Evaluation of *Justicia adhatoda*-Incorporated Tin Oxide Nanoparticles and CuSe-Doped SnO_2 Nanocomposites: Characterization and Enhancement of Antibacterial, Antioxidant, and Cytotoxic Activities in an In Vitro Approach

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2 Material and Methodology

A brief description of the materials, instrumentation, and analytical procedures is provided in the Electronic Supporting Information (ESI)

2.1. Materials

The Malabar nut (*J. adhatoda*) plant leaves were collected from the cultivated garden near SRM University in Chennai and Tin (II) Chloride Dihydrate ($SnCl_2.2H_2O$) Extra pure AR (99%), Selenium Metal Powder Ultrapure (99%), and Cupric Acetate Monohydrate (99%) were acquired from SRL Chemicals, India. A 25% NH_3 Solution purchased from Merck Life Sciences was utilized, and the entire synthesis process was conducted with double distilled water. The Strains of bacteria, including the positive strain Staphylococcus aureus (MTCC 96) and the negative strains Klebsiella pneumoniae (MTCC 618), Escherichia coli (MTCC 443), and Pseudomonas aeruginosa (MTCC 1688), were obtained from the Microbial Type Culture and Collection (MTCC) located in Chandigarh, India.

2.2 Characteristic Analysis

The crystalline structure of the synthesized materials was examined using PANalytical Powder X-Ray Diffraction (P-XRD) with a Cu Kα radiation source (λ = 1.54178 Å) operated at 40 kV, covering a scanning range of 20° to 80°. Surface topography was analysed using the Thermo Scientific Apreo S High-Resolution Scanning Electron Microscope (HR-SEM). Inner-surface morphology and selected area electron diffraction (SAED) patterns were studied using the JEOL JEM-2100 Plus High-Resolution Transmission Electron Microscope (HR-TEM). Optical properties of the samples were characterized using a SHIMADZU UV-3600 PLUS UV-visible spectrophotometer. Functional groups and chemical characteristics of the

nanomaterials were investigated using the ATR technique with SHIMADZU IRTracer-100 Fourier Transform Infrared (FT-IR) spectrometers.

2.2.1 Antibacterial analysis of Nanocomposites

To investigate the vulnerability of *Phyto*-SnO_2 NPs and copper selenide-doped *Phyto*-SnO_2 nanocomposites against different types of bacteria by disk diffusion method. The study included three-gram negative bacteria, specifically Klebsiella pneumoniae (MTCC 618), Escherichia coli (MTCC 443), and Pseudomonas aeruginosa (MTCC 1688), as well as one-gram positive bacterium, Staphylococcus aureus (MTCC 96). The bacterial strains were sourced from Chandigarh, India. To prepare for testing, 10 ml of Mueller-Hinton agar medium was poured into sterile petri dishes (60 mm in diameter) and inoculated with the test organism. Sterile filter paper discs impregnated with sample concentrations of 5, 15, 25, 50, 75, and 100 µg/ml were placed on the agar surface. A filter paper disc impregnated with 5 µg of amoxicillin was used as the positive control. The plates were incubated at 37 °C for 24 hours, after which the inhibition zones were measured in millimetres. Each experiment was conducted in duplicate for precision.

2.2.2 Antioxidant activity of DPHH Assay

We examined the antioxidant activity of the nanocomposites was assessed using the stable DPPH free radical assay. An ethanolic DPPH solution (0.05 mM, 500 µl) was mixed with 1000 µl of samples at varying concentrations (20–100 µl). The prepared DPPH solution was stored in the dark at 4°C to maintain stability. Subsequently, 1000 µl of 96% ethanol added and Shaked vigorously. The mixture was left to stand for 5 minutes, after which the absorbance was measured spectrophotometrically at 540 nm. Ethanol was used as the baseline to set the absorbance to zero. A blank containing the same amount of ethanol and DPPH was prepared to serve as a control. All experiments were performed in triplicate. The free radical scavenging activity of the samples was represented as a percentage of inhibition.

$$percentage\ inhibition\ of\ DPHH\ assay = \left[\frac{A-B}{A}\right] * 100$$

Where A and B represent the absorbance values of the blank and the sample, respectively. A graph was plotted with sample concentration on the x-axis and percentage inhibition on the y-axis. The concentration required to achieve 50% inhibition (IC_{50}) was then determined from the curve.

2.2.3 Invitro Cytotoxicity assay of MTT ASSAY- HepG2 Cell line

We used 3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide (MTT) to do the MTT assay according to Mossman (1983). The MTT assay relies on the metabolic activity of mitochondrial succinate dehydrogenase and reductase enzymes in viable cells to reduce MTT into insoluble purple formazan crystals. The amount of formazan produced is directly proportional to the number of viable cells and inversely related to the level of cytotoxicity. In this procedure, trypsinized HepG2 cells were seeded into a 96-well plate and incubated at 37°C for 24 hours to allow attachment. Subsequently, the cells were treated with varying concentrations of the test samples and incubated for an additional 24 to 48 hours. After the treatment period, the media were carefully discarded, and 100 µl of MTT solution (5 mg/ml in phosphate-buffered saline) was added to each well. The plate was then incubated at room temperature for 4 hours to facilitate the formation of formazan crystals. The formed formazan crystals were solubilized by adding 100 µl of dimethyl sulfoxide (DMSO) to each well. The absorbance of the resulting solution was measured at 570 nm using a microplate reader. To prepare the test samples, 1 mg of each was initially dissolved in sterile complete media and then serially diluted to obtain the desired concentrations.

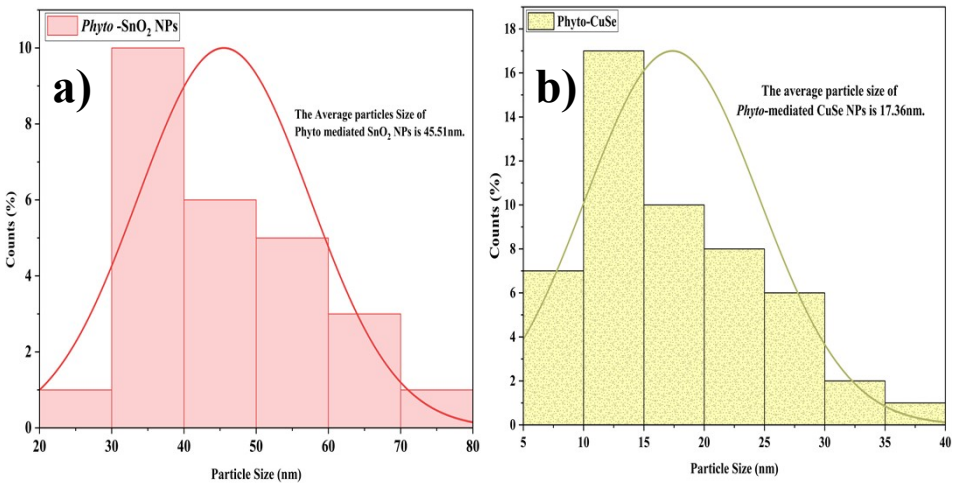

Figure S1 Particle size distribution of a) Phyto-SnO_2 NPs b) Phyto-CuSe NPs.