

Biosynthesis of CuO Nanoparticles Using Plant Extract as a Precursor: Characterization, Antibacterial, and Antioxidant Activity

Pawar Abhimanyu¹✉, Mungole Arvind², Naktode Kishor¹

¹ Department of Chemistry, NevjabaiHitkarini College, Bramhapuri, Maharashtra 441 206, India

² Department of Botany, NevjabaiHitkarini College, Bramhapuri, Maharashtra 441 206, India

✉ Corresponding author. E-mail: abhaypawar1988@gmail.com

Received: Dec. 29, 2022; **Revised:** May 20, 2023; **Accepted:** Jun. 20, 2023

Citation: P. Abhimanyu, M. Arvind, N. Kishor. Biosynthesis of CuO nanoparticles using plant extract as a precursor: characterization, antibacterial, and antioxidant activity. *Nano Biomedicine and Engineering*, 2023, 15(4): 369–377.

<http://doi.org/10.26599/NBE.2023.9290027>

Abstract

Biosynthesis of metal nanoparticles presents a promising approach for their efficient and environmentally friendly production. In this study, CuO nanoparticles were successfully synthesized by using *Rumex nepalensis* Spreng. as a bio-reducing agent. The spectroscopic analysis confirmed the crystalline monoclinic structure of the synthesized CuO NPs, with particle sizes ranging from 21 to 97 nm. These biosynthesized CuO NPs exhibited notable antimicrobial activity against diverse microorganisms, suggesting their potential for antimicrobial applications. Moreover, the CuO NPs displayed significant antioxidant activity, demonstrated by their effective scavenging of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radicals. This study highlights the straightforward, cost-effective, non-toxic, and robust nature of CuO NPs synthesis using *Rumex nepalensis* Spreng., offering insights into their potential applications in antimicrobial and antioxidant fields.

Keywords: antimicrobial activity; antioxidant activity; X-ray diffraction (XRD); transmission electron microscopy (TEM); CuO nanoparticles (NPs)

Introduction

Numerous particles of varying shape and size exist on Earth, and those falling within the 1–100 nm (nanometer) range are classified as nanoparticles (NPs) [1]. Nanotechnology, a field focused on designing environmentally friendly synthesis methods, faces challenges in nanoparticle characterization and application. By manipulating materials at the atomic level, nanotechnology enables the creation of nanostructured materials with unique properties tailored for specific functions [2]. Nanomaterials serve as

fundamental building blocks and have garnered attention due to their wide-ranging impact in fields such as cosmetics, energy, antimicrobial agents, electronics, food and agriculture, medicine, paints, polymers, textiles, and catalysis [3, 4]. As a result, researchers are increasingly interested in nanoparticle synthesis. Various synthesis methods, including chemical techniques such as electrochemical reduction, sonochemical methods, thermal decomposition, chemical precipitation, microwave irradiation, gas phase evaporation, and sol-gel processes, have been employed to control the size and shape of synthesized CuO nanoparticles. However, these methods have drawbacks

such as the use of toxic substances and the generation of hazardous by-products, posing risks to the environment [5–12]. To overcome these challenges, there is a need to develop environmentally friendly green or biosynthetic methods that employ secure, rapid, cost-effective, biocompatible, eco-friendly, and non-hazardous chemicals. Such methods should yield large quantities of metal-based nanoparticles with well-defined morphology, homogenous distribution of nanoparticle constituents, and without inherent toxicity. One approach involves combining transition metals with plant extracts to form metal oxide nanoparticles, such as NiO, AgO, ZnO, CoO, Fe₃O₄, CuO, TiO₂, MnO, CeO₂, Al₂O₃, etc. These metal-oxide nanoparticles have demonstrated essential roles in diverse sectors [13, 14] and daily life applications [15] due to their unique surface properties. Among these transition metal oxide nanoparticles, CuO nanoparticles synthesized using plant-mediated biosynthesis methods have garnered significant attention. This is attributed to their crucial physicochemical properties, including being a p-type semiconductor with a narrow band gap [16], catalytic activity [17], and optical and magnetic properties [18]. CuO NPs can be applied to applications in various domains such as dye degradation [19], energy storage [20], chemical industries [21], solar cells [22], antifungal and antibacterial treatments [23], anti-inflammatory effects [24], antioxidant properties [25], and anticancer research [26]. Reports exist on the synthesis of CuO nanoparticles from different plant species, including *Annona squamosa* [27], *Psidium guajava* [28], *Bougainvillea* [29], *Catharanthus roseus* [30], *Houttuynia cordata* [31], *Justicia gendarussa* [32], *Aerva javanica* [33], *Cissus vitifolia* [34], *Colocasia esculenta* [35], *Lantana camara* L., and *Nerium oleander* L. [36] etc. These plants contain abundant quantities of phytochemical constituents or biomolecules such as polyphenols, alkaloids, proteins, flavonoids, and terpenoids. These biomolecules act as capping and reducing agents for metal ions (from metal salts to metal NPs), preventing nanoparticles agglomeration and enhancing their biological potential. In this study, we present a clean, economically viable, biocompatible, eco-friendly, and suitable biosynthesis method for preparing CuO nanoparticles by using *Rumex nepalensis* Spreng. We investigate the antimicrobial and antioxidant activities of the synthesized nanoparticles. The nanoparticles are thoroughly analyzed by using techniques such as X-ray diffraction (XRD), Fourier-transform infrared

spectroscopy (FTIR), energy-dispersive X-ray spectroscopy (EDX), ultraviolet (UV)–visible (Vis) spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), etc.

Experimental

Materials and methods

The preferred plant *Rumex nepalensis* Spreng. was collected from the Bramhapuri area of Chandrapur district in Maharashtra state, India, and used to prepare an extract. Silver nitrate (AgNO₃), 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyl toluene (BHT), were procured from Sigma-Aldrich chemical.

Preparation of extracts from whole plant material

The harvested plant material was carefully cleaned by deionized water, repeating the process 2–3 times to ensure the removal of all debris and discarded materials from the surface. Subsequently, the plant material was sliced into small pieces and dried under shade. The entire parts of the *R. nepalensis* Spreng. plant were then powdered using a mortar and pestle. A quantity of 20 g of the plant powder was added to a clean and dry round-bottom flask containing 200 mL of distilled water. The flask was ensured to be neat, clean, and dry. The mixture was then boiled at a temperature range of 60–70 °C for a minimum of 30 min and subsequently allowed to cool down to room temperature. The solution was filtered by using Whatman No. 41 filter paper to obtain a clear filtrate. The obtained filtrate was stored in a refrigerator for further utilization in the preparation of CuO NPs.

Bio-synthesis of CuO NPs

CuO NPs were formed using a method previously reported by Sharma et al. [37]. A round bottom flask (RBF) containing 100 mL of the extract obtained from *R. nepalensis* Spreng. plant was heated using a magnetic stirrer at a temperature range of 70–80 °C. Subsequently, a 30 mL of aqueous solution of Cupric nitrate trihydrate (3 g) was slowly added to the flask under constant stirring. The resulting mixture was then boiled until a greenish colored glue-like substance was obtained. This glue was carefully collected and transferred into a ceramic crucible. It was subjected to calcination in a furnace at 400 °C for 3 h. Finally, fine black-colored particles of CuO

NPs were obtained and utilized for further characterization.

Characterization of nanoparticles

The nanoparticles were characterized by various methods described in the literature review [38]. Elemental analysis, electronic structure, and elemental composition were determined using X-ray absorption spectrometry [39]. Size and morphology analyses were conducted using scanning electron microscopy [40]. FTIR was employed for optical characterization, functional group analysis, and identification of functional groups [41]. Size analysis was performed using TEM [42]. Chemical composition and purity were assessed using energy dispersive X-ray elemental analysis [43]. Nanoparticle formation and synthesis confirmation were investigated using UV–Vis spectroscopy [44]. The characterization of copper oxide nanoparticles, produced through the reduction of copper salt with a plant extract, was conducted using various analytical techniques. The UV–Vis diffuse reflectance (DRS) spectra of nanomaterial are recorded using Thermo scientific evolution 300 UV–Vis spectrophotometer. FTIR analysis was performed on samples using a Thermo Nicolet iS50 FTIR Spectrometer within the 4 000–100 cm^{-1} range. Crystallographic and structural analyses of the prepared copper oxide nanoparticles were carried out by using the Bruker AXS D8 X-ray diffraction technique with copper as the X-ray source. For size, morphology, and composition analysis of the copper oxide nanoparticles, TEM (Jeol/JEM, 2100, at an accelerating voltage of 200 kV) and SEM-EDX (Jeol 6390la/OXFORD XMX N) were utilized. All of the analyses were performed by SAIF, Kochi (India).

Antibacterial and antioxidant activity of nanoparticles

The antibacterial activity and antioxidant activity will be evaluated following the methods described by Perez et al. and Ahmed et al., respectively [45, 46]. For the antimicrobial assessment, the synthesized CuO NPs at three different concentrations (25, 50, and 100 μL) were tested against four bacterial organisms: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*. The well diffusion method was employed, and the antimicrobial activity was determined by measuring the zone of inhibition around the wells. Furthermore,

the antimicrobial activity of CuO NPs was compared with the standard antibiotic Amikacin 30 μg . The antioxidant activity of the synthesized CuO NPs was evaluated by measuring the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity. To assess the potential of the prepared CuO NPs synthesized from *R. nepalensis* Spreng., various concentrations (20, 40, 60, 80, and 100 $\mu\text{g}/\text{mL}$) were prepared by mixing 3 mL of methanol and 1 mL of 4% DPPH solution in equal proportions at room temperature. The mixture was kept in a dark place for 30 min before measuring the absorbance at 517 nm using a visible spectrophotometer. The results were compared with butylated hydroxytoluene (BHT) as a reference compound. The percentage of antioxidant activity (%RSA) was determined as the following formula:

$$\% \text{RSA} = \frac{[\text{Abs}_{517 \text{ nm of control}} - \text{Abs}_{517 \text{ nm of sample}}]}{\text{Abs}_{517 \text{ nm of control}}} \times 100$$

where %RSA is the percentage radical scavenging activity and Abs is the absorbance)

Results and Discussion

X-ray diffraction analysis

The XRD pattern of the synthesized CuO NPs via *R. nepalensis* Spreng. is shown in Fig. 1. The XRD peaks were observed at 2θ values of 32.439°, 35.458°, 38.678°, 48.706°, 53.20°, 58.270°, 61.507°, 66.246°, 68.12°, 72.288°, and 75.101°, which corresponded well with the (110), (002), (111), (202), (020), (202), (113), (311), (220), (311), and (004) hkl planes, respectively. These patterns closely matched the standard values from the JCPDS file No. 89–5895 [47]. Each peak of the prepared CuO NPs exhibited good agreement with the diffraction peaks of monoclinic crystal structures. The average crystallite size of the formed CuO nanoparticles was estimated using the Debye–Scherrer's relation: $D = k \cdot \lambda / (\beta \cos \theta)$, where D represents the average crystallite size, k is the shape factor with a value of 0.94, λ is the X-ray wavelength ($\lambda = 1.54 \text{ \AA}$), β is the full width half maximum (FWHM) value in radians, and θ is the Bragg's angle. The calculated average crystallite size for the synthesized CuO NPs was 15.12985 nm. Additionally, the crystallinity of the nanoparticles was found to be 71.0372%, the average dislocation density was 0.006591, and the individual interplanar

spacing (d) values were determined as presented in Table 1.

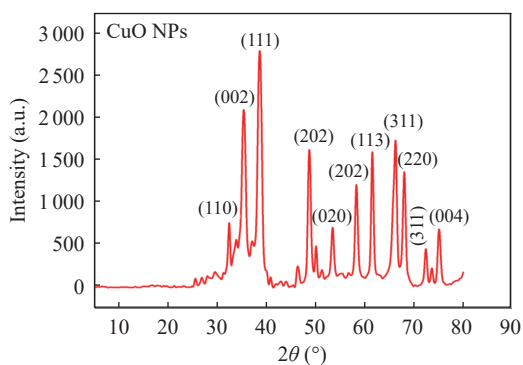


Fig. 1 XRD pattern of CuO NPs formed.

FTIR analysis

FTIR is an important instrument used to recognize the functional groups of substances. In the present study, FTIR was used to identify the functional groups of plant-based nanoparticles. The FTIR spectra of CuO NPs are exhibited in Fig. 2. The peaks noted at 3446.17, 2357.55, 1643.05, 1551.45, 1384.64, 1181.19, 1135.87, 1038.48, 986.411, 869.739, and 533.221 cm^{-1} , respectively. The peaks at 3446.17, and 2357.55 cm^{-1} illustrate the O-H (hydroxyl group) and alkynes bands. This hydroxyl group is obtained from water molecules, and the surface of CuO NPs may absorb water molecules from the atmosphere [48]. Moreover, the FTIR bands at 1643.05, 1551.45, 1384.64, 1181.19, 1135.87, and 1038.48 cm^{-1} correspond to -C=O (carbonyl), -N=O (nitro compound), CN group, C-OH, C-O, and $\text{CH}_2\text{-O}$, which represent bending or stretching vibrations, respectively. All these bands may be attributed to the presence of these groups in

the *Rumex nepalensis* Spreng. plant. Additionally, absorption bands detected at 986.41 and 869.73 cm^{-1} are correlated with strong C=C bending (distributed in trans) and C-Cl (halo compound). Especially, the intense and sharp band at 533.22 cm^{-1} confirms the existence of the Cu-O bond in the synthesized CuO NPs from the plant extract of *Rumex nepalensis* Spreng. via a biological method [49].

Solid UV-Vis-diffuse reflectance (DR) analysis

UV-Vis spectrum is a technique used to recognize the optical absorption properties and energy structure of nanoscale substances. Fig. 3(a) demonstrates the DR solid UV-Vis spectrum of synthesized CuO NPs from *R. nepalensis* Spreng. From the figure, it can be observed that there is a broad absorption band edge ranging from 400 to 850 nm [50]. The Kubelka-Munk (K-M) function is employed to analyze the diffuse reflectance spectra (DRS) obtained from poorly absorbing materials and is applied to convert the DRS values into absorbance [51]. The bandgap energy was determined by plotting $(F(R)hv)^2$ against hv (energy in eV) for the biosynthesized CuO NPs from *Rumex nepalensis* Spreng., as illustrated in Fig. 3(b). The calculated bandgap energy for the biosynthesized CuO NPs was found to be 3.13 eV.

TEM analysis

TEM is a crucial tool for determining the size, morphology, and structural orientation of materials. The distinctive images of the prepared CuO NPs are presented in Figs. 4(a) and 4(b). The results indicate

Table 1 Individual crystallite size and interplanar spacing (d) of formed CuO NPs were calculated

Plane	2θ value ($^\circ$)	FWHM	Crystallite size (nm)	θ value ($^\circ$)	Interplanar spacing (d) (\AA)	Dislocation density $\delta = 1/D^2$	Crystallinity
(110)	32.4398	0.29998	26.55879	16.21990	2.7577	0.001418	
(002)	35.4575	0.37788	20.91488	17.72875	2.5296	0.002286	
(111)	38.68257	0.44088	17.75786	19.34128	2.3258	0.003171	
(202)	48.71432	0.44235	17.08791	24.35716	1.8677	0.003425	
(020)	53.20134	0.48673	15.24271	26.60067	1.7203	0.004304	
(202)	58.27321	0.49755	14.56624	29.13660	1.5820	0.004713	71.0372
(113)	61.50687	0.42576	16.74783	30.75343	1.5064	0.003565	
(311)	66.2465	0.84717	8.202629	33.12325	1.4096	0.014863	
(220)	68.12047	0.5928	11.59573	34.06023	1.3753	0.007437	
(311)	72.28847	0.89486	7.487667	36.14423	1.3059	0.017836	
(004)	75.10317	0.64077	10.26608	37.55158	1.2638	0.009488	

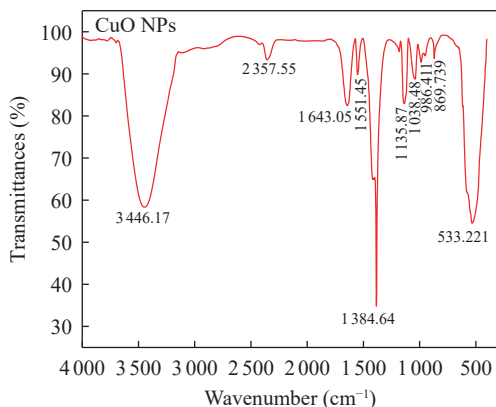


Fig. 2 FTIR spectrum of CuO NPs formed.

that the designed CuO NPs exhibited a spherical shape with an average size ranging between 21 and 97 nm. The increase in size can be attributed to surface agglomeration, which depends on the concentration of the plant extract used during the nanoparticle formation. The selected area electron diffraction (SAED) pattern confirms that the prepared CuO NPs from *R. nepalensis* Spreng. exhibit a crystalline nature, as shown in Fig. 5(a).

EDX Analysis

EDX is a technique used to investigate the chemical composition or elemental analysis of nanomaterials. Figure 5(b) displays the EDX spectrum of the

synthesized CuO NPs via *R. nepalensis* Spreng. The EDX spectrum exhibits two important characteristic signals corresponding to copper and oxygen. These signals confirm the presence of copper and oxygen in the form of CuO, as depicted in Fig. 5(b). The signals for copper show a weight percentage of 41.51% and an atomic percentage of 27.62%, while for oxygen, the weight percentage is 16.23% and the atomic percentage is 42.89%. Additionally, a few other peaks are observed due to photochemical constituents present in the plant.

SEM analysis

SEM is used to examine the surface morphology of nanoparticles. Figs. 6(a) and 6(b) display SEM images of the synthesized CuO NPs from *R. nepalensis* Spreng. at different magnifications. The images reveal that the prepared CuO NPs exhibit an almost spherical shape and also form sponge-like clusters due to surface agglomeration. The size of the nanoparticles is influenced by factors such as the concentration of reducing agents, the procedure and conditions of synthesis, and the presence of various phytochemical species in the plant [52].

Antimicrobial activity

From Fig. 7, it is observed that *E. coli* is sensitive to Amikacin 30 μ g (AK30), exhibiting a zone of

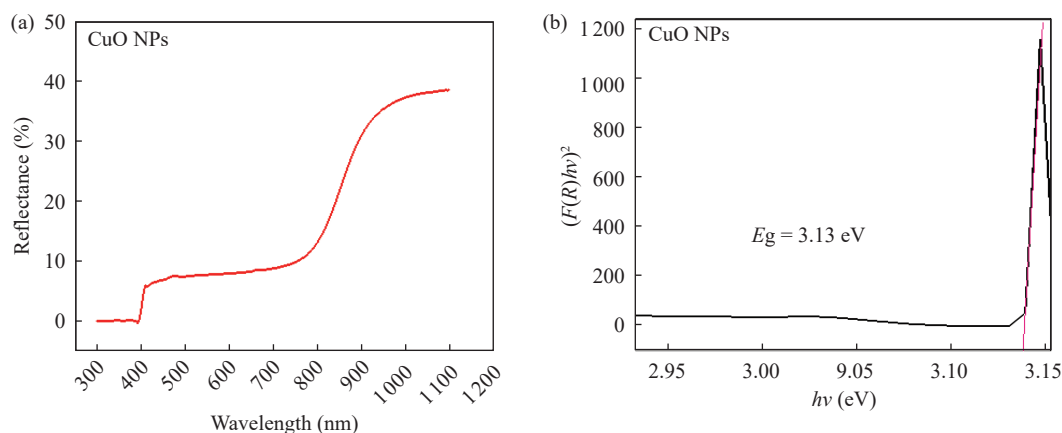


Fig. 3 (a) Diffuse reflectance spectrum (b) Plot of $(F(R)hv)^2$ versus hv (eV) for CuO NPs prepared.

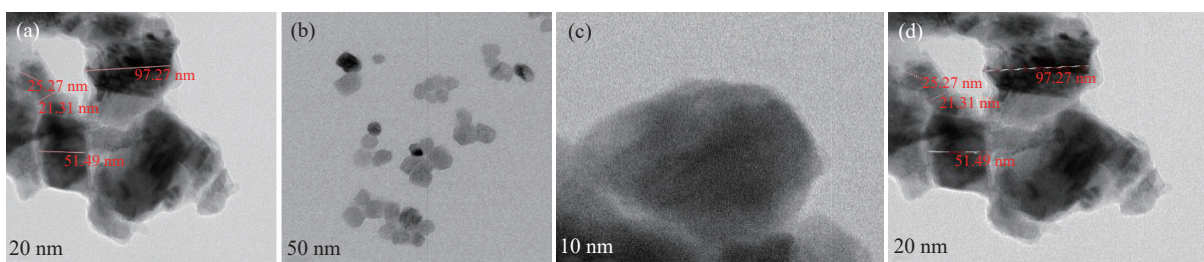


Fig. 4 TEM pictures of CuO NPs formed. (a) Showing a size of 21–97 nm; (b) Magnified picture at a scale of 50 nm; (c) Magnified picture at a scale of 10 nm; (d) magnified picture at a scale of 20 nm.

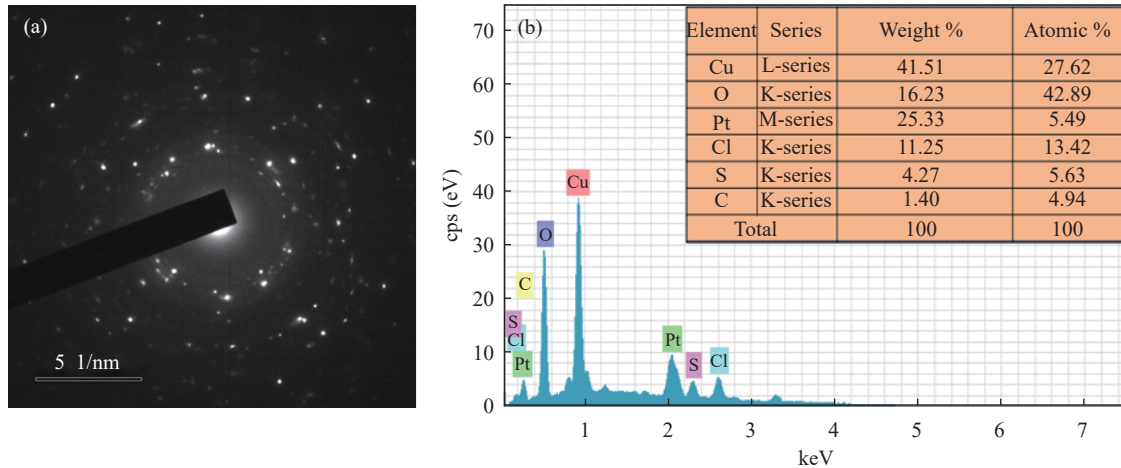


Fig. 5 (a) SAED pattern picture and (b) EDX spectrum of synthesized CuO NPs.

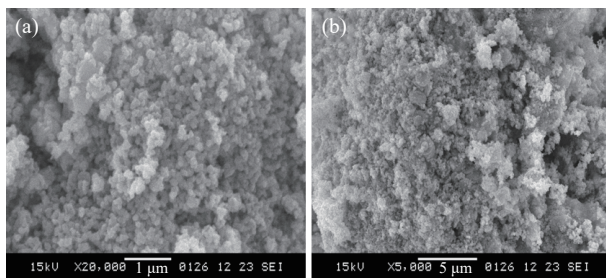


Fig. 6 SEM result of CuO NPs prepared. (a) Spherical in nature; (b) Looking like sponge bunch.

inhibition of 21 mm. However, CuO NPs show no inhibition in 25 and 50 μL , but a zone of inhibition of 14 mm is observed in 100 μL . For *S. aureus* and *P. aeruginosa*, the zone of inhibition is recorded as 28 and 10 mm, respectively, for Amikacin 30 μg (indicating sensitivity). CuO NPs show no inhibition in 25 and 50 μL for *S. aureus*, while for *P. aeruginosa*, a zone of inhibition of 15 mm is observed in 100 μL only. Similarly, *K. pneumoniae* is sensitive to the antibiotic AK30 μg , resulting in a zone of inhibition of 20 mm, while CuO NPs exhibit no

inhibition in 25, 50, or 100 μL . Notably, at high concentrations (100 μL) of the formed CuO NPs, moderate to strong antimicrobial activity is observed only for two organisms (*E. coli* and *K. pneumoniae*) (Table 2).

Anti-oxidant activity

The results indicate the free radical scavenging activity of the synthesized CuO NPs on DPPH, as shown in Fig. 8. It is observed that the CuO NPs exhibit strong antioxidant activity, and the percentage inhibition values gradually increase with the rising concentration of CuO NPs. at concentrations of 50, 100, 150, 200, and 250 $\mu\text{g/mL}$, the percentage inhibition values are 8.47%, 19.83%, 30.89%, 41.72%, and 55.07%, respectively. The highest antioxidant activity is observed at 55.07% for the formed CuO NPs at a concentration of 250 $\mu\text{g/mL}$. The antioxidant activity of CuO NPs is attributed to the presence of alkaloids, phenolic compounds, flavonoids, and other bioactive components present in

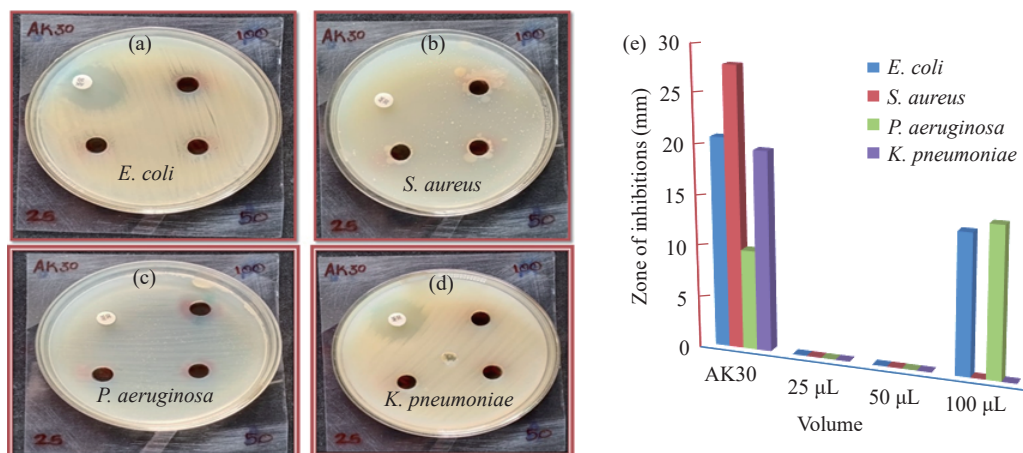


Fig. 7 Zone of inhibitions occurred by prepared CuO NPs against (a) *E. coli*, (b) *S. aureus*, (c) *P. aeruginosa*, and (d) *K. pneumoniae* and. (e) Comparative antibacterial activity of biosynthesized CuO NPs and AK30 μg antibiotic as standard.

Table 2 Zone of inhibition of organisms at various concentrations

Organisms	Volume			
	AK30	25 μ L	50 μ L	100 μ L
<i>E. coli</i>	21	NI	NI	14
<i>S. aureus</i>	28	NI	NI	NI
<i>P. aeruginosa</i>	10	NI	NI	15
<i>K. pneumoniae</i>	20	NI	NI	NI

Note: NI is no inhibition; AK30 is Amikacin 30 μ g.

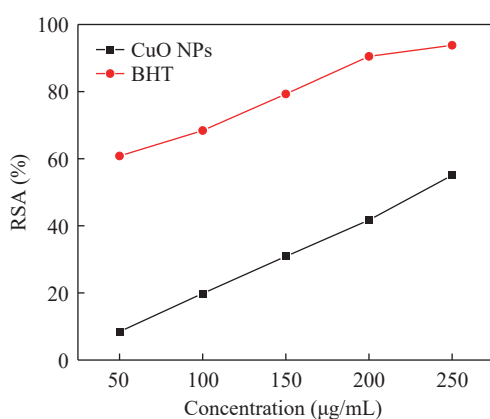


Fig. 8 Antioxidant activity of CuO NPs biosynthesized.

R. nepalensis Spreng., which are effectively coupled with the nanomaterials. Therefore, CuO NPs hold potential in the treatment of various untreatable diseases.

Conclusion

In summary, we have successfully employed a safe, cost-effective, and environmentally friendly biosynthesis method to produce CuO NPs using *Rumex nepalensis* Spreng. The plant extract acts as a rich source of bioactive compounds that not only reduce metal ions but also stabilize the resulting nanoparticles. Spectroscopic techniques confirmed the structural characteristics of the formed CuO NPs, which exhibited a spherical shape with sizes ranging from 21 to 97 nm. The biosynthesized CuO NPs demonstrated a band gap of 3.13 eV and displayed potent antimicrobial activity against human pathogenic bacteria, along with significant antioxidant activity. This research highlights the potential of CuO NPs for diverse applications while promoting a sustainable and eco-friendly synthesis approach.

CRedit Author Statement

Pawar Abhimanyu: Synthesis, analysis, writing, review, and editing. **Mungole Arvind:** data collection of plant material and identification. **Naktode Kishor:** supervision, analysis, writing, and editing.

Acknowledgment

We would like to express our sincere gratitude to the Principal of NevjabaiHitkarini College, Bramhapuri, Maharashtra (India), for their unwavering support, encouragement, and provision of facilities to carry out this research. Additionally, we extend our thanks to Sophisticated Test and Instrumentation Centre (STIC) at Cochin University of Science and Technology, Cochin, Kerala, India, for their assistance in characterizing the samples.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

- [1] I.M. Chung, I. Park, K. Seung-Hyun, et al. Plant-mediated synthesis of silver nanoparticles: Their characteristic properties and therapeutic applications. *Nanoscale Research Letters*, 2016, 11: 40. <https://doi.org/10.1186/s11671-016-1257-4>
- [2] Payal, P. Pandey. A review of recent developments and patents. *Recent Patents on Nanotechnology*, 2022, 16(1): 45–66. <https://doi.org/10.2174/1872210515666210120114504>
- [3] S. Kargozar, M. Mozafari. Nanotechnology and nanomedicine: Start small, think big. *Materials Today: Proceedings*, 2018, 5(7): 15492–15500. <https://doi.org/10.1016/j.matpr.2018.04.155>
- [4] V.K. Yemmireddy, Y.C. Hung. Using photocatalyst metal oxides as antimicrobial surface coatings to ensure food safety—opportunities and challenges. *Comprehensive*

- Reviews in Food Science and Food Safety*, 2017, 16(4): 617–631. <https://doi.org/10.1111/1541-4337.12267>
- [5] M.E. Grigore, E.R. Biscu, A.M. Holban, et al. Methods of synthesis, properties and biomedical applications of CuO nanoparticles. *Pharmaceuticals*, 2016, 9(4): 75. <https://doi.org/10.3390/ph9040075>
- [6] N. Verma, N. Kumar. Synthesis and biomedical applications of copper oxide nanoparticles: An expanding horizon. *ACS Biomaterials Science & Engineering*, 2019, 5(3): 1170–1188. <https://doi.org/10.1021/acsbiomaterials.8b01092>
- [7] N. Silva, S. Ramírez, I. Díaz, et al. Easy, quick, and reproducible sonochemical synthesis of CuO nanoparticles. *Materials*, 2019, 12(5): 804. <https://doi.org/10.3390/ma12050804>
- [8] Y.K. Abdel-Monem, S.M. Emam, H.M.Y. Okda. Solid state thermal decomposition synthesis of CuO nanoparticles from coordinated pyrazolopyridine as novel precursors. *Journal of Materials Science: Materials in Electronics*, 2017, 28(3): 2923–2934. <https://doi.org/10.1007/s10854-016-5877-3>
- [9] M. Chauhan, B. Sharma, R. Kumar, et al. Green synthesis of CuO nanomaterials and their proficient use for organic waste removal and antimicrobial application. *Environmental Research*, 2019, 168: 85–95. <https://doi.org/10.1016/j.envres.2018.09.024>
- [10] K.N. Thakkar, S.S. Mhatre, R.Y. Parikh. Biological synthesis of metallic nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*, 2010, 6(2): 257–262. <https://doi.org/10.1016/j.nano.2009.07.002>
- [11] F.C. Lee, Y.F. Lu, F.C. Chou, et al. Mechanistic study of gas-phase controlled synthesis of copper oxide-based hybrid nanoparticle for CO oxidation. *The Journal of Physical Chemistry C*, 2016, 120(25): 13638–13648. <https://doi.org/10.1021/acs.jpcc.6b05200>
- [12] V.U. Siddiqui, A. Ansari, R. Chauhan, et al. Green synthesis of copper oxide (CuO) nanoparticles by *Punica granatum* peel extract. *Materials Today: Proceedings*, 2021, 36: 751–755. <https://doi.org/10.1016/j.matpr.2020.05.504>
- [13] A. Azam, A.S. Ahmed, M. Oves, et al. Size-dependent antimicrobial properties of CuO nanoparticles against Gram-positive and-negative bacterial strains. *International Journal of Nanomedicine*, 2012, 2012(7): 3527–3535. <https://doi.org/10.2147/IJN.S29020>
- [14] A. A. Annu, S. Ahmed. Green Synthesis of Metal, Metal Oxide Nanoparticles, and Their Various Applications. In: *Handbook of Ecomaterials*. Cham: Springer, 2018: 1–45. https://doi.org/10.1007/978-3-319-48281-1_115-1
- [15] S. Ramya, G. Viruthagiri, R. Gobi, et al. Synthesis and characterization of Ni²⁺ ions incorporated CuO nanoparticles and its application in antibacterial activity. *Journal of Materials Science: Materials in Electronics*, 2016, 27(3): 2701–2711. <https://doi.org/10.1007/s10854-015-4080-2>
- [16] Y.Y. Lv, J. Liu, Z.Y. Zhang, et al. Green synthesis of CuO nanoparticles-loaded ZnO nanowires arrays with enhanced photocatalytic activity. *Materials Chemistry and Physics*, 2021, 267: 124703. <https://doi.org/10.1016/j.matchemphys.2021.124703>
- [17] A. Benhammada, D. Trache, S. Chelouche, et al. Catalytic effect of green CuO nanoparticles on the thermal decomposition kinetics of ammonium perchlorate. *Zeitschrift Für Anorganische Und Allgemeine Chemie*, 2021, 647(4): 312–325. <https://doi.org/10.1002/zaac.202000295>
- [18] R.V. Poonguzhali, E.R. Kumar, N. Arunadevi, et al. Natural citric acid assisted synthesis of CuO nanoparticles: Evaluation of structural, optical, morphological properties and colloidal stability for gas sensor applications. *Ceramics International*, 2022, 48(18): 26287–26293. <https://doi.org/10.1016/j.ceramint.2022.05.311>
- [19] K. Dulta, G.K. Ağçeli, P. Chauhan, et al. Multifunctional CuO nanoparticles with enhanced photocatalytic dye degradation and antibacterial activity. *Sustainable Environment Research*, 2022, 32(1): 2. <https://doi.org/10.1186/s42834-021-00111-w>
- [20] M. El Batouti, H.A. Fetouh. A facile new modified method for the preparation of a new cerium-doped lanthanum cuperate perovskite energy storage system using nanotechnology. *New Journal of Chemistry*, 2021, 45(19): 8506–8515. <https://doi.org/10.1039/D1NJ00455G>
- [21] K. Pagar, S.K. Ghotekar, T. Pagar, et al. Antifungal activity of biosynthesized CuO nanoparticles using leaves extract of *Moringa oleifera* and their structural characterizations. 2020, 3(1): 15–23. <https://doi.org/10.26655/AJNANOMAT.2020.1.2>
- [22] T. Velusamy, A. Liguori, M. Macias-Montero, et al. Ultra-small CuO nanoparticles with tailored energy-band diagram synthesized by a hybrid plasma-liquid process. *Plasma Processes and Polymers*, 2017, 14(7): 1600224. <https://doi.org/10.1002/ppap.201600224>
- [23] A.A. Mohamed, M. Abu-Elghait, N.E. Ahmed, et al. Eco-friendly mycogenic synthesis of ZnO and CuO nanoparticles for *in vitro* antibacterial, antibiofilm, and antifungal applications. *Biological Trace Element Research*, 2021, 199(7): 2788–2799. <https://doi.org/10.1007/s12011-020-02369-4>
- [24] V. Balakrishnan, K. Thangaraj, M. Palani, et al. Green synthesis of copper oxide nanoparticles using *Euphorbia hirta* leaves extract and its biological applications. *Inorganic and Nano-Metal Chemistry*, 2022, 52(6): 809–818. <https://doi.org/10.1080/24701556.2021.1952260>
- [25] A. Waris, M. Din, A. Ali, et al. A comprehensive review of green synthesis of copper oxide nanoparticles and their diverse biomedical applications. *Inorganic Chemistry Communications*, 2021, 123: 108369. <https://doi.org/10.1016/j.inoche.2020.108369>
- [26] H. Al-Jawhari, H. Bin-Thiyab, N. Elbially, et al. *In vitro* antioxidant and anticancer activities of cupric oxide nanoparticles synthesized using spinach leaves extract. *Nano-Structures & Nano-Objects*, 2022, 29: 100815. <https://doi.org/10.1016/j.nanoso.2021.100815>
- [27] P. Singh, K.R. Singh, J. Singh, et al. Tunable electrochemistry and efficient antibacterial activity of plant-mediated copper oxide nanoparticles synthesized by *Annona squamosa* seed extract for agricultural utility. *RSC Advances*, 2021, 11(29): 18050–18060. <https://doi.org/10.1039/D1RA02382A>
- [28] S. Sathiyavimal, S. Vasantharaj, V. Veeramani, et al. Green chemistry route of biosynthesized copper oxide nanoparticles using *Psidium guajava* leaf extract and their antibacterial activity and effective removal of industrial dyes. *Journal of Environmental Chemical Engineering*, 2021, 9(2): 105033. <https://doi.org/10.1016/j.jece.2021.105033>
- [29] M.W. Shammout, A. Awwad. A novel route for the synthesis of copper oxide nanoparticles using *Bougainvillea* plant flowers extract and antifungal activity evaluation. *Chemistry International*, 2021, 7(1): 71–78. <https://doi.org/10.5281/zenodo.4042902>
- [30] A. Verma, N. Bharadvaja. Plant-mediated synthesis and characterization of silver and copper oxide nanoparticles: Antibacterial and heavy metal removal activity. *Journal of Cluster Science*, 2022, 33(4): 1697–1712. <https://doi.org/10.1007/s10876-021-02091-8>
- [31] H.M. Chen, X.J. Feng, L. Gao, et al. Inhibiting the PI3K/AKT/mTOR signalling pathway with copper oxide nanoparticles from *Houttuynia cordata* plant: Attenuating

- the proliferation of cervical cancer cells. *Artificial Cells, Nanomedicine, and Biotechnology*, 2021, 49(1): 240–249. <https://doi.org/10.1080/21691401.2021.1890101>
- [32] S. Vasantharaj, P. Shivakumar, S. Sathiyavimal, et al. Antibacterial activity and photocatalytic dye degradation of copper oxide nanoparticles (CuONPs) using *Justicia gendarussa*. *Applied Nanoscience*, 2023, 13(3): 2295–2302. <https://doi.org/10.1007/s13204-021-01939-9>
- [33] F. Amin, B. Khattak, A. Alotaibi, et al. Green synthesis of copper oxide nanoparticles using aerva javanica leaf extract and their characterization and investigation of *in vitro* antimicrobial potential and cytotoxic activities. *Evidence-Based Complementary and Alternative Medicine*, 2021, 2021: 5589703. <https://doi.org/10.1155/2021/5589703>
- [34] M.A. Thakar, S.S. Jha, K. Phasinam, et al. X ray diffraction (XRD) analysis and evaluation of antioxidant activity of copper oxide nanoparticles synthesized from leaf extract of *Cissus vitiginea*. *Materials Today: Proceedings*, 2022, 51: 319–324. <https://doi.org/10.1016/j.matpr.2021.05.410>
- [35] K. Barman, P. Dutta, D. Chowdhury, et al. Green biosynthesis of copper oxide nanoparticles using waste *Colocasia esculenta* leaves extract and their application as recyclable catalyst towards the synthesis of 1, 2, 3-triazoles. *BioNanoScience*, 2021, 11(1): 189–199. <https://doi.org/10.1007/s12668-021-00826-5>
- [36] K.S. Shiny, S. Nair, N. Mamatha, et al. Decay resistance of wood treated with copper oxide nanoparticles synthesised using leaf extracts of *Lantana camara* L. and *Nerium oleander* L. *Wood Material Science & Engineering*, 2022, 17(6): 727–733. <https://doi.org/10.1080/17480272.2021.1934728>
- [37] J.K. Sharma, P. Srivastava, G. Singh, et al. Catalytic thermal decomposition of ammonium perchlorate and combustion of composite solid propellants over green synthesized CuO nanoparticles. *Thermochimica Acta*, 2015, 614: 110–115. <https://doi.org/10.1016/j.tca.2015.06.023>
- [38] S.A. Akintelu, A.S. Folorunso, F.A. Folorunso, et al. Green synthesis of copper oxide nanoparticles for biomedical application and environmental remediation. *Heliyon*, 2020, 6(7): e04508. <https://doi.org/10.1016/j.heliyon.2020.e04508>
- [39] C. Lamberti, G. Agostini. *Characterization of Semiconductor Heterostructures and Nanostructures*, 2nd Ed. Elsevier Science: Amsterdam, 2013. <https://doi.org/10.1016/C2011-0-07690-1>
- [40] P. Ananthi, S.M.J. Kala. Plant extract mediated synthesis and characterization of copper nanoparticles and their pharmacological activities. *International Journal Innovative Research in Science, Engineering and Technology*, 2017, 6(7): 13455–13465.
- [41] J.A. Wisam, A.J. Haneen. A new paradigm shift to prepare copper nanoparticles using biological synthesis and evaluation of antimicrobial activity. *Plant Archives*, 2018, 18(2): 2020–2024.
- [42] P. Kuppusamy, S. Ilavenil, S. Srigopalram, et al. Treating of palm oil mill effluent using *Commelina nudiflora* mediated copper nanoparticles as a novel bio-control agent. *Journal of Cleaner Production*, 2016, 141: 1023–1029. <https://doi.org/10.1016/j.jclepro.2016.09.176>
- [43] M. Maruthupandy, Y. Zuo, J.S. Chen, et al. Synthesis of metal oxide nanoparticles (CuO and ZnO NPs) via biological template and their optical sensor applications. *Applied Surface Science*, 2017, 397: 167–174. <https://doi.org/10.1016/j.apsusc.2016.11.118>
- [44] S.M.H. Akhter, F. Mohammad, S. Ahmad. Terminalia belerica mediated green synthesis of nanoparticles of copper, iron and zinc metal oxides as the alternate antibacterial agents against some common pathogens. *BioNanoScience*, 2019, 9(2): 365–372. <https://doi.org/10.1007/s12668-019-0601-4>
- [45] C. Perez, M. Pauli, P. Bazeuque. An antibiotic assay by agar well diffusion method. *Acta Biologicae et Medicinae Experimentalis*, 1990, 15: 113–115.
- [46] M.I. Ahmed, M.S. Hasan, S.J. Uddin, et al. Antinociceptive and antioxidant activities of the ethanolic extract of *Excoecaria indica*. *Dhaka University Journal of Pharmaceutical Sciences*, 2007, 6(1): 51–53. <https://doi.org/10.3329/dujps.v6i1.344>
- [47] M. Ahamed, H.A. Alhadlaq, M.A.M. Khan, et al. Synthesis, characterization, and antimicrobial activity of copper oxide nanoparticles. *Journal of Nanomaterials*, 2014, 2014: 637858–434. <https://doi.org/10.1155/2014/637858>
- [48] T.B. Vidovix, H.B. Quesada, E.F.D. Januário, et al. Green synthesis of copper oxide nanoparticles using *Punica granatum* leaf extract applied to the removal of methylene blue. *Materials Letters*, 2019, 257: 126685. <https://doi.org/10.1016/j.matlet.2019.126685>
- [49] O. Antonoglou, K. Lafazanis, S. Mourdikoudis, et al. Biological relevance of CuFeO₂ nanoparticles: Antibacterial and anti-inflammatory activity, genotoxicity, DNA and protein interactions. *Materials Science and Engineering: C*, 2019, 99: 264–274. <https://doi.org/10.1016/j.msec.2019.01.112>
- [50] M. Parthibavarman, V. Sharmila, P. Sathishkumar, et al. A rapid one-pot synthesis of CuO rice-like nanostructure and its structural, optical and electrochemical performance. *Journal of Electronic Materials*, 2018, 47(9): 5443–5451. <https://doi.org/10.1007/s11664-018-6435-y>
- [51] G.T. Anand, S.J. Sundaram, K. Kanimozhi, et al. Microwave assisted green synthesis of CuO nanoparticles for environmental applications. *Materials Today: Proceedings*, 2021, 36: 427–434. <https://doi.org/10.1016/j.matpr.2020.04.881>
- [52] A.K. Mittal, Y. Chisti, U.C. Banerjee. Synthesis of metallic nanoparticles using plant extracts. *Biotechnology Advances*, 2013, 31(2): 346–356. <https://doi.org/10.1016/j.biotechadv.2013.01.003>

© The author(s) 2023. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY) (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.