



An Artificial Neural Network-based Model for Antibacterial Activity of CuO/Al₂O₃/Chitosan Nanocomposites

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ABSTRACT

The increasing contamination of aquatic environments by industrial effluents, agricultural runoff, and domestic waste has created a critical demand for advanced antibacterial materials with reliable predictive capabilities. A ternary CuO/Al₂O₃/chitosan (CS) nanocomposite was developed to address this challenge, where CuO provides photocatalytic activity, Al₂O₃ enhances dispersion and structural stability, and chitosan contributes as a biopolymeric antibacterial matrix. The nanocomposite beads were synthesized *via* an *ex situ* mechanical mixing followed by ionic gelation using NaOH, and their antibacterial performance was evaluated against *Escherichia coli* and *Pseudomonas aeruginosa* using the disc diffusion method. An Artificial Neural Network integrated with Bayesian Optimization (ANN-BO) was employed to model and optimize the nonlinear relationship between material composition and antibacterial activity. The results demonstrated inhibition zones ranging from 1.8 to 6.1 mm for *E. coli* and 3.0 to 10.0 mm for *P. aeruginosa* with predicted values closely matching experimental outcomes (residuals within -1.80 to +0.93 mm and -1.01 to +1.79 mm). The optimized CuO/Al₂O₃/CS (2:2:1) nanocomposite exhibited the highest antibacterial activity with a maximum inhibition zone of 10.0 mm against *P. aeruginosa*, while the 2:1:1 composition showed optimal performance against *E. coli* with an inhibition zone of 6.1 mm. The ANN-BO model exhibited high predictive accuracy ($R \approx 0.935$). The developed framework demonstrates strong potential for the design of efficient antibacterial nanocomposites for water treatment and antimicrobial coating applications.

Keywords: CuO/Al₂O₃/chitosan nanocomposite; Artificial neural network-Bayesian optimization; Antibacterial activity; *Escherichia coli*.

1. INTRODUCTION

The increasing concentration of inorganic and organic pollutants in aquatic environments, agricultural runoff, industrial effluents, and household waste, generates an immediate global need for advancement of innovative and effective methods to address water pollution challenges. The growing demand for clean and sustainable water resources has intensified research on advanced materials and hybrid technologies for efficient wastewater treatment and desalination, as conventional techniques often suffer from low efficiency, high operational cost, and limited adaptability to complex contaminants. Hybrid nanofluids have emerged as a promising solution for enhancing thermal systems used in water purification, where the incorporation of

Al₂O₃/SiO₂, SiO₂/CuO, and Al₂O₃/CuO nanofluids into flat plate collectors significantly improved solar desalination performance, achieving thermal and exergy efficiencies of 61.4% and 6.7%, respectively (Venkatesh *et al.* 2024). In parallel, computational intelligence techniques such as hybrid ANN-GA (Genetic Algorithm) models have enabled efficient optimization of process parameters, demonstrating high predictive accuracy in chitosan production and dye removal processes, thereby confirming their robustness for nonlinear environmental systems (Bello and Olafadehan, 2021). Among various adsorbent materials, chitosan-based nanocomposites have gained considerable attention due to their biodegradability and high adsorption capacity, where an AgMgO-based chitosan nanocomposite exhibited approximately 80% fluoride removal governed by

pseudo-second-order kinetics and Langmuir isotherm behavior, along with notable antibacterial activity (Ayinde *et al.* 2020).

Similarly, modified systems such as CS-Tp@Fe₃O₄ demonstrated a high adsorption capacity of 440.24 mg/g with strong model correlation under Response Surface Methodology (RSM) optimization (Akinremi *et al.* 2021), while graphene oxide-incorporated chitosan (CS)-PVA (polyvinyl acetate) hydrogels achieved 88.17% dye removal efficiency with ANN-supported modeling (Das *et al.* 2020). Furthermore, multifunctional chitosan-isatin Schiff base derivatives achieved removal efficiencies of 98.74% for dyes and 92.33% for Cr (VI), highlighting their dual-functionality and environmental applicability (Elgamel *et al.* 2025). In addition to adsorption-based approaches, catalytic and photocatalytic systems have shown significant potential in degrading persistent pollutants, where CuO-supported catalysts demonstrated superior performance in 4-nitrophenol reduction with CuO/ZrO₂ exhibiting the highest activity and a rate constant of $15.97 \times 10^{-3} \text{ s}^{-1}$ (Coşkuner Filiz, 2020). Advanced photocatalysts such as Fe₂Ni_{0.5}Cu_{0.5}O₄-chitosan microspheres achieved degradation efficiencies of 93% for methylene blue and 90% for rhodamine B under visible light (Ali *et al.* 2025), while structured systems like aACF/ZnO/CuO@Al₂O₃ exhibited enhanced surface morphology and catalytic activity (Zamani *et al.* 2025). Catalytic ozonation systems including Cu-Ce@ γ -Al₂O₃, achieved 63.4% COD removal through hydroxyl radical-mediated reactions (Sun *et al.* 2025), whereas CuO/ γ -Al₂O₃ catalysts demonstrated 90.72% decolorization with excellent reusability (Zhao *et al.* 2021), and CuO/Al₂O₃ and MnO₂/Al₂O₃ systems also exhibited high removal efficiencies for organic pollutants (Zhou *et al.* 2025).

Sustainable materials derived from industrial waste have also shown strong potential, where fly ash-derived zeolite A exhibited high adsorption capacities for Pb²⁺ (277.78 mg/g) and Cd²⁺ (87.73 mg/g) (Sai and Matli, 2025), and fly ash-based catalysts achieved removal efficiencies of 95.62% for acrylonitrile and 88.95% for COD under optimized conditions (Kumar *et al.* 2021). Moreover, surfactant-modified NaA zeolite demonstrated an exceptional adsorption capacity of 769.23 mg/g with 98.92% removal efficiency (Haghjoo *et al.* 2023), while MOF-2@Al₂O₃ composites showed high uranium adsorption capacity of 300.3 mg/g with strong reusability (Koppula *et al.* 2022). Additionally, γ -AlOOH and γ -Al₂O₃ adsorbents achieved adsorption capacities of 344.44 mg/g and 421.94 mg/g, respectively, confirming their effectiveness in dye removal applications (Ballou *et al.* 2024). Nanocomposites have emerged as promising antimicrobial materials due to their enhanced surface reactivity, reactive oxygen species (ROS) generation and synergistic heterostructure effects. Studies have shown that MnO₂/Al₂O₃ nanocomposites exhibit superior

antibacterial activity owing to improved charge separation and ROS production (Janani *et al.* 2021).

Similarly, CuO/Al₂O₃ composite demonstrated strong antimicrobial performance, where CuO morphology and loading significantly influenced bacterial inhibition, particularly against *E. coli* and *S. aureus* (Bayat *et al.* 2025). SnO₂/Al₂O₃ nanocomposites further reveal concentration-dependent antibacterial activity against both Gram-positive and Gram-negative bacteria, indicating their suitability for coating applications (Khattar *et al.* 2025). In addition, hybrid fiber-reinforced composites incorporating SiO₂ and Al₂O₃ nanofillers achieve enhanced antimicrobial efficiency alongside improved mechanical properties (Lakshmaiya *et al.* 2025). Metal-capped Co/ZnO nanocomposites also demonstrate improved antibacterial activity due to increased ROS generation and surface reactivity (Riaz, 2025).

This study presents a novel integration of material design and artificial intelligence by developing a ternary CuO/Al₂O₃/chitosan nanocomposite with enhanced antibacterial performance through synergistic interactions of photocatalysis, improved dispersion, and polymer-induced membrane disruption. Unlike conventional studies that focus either on material synthesis or predictive modeling, this work uniquely combines experimental nanocomposite fabrication with an ANN-BO framework to accurately predict antibacterial activity and capture complex nonlinear structure-property relationships. Furthermore, the transformation of nanomaterials into bead-structured systems for realistic application conditions, along with demonstrated improvements in dispersion and reliable predictive accuracy establishes a new pathway for the rapid design and optimization of multifunctional antibacterial materials for water treatment applications.

2. MATERIALS AND METHODOLOGY

2.1 Materials

CuO nanoparticles and Al₂O₃ nanoparticles (99.5% purity, Sigma-Aldrich) were used as the starting materials for the preparation of CuO/chitosan and CuO/Al₂O₃/chitosan nanocomposites. Acetic acid, chitosan flakes, and sodium hydroxide (Sigma-Aldrich) were also used in the study. All materials were used as received without further purification.

2.2 Synthesis of CuO/Chitosan and CuO/Al₂O₃/Chitosan Nanocomposites

The CuO/chitosan and CuO/Al₂O₃/chitosan were synthesized by an *ex situ* mechanical mixing approach with controlled dispersion and precipitation steps. Initially, predetermined amounts of CuO, Al₂O₃, and CS powders were individually dispersed in 50 mL of

2 wt% acetic acid solution under continuous magnetic stirring for 3 hours to ensure uniform dispersion (Upadhyay *et al.* 2026), (Georgy *et al.* 2025). CuO and Al₂O₃ particles were well suspended in the acidic medium, while CS underwent protonation of amine groups, resulting in the formation of a homogeneous viscous polymeric solution. Subsequently, the individual dispersions were combined according to the required weight ratios, CuO/Al₂O₃/CS (1:2:1) and CuO/CS (2:1), and further magnetically stirred for 1 day at ambient temperature to achieve stable and homogeneous precursor mixture with effective interfacial interaction between metal oxides and biopolymer matrix. The resulting precursor solution was then introduced dropwise into a 2 M NaOH solution by syringe under

gentle stirring. This process induced ionic gelation and instantaneous precipitation due to deprotonation of chitosan, leading to the formation of spherical nanocomposite beads with embedded CuO and Al₂O₃ nanoparticles (Soliman *et al.* 2021). The formed beads were collected and washed multiple times by deionized water to eliminate residual NaOH until neutral pH was attained. Finally, the nanocomposite catalysts were dried at room temperature and stored for further analysis. The synthesized materials were subsequently subjected to physicochemical characterization techniques to evaluate their structural integrity, surface morphology, and compositional properties. The overall experimental and modeling workflow adopted in this study is illustrated in Fig. 1.

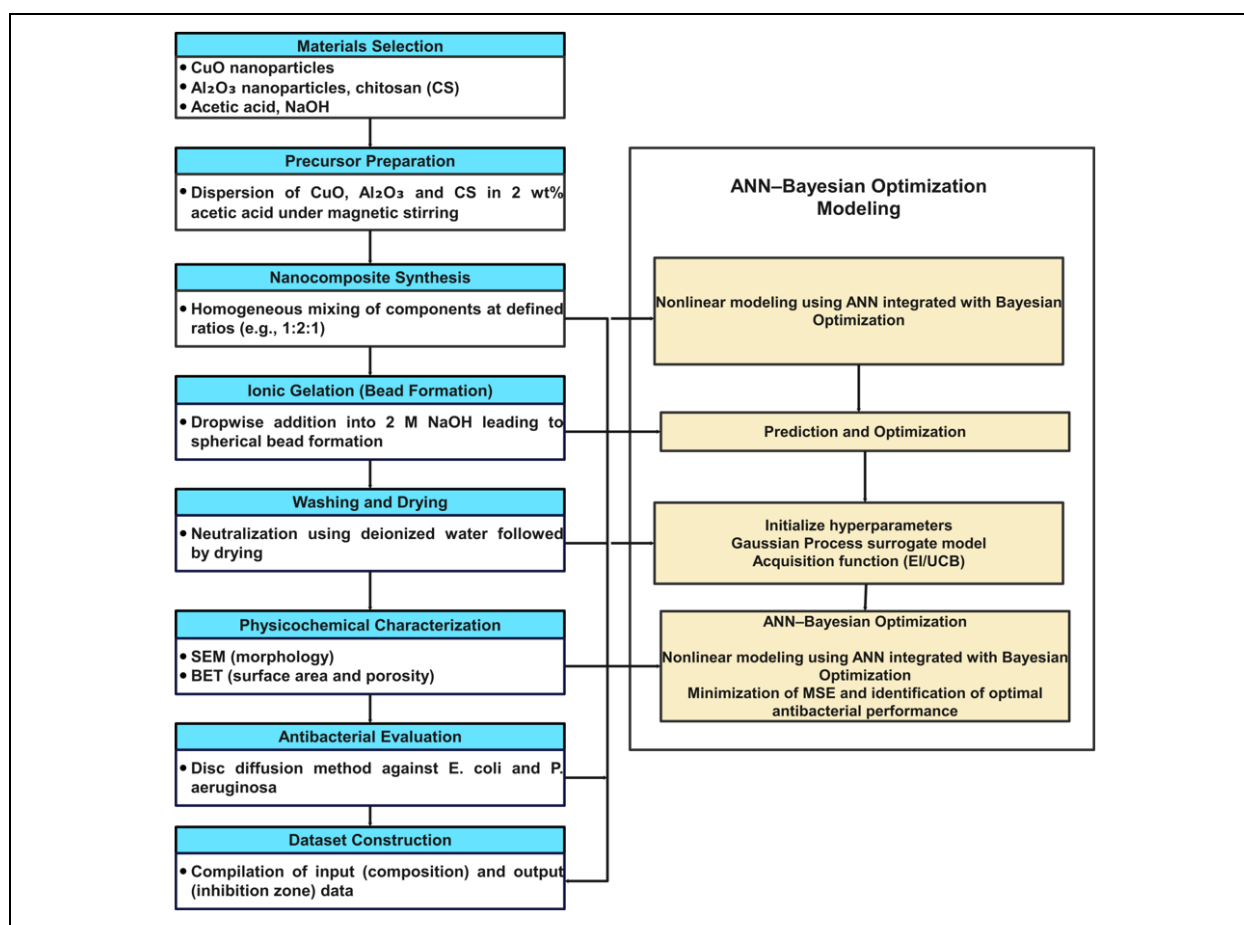


Fig. 1: Process flow diagram illustrating the synthesis, characterization, antibacterial evaluation, and ANN-BO modeling of CuO/Al₂O₃/CS nanocomposite

2.3 Characterization

Surface morphology and microstructural behaviour of synthesized CuO/CS and CuO/Al₂O₃/CS nanocomposite beads were examined using a Zeiss Gemini SEM. The acquired high-resolution micrographs were used to evaluate surface morphology, particle dispersion, and effective addition of CuO and Al₂O₃ nanoparticles within the chitosan matrix. For sample

preparation, a small quantity of the nanocomposite was dispersed in ethanol and subjected to ultrasonication for 10 min at ambient conditions to ensure uniform suspension. A droplet of the dispersion was then carefully deposited onto 400-mesh copper grid and allowed to dry under controlled conditions for 72 hours prior to imaging.

Textural properties including pore characteristics and specific surface area were determined

through BET adsorption–desorption using a Micromeritics ASAP Surface Area Analyzer. Samples were degassed at 120 °C for 9 hours to remove physically adsorbed moisture and impurities. The pore size was subsequently calculated from desorption branch of isotherm employing Barrett–Joyner–Halenda technique. Therefore, in the current study, emphasis was given to morphological, surface, and functional performance analyses relevant to antibacterial activity and subsequent ANN-based modelling, thereby avoiding duplication of previously established findings.

2.4 Antibacterial Analysis

2.4.1 Preparation of Microbial Media

Antibacterial evaluation was carried out by two representative Gram-negative bacterial strains: *E. coli* and *P. aeruginosa*. Previous studies have demonstrated that nanocomposites exhibit significant antibacterial activity against waterborne pathogens such as *E. coli* and *P. aeruginosa*, demonstrating their effectiveness for wastewater disinfection applications (Abou *et al.* 2020). These microorganisms were maintained and grown using sterile nutrient agar and nutrient broth as the growth media. Initially, preserved bacterial stocks were aseptically transferred onto nutrient agar plates and incubated to obtain well-isolated colonies. A single discrete colony from each strain was then carefully picked using a sterile inoculation loop and transferred into nutrient broth to prepare fresh bacterial suspensions. The inoculated broth cultures were incubated under controlled conditions at 36 °C for 20–24 hours to achieve sufficient bacterial growth and reach the logarithmic phase, ensuring active and uniform cell populations for subsequent experimental analysis.

2.4.2 Disc Diffusion Method

Antimicrobial performance of synthesized CuO/CS and CuO/Al₂O₃/CS nanocomposite beads was assessed through an *in vitro* agar diffusion method, with streptomycin (100 µg/mL) as the reference antibiotic for comparative analysis. The study was conducted against Gram-negative bacterial strains, namely *Pseudomonas aeruginosa* and *Escherichia coli*. Sterile Mueller–Hinton plates were uniformly inoculated by spreading 10⁸ CFU/mL of freshly prepared bacterial suspension using a sterile cotton swab to ensure the formation of a consistent microbial lawn. Subsequently, individual nanocomposite beads were aseptically placed at the center of the agar plate. The plates were then incubated at 36 °C for 18–24

hours under standard laboratory conditions. Following incubation, antibacterial activity was quantified by measuring diameter of clear inhibition zones. The experiments were conducted in triplicate to ensure reproducibility, and results are presented as mean values accompanied by standard deviation. The error bars in graphical representations correspond to the variation among three independent experimental runs. All antibacterial studies were carried out under ambient laboratory lighting without specific light-controlled conditions. Among the investigated formulations, the 1:2:1 CuO/Al₂O₃/CS was identified as the optimal composition based on systematic comparative analysis of multiple compositions (1:1:1, 2:1:1, 1:2:1, and 2:2:1). The selection was based on integrated performance metrics, including inhibition efficiency, stability, and reproducibility, rather than reliance on single parameter.

2.5 Artificial Neural Network-Bayesian Optimization (ANN-BO) Model

The ANN-BO model was developed to capture the nonlinear relationship between compositional inputs and antibacterial responses (Elsoudy *et al.* 2024). The transformation from input to output layer is governed by the following equations. The ANN-BO model was developed using three input parameters, namely CuO (wt%), Al₂O₃ (wt%), and chitosan content to predict antibacterial performance. The output responses considered were the inhibition zone diameters against *Escherichia coli* and *Pseudomonas aeruginosa*. A total dataset of 38 experimental samples was used for model development, ensuring sufficient data representation for capturing nonlinear relationships between compositional variables and antibacterial activity.

The architecture of the ANN-BO model is illustrated in Fig. 2. The model consists of three input neurons corresponding to CuO (wt%), Al₂O₃ (wt%), and chitosan content, which are fed into a hidden layer with an optimized number of neurons to capture complex nonlinear relationships. The output layer comprises two neurons representing the predicted inhibition zone diameters against *Escherichia coli* and *Pseudomonas aeruginosa*. Bayesian Optimization is integrated with the ANN to iteratively tune hyperparameters such as the number of hidden neurons, learning rate, and network weights by minimizing the mean squared error (MSE). This hybrid framework enhances prediction accuracy and ensures robust generalization of antibacterial performance.

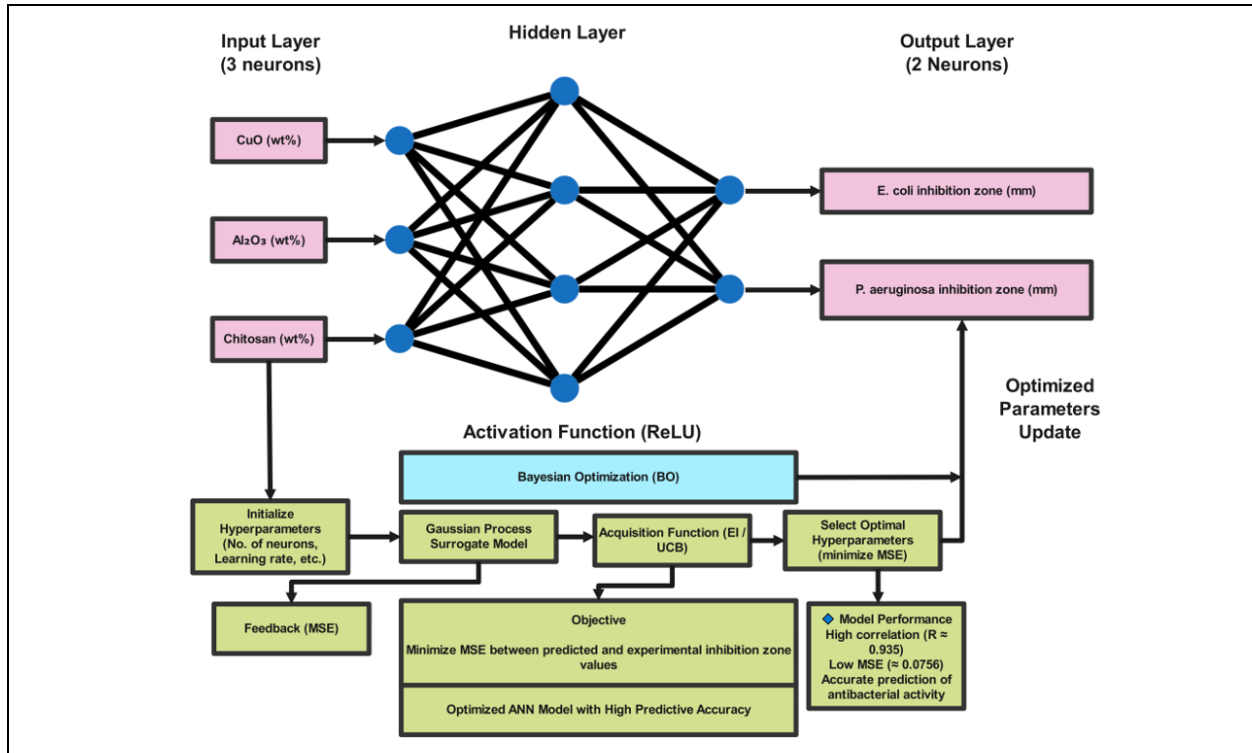


Fig. 2: ANN-BO architecture for predicting antibacterial activity of CuO/Al₂O₃/CS nanocomposites

The output of the hidden layer neurons is expressed as:

$$h_j = f\left(\sum_{i=1}^n w_{ij}x_i + b_j\right) \quad \dots (1)$$

where x_i represents input variables, w_{ij} denotes weights connecting input and hidden layers, and b_j is the bias term. The activation function $f(\cdot)$ introduces nonlinearity into the model.

The final network output is obtained as:

$$y_k = f\left(\sum_{j=1}^m w_{jk}h_j + b_k\right) \quad \dots (2),$$

where w_{jk} and b_k represent weights and biases between hidden and output layers, respectively, and y_k corresponds to predicted antibacterial activity.

A tangent sigmoid activation function was employed to effectively model nonlinear interactions:

$$f(x) = \frac{2}{1+e^{-2x}} - 1 \quad \dots (3)$$

The prediction error between experimental and estimated outputs is defined as:

$$e_i = d_i - y_i \quad \dots (4),$$

where d_i and y_i denote experimental and predicted values, respectively. The performance of the ANN-BO model was evaluated using mean squared error (MSE), given by:

$$MSE = \frac{1}{N} \sum_{i=1}^N (d_i - y_i)^2 \quad \dots (5)$$

and the coefficient of determination (R^2):

$$R^2 = 1 - \frac{\sum_{i=1}^N (d_i - y_i)^2}{\sum_{i=1}^N (d_i - \bar{d})^2} \quad \dots (6),$$

where N is the number of observations and $\bar{d}$ is the mean of experimental values. During training, the network weights were iteratively updated using backpropagation:

$$w^{\text{new}} = w^{\text{old}} - \eta \frac{\partial E}{\partial w} \quad \dots (7),$$

where η is the learning rate and E represents the error function.

The minimum required dataset size for reliable ANN training is estimated as:

$$T_n = (i_n + 1)h_n + (h_n + 1)o_n \quad \dots (8),$$

where i_n , h_n , and o_n represent the number of input neurons, hidden neurons, and output neurons, respectively.

To enhance model performance, Bayesian Optimization was integrated to identify optimal hyperparameters by minimizing the objective function:

$$\min f(\theta) = \text{MSE}(\theta) \quad \dots (9),$$

where θ represents the set of ANN hyperparameters. The selection of optimal parameters is guided by the Expected Improvement (EI) criterion:

$$EI(x) = E[\max(0, f_{\text{best}} - f(x))] \quad \dots (10)$$

which balances exploration and exploitation during the optimization process.

The integration of ANN with BO enhances predictive accuracy, accelerates convergence, and ensures robust generalization for modeling antibacterial activity of nanocomposites.

3. RESULTS AND DISCUSSION

3.1 Surface and Morphology Evaluation

Scanning electron microscopy (SEM) analysis reveals the microstructural evolution and dispersion behavior of the synthesized nanocomposites. The CuO/CS sample (Fig. 3a) exhibits a highly agglomerated morphology characterized by irregular clusters of CuO

nanoparticles with significant void regions and non-uniform distribution. The surface appears loosely packed with noticeable inter-particle gaps, indicating weak interfacial interaction between CuO and the chitosan matrix, which may lead to reduced structural integrity. In contrast, the CuO/Al₂O₃/CS nanocomposite (Fig. 3b) demonstrates a comparatively dense, homogeneous, and finely distributed nanoparticle network. The incorporation of Al₂O₃ nanoparticles effectively reduces agglomeration, resulting in a more compact microstructure with minimized porosity and improved particle packing. The surface texture becomes more uniform and interconnected, suggesting enhanced interfacial bonding and compatibility within the polymer matrix. This transition from a coarse, clustered morphology to a refined and integrated structure confirms the significant role of Al₂O₃ in promoting better dispersion, structural uniformity, and overall composite performance. In a previous research, SEM analysis revealed a well-developed porous structure with uniform dispersion of γ -Al₂O₃/CuO particles, enhancing surface roughness and active sites (Chai *et al.* 2024).

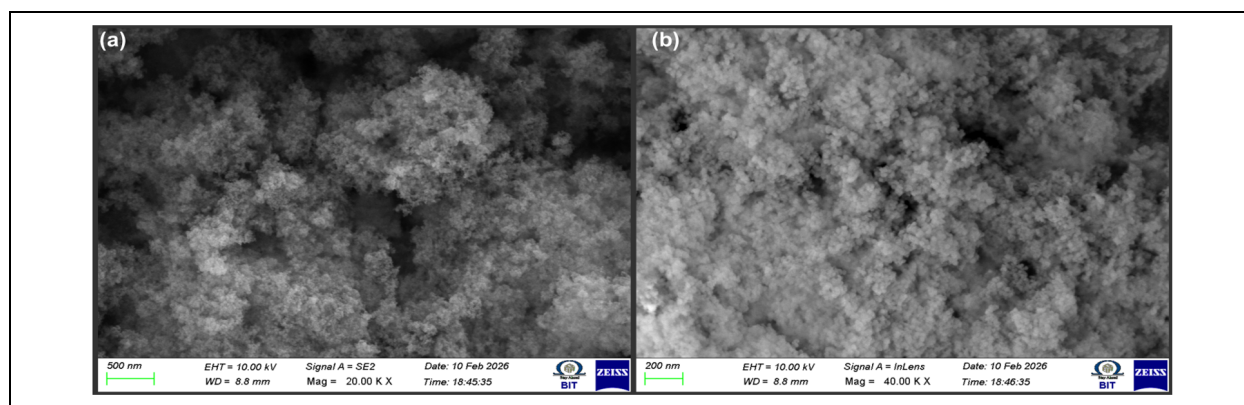


Fig. 3: SEM micrographs of (a) CuO/CS nanocomposite and (b) CuO/Al₂O₃/CS nanocomposite

The enhanced structural uniformity of the CuO/Al₂O₃/CS nanocomposite significantly influences its functional behavior. The relatively smoother and well-organized mesoporous surface facilitates improved light interaction while reducing surface irregularities that can hinder effective activation. Additionally, the presence of Al₂O₃ promotes better dispersion of CuO particles, which supports uniform generation and distribution of reactive oxygen species (ROS) and enhances interfacial electron transfer by limiting particle agglomeration. These characteristics contribute to improved photocatalytic efficiency and strengthen antibacterial performance by enabling effective interaction with the outer cell membranes of Gram-negative bacteria. The improved particle distribution, reduced surface roughness and consistent bead size, collectively show clear structural basis for the superior activity of the CuO/Al₂O₃/CS system. These findings highlight the effectiveness of the ternary composite design for advanced photocatalytic and antimicrobial applications.

Pore size distributions and nitrogen adsorption-desorption isotherms were studied to clarify the textural features of the produced materials (Fig. 4). Pristine CuO (Fig. 4a) displays IV isotherm by H3 hysteresis loop, signifying the presence of mesoporous structures formed by aggregated, plate-like particles. This behavior aligns with its considerable BET (8.0 cm²/g) and a restricted pore size centered at 12 nm, affirming well-defined mesoporous structure that enhances reactant transport and accessibility to catalytic sites. Al₂O₃ exhibits mesoporous properties, possessing BET surface of 86.0 m²/g and pore width of 10 nm. These attributes underscore their capacity to facilitate routes for sufficient exposure of active surfaces and mass transfer for catalytic activities.

The CuO/CS (2:1) hybrid (Fig. 4b) displays a type II adsorption-desorption isotherm, defined by negligible nitrogen adsorption and the lack of a noticeable hysteresis loop, indicating a primarily non-

porous to macroporous structure with surface-limited adsorption. The markedly diminished BET ($8.5 \text{ m}^2/\text{g}$) and broad pore size distribution centered at 38 nm show that dense chitosan phase effectively encases CuO particles, blocks pores and inhibits intrinsic mesoporous structure. This compact morphology obstructs molecular transport and hence reduces the photocatalytic effectiveness compared to clean CuO.

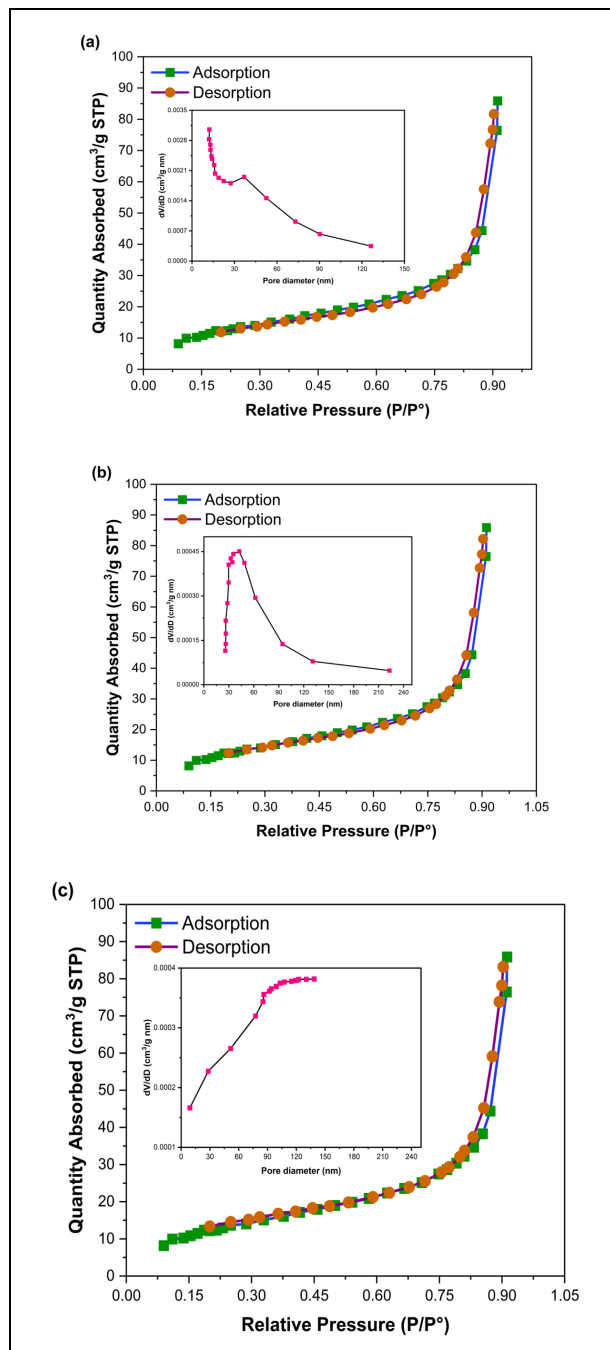


Fig. 4: (a) Isotherms and particle size distribution of CuO, (b) CuO/CS and (c) CuO/Al₂O₃/CS

The CuO/Al₂O₃/CS (1:2:1) nanocomposite (Fig. 4c) displays mesoporous properties, shown by type IV

adsorption isotherm significant H₃ loop and noticeable curvature at elevated relative pressures. The BET area ($8.0 \text{ m}^2/\text{g}$) exhibits a slight increase relative to the CuO/CS system, although average pore size (120 nm) distinctly falls inside mesoporous range. This behavior indicates Al₂O₃ nanoparticles serve interfacial separators, reducing matrix compaction and maintaining pore architecture by creating slit-like spaces. The resultant hierarchical pore network amplifies photon interaction and promotes efficient passage of reactant molecules to catalytic sites, ultimately enhancing both photocatalytic activity and antimicrobial efficacy. In previous studies, biochar was incorporated into a PVA/chitosan matrix, exhibiting a high BET surface area of $176.21 \text{ m}^2/\text{g}$ with enhanced porosity. This led to superior adsorption performance, achieving 1795.007 mg/g capacity and 98.52% levofloxacin removal efficiency (Mosaffa *et al.* 2025).

Pristine CuO displays a distinct mesoporous structure, but inclusion of CS lowers specific surface area and alters pore distribution as larger, macroporous regions. Use of Al₂O₃ mitigates effect by improving the internal porosity and restoring mesoporosity of the composite matrix. Similar behavior has been observed in CuO–Al₂O₃ systems, where Al₂O₃ mitigates pore collapse and facilitates uniform particle dispersion, thereby enhancing catalytic performance. These findings underscore the importance of Al₂O₃ in optimizing the textural properties of the composite, resulting in an ideal balance of pore structure, surface area, and dispersion crucial for efficient antibacterial and photocatalytic performance.

To avert the overestimation of governing parameters, the effects of Al₂O₃ loading and specific surface area are explained based on statistical evidence and mechanistic analysis. The content of Al₂O₃ in the examined compositions demonstrates a continuous positive association by antibacterial efficacy, against *P. aeruginosa*, as evidenced by correlation analyses that account for experimental variability and regression intervals. In contrast, SBET was assessed solely for specific samples, thereby offering predominantly qualitative insights into structure–property relationships. The inclusion of Al₂O₃ increases accessible active sites and partially restores mesoporosity relative to CuO/CS, facilitating ROS, enhancing diffusion, and extending interaction with bacterial cells. Since SBET was not assessed for all formulations, no uniform statistical correlation between surface area and activity is claimed; rather, it is regarded as a supplementary parameter that supports composition–performance trends. Consequently, results were limited with few experimental samples, extrapolation to other synthesis settings, microbiological systems, or aquatic environments necessitate additional confirmation by expanded datasets and characterisation.

The intentional alteration of textural characteristics in these composites is beneficial from a materials engineering perspective. The addition of CuO and Al₂O₃ into CS framework increases pore formation while maintaining mesostructural integrity. The concomitant raises in pore volume and surface area improves accessibility of active sites, hence facilitating formation of ROS and improving photocatalytic effectiveness. This promotes more robust interactions with microbial cell walls, resulting in improved antibacterial performance. These enlarged pore network facilitates efficient transport of oxygen and light across the matrix, allowing for consistent photocatalytic activity across the material. These enhancements in structural features collectively improve the composite's performance and durability, hence enhancing its potential for scaled environmental and biomedical applications.

The textural properties were crucial for antibacterial efficacy against *Pseudomonas aeruginosa* and *Escherichia coli*, Gram-negative species with resilient outer walls that restrict permeability. The CuO/Al₂O₃/CS nanocomposite establishes a conducive milieu that facilitates the effective passage of ROS and extended interaction with microbial cells, resulting in membrane rupture and oxidative inactivation. The gas adsorption–desorption findings thus validate the structure–performance relationship in this photocatalytic systems. The existence of mesopores, increased surface area, and hierarchical pore distribution at CuO/Al₂O₃. Chitosan substantially enhances antibacterial efficacy, validating its role as a multifunctional photocatalytic antimicrobial agent.

3.2 Antibacterial Analysis

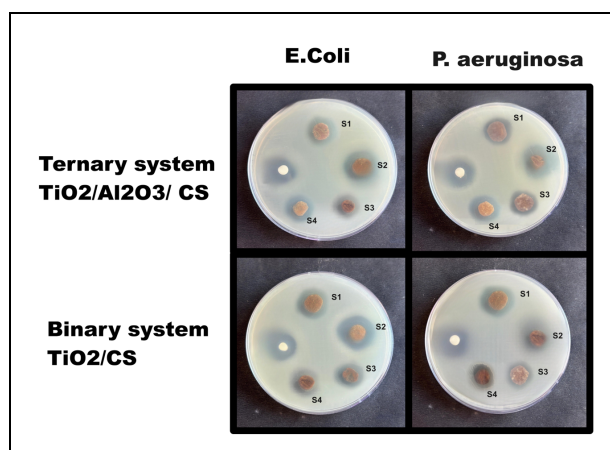


Fig. 5: Antibacterial efficacy of CuO-based nanocomposite systems

The efficacy of CS nanocomposites were assessed via disc diffusion against *Pseudomonas aeruginosa* and *Escherichia coli*. The observed outcomes indicate moderate antibacterial activity rather than insignificance, as distinct zones of inhibition were still

identified. Despite the narrower inhibition zones relative to the standard antibiotic streptomycin, the results confirm that CuO/CS demonstrates a significant antimicrobial effect (Fig. 5).

The study focuses on bead-structured CuO/Al₂O₃/CS nanocomposites, wherein antimicrobial properties result from the synergistic interaction between CS matrix and photocatalytic oxides, rather from effects of individual components. Direct comparison with isolated powders is not appropriate because of changes in shape, mass transport pathways, available surface area, and effective contact interfaces, substantially influence inhibition zone results. Although prior research has demonstrated antibacterial efficacy of CuO, Al₂O₃, and CS separately, their combination as stable composite produces novel features not readily deduced from single-phase systems. The investigation was limited to composite materials, which more accurately reflect realistic water treatment conditions. Table 1 presents the evaluation framework by specifying the material configurations, experimental methodologies, and functional responsibilities of both composite structures and individual components examined in this research.

Table 1. Assessment of materials characterization and antibacterial performance

System Composition	Microbiological Assay Method	Morphological State	Functional Role in Research
CS	Reported elsewhere	Polymer	Cationic antibacterial agent
CuO	Reported elsewhere	Powder	Baseline photocatalyst
Al ₂ O ₃	Reported elsewhere	Powder	Intrinsic antibacterial oxide
CuO/CS	This study	Beads	Synergistic binary system
CuO/Al ₂ O ₃ /CS	This study	Beads	Optimized ternary antibacterial platform

The integrated ANN–BO model demonstrated strong capability in predicting and optimizing the antibacterial performance of CuO/Al₂O₃/CS nanocomposites against *Escherichia coli* and *Pseudomonas aeruginosa*. The experimental inhibition zones for *E. coli* ranged from 1.8 to 6.1 mm, while ANN-predicted values varied between 2.70 and 6.50 mm, with residual errors within −1.80 to +0.93 mm. Similarly, for *P. aeruginosa*, the observed inhibition zones ranged from 3.0 to 10.0 mm, whereas predicted values ranged from 3.26 to 8.21 mm with residuals between −1.01 and +1.79 mm. The ANN model exhibited high prediction accuracy in intermediate composition regions (CuO = 1–1.5, Al₂O₃ = 1–1.5), where residuals were confined within ±0.2 to ±0.5 mm, indicating strong agreement between experimental and predicted results.

The BO algorithm further enhanced the model by identifying optimal compositional regions that maximize antibacterial activity. The optimal condition for *E. coli* inhibition was observed near CuO: Al₂O₃:CS = 2:1:1, yielding a maximum zone of 6.1 mm, whereas for *P. aeruginosa*, the BO-assisted optimization indicated superior performance at 2:2:1, achieving a maximum inhibition of 10.0 mm. Despite slight deviations at extreme compositions, particularly at higher oxide loadings where residuals increased, the ANN–BO framework effectively captured the nonlinear interactions between CuO, Al₂O₃, and CS. Overall, the hybrid ANN–BO approach provides a reliable and efficient tool for predictive modeling and optimization of antibacterial nanocomposite systems.

Antibacterial efficacy of ternary and binary nanocomposites were regulated by photocatalytically produced oxygen species, including hydroxyl radicals and superoxide anions, which affect intracellular components and cell membranes. The CS matrix, because of polycationic nature, destabilizes microbial membranes, restricts electron-hole recombination and promotes CuO dispersion, thus facilitating ROS generation. The robust electrostatic interactions among nanomaterials and bacterial cells promote localized ROS formation, resulting in oxidative damage and cell death. The enhanced antibacterial efficacy of the ternary system results from the synergistic interplay of its constituents. CuO serves as an effective photocatalyst, generating ROS including hydrogen peroxide and hydroxyl radicals upon illumination, which leads to the degradation of cellular structures. Al₂O₃ improves antibacterial efficacy by facilitating superior particle dispersion and surface interface, therefore enhancing effective contact and catalytic activity. Simultaneously, CS facilitates electrostatic interaction with negatively charged bacterial membranes, enhancing permeability and causing release of intracellular contents. The CuO/Al₂O₃/CS nanocomposite shows superior antibacterial efficacy attributed to synergistic effects of ROS-mediated oxidation, increased surface contacts, and membrane instability. The improved 2:1:1 formulation offers a harmonious synergy among these mechanisms, underscoring its potential for use at water purification systems and antimicrobial coatings.

The ANN model was constructed using experimentally derived inhibition zone widths, which are widely acknowledged as reliable metrics for evaluating and forecasting antimicrobial efficacy in comparative studies.

CuO/Al₂O₃/CS nanocomposite (1:2:1 weight ratio) was selected through systematic comparison of four principal formulations, evaluated by several assessment criteria. The antibacterial activity, assessed

using zone measurements demonstrated that 1:2:1 composition exhibited most dependable and consistent efficacy. The BET characterization indicated that the formulation preserved mesoporous characteristics with advantageous pore distribution, facilitating efficient mass transfer and surface exposure. The SEM measurements further validated the homogeneous distribution of CuO and Al₂O₃ inside CS matrix, minimizing particle agglomeration and improving contact. The 1:2:1 ratio guarantees sufficient metal oxide concentration for continuous ROS formation and ionic effects, while maintaining appropriate CS to uphold structural integrity and electrostatic interaction within bacterial cells. This adjusted balance with prior photocatalytic studies in dye degradation, where composition exhibited improved efficiency, underscores the significance of regulated interfacial synergy and oxide loading at ROS-mediated processes.

3.2.1 Antibacterial Mode of Action of CuO/Al₂O₃/CS

The CuO/Al₂O₃/Chitosan composite exploits synergistic antibacterial actions when exposed to light. The mechanistic interpretation provided is based on observed performance patterns and documented material behavior, than direct mechanistic measurements. The irradiated CuO functions as a photoactive element that produces electron-hole pairs, whilst Al₂O₃ aids in improving charge separation and surface stability. These interactions facilitate surface redox processes that result in generation of reactive oxygen species, like hydroxyl superoxide ions and radicals, which provoke oxidative stress in bacterial cells. ROS-driven activities are recognized for compromising membrane integrity, enhancing permeability, and damaging vital macromolecules, including nucleic acids and proteins.

The CS phase improves antibacterial efficacy owing to its polycationic properties. The protonated amine groups in chitosan facilitate robust electrostatic adhesion to negatively charged bacterial cell surfaces, hence enhancing localized interactions among microorganisms and catalytic interface. Furthermore, Al₂O₃-based materials have been documented to possess intrinsic antibacterial properties, typically ascribed to surface-mediated ionic interactions that can interfere with cellular functions. The combination of CuO, Al₂O₃, and CS creates a multifunctional antibacterial system where electrostatic adhesion, ROS-mediated photocatalytic activity, and inherent oxide-related effects function synergistically.

The generation of reactive oxygen species in CS nanocomposites is influenced by surface-driven mechanisms, ionic interactions with Al₂O₃, and electrostatic attraction among negatively charged bacterial membranes and protonated CS. Porous bead structure enhances close interaction among bacteria and

reactive spots, leading to intracellular oxidative consequences and membrane damage.

Photoluminescence results from our prior photocatalytic studies showed low electron–hole pairs in CuO/Al₂O₃/CS system when exposed to light. In this context, these observations are cited solely to emphasize effective interfacial interaction within the composite, and not to any light-induced impact.

3.3 Limitation

This research validates antibacterial performance of CuO/Al₂O₃/CS and underscores the use of ANN-based prediction, while significant limitations suggest avenues for future investigation. The integration of quantitative antimicrobial parameters, including minimum bactericidal concentration and minimum inhibitory concentration, would provide accurate assessment among qualitative diffusion study. The direct measurement of reactive oxygen species, such as using DCFDA fluorescence techniques, will provide enhanced understanding of the relationship between ROS production and antibacterial efficacy. Additionally, controlled experiments utilizing individual CuO and Al₂O₃ at lit and dark circumstances are necessary to confirm synergistic effects within the composite system. Subsequent research should combine comprehensive physicochemical characterization with cellular-level biological evaluations to further the understanding of antibacterial processes. Parameters including amino group and surface charge density can be associated with light/dark conditions and ion analysis to differentiate roles in electrostatic, catalytic oxidation, and intrinsic chitosan activity. These interpretations can be further substantiated by comparing ANN performance with SEM observations, intracellular and colony-forming assessments, and ROS detection. Furthermore, assessing performance in low-light and dark will validate real-world application of wastewater, showing transient photocatalytic responses.

3.4 Analyzing Predictive Neural Networks

3.4.1 Regression and MSE Evaluation

The performance of the developed ANN–BO model was evaluated using regression analysis and error distribution, as shown in Fig. 6a and b. The regression plots indicate a strong correlation between predicted and experimental values, with correlation coefficients of 0.95005 (training), 0.93119 (validation), 0.91857 (testing), and 0.93538 (overall), demonstrating good predictive accuracy and consistency across all datasets. The regression lines closely follow the ideal $y=x$ relationship, confirming that the ANN–BO model effectively captures the underlying nonlinear patterns in the data. Additionally, the error histogram reveals that most residuals are concentrated around zero with a nearly

symmetric distribution across training, validation, and testing sets, indicating stable model performance and minimal overfitting. The incorporation of Bayesian Optimization enhances hyperparameter tuning, leading to improved generalization and reliable prediction capability of the ANN–BO framework.

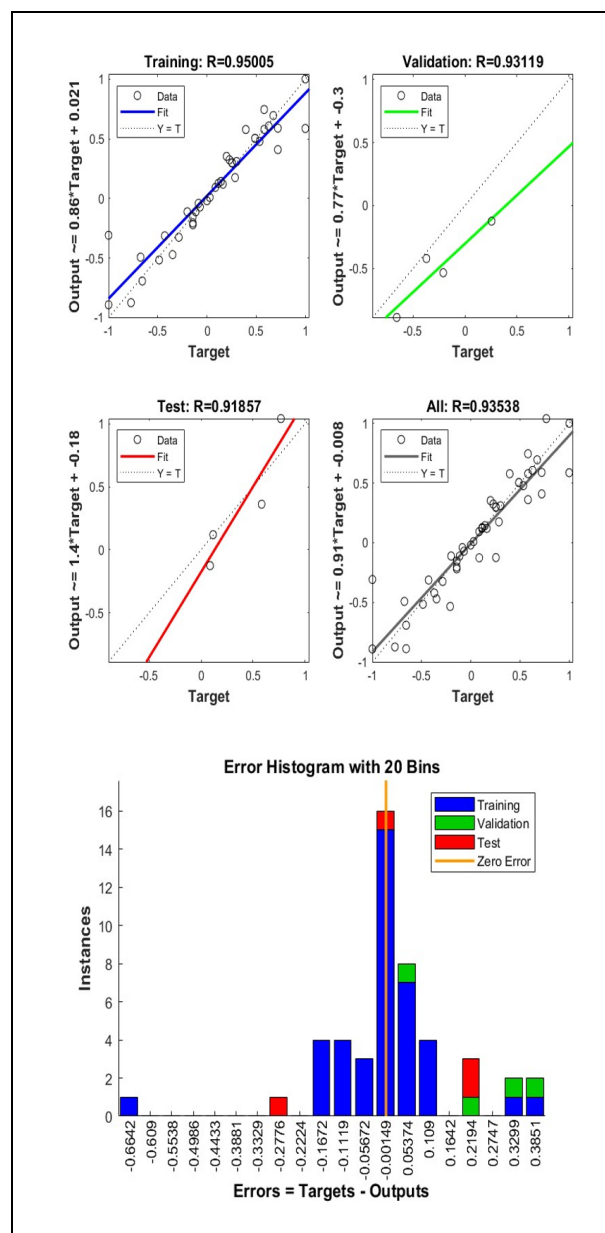


Fig. 6: (a) Neural network regression and (b) Neural network MSE evaluation

The MSE convergence curve of the ANN–BO model as shown in Fig. 7 illustrates the training, validation, and testing performance over 11 epochs. Initially, all three curves exhibit relatively high error values, indicating large prediction deviations during the early learning phase. As training progresses, a sharp reduction in MSE is observed, confirming effective weight adjustment and hyperparameter tuning facilitated

by Bayesian Optimization. The best validation performance is achieved at epoch 5 with a minimum MSE of 0.076583, representing the optimal generalization point of the model. Beyond this epoch, the training error continues to decrease, while the validation error shows a gradual increase, suggesting the onset of slight overfitting. The testing curve remains stable with low error values, indicating consistent predictive capability. Thus, the ANN-BO model demonstrates efficient learning behavior, rapid convergence, and reliable generalization performance, with epoch 5 identified as the optimal stopping point for achieving balanced accuracy across all datasets.

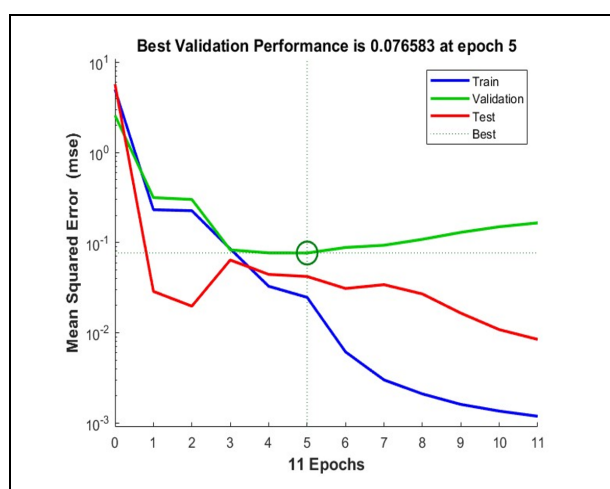


Fig. 7: Neural network training and error convergence profiles

The training state performance of the ANN-BO model, illustrated in Fig. 8, demonstrates the optimization and convergence behavior through gradient, adaptive learning parameter (μ), and validation checks over 11 epochs. The gradient shows a consistent decreasing trend from higher initial values to 0.0020182 at epoch 11, indicating effective minimization of the objective function and stable convergence of the network. The adaptive learning parameter μ , governed by the Levenberg–Marquardt mechanism within the ANN framework and optimized *via* Bayesian tuning, initially varies to allow faster learning and subsequently stabilizes at 0.0001, enabling fine weight adjustments near the optimal solution. Additionally, the validation checks increase gradually and reach a maximum of 6 at epoch 11, indicating no further improvement in validation performance and triggering early stopping to prevent overfitting. The ANN-BO model exhibits controlled learning dynamics, efficient convergence, and strong generalization capability through optimized parameter tuning and stable training behavior.

3.4.2 Comparative Predictive Model Assessment

The obtained results further validate the effectiveness of the ANN-BO approach in comparison

with existing ANN-based studies, while highlighting its distinct advantages. The developed model achieved an overall correlation coefficient of $R=0.93538$, which is significantly higher than previously reported ranges (approximately $R \approx 0.22-0.11$) for antibiotic prediction, indicating superior predictive accuracy. This improvement can be attributed to the integration of Bayesian Optimization for optimal hyperparameter tuning along with a more informative and feature-rich dataset.

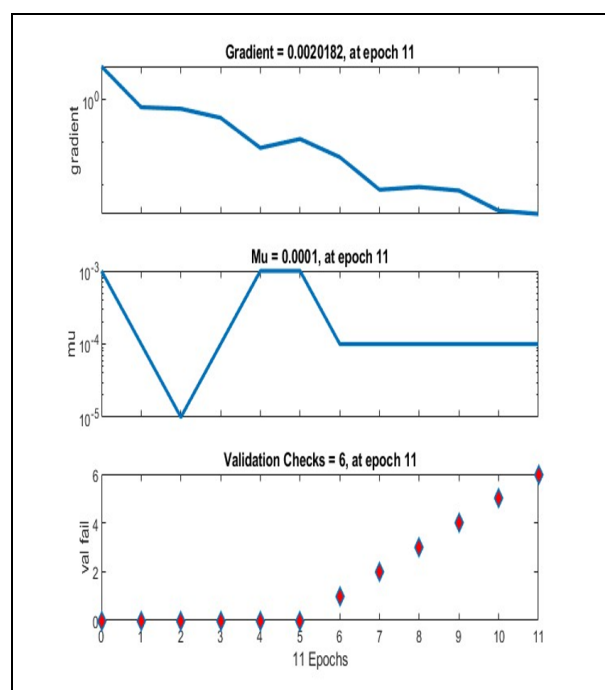


Fig. 8: Neural network convergence for antibacterial prediction

3.4.3 Prediction of Antibacterial using ANN

The predictive capability of the developed ANN-BO model was evaluated using a dataset of 38 experimental observations comprising varying compositions of CuO, Al₂O₃, and chitosan (Fig. 9). These input parameters were employed in the MATLAB-based model to simultaneously predict the inhibition zone diameters of *Escherichia coli* and *Pseudomonas aeruginosa*. The actual versus predicted plots demonstrate a strong agreement between experimental and model outputs for both bacterial strains, indicating high prediction accuracy and consistent trend capture across all samples. Minor deviations were observed at certain points. However, the overall prediction curves closely follow the experimental values, confirming effective learning of nonlinear relationships. Furthermore, the residual plots show that most errors are distributed around zero with limited dispersion, highlighting the absence of systematic bias and good generalization capability of the model. The incorporation of BO enhances the selection of optimal network

parameters, thereby improving prediction performance. Overall, the ANN–BO framework serves as a reliable and efficient alternative to conventional experimental

methods for rapid estimation of antibacterial activity based on nanocomposite composition.

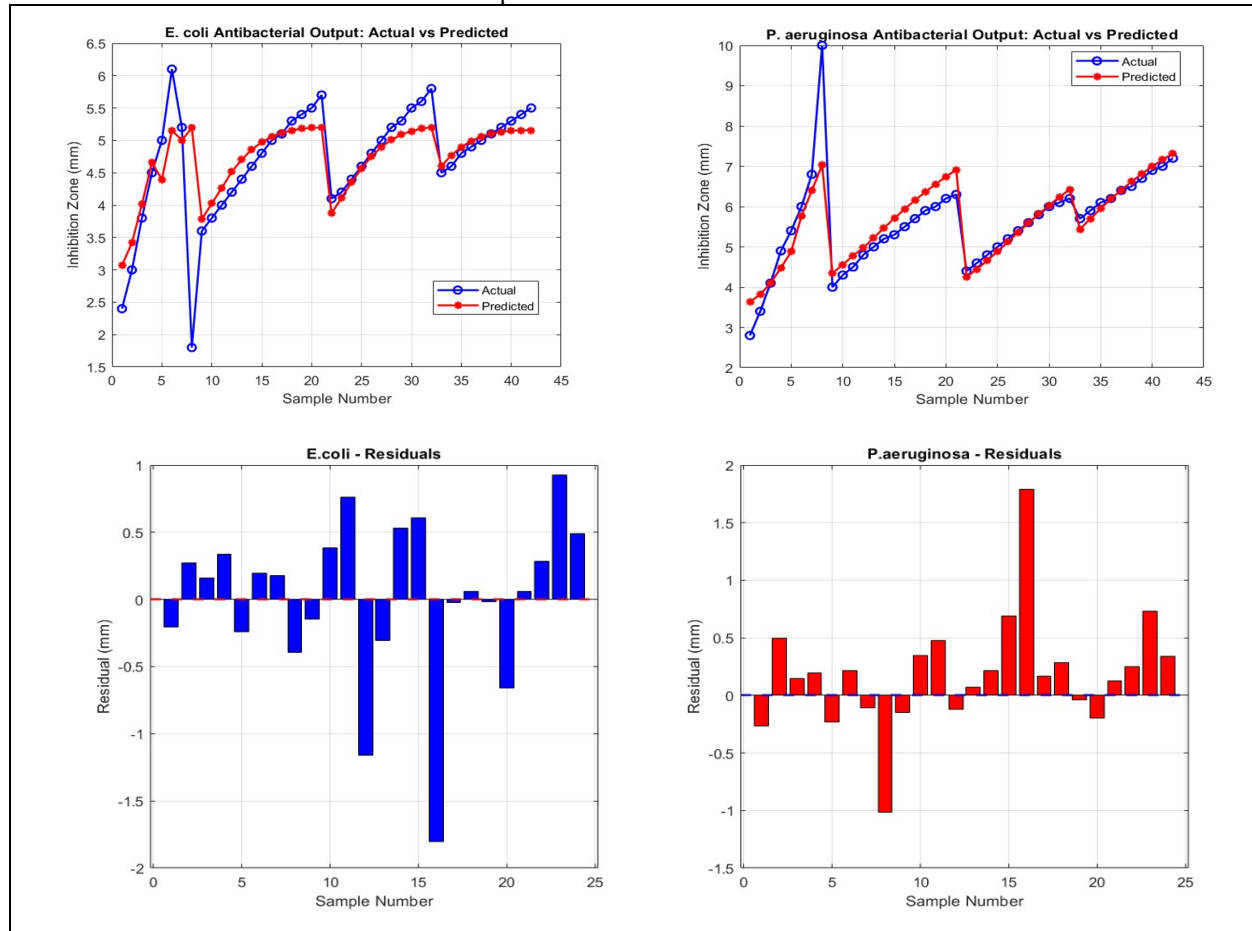


Fig. 9: Comparison of experimental and ANN-predicted antibacterial activity for *E. coli* and *P. aeruginosa* with corresponding residual error analysis

Table 2 presents the prediction performance of the ANN–BO model by comparing experimental and predicted inhibition zones for *Escherichia coli* and *Pseudomonas aeruginosa*. The predicted values show strong agreement with experimental results across most compositions, confirming the high accuracy and reliability of the model. For lower filler concentrations (e.g., CuO = 0.5–1 and Al₂O₃ = 0.5–1), the residuals are minimal, typically within ± 0.3 mm, indicating precise prediction even at lower antibacterial activity levels. The model also maintains consistent performance for moderate compositions, effectively capturing the combined influence of CuO, Al₂O₃, and chitosan with only slight deviations observed. However, larger discrepancies are noted at certain compositions, particularly at higher filler loadings (e.g., CuO = 2, Al₂O₃

= 2), where the model shows higher residuals (up to ± 1.8 mm), suggesting localized prediction sensitivity. Similarly, some intermediate compositions exhibit moderate overestimation or underestimation, indicating nonlinear complexity in specific regions of the dataset. Despite these deviations, the residual distribution remains largely balanced around zero, demonstrating that the ANN–BO model does not exhibit systematic bias and retains good generalization capability. Overall, the model effectively predicts antibacterial activity across a wide compositional range, making it a robust tool for nanocomposite design and optimization. In a previous investigation the hybrid RSM–BO framework was used to optimize ligand ratios in CuO nanolubricants. This approach enhanced dispersion stability and reduced friction by up to 60.6% (Elsoudy *et al.* 2025).

Table 2. ANN predicted microbial inhibition zones

CuO	Al ₂ O ₃	CS	Actual <i>E. coli</i> (mm)	Predicted <i>E. coli</i> (mm)	Residual <i>E. coli</i> (mm)	Actual <i>P. aeruginosa</i> (mm)	Predicted <i>P. aeruginosa</i> (mm)	Residual <i>P. aeruginosa</i> (mm)
0.5	0.5	1	2.5	2.704	-0.204	3	3.263	-0.263
1	0.5	1	3.2	2.93	0.27	3.8	3.3	0.5
1.5	0.5	1	3.8	3.64	0.16	4.2	4.051	0.149
2	0.5	1	4.5	4.163	0.337	4.8	4.604	0.196
0.5	1	1	3.5	3.739	-0.239	4.2	4.433	-0.233
1	1	1	4.5	4.302	0.198	5.2	4.987	0.213
1.5	1	1	5.2	5.024	0.176	5.8	5.909	-0.109
2	1	1	6.1	6.496	-0.396	6	7.014	-1.014
0.5	1.5	1	4.2	4.346	-0.146	5	5.151	-0.15
1	1.5	1	5	4.617	0.383	6.2	5.85	0.35
1.5	1.5	1	5.5	4.737	0.763	6.8	6.321	0.479
2	1.5	1	5.2	6.357	-1.157	7.2	7.318	-0.118
0.5	2	1	4.5	4.803	-0.303	6	5.929	0.071
1	2	1	5.2	4.666	0.534	6.8	6.583	0.217
1.5	2	1	4.8	4.191	0.609	7.5	6.812	0.688
2	2	1	1.8	3.602	-1.802	10	8.213	1.787
0.8	1.2	1	4.3	4.325	-0.025	5.5	5.335	0.165
1.2	1.2	1	5.1	5.038	0.062	6.1	5.814	0.286
1.6	1.2	1	5.4	5.418	-0.018	6.6	6.64	-0.04
1.8	1.2	1	5.6	6.261	-0.661	6.9	7.097	-0.197
0.7	1.7	1	4.6	4.538	0.062	6	5.874	0.126
1.1	1.7	1	5	4.719	0.281	6.5	6.248	0.252
1.5	1.7	1	5.3	4.373	0.927	7	6.27	0.73
1.9	1.7	1	5.5	5.009	0.491	7.3	6.964	0.336

The developed ANN–BO model demonstrated strong predictive performance, with most deviations confined within a narrow range and the majority of errors remaining within ± 1 mm, indicating high reliability across diverse input conditions. The close agreement between predicted and experimental values confirms the robustness of the model in capturing complex nonlinear relationships governing antimicrobial activity. Although a few outliers are observed at specific compositions, these deviations highlight localized sensitivity rather than systematic error, suggesting scope for further refinement through dataset expansion and model tuning. The ANN–BO framework implemented in MATLAB proves to be an effective computational tool for predicting antibacterial behavior, significantly reducing dependence on extensive experimental trials while offering a time-efficient and cost-effective approach for material design. Incorporation of additional experimental data and continued optimization is expected to further enhance model accuracy, stability, and generalization capability. A dependable ANN implementation essentially relies on adequate data coverage and systematic training to

guarantee robust generalization while preventing overfitting.

Fig. 9 demonstrates the strong predictive capability of the developed ANN–BO model in estimating the antibacterial efficacy of nanocomposites against *Escherichia coli* and *Pseudomonas aeruginosa*. The comparison between experimental and predicted inhibition zones (Fig. 9a) reveals a close agreement for both bacterial strains, confirming that the model effectively captures the nonlinear behavior of the system. For *Escherichia coli*, the ANN–BO model accurately follows the experimental trend with only minor deviations, while for *Pseudomonas aeruginosa*, a gradual increase in inhibition zone is well predicted, with slight underestimation observed at higher values but still within acceptable limits. The residual analysis (Fig. 9b) further supports the model's accuracy, showing that most residuals are centered around zero with limited dispersion, indicating minimal prediction error and absence of systematic bias. Although a few larger deviations are observed at specific compositions, the

overall error distribution remains balanced, confirming good generalization performance. The strong regression behavior, characterized by high correlation between predicted and experimental values, validates the robustness of the ANN–BO framework. Minor underprediction at higher inhibition levels may be attributed to increased nonlinear complexity or boundary effects within the training data range. Nevertheless, the model consistently provides reliable predictions, establishing it as an efficient computational tool for rapid evaluation and design of antimicrobial nanomaterials.

3.4.5 Operational Implications and Implementation

The ANN model serves both as a predictive framework and as a crucial element in an efficient material design workflow. The model can be used to swiftly assess many nanocomposite formulations computationally, thus reducing the need for laborious synthesis and experimental trials. Compositions anticipated to demonstrate enhanced antibacterial efficacy, exemplified by greater inhibition zones against specific pathogens, may then selectively be prioritized for laboratory validation. This method diminishes material and time demands, facilitating a transition from trial-and-error techniques targeted, data-driven design strategy for quick antimicrobial material formulation.

4. CONCLUSION

The persistent challenge of water contamination caused by complex organic pollutants and microbial pathogens necessitates the development of efficient antibacterial materials with predictive design capability. In this study, a ternary CuO/Al₂O₃/chitosan nanocomposite was developed, where CuO acted as a photocatalyst, Al₂O₃ improved dispersion and structural stability, and chitosan functioned as a biopolymeric antibacterial matrix. The nanocomposite beads were synthesized *via ex situ* mechanical mixing followed by ionic gelation. Their antibacterial performance was evaluated using the disc diffusion method, while an ANN–BO framework was employed to model and optimize the nonlinear interactions between the composition and antibacterial activity. The results demonstrated inhibition zones ranging from 1.8–6.1 mm for *E. coli* and 3.0–10.0 mm for *P. aeruginosa*. The optimized CuO/Al₂O₃/CS (2:2:1) nanocomposite exhibited the highest antibacterial activity with a maximum inhibition zone of 10.0 mm against *P. aeruginosa*, while the 2:1:1 composition showed optimal performance against *E. coli* with 6.1 mm. The ANN–BO model exhibited strong predictive accuracy ($R \approx 0.935$). The synergistic interaction among CuO, Al₂O₃, and CS significantly enhanced antibacterial efficacy through improved dispersion, mesoporosity, and reactive oxygen species generation. The developed system shows strong potential for applications in water purification,

antimicrobial coatings, and an intelligent design of advanced antibacterial nanomaterials.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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