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Biogenic synthesis of copper oxide nanoparticles (CuONPs) using *Nerium Oleander*: *in-vitro* antimicrobial potential and photocatalytic studies

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Abstract

The present study is on the green synthesis of CuONPs of *Nerium oleander* as a bioreducing and stabilizing agent. This approach is a green and nominal cost alternative to existing methods. The structural determination of the synthesized material was done by UV, FTIR, SEM, TEM, XRD and EDX. Formation of crystalline, spherical CuONPs in the range from 15 to 40 nm is identified. Whereas XRD pattern matched the monoclinic phase of CuONPs. The calculated band gap using Tauc plot arrived as 2.10 eV infer nanoscale particle formation in the present green synthesis. The antimicrobial efficacy of the CuONPs was evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Salmonella typhi* using the agar well diffusion method. A concentration-dependent inhibition was observed, with significant zones of inhibition recorded, indicating appreciable antibacterial activity of the synthesized nanoparticles. To be mentioned activity against *S. aureus* was observed as 18.6 ± 0.5 mm compared to the standard with zone of inhibition of 23.2 ± 0.6 mm. Furthermore, the photocatalytic potential of CuONPs was examined by the decay of methylene blue, crystal violet, and eosin yellow under sunlight. The nanoparticles exhibited efficient dye degradation, with over 90% degradation of methylene blue, eosin yellow and crystal violet with maximum time limit of 180 min exposure to sunlight. The prolong time not gave any additional degradation and hence the maximum degradation achieved can be only 90%, following pseudo-first-order kinetics. These findings demonstrate that *N. oleander*-mediated CuONPs possess excellent antimicrobial and photocatalytic activity favours in the applications of biomedical coatings, environmental remediation, and sustainable nanomaterial development.

Keywords CuONPs, *Nerium oleander*, Eco-friendly green synthesis, XRD analysis, Photo degradation



1 Introduction

The synthesis of nanomaterials through green and sustainable methods have attracted the interest of scientists. Utilization of plant extracts is an alternate to existing methods due to its nominal cost, environmental safety, and the use of naturally occurring phytochemicals for the synthesis [1]. These bio-mediated approaches eliminate the toxic reagents and yielding nanoparticles that are often more biocompatible and functionally versatile [2, 3].

Among the various metal oxide nanoparticles, the aim to synthesize CuONPs [4] is due to their unique physicochemical properties, including the band gap (1.2–1.9 eV) [1], high surface reactivity, and potent biological activities [5]. The diverse applications of CuONPs are such as antimicrobial agents [6], catalysts, and photocatalysts for degradation of dyes [7, 8]. The photocatalytic activity of CuONPs, particularly under visible light, makes it an attractive candidate for environmental remediation. Additionally, the ability of CuONPs to disrupt microbial membranes and induce reactive oxygen species (ROS) generation underlies their activity [9].

The green synthesis of CuONPs using *Psidium guajava*, *Azadirachta indica*, *Moringa oleifera*, *Vitex negundo* and *Murraya Koenigii* is reported in the literature [10–16]. These studies have demonstrated that plant-derived phytochemicals can facilitate formation and size of the nanoparticles. The potential of many species remains underexplored in nanomaterial synthesis.

N. oleander, a widely distributed ornamental plant is reported as rich in secondary metabolites [17]. These compounds exhibit strong reducing and stabilizing capacities, making the plant a promising candidate for green nanotechnology applications. Nevertheless, there is a distinct lack of comprehensive studies that utilize *N. oleander* leaf extract for the biogenic synthesis of CuONPs, particularly with respect to evaluating their antimicrobial and photocatalytic efficacy. While *N. oleander* has been studied for its therapeutic properties, its application in nanomaterials synthesis remains largely unexplored.

The reactive dyes as an effluent from various industries become a threat to health. Various conventional and biological methods employed for degradation of the dyes. Nanomaterials and nanocomposites mediate photocatalytic degradation is in current use. Being heterogeneous photocatalysis, the photocatalyst can be separated from the reaction medium and hence the possibility of reuse [18].

Photocatalytic degradation of organic dyes using semiconductor oxides as catalysts under sunlight is being used currently. An enhanced photocatalytic activity of ZnO-TiO₂ mixture in nanocrystalline form was reported in the literature [19]. Noble metal incorporation into metal oxide composites to enhance the lifetime of photogenerated charge carriers in TiO₂-Ag-ZnO composite was another milestone in photocatalytic dye degradation [20]. CeO₂ nanocomposites for dye degradation after exposure to sunlight received energy greater than the energy of the band gap produce electron–hole pairs, which are required to start the photocatalytic process [21]. The oxidation state transition between Ce³⁺ and Ce⁴⁺ found on the surface of CeO₂-NPs is responsible for the catalytic activity of this nanoparticle [22]. It is worth to mention the Chitosan/CeO₂/WO₃ hybrid nanocomposite exhibits remarkable stability (95.5% after 5 cycles) during the short-term (3 min) elimination of MB dye pollution [23].

Although green synthesis of CuONPs has been widely investigated with various plant systems, the use of *N. oleander* leaf extract remains scarcely reported. Furthermore, very few studies have combined detailed structural characterization with both antimicrobial and photocatalytic performance analysis of CuONPs synthesized via this route. This represents a notable gap in the literature that this study aims to fill.

The objective is to synthesize CuONPs using *N. oleander* leaf extract through green pathway. The structure was arrived using XRD, SEM, TEM, FTIR [4] and UV. The antimicrobial potential of the CuONPs is assessed against clinically relevant bacterial strains, while their photocatalytic activity is evaluated by dye degradation under visible light. This work not only contributes to expanding the library of green-synthesized CuONPs but also explores the untapped potential of *N. oleander* in nanotechnology, offering insight into its dual utility in biomedical and environmental applications.

2 Experimental methods

2.1 Materials

We used some chemicals and reagents used in this study. All the chemicals used were of analytical grade and are listed in the Table 1 [24]. Distilled water was used throughout the experiments [25] [26].

2.2 Preparation of *N. oleander* leaf extract

The plant is a cultivating plant and it was gathered from Dr. N.G.P Arts and Science College, Coimbatore. The plant is cultivated inside the campus. The aerial part (leaf) of the plant only has been used for research purposes. The plant is not an endangered species and it has been identified by Dr. S. Jeyendran, Assistant professor of Botany, Government Arts College, Udumalpet. A specimen copy (ANO 210190) of the plant is available in the Department of Botany, Government Arts College, Udumalpet. *N. oleander* leaf was cleaned with water and dried [9, 13, 27].

The decoction of the plant *N. oleander* was prepared by boiling 50 g of dry powdered leaf in 250 mL of distilled water by boiling. The decoction was filtered through Whatman filter paper before use [21].

2.3 Biogenic synthesis of CuONPs

50 mL of 0.1 M aqueous Copper(II) sulphate [24] solution was mixed slowly dropwise with 250 mL of the *N. oleander* leaf extract at ambient temperature. A visible colour changes from blue to brown inferred the formation of CuONPs [8, 11, 28].

The solution was centrifuged at 6,000 rpm for 15 min to collect the nanoparticles. The thorough washing was ensured with distilled water and once with ethanol to remove

Table 1 Chemicals and reagents

S. No	Chemical/Reagent	Grade	Purity	Supplier/Manufacturer
1.	Copper(II) sulphate pentahydrate ($\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$)	Analytical grade	$\geq 98\%$	Merck Life Science Pvt. Ltd.
2.	Sodium hydroxide (NaOH)	Analytical grade	$\geq 97\%$	Sigma-Aldrich
3.	Ethanol ($\text{C}_2\text{H}_5\text{OH}$)	Analytical grade	$\geq 99.5\%$	HiMedia Laboratories Pvt. Ltd.
4.	Methylene blue (dye)	Laboratory grade	$\geq 98\%$	Loba Chemie Pvt. Ltd.
5.	Crystal Violet (dye)	Laboratory grade	$\geq 95\%$	SRL (Sisco Research Lab), India
6.	Eosin Yellow (dye)	Laboratory grade	$\geq 95\%$	SRL (Sisco Research Lab), India
7.	Distilled water	Laboratory reagent	-	Prepared in-house

unreacted plant residues [29]. Thus arrived CuONPs was calcinated in a muffle furnace at 800 °C for 5 h [30]. From 1250 mg of $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ 356 mg of CuONPs was arrived.

The synthesis yield was calculated using the formula:

The theoretical yield was approximately 398 mg. Therefore, the average yield of CuONPs obtained is ~89.4%.

The consistency was evaluated by performing the synthesis in triplicate. The structure was arrived by UV, FTIR, XRD, SEM, EDX and TEM analyses [6]. Similarly, both the antimicrobial activity and photocatalytic degradation tests were also performed in triplicate.

2.4 Characterization techniques of synthesized cuonps

After the synthesis process of CuONPs, we used to carry out some basic spectroscopic techniques to confirm the prepared nanoparticles. Structure, morphology and composition of CuONPs identified by the following methods:

$$\text{Yield (\%)} = \left(\frac{\text{Mass of dried CuO nanoparticles}}{\text{Theoretical mass of CuO from precursor}} \right)$$

2.4.1 UV-Visible spectroscopy (UV)

This method is used to detect optical absorption the new CuONPs due to SPR [31] between the range of 200–800 nm [32, 33] using SHIMADZU spectrophotometer.

2.4.2 Fourier-Transform infrared spectroscopy (FTIR)

To identify the type of Cu-O stretching frequency present in the new CuONPs Bruker Tensor 27 FTIR spectrometer was used [34, 35].

2.4.3 X-ray diffraction (XRD)

The nature and phase purity of the CuONPs measured by PANalytical X'Pert PRO diffractometer with Cu $K\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$) at 40 kV and 30 mA [34, 36] and Debye–Scherrer [37, 38] equation is used to calculate average crystallite size.

2.4.4 Scanning electron microscopy (SEM)

The morphology and approximate particle size of the synthesized CuONPs measured by SEM using a MIRA3 TESCAN at an operating voltage of 15–20 kV [12] and SEM images revealed quasi-spherical particles with moderate aggregation, typical of biogenically synthesized nanoparticles [39].

2.4.5 Transmission electron microscopy (TEM)

The internal crystallinity of CuONPs identified by TEM using a JEOL JEM-2100 instrument at 200 kV. The nanoparticles exhibited spherical to elliptical shapes in the range from 20 to 50 nm [40]. The crystalline nature of the CuONPs was confirmed by SAED pattern [41].

2.4.6 Energy dispersive X-ray spectroscopy (EDX)

The purity and composition of CuONPs [4] confirmed by EDX using an Oxford Instruments EDS detector. The characteristic peaks of Cu ($K\alpha$ at ~ 8.0 keV) and O ($K\alpha$ at ~ 0.5 keV) confirmed the successful formation of CuONPs without extraneous elements [42].

2.5 Evaluation of antibacterial activity

The antibacterial activity against *S. aureus*, *E. coli*, *B. subtilis* and *S. typhi* [9, 43] was measured by inoculating in Muller Hinton Agar (MHA) [44, 45]. CuONPs suspension prepared in sterile distilled water. Ciprofloxacin was a positive control. Each test was performed in triplicate and the mean Zone of Inhibition (ZOI) values were recorded.

2.5.1 Evaluation of photocatalytic activity

The photocatalytic activity of the biosynthesized CuONPs was examined by the decay of dyes namely methylene blue (MB), crystal violet (CV) and eosin yellow (EY) under sunlight [46]. The degradation efficiency was determined using UV by monitoring the decrease in absorbance of each dye at their respective maximum absorbance wavelengths. Working solutions of 10 mg/L were prepared and used for photocatalytic experiments [47].

2.5.2 Photocatalytic degradation setup

A measured quantity of 100 mg of CuONPs was dispersed in the prepared dye solution. The CuONPs-dye mixture was exposed to direct sunlight in order to assess its photo degradation property [48]. Dye degradation of the solution was observed in different time intervals.

2.5.3 Calculation of degradation efficiency

The degradation efficiency (%) was calculated using the formula:

$$\text{Degradation (\%)} = \left(\frac{C_0 - C_t}{C_0} \right) \times 100$$

Where:

- C_0 = initial concentration or absorbance.
- C_t = concentration or absorbance at time t .

2.5.4 Reusability test

The reusability of the CuONPs was evaluated by recovering the catalyst after the first photocatalytic cycle via centrifugation, washing with distilled water, drying at 110°C , and reusing for up to 3 successive cycles. The degradation efficiency was recorded for each cycle.

3 Results and discussion

3.1 UV-Visible spectroscopy (UV) analysis of cuonps

The generation of CuONPs was identified by a distinct color change from blue to brown upon mixing *N. oleander* decoction with solution of copper sulphate (Fig. 1). This change suggests the generation of nanoparticles, driven by phytochemicals in the plant extract.

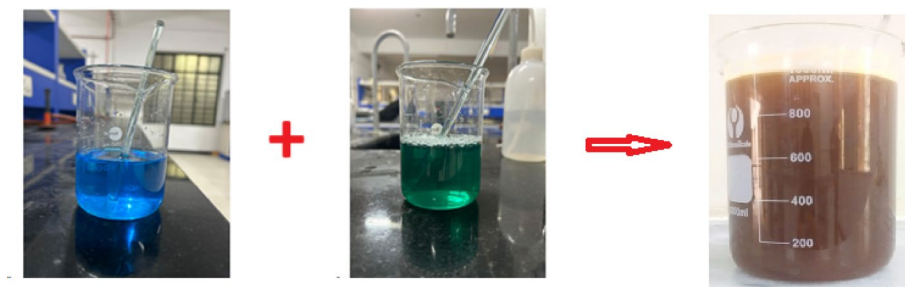


Fig. 1 Color change observed during green synthesis of CuONPs

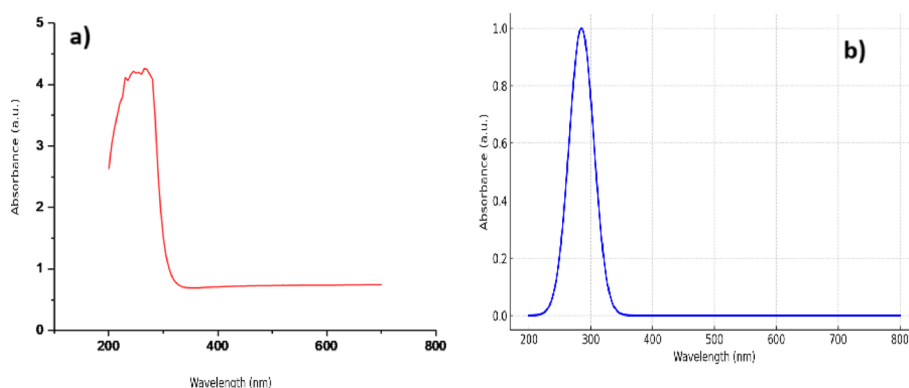


Fig. 2 UV spectra of CuONPs (a) observed graph (b) theoretical graph

The formation of CuONPs is identified from distinct absorption peak at 285 nm (Fig. 2), This peak corresponds to interband transitions characteristic of Cu-O and indicates good monodispersity and size uniformity. This is consistent with previous studies that reported SPR peaks for CuONPs in the range of 260–330 nm [49].

3.1.1 Band gap Estimation via Tauc plot

The calculated band gap was arrived at approximately 2.10 eV [4] using Tauc's method by extrapolating the plot to x-axis (Fig. 3) between the $(\hbar\nu)^2$ versus photon energy ($\hbar\nu$). The higher E_g value confirms the nanoscale nature and effective synthesis of CuONPs using the plant-mediated approach.

3.2 GC-MS analysis of *N. Oleander* leaf extract

From literature the presence of secondary metabolites identified and reported in *N. Oleander* leaf extract are 1-Dodecanol, 1-beta-d-Ribofuranosyl-3-[5-tetraazoyl]-1,2,4-triazole, N-Acetyl-N'-butyrylurea, 2-Chloroethyl vinyl sulfide, Hexadecanoic acid methyl ester, 9,12,15-Octadecatrienoic acid methyl ester, Phenol, 4,4'-(1-methylethylidene)bis- 1,8,9-Anthracenetriol, 3-methyl, 10-Anthracenedione, 1,8-dihydroxy-3-methyl, P-(Adamantyl-1)-ethylthiobenzene, 4-[(2-Methyl-5-nitrophenylimino)-methyl]-benzene-1,3-diol, beta-Tocopherol, dl-alpha-Tocopherol, Ethanone, 2-(2-benzothiazolylthio)-1-([50].

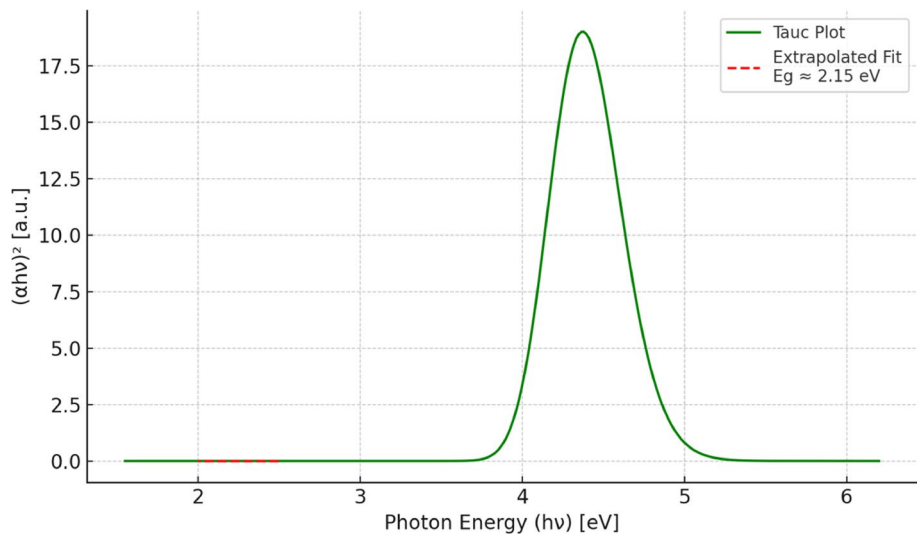


Fig. 3 Tauc plot for band gap determination of CuONPs

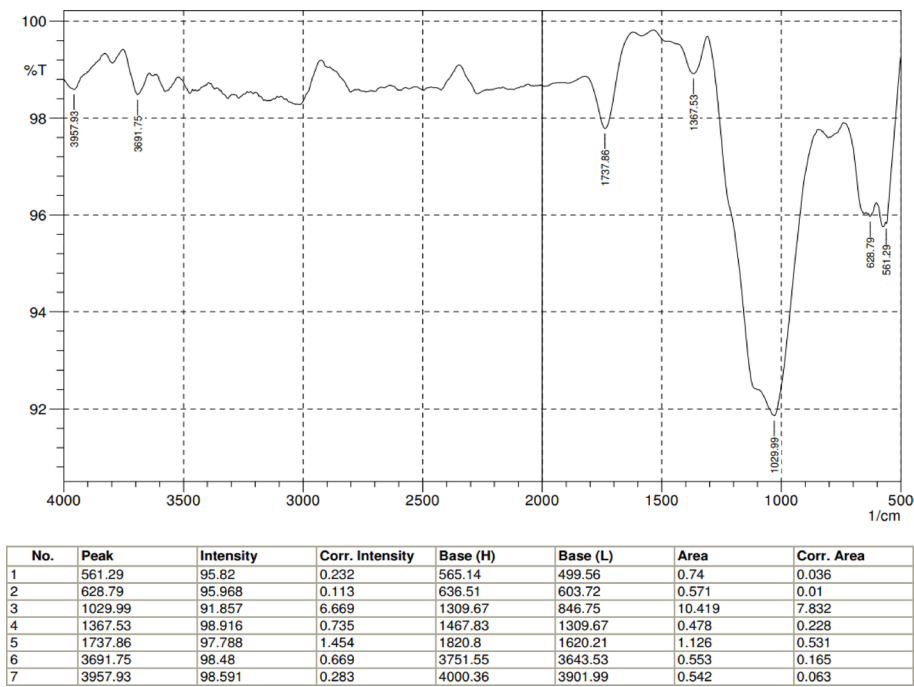


Fig. 4 FT-IR spectrum of CuONPs

3.3 FT-IR spectroscopy (FT-IR) analysis of cuonps

The FTIR spectrum revealed Cu-O stretching vibration of CuONPs at 628 and 561 cm^{-1} [4, 51], when plotted parentage transmittance (%T) against wavelength in cm^{-1} (Fig. 4).

3.4 X-ray diffraction (XRD) analysis of cuonps

The crystalline nature, phase composition and average crystallite size of CuONPs synthesized using *N. oleander* leaf extract was measured using XRD drawn as intensity in a.u. at y-axis and angle of diffraction 2θ at x-axis. The prominent peaks consistent with the monoclinic structure of CuONPs (Fig. 5).

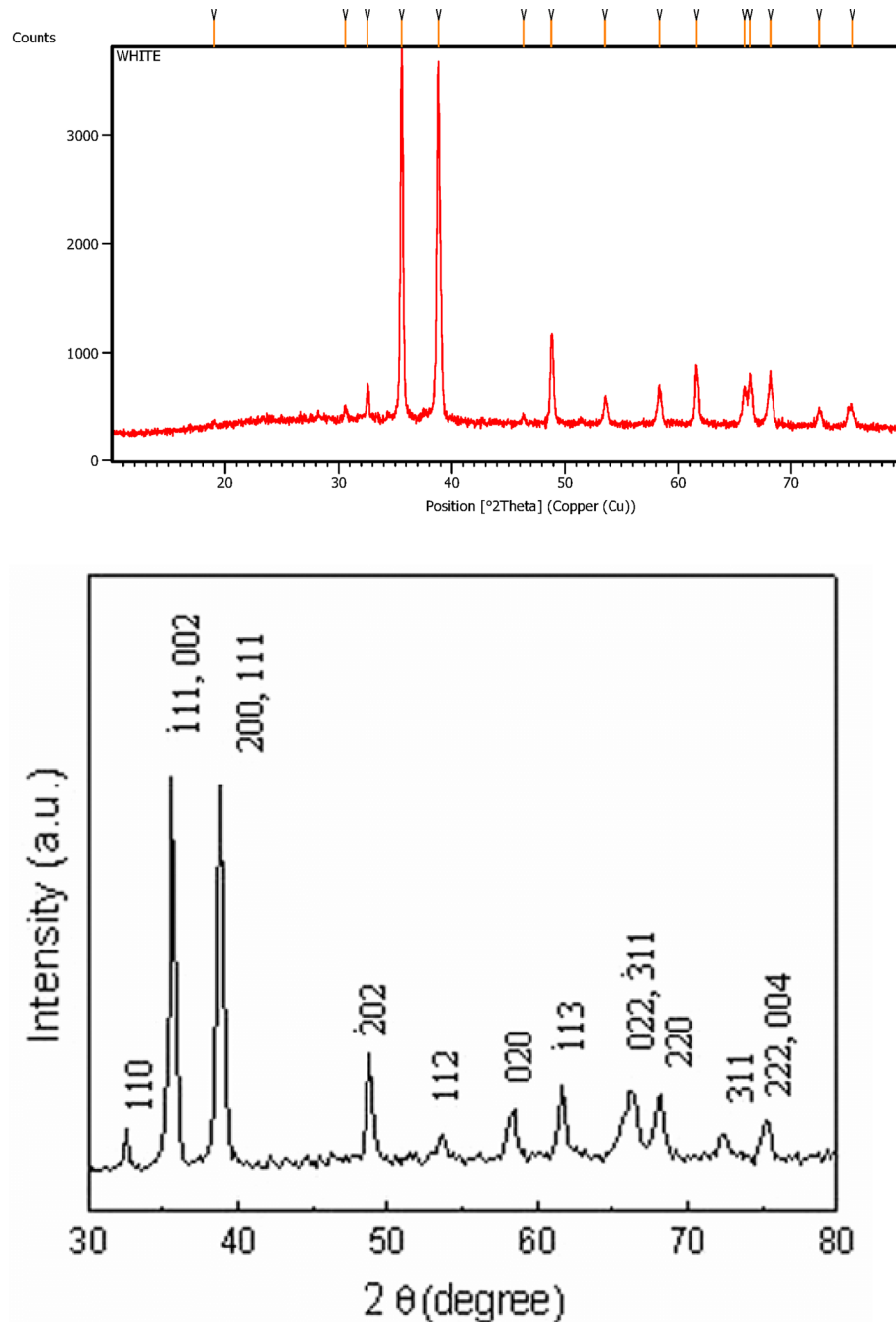


Fig. 5 XRD pattern of CuONPs (JCPDS No. 80–1917)

The 2θ values of sharp peaks observed at 32.58° , 35.60° , and 38.79° correspond to the (110), (-111), and (111) planes of monoclinic structure (JCPDS No. 80–1917) [52] and the detailed data clearly tabulated in the following Table 2.

3.4.1 Crystallite size Estimation

The Scherrer equation was applied to estimate the average crystallite size D :

$$D = \frac{0.9\lambda}{\beta \cos \theta}$$

Table 2 XRD peaks and structural analysis of cuonps

2θ (°)	Peak Intensity (cts)	d-spacing (Å)	FWHM (°)	Relative Intensity (%)	Assigned Plane (hkl)
32.59	321.92	2.748	0.1673	9.31	(110)
35.60	3457.53	2.522	0.1840	100.00	(-111)
38.80	3281.94	2.321	0.1506	94.92	(111)
48.80	792.49	1.866	0.1171	22.92	(-202)
53.51	238.02	1.713	0.1673	6.88	(020)
58.35	329.83	1.581	0.1673	9.54	(202)
61.63	523.62	1.505	0.1338	15.14	(-113)
66.33	430.97	1.409	0.1673	12.46	(-311)
68.14	460.87	1.376	0.1673	13.33	(220)
72.46	146.58	1.304	0.3346	4.24	(311)

$$D = \frac{0.9 \times 0.15406}{0.1840 \times \cos(17.80^\circ)} \approx 23.7 \text{ nm}$$

This value suggests the particles are nanocrystalline, consistent with TEM and SEM observations.

The diffraction peaks compared to standard reference (JCPDS No. 80–1917) infers monoclinic nature of CuONPs. No peaks corresponding to Cu and Cu₂O [53, 54].

The sharp and intense peaks reflect a high degree of crystallinity, and the average crystallite size (~ 23.7 nm) confirms the nanoscale dimensions [37]. These features are crucial for enhancing the surface area and reactivity of CuONPs.

XRD analysis ensured the synthesis of phase-pure, monoclinic CuONPs using *N. oleander* leaf extract. The average crystallite size was ~ 23.7 nm calculated by Scherrer equation consistent with SEM and TEM findings.

3.5 Scanning electron microscopy (SEM) analysis of cuonps

Shape, aggregation behavior, surface topology and size distribution of CuONPs were identified [4] using SEM. The image shows quasi-spherical nanoparticles with mild agglomeration. Particle boundaries are distinguishable, indicating effective phytochemical-mediated synthesis and stabilization (Fig. 6).

3.6 Morphological observations

Spherical to quasi-spherical in shape with smooth surfaces of CuONPs revealed by the SEM image [31]. Particles are observed to be relatively well-dispersed, though some degree of mild agglomeration is present and likely due to van der Waals interactions or insufficient capping at certain points. This morphology is consistent with previously reported green-synthesized CuONPs [55] and the neat observation tabulated in the following Table 3.

XRD calculations revealed ~ 23.7 nm crystallite size of the CuONPs was also observed in SEM image. However, SEM captures larger diameters due to particle boundaries and possible aggregation, indicating that individual crystallites may form larger clusters capped by organic compounds from the leaf extract.

The observed moderate polydispersity in size is common in biogenic syntheses, where diverse phytochemicals interact differently with the metal precursor. Nonetheless, the particles remain largely monodisperse within the 30–60 nm range [1, 55].

SEM analysis confirms that new CuONPs [1] using *N. oleander* decoction are spherical to quasi-spherical, Nanometric in size (~ 30–60 nm), Moderately agglomerated, with

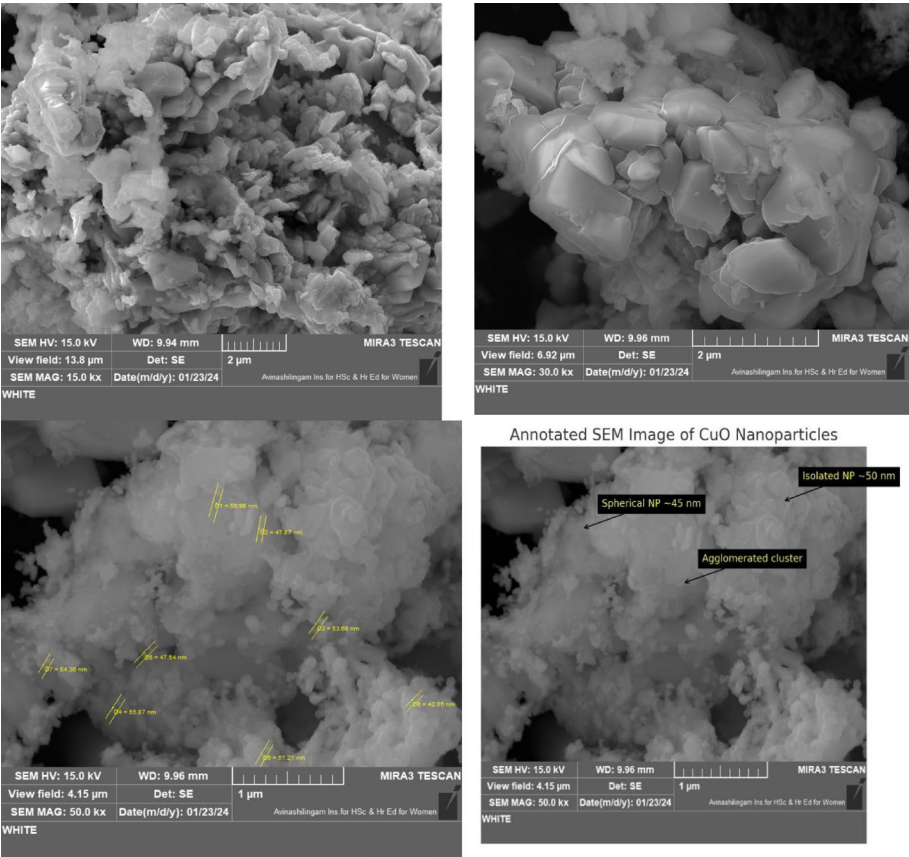


Fig. 6 SEM micrograph of CuONPs

Table 3 SEM-Based morphological data of cuonps

S. No	Parameter	Observation
1.	Particle shape	Mostly spherical to quasi-spherical
2.	Particle Size range (from SEM)	~30–60 nm (estimated)
3.	Average particle size	~45 nm (visual estimation; confirmed by histogram if needed)
4.	Surface texture	Smooth
5.	Aggregation observed?	Yes, Mild aggregation due to natural capping agents
6.	Capping evidence	Smooth surface boundaries, suggesting phytochemical capping

visible boundaries and stabilized by plant-based reducing agents. These structural features make the nanoparticles suitable candidates for applications in microbial inhibition and photocatalytic degradation of dyes [29].

3.7 Energy dispersive X-ray spectroscopy (EDX) analysis of cuonps

The composition of Copper (Cu) and Oxygen (O) of the CuONPs was arrived using EDX spectrum by plotting the x-axis as the energy of the X-rays (in keV) and the y-axis as X-ray counts or intensity. The presence of minor impurities such as Silicon (Si), Molybdenum (Mo) and Iron (Fe) showed in the EDX spectrum and presented in Table 4; Figs. 7 and 8.

Formation of CuONPs is confirmed from prominent peaks for Cu and O observed in EDX. The Cu peaks were observed around 1 keV and 8 keV, corresponding to Cu Lα and Kα lines, respectively, while the O Kα peak appeared near 0.5 keV.

Table 4 Elemental composition of cuonps by EDX

S. No	Element	Weight%	Atomic %	Remark
1.	O	15.00	40.79	Oxide content
2.	Si	1.65	2.55	Likely from plant capping agents
3.	Mo	2.64	1.20	TEM grid material and substrate
4.	Fe	1.87	1.46	Likely from plant capping agents
5.	Cu	78.85	54.00	Major component of CuO

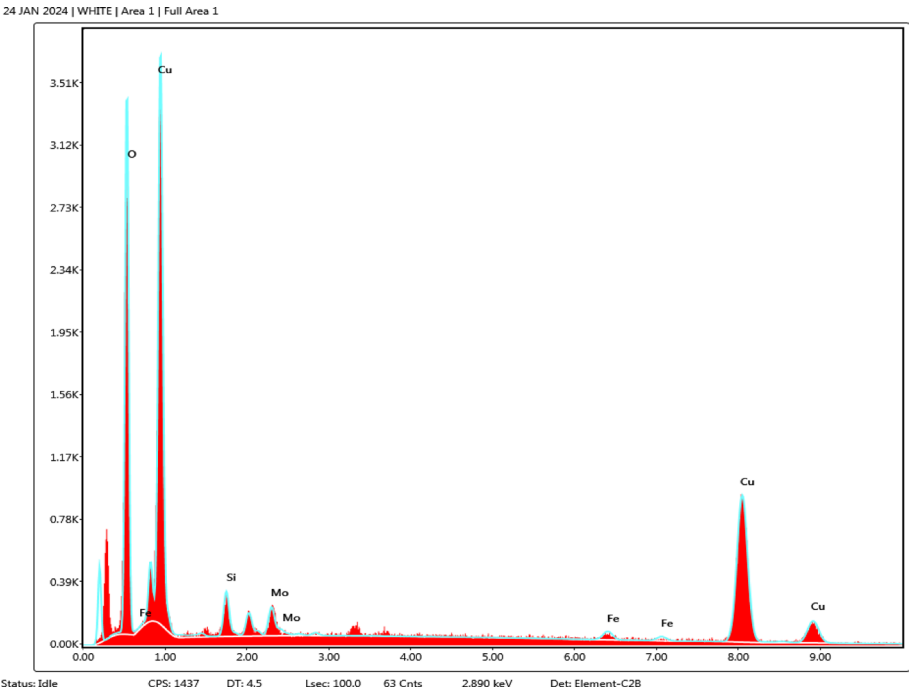


Fig. 7 EDX Spectrum of CuONPs

The presence of Mo and Si is attributed to the TEM grid material and substrate, while Fe and Si traces may also originate from the phytochemicals of *N. oleander*. These findings are consistent with previous studies reporting that [1] biogenic CuONPs show minimal impurities and effective phytochemical surface stabilization [36, 55].

3.8 Transmission electron microscopy (TEM) analysis

The size, shape, distribution and crystallinity [1] of CuONPs measured using TEM. TEM provides nanoparticles at the nanometer scale, which complements SEM and XRD findings (Fig. 9).

The particles appear mostly spherical to quasi-spherical with clear boundaries in the range between 15 and 40 nm. Mild agglomeration is visible, likely due to van der Waals interactions [36].

The TEM image shows dense packing of nanoparticles with well-defined, mostly spherical morphology is presented in Table 5. The particle size appears to vary between 15 and 40 nm, with an average diameter around 25 nm, in agreement with calculated value (~ 23.7 nm) by Scherrer equation. This consistency confirms the formation of single or few-domain nanocrystals [29].

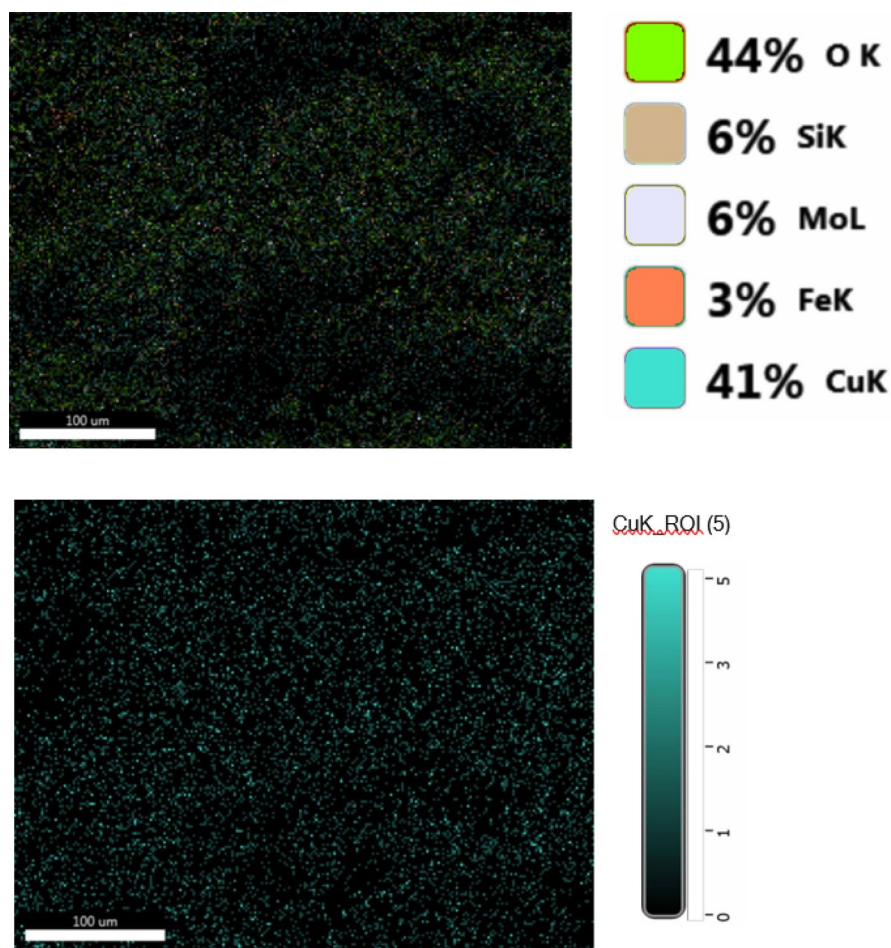


Fig. 8 Element Overlay and EDX APEX of CuONPs

The particles appear to be slightly agglomerated, which is expected in biogenically synthesized nanoparticles where complete steric stabilization may be limited. Nevertheless, the smooth boundaries of most particles indicate the presence of phytochemicals from the *N. oleander* extract that act as capping agents, preventing uncontrolled aggregation and aiding in shape regulation [25].

TEM images displayed crystallinity of the CuONPs. The interplanar spacing measured from the fringes (e.g., 0.232 nm) consistent with XRD results and to the plane (111).

The observation of such lattice planes further supports the formation of monocrystalline or highly ordered nanocrystals, critical for applications such as photocatalysis and antimicrobial activity, where surface atomic arrangement governs reactivity [40].

3.9 Antibacterial efficacy of cuonps

The antibacterial activity of CuONPs was performed against four selected bacterial strains. The efficacy of the CuONPs was compared with standard antibiotics [56] (Fig. 10).

The biological activity of CuONPs were explored largely in the literature. In particular antibacterial activity is having greater attention due to its activity towards many of the pathogens. Better activity of the CuONPs reported in the literature towards the strains *S. aureus* [57], *E. coli* [58], *B. subtilis* and *P. aeruginosa* [59]. Among the tested strains

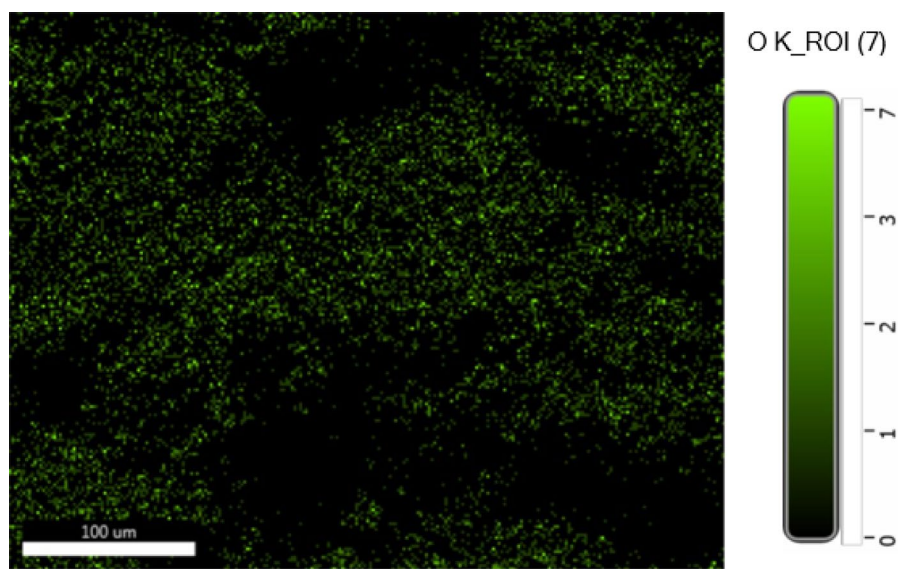


Fig. 8 (continued)

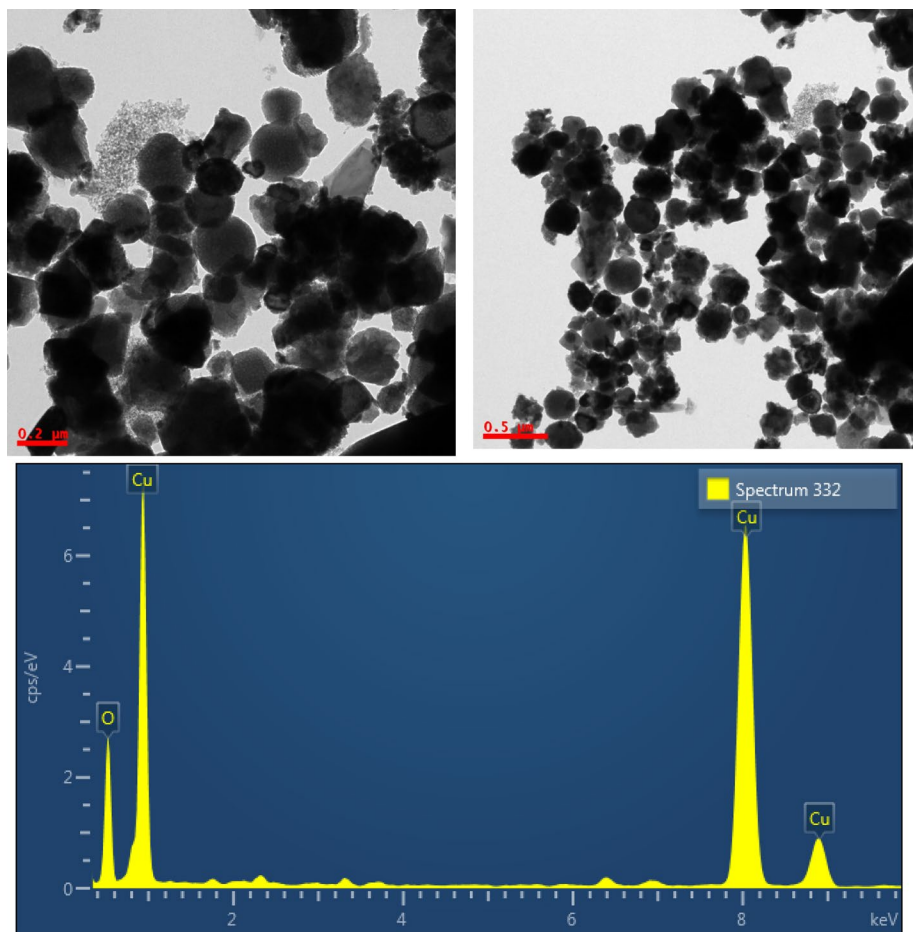


Fig. 9 TEM images and EDX spectrum of CuONPs

Table 5 TEM-Based morphological analysis of cuonps

S. No	Parameter	Observation
1.	Particle shape	Mostly spherical to quasi-spherical
2.	Particle Size range (from SEM)	~ 15–40 nm (estimated)
3.	Average particle size	~ 25 nm (visual estimation; confirmed by histogram if needed)
4.	Surface Morphology	Smooth
5.	Aggregation	Mild to moderate
6.	Crystallinity Inference	Correlated with XRD data (high crystallinity confirmed)

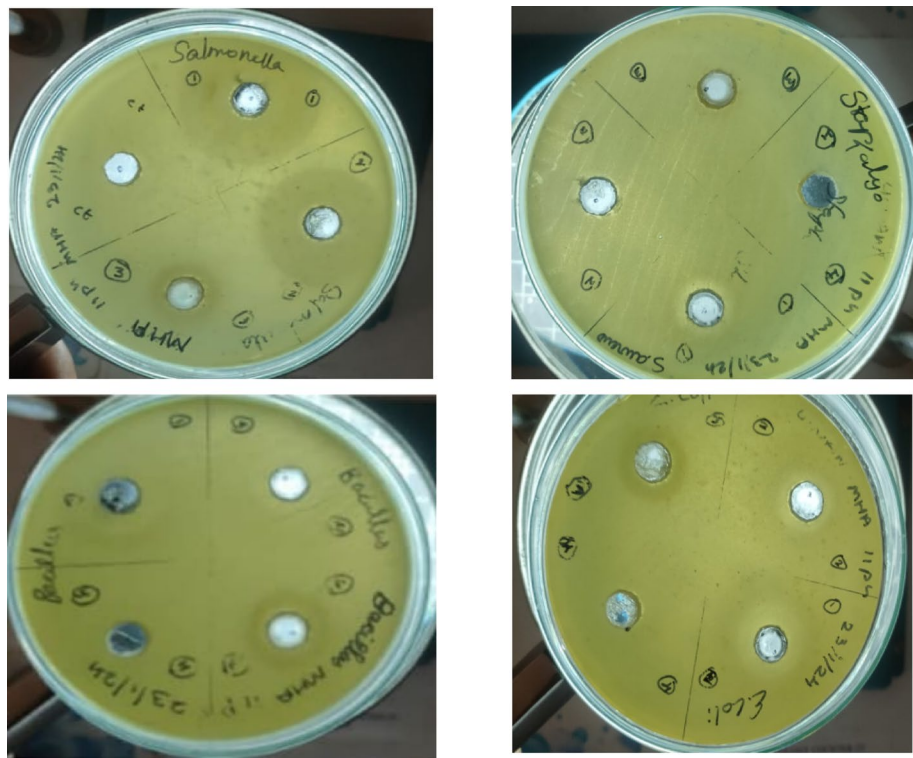


Fig. 10 Zone of inhibition exhibited by CuONPs against bacterial strains: *S. typhi*, *S. aureus*, *B. subtilis* and *E. coli*

in our laboratory, we found the activity against the strains as follows. *Staphylococcus aureus* exhibited the highest sensitivity of 18.6 mm, followed by *Escherichia coli* 17.1 mm, *Bacillus subtilis* 16.4 mm and *Salmonella typhi* 15.2 mm. From the literature it was observed that CuONPs prepared using *N. oleander* also showing better activity against the tested strains.

A comparison of antibacterial activity of CuONPs [4] derived from few plant sources is presented in Table 6 and from the data it was found that CuONPs from *N. oleander* exhibits appreciable activity against the two strains namely *B. subtilis* and *S. typhi* compare to other plant derived CuO NPs. Hence eco-friendly nanoparticle synthesis offers a biocompatible, eco-friendly alternative with potential for coatings, wound dressings, and environmental disinfection applications.

3.10 Photocatalytic degradation of organic dyes

The photocatalytic activity of CuONPs [26] was assessed by evaluating their ability to degrade the following dyes: Methylene Blue (MB), Crystal Violet (CV) and Eosin Yellow (EY) under sunlight. The concentration of dye remaining at time intervals was calculated

Table 6 Comparison on antibacterial activity of cuonps derived from *N. oleander* and other plant species from literature

S. No	Copper oxide nanoparticles and Plant Material	Bacterial strains with inhibition zone (mm)	References
1	<i>Gloriosa superba</i> L. extract mediated CuO NPs	<i>K. aerogenes</i> (15.67 mm), <i>E. coli</i> (13.67 mm) <i>S. aureus</i> (6.00 mm) and <i>P. desmolyticum</i> , (5.33 mm)	[57]
2	<i>Phyllanthus amarus</i> leaf extract mediated CuO NPs	<i>E.coli</i> (24 mm), <i>P.aeruginosa</i> (25 mm), <i>B.subtilis</i> (31 mm) and <i>S.aureus</i> (28 mm)	[58]
3	<i>Saccharum officinarum</i> juice mediated CuO NPs	<i>E. coli</i> (5 mm), <i>P. aeruginosa</i> (7 mm), <i>S. aureus</i> (8 mm) and <i>B. subtilis</i> (9 mm)	[59]
4	<i>Sesbania grandiflora</i> CuO NPs	<i>P.Aeruginosa</i> 19 mm, <i>S.Aureus</i> 15 mm	[60]
5	<i>Cedrus deodara</i> CuO NPs	<i>E. coli</i> 29 mm	[61]
6	<i>Justicia Schimperiana</i> CuO NPs	<i>salmonella typhimurium</i> 18.6 mm	[62]
7	<i>Hagenia abyssinica</i> CuO NPs	<i>S. aureus</i> 14.7 mm	[63]
8	<i>Abutilon indicum</i> CuO NPs	<i>B. subtilis</i> 15 mm	[64]
9	<i>Catha edulis</i> CuO NPs	<i>S. aureus</i> 22 mm, <i>S. pyogenes</i> 24 mm, <i>E.Coli</i> 32 mm, <i>K. pneumonia</i> 29 mm	[65]
10	<i>N. Oleander</i> mediated CuONPs	<i>S.aureus</i> (18.6 mm), <i>E. coli</i> (17.1 mm), <i>B. subtilis</i> (16.4 mm), <i>S. typhi</i> (15.2 mm)	Current work

using UV measurement between absorbance (a.u.) at y-axis and wavelength in nm at x-axis. Figure 11 clearly showed the absorption of dyes at zero minutes and absorption of CuONPs-dye solution at different time intervals. Methylene blue a reactive dye was degraded to the extent of 90% upon exposure to sunlight in the presence of our nano catalyst within the experimental time duration of to the maximum of 3 h. Comparatively the other two dyes examined Eosin yellow observed to be degraded 60% and Crystal violet was degraded to 80%.

3.11 Mechanism of photocatalytic activity

The research on many metal oxide nanoparticles and their applications as a heterogeneous catalyst for dye degradation has been reported in the literature [49]. As for as mechanism of action is concern the formation of reactive species in the form of radicals or excited species believed to induce reaction with non-biodegradable pigments like Methylene Blue (MB), Crystal Violet (CV) and Eosin Yellow (EY) in the polluted water.

The CuONPs act as a semiconductor photocatalyst, absorbing visible light and generating electron-hole pairs (e^-/h^+) which degrade dye molecules into non-toxic products (Fig. 12).

Key factors for high activity:

- Small particle size (~ 25 nm) $\rightarrow$ large surface area.
- High crystallinity $\rightarrow$ low electron-hole recombination.
- Phytochemical capping $\rightarrow$ stabilizes particle dispersion and charge transfer.

4 Conclusion

In this study, CuONPs was synthesized via a green, eco-friendly route using *N. oleander* leaf extract. Structural confirmation using UV, FTIR, SEM, TEM, XRD, and EDX confirmed the formation of spherical, crystalline CuONPs of 15 to 40 nm. XRD and TEM confirmed high crystallinity and nanometric structure. The new CuONPs possessed appreciable antibacterial activity against four selected bacterial strains, with dose-dependent inhibition zones. The greater efficacy of the CuONPs upon *E. coli* was observed as it was reported in the literature prepared from other plant source. Similarly,

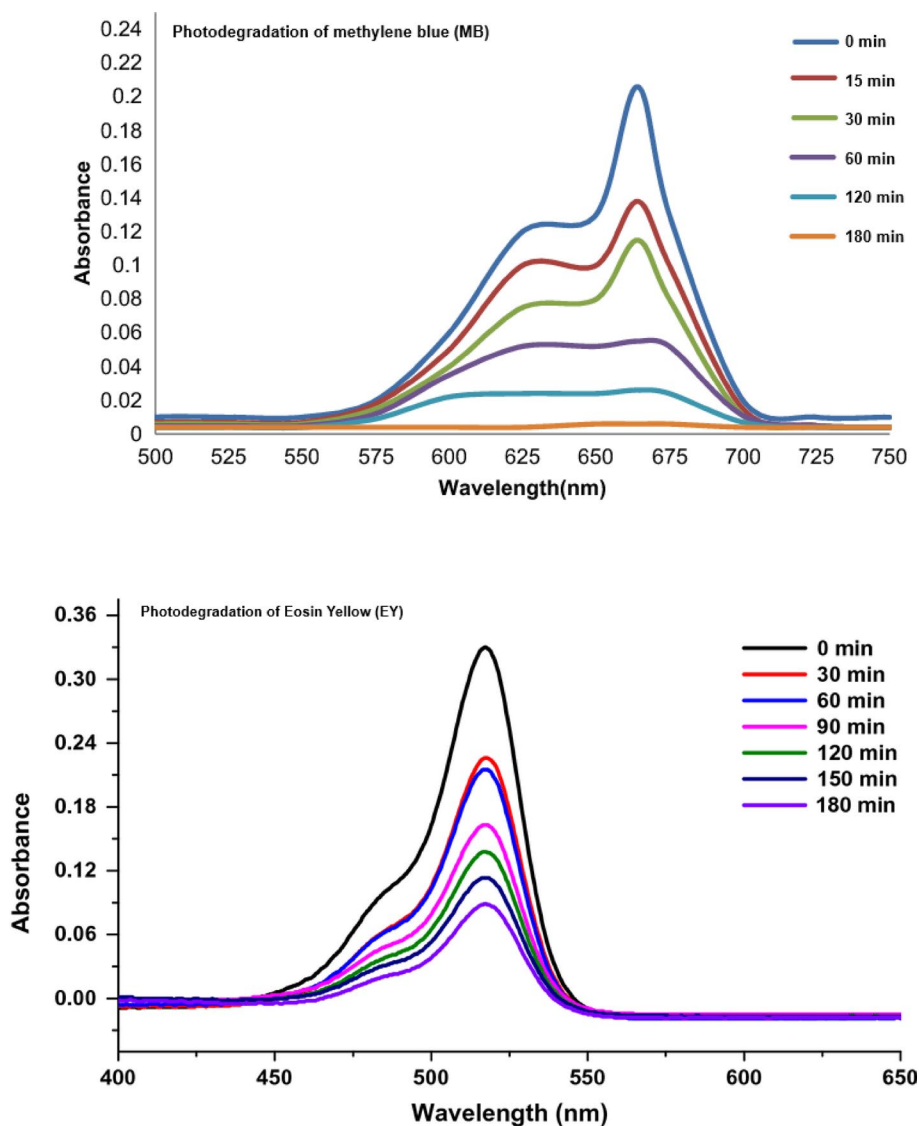


Fig. 11 Photodegradation property of CuONPs against Methylene Blue (MB), Crystal Violet (CV) and Eosin Yellow (EY)

a very less report upon the activity on *S. typhi* was observed. In our study *S. typhi* was tested and the activity in terms of zone of inhibition is reported.

The usage of dye stuffs and the contaminated effluent discharged into the water bodies have become a universal issue because the non-biodegradable nature of the active organic molecules otherwise called pigments and dyes and also their unusual influence results in genetic damage, cancer, bioaccumulation and spoiling of biodiversity by changing the BOD and COD of water finally results in human health issues. So, invention of a material other than conventionally used carbon-based adsorbents and microbes used in biogenic method used to purify water is an inevitable requirement. Many metal oxide nanoparticles have been reported in the literature and studied for their purification of water. In this connection we demonstrated a biogenic synthesis of CuONPs in our laboratory and observed that appreciable photocatalytic activity against three selected dyes. The degradation kinetics followed pseudo-first-order behavior, highlighting the nanoparticles efficacy in environmental remediation.

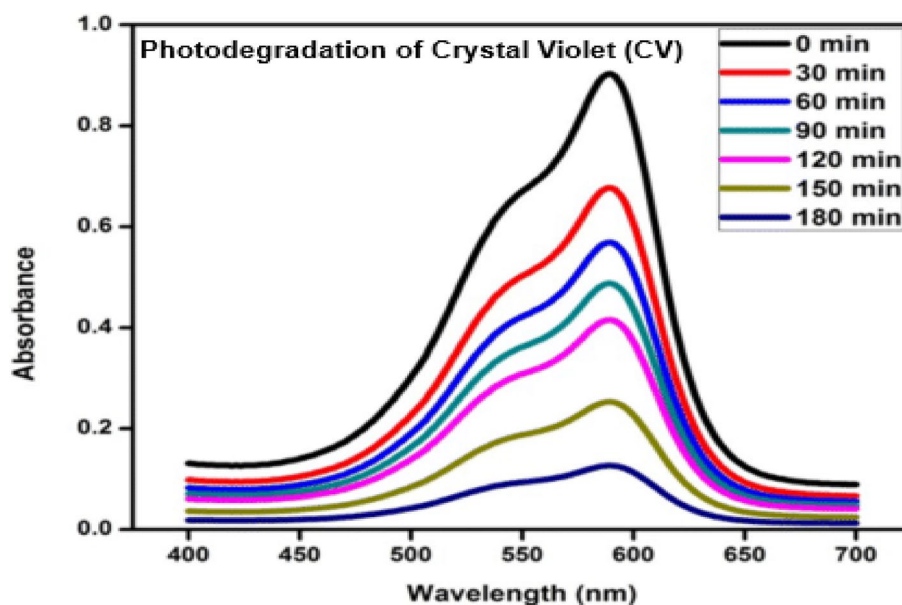


Fig. 11 (continued)

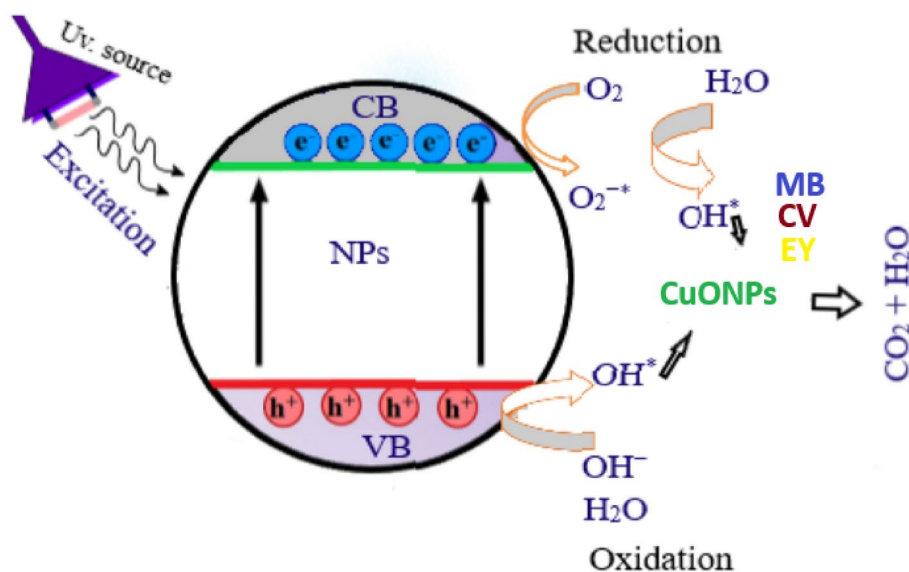


Fig. 12 Schematic diagram of mechanism of photodegradation property of CuONPs against Methylene Blue (MB), Crystal Violet (CV) and Eosin Yellow (EY)

In summary, this work demonstrates that biogenically synthesized CuONPs are promising multifunctional agents with potential applications in antibacterial formulations, wastewater treatment, and green nanotechnology. The use of *N. oleander* decoction as a bioresource offers a cost-effective and sustainable alternative for nanoparticle production.

Author contributions

Dinesh Kumar Matheshwaran: Writing—original draft, review & editing, Experiment, Validation, Supervision, Data curation, Conceptualization. Vishnu Krishna Mohan: Writing—original draft, Experiment, Methodology, Software, Resources, Formal analysis. Padma Kantharaj: Writing—original draft, Experiment, Validation, Methodology, Data curation, Conceptualization. Jagadeesh Kumar Alagarasan: Validation, Supervision, Project administration, Funding acquisition, Conceptualization. Ravikumar Raju: Writing—original draft, Experiment, Validation, Supervision, Data curation, Conceptualization. Chintala Muralikrishna: Methodology, Data curation, Conceptualization.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations**Ethics approval and consent to participate**

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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