLab, USA) and Microsoft Excel software (version 2021, Microsoft, USA). The significance differences among results was carried out using the IBM SPSS Statistics 20 (IBM, USA). All experiments were performed in triplicate replication, and the results were given as the mean of measurements ± standard deviation.

References

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