JOTCS A
Journal of the Turkish Chemical Society
Section: A
Chemistry
TURKISH CHEMICAL SOCIETY

Biogenic Copper Oxide Nanoparticles Synthesized from Whole Plant Extract of *Nicotiana plumbaginifolia* Viv.: Characterization, Antibacterial, and Antioxidant Properties

Abhimanyu Pawar[1*], Arvind Mungole[2], Kishor Naktode[1]

[1]Department of Chemistry, Nevjabai Hitkarini College, Bramhapuri, Maharashtra 441 206, India.
[2]Department of Botany, Nevjabai Hitkarini College, Bramhapuri, Maharashtra 441 206, India.

Abstract: Nanoparticles crafted through biological processes show potential for advancing medicine. Plant-derived compounds, produced through environmentally friendly green synthesis, present distinctive and beneficial applications in the field of nanomedicine. This study describes an easy, sustainable, environmentally friendly, and cost-efficient method to create copper oxide nanoparticles (CuO NPs) using whole part of *Nicotiana plumbaginifolia* Viv. extract. The characterization involved various techniques like solid UV-Visible-DR analysis, Fourier transform infrared (FTIR), EDAX analysis, X-ray diffraction (XRD), transmitted electron microscopy (TEM), and scanning electron microscopy (SEM). The copper oxide nanoparticles (CuO NPs) were found to be quasi-spherical pattern, with sizes ranging from 12 to 14 nm, and exhibited a crystal structure identified as monoclinic. The resulting copper oxide nanoparticles (CuO NPs) were examined for antimicrobial and antioxidant properties. It showed suppressing bacterial growth against tested human pathogenic bacteria, emphasizing their potential as antimicrobial agents. Results revealed that the maximum zone of inhibition was observed when the concentrations (25, 50, and 100 µL,) of NPs is increased against *S. aureus* i.e. 17 mm, 20 mm and 22 mm respectively. Whereas findings also reveal potent antioxidant activity, with escalating CuO nanoparticle concentrations correlating to increased percentage inhibition 50 µg/mL – 1.68%, 100 µg/mL – 10.45%, 150 µg/mL – 18.54%, 200 µg/mL – 37.83%, and 250 µg/mL – 51.72%. The highest activity, at 51.72%, occurs at 250 µg/mL.

Keywords: Biogenic synthesis, CuO nanoparticles, Antimicrobial activity, Antioxidant activity.

Submitted: January 20, 2024. **Accepted:** May 13, 2024.

Cite this: Pawar A, Mungole A, Naktode K. Biogenic Copper Oxide Nanoparticles Synthesized from Whole Plant Extract of *Nicotiana plumbaginifolia* Viv.: Characterization, Antibacterial, and Antioxidant Properties. JOTCSA. 2024;11(3): 1005-16.

DOI: https://doi.org/10.18596/jotcsa.1422924

***Corresponding author's E-mail:** abhaypawar1988@gmail.com

1. INTRODUCTION

In contemporary times, Nano biotechnology has evolved as a fundamental domain of modern science, representing a novel era in material science. Its myriad applications have garnered global attention. This interdisciplinary field, at the intersection of nanotechnology and biology, holds immense promise for advancements in various scientific disciplines. Crucial research in this field centers on synthesizing nanoparticles with varied shapes, sizes, chemical compositions, and controlled dispersions for exploration (1,2). Nanomaterials, or nanoparticles, serve as crucial building blocks, attracting considerable attention for their positive impact across diverse domains such as detergents, catalysis, energy, shampoos, polymers, soaps, cosmetics, toothpaste, food and agriculture, medicine, soaps, antimicrobial agents, paints, footwear, textiles, and electronics. This has spurred research interest in nanoparticle synthesis (3,4).

Metal oxide nanoparticles have emerged as a focal point in scientific research, owing to their diverse applications that captivate the attention of investigators keen on exploring their unique properties. Notably, copper oxide nanoparticles have garnered particular interest among transition metal oxides due to their efficacy in various domains, such as antimicrobial applications, sensors, nanofluids, energy storage systems, antioxidant activity, anticancer agents, and catalysis (5-11). The distinctive capability of copper oxide nanoparticles to modulate the physical, optical, and electronic properties of compounds further accentuates their significance in contemporary research. Numerous methods have been employed for the synthesis of copper oxide nanoparticles, encompassing physical, biological,

and chemical approaches (12). However, conventional methods often entail the use of expensive and toxic chemicals, rendering them unsuitable for biomedical applications (13). Consequently, there is a growing impetus to explore synthetic methods rooted in naturally occurring biomaterials, presenting an alternative avenue for obtaining copper oxide nanoparticles tailored for biological applications. This shift towards biocompatible synthesis methods not only aligns with the principles of sustainability but also underscores the importance of developing nanoparticle formulations suitable for integration into biomedical contexts (14,15).

The biosynthesis or green synthesis of copper oxide nanoparticles utilizing various plant extracts, such as *Ephedra alata, Rubia cordifolia* bark*, Spinacia oleracea* leaf*, Eichhornia Crassipes* leaf*, Abelmoschus esculentus, Berberis vulgaris* leaf*, Sesbania grandiflora* leaf*, Bombax ceiba* plant*, Rumex nepalensis* (16-26) etc. has been extensively documented in scientific literature. This approach harnesses the inherent properties of plant extracts to facilitate the reduction and stabilization of copper ions, ultimately yielding nanoparticles with tailored characteristics. Notably, the utilization of plant extracts in copper oxide nanoparticle synthesis offers several key advantages. First and foremost, these extracts are readily accessible, contributing to the economic feasibility of the synthesis process. Additionally, the use of plant extracts is generally considered safe and non-toxic, aligning with the principles of green chemistry (27-30). The avoidance of harmful chemicals in the synthesis process is pivotal, especially in the context of biomedical applications. *Nicotiana plumbaginifolia* Viv. is an erect annual plant up to 1 m tall. It belongs to Solanaceae family, which is generally known as night shade family, native to the west Indies and Mexico and is usually found in waste places near water. The antimicrobial and antioxidant potential of plants is due to the presence of several secondary metabolites that have different mechanisms of action; some are proteins and enzymes, while others are vitamins, anthocyanins, alkaloids, flavonoids, carotenoids, and other phenolic compounds. Solanaceae family is the rich source of alkaloid content as the Nicotine is reported from *Nicotiana tobacum*. There no work has been reported from this plant material in the field of green synthesis of copper oxide nanoparticles.

Copper oxide nanoparticles, synthesized through biogenic or green methods, have demonstrated superior microbial toxicity related to other metal oxides (31). This heightened antimicrobial activity renders them particularly attractive for applications in areas such as healthcare and biomedical research. The specific mechanisms underlying the enhanced microbial toxicity of copper oxide nanoparticles warrant further investigation, opening avenues for elucidating their potential in combating microbial threats.

This study represents a pioneering synthesis of copper oxide nanoparticles utilizing the *Nicotiana plumbaginifolia* Viv. The methodology employed underscores an environmentally conscious approach, emphasizing the adoption of greener protocols. We introduce a clean, cost-effective, non-toxic chemical, less time and eco-friendly technique for the fabrication of copper oxide nanoparticles, with *Nicotiana plumbaginifolia* Viv*.* serving as a key component in the synthesis process.The synthesized nanoparticles were comprehensively characterized through X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Energy Dispersive X-ray Spectroscopy (EDX), UV-Visible spectroscopy, Transmission Electron Microscopy (TEM), and Scanning Electron Microscopy (SEM). These biocompatible nanoparticles demonstrated notable antimicrobial and antioxidant activities.

2. EXPERIMENTAL SECTION (All capitals, one space before and after the heading)

2.1. Materials and Methods

The selected botanical specimen, *Nicotiana plumbaginifolia* Viv*.* was sourced from the proximate Bramhapuri region in the Chandrapur district of Maharashtra state, India. The plant material was meticulously collected and utilized in extract preparation. The following chemicals have been used under study, ensuring high-grade chemical standards for the experiments:

1. Sigma-Aldrich supplied Cupric nitrate trihydrate
2. 1,1-Diphenyl-2-picrylhydrazyl (DPPH)
3. Butylated Hydroxytoluene (BHT).

2.2. Preparation of Aqueous Extracts from Whole Plant Material

The harvested *Nicotiana plumbaginifolia* Viv*.* plant was meticulously cleansed with de-ionized water, eliminating debris and extraneous material. Subsequently, the plant material was sliced into small fragments and air-dried under shade. The entirety of the plant components were pulverized into a fine powder using a mortar and pestle. A measured quantity (20 g) of the plant powder was introduced into a 200 mL distilled water-filled, clean, and dry round-bottom flask. The resultant mixture underwent boiling at 60–70 °C for a minimum of 30 minutes, followed by natural cooling to room temperature. The solution was then meticulously filtered using Whatman number 41 filter paper. The resulting filtrate was refrigerated and earmarked for subsequent utilization in the synthesis of CuO NPs.

2.3. Biogenic Synthesis of CuO NPs

The synthesis of CuO NPs followed the established method by Sharma et al. (32). A round- bottom flask (RBF) containing 100 mL of *Nicotiana plumbaginifolia* Viv. plant extract underwent heating at 70-80 °C using a magnetic stirrer. Subsequently, a 30 mL aqueous solution containing 3 g of cupric nitrate trihydrate was gradually introduced with continuous stirring. The resulting mixture was boiled until a greenish-colored gel formed. This gel was collected, transferred into a ceramic crucible, and subjected to calcination in a furnace at 400 °C for 3 hours. The culmination of this process yielded finely dispersed black-colored CuO NPs, which were then utilized for subsequent characterization.

2.4. Characterizations

The nanoparticles underwent a comprehensive

characterization employing methodologies outlined in the literature review (33). Scanning electron microscopy (34) was employed for size and morphology analysis. Fourier transmission infrared spectroscopy (35) was utilized for optical characterization, functional group analysis, and identification. Transmission electron microscopy (36) enabled size analysis. Energy dispersive X-ray elemental analysis (37) assessed chemical composition and purity. UV-visible spectroscopy (38) was employed to investigate nanoparticle formation and confirm synthesis. X-ray absorption spectrometry (39) facilitated the determination of elemental analysis, electronic structure, and elemental composition.The copper oxide nanoparticles, derived from the reduction of copper salt using an extract of plant materials, underwent meticulous characterization through diverse analytical techniques. UV–visible diffuse reflectance spectra were acquired using the Thermo Scientific Evolution 300 UV–visible spectrophotometer. FT-IR analysis within the 4000-100 cm^{-1} range was executed with the Thermo Nicolet iS50 FTIR Spectrometer. Crystallographic and structural analyses were conducted using the Brucker AXS D8 X-ray diffraction technique with copper as the X-ray source. For size, morphology, and composition analysis, TEM (Jeol/JEM, 2100, at 200 kV) and SEM-EDX (Jeol 639Ola/OXFORD XMX N) were employed. All analyses were performed by SAIF, Kochi (India).

2.5. Antimicrobial Activity

The antimicrobial efficacy of synthesized CuO nanoparticles was examined at concentrations of 25, 50, and 100 µL against *E. coli, S. aureus, P. aeruginosa,* and *K. pneumonia,* employing the well diffusion method. The assessment centered on zones of inhibition observed in Figure 9 and summarized in Table 1. Comparative analysis with the standard antibiotic, amikacin (30 µg), depicted in figure 10, offered contextual insights. The observed zones of inhibition serve as indicators of CuO nanoparticles effectiveness in suppressing bacterial growth, emphasizing their potential as antimicrobial agents.

2.6. Anti-Oxidant Activity

The antioxidant efficacy of the synthesized CuO nanoparticles was evaluated through the assessment of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity. Varied concentrations (20, 40, 60, 80, and 100 µg/mL) of the prepared CuO nanoparticles from *Nicotiana plumbaginifolia* Viv. were formulated by combining 3 mL of methanol and 1 mL of 4% DPPH solution equally at room temperature. The mixture was left undisturbed in a dark environment for 30 minutes before measuring absorbance at 517 nm using a visible spectrophotometer, with butylated hydroxytoluene (BHT) serving as the standard. The percentage of radical scavenging activity (% RSA) was calculated using the formula:

$$\%\ RSA = [(abs_{517\ nm\ of\ control} - abs_{517\ nm\ of\ sample}) / abs_{517\ nm\ of\ control}] \times 100 \quad \text{(Eq.1)}$$

(% RSA- percentage radical scavenging activity, Abs -absorbance) (Eq.2)

3. RESULTS AND DISCUSSION

Solid UV-Visible-DR analysis is a technique employed to discern the optical absorption properties and energy structure of nanoscale substances. This method involves measuring the diffuse reflectance of a solid material across the ultraviolet and visible spectra, allowing for the characterization of its electronic transitions and absorption features. The analysis provides valuable insights into the optical behavior and energy band structure of the nanomaterial under investigation. Figure 1 presents the solid UV–Visible spectrum of synthesized CuO nanoparticles through *Nicotiana plumbaginifolia* Viv. as manifested by the diffuse reflectance (DR) analysis. The spectrum reveals a broad absorption band edge scanning the range of 200 to 730 nm (40). The Kubelka–Munk (K-M) function, utilized for analyzing diffuse reflectance spectra (DRS) from weakly absorbing materials, is employed to convert DRS values into absorbance (41,42). The bandgap energy assessment involved plotting $(F(R)\ h\nu)^2$ against $h\nu$ (energy in eV) for the biosynthesized CuO nanoparticles from *Nicotiana plumbaginifolia* Viv.as depicted in Figure 2. The determined bandgap energy for the biosynthesized CuO nanoparticles was 5.16 eV.

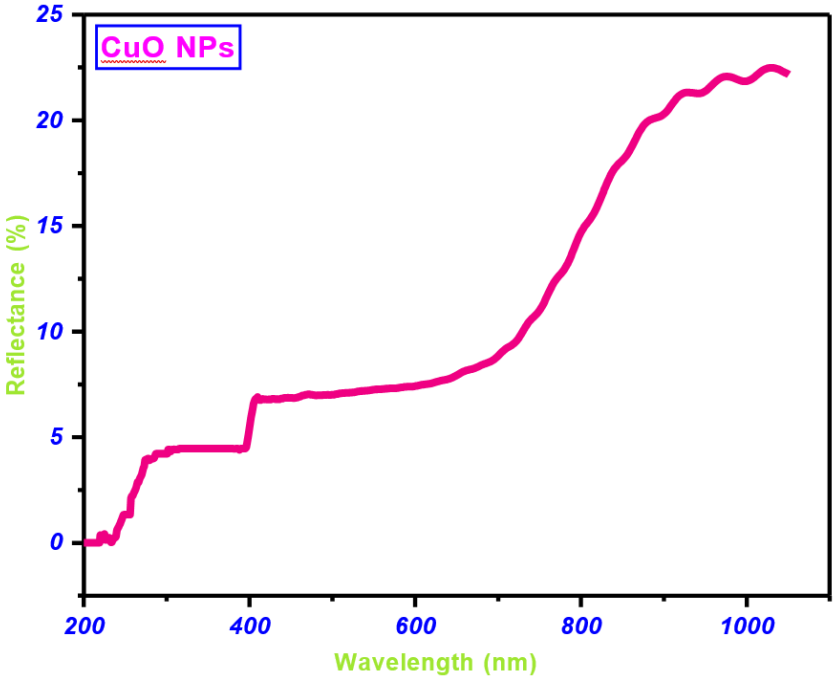

Figure 1: Diffuse reflectance spectrum of CuO NPs synthesized.

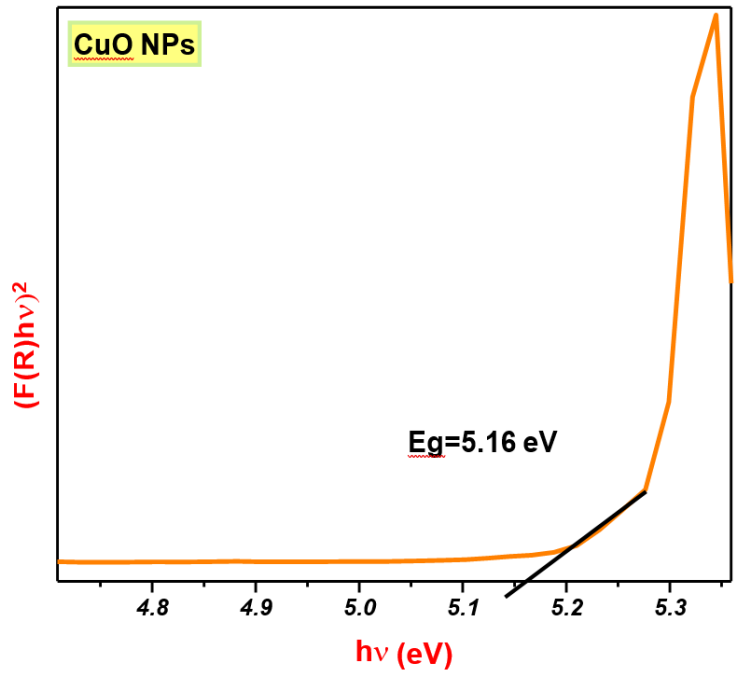

Figure 2: Plot of $(F(R)h\nu)^2$ versus hν (eV) for CuO NPs synthesized.

Figure 3 displays the FTIR measurements of the synthesized copper oxide nanoparticles, facilitating the identification of potential biomolecules serving as both reducing and capping agents in the synthesis process.The FTIR spectrum exhibits distinctive peaks at various wavenumbers, offering valuable insights into the molecular composition of the synthesized copper oxide nanoparticles. At 3432.67 cm^{-1}, the presence of O-H (hydroxyl group) is indicated, while the peak at 1629.55 cm^{-1} corresponds to -C=O (carbonyl) vibrations. Notably, peaks at 1382.71, 1141.65, and 1029.8 cm^{-1} signify CN group, C-O, and CH_2-O bending or stretching vibrations, respectively, shedding light on specific molecular functionalities. The presence of C-Cl (halo compound) is indicated by the peak at 827.31 cm^{-1} (43-44). Furthermore, distinctive peaks at 673.03 and 518.75 cm^{-1} are attributed to the Cu–O vibration in the copper oxide nanoparticles (45,46). These observations collectively unravel the intricate molecular details of the synthesized nanoparticles, providing a comprehensive understanding of their composition and functional groups.

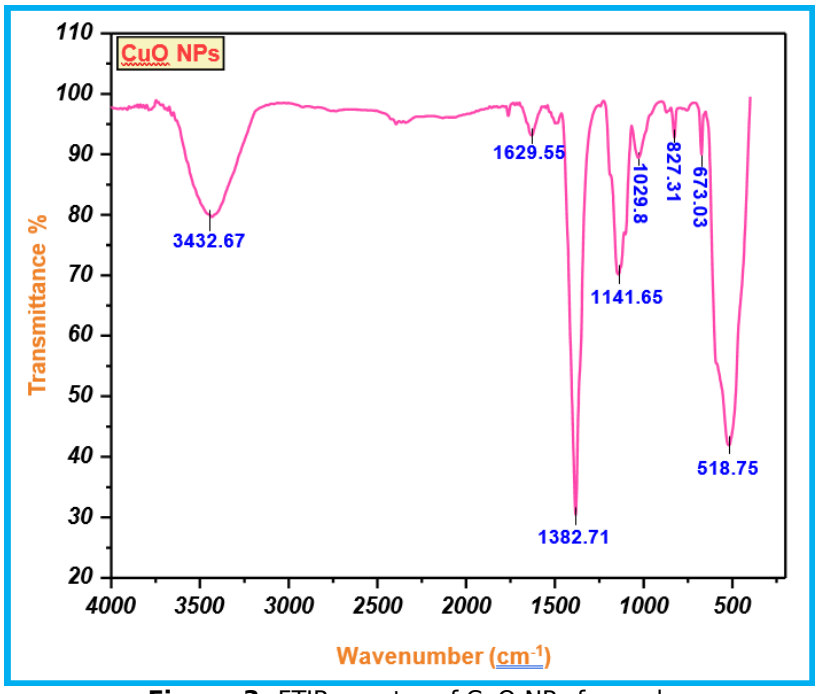

Figure 3: FTIR spectra of CuO NPs formed.

Figure 4 depicts the X-ray diffraction (XRD) pattern of CuO nanoparticles synthesized via *Nicotiana plumbaginifolia* Viv. The identified XRD peaks at 2-theta values of 32.615°, 35.655°, 38.872°, 48.792°, 53.803°, 58.609°, 61.718°, 66.394°, 68.344°, 72.28847°, and 75.278° precisely correspond to the (110), (002), (111), (202), (020), (202), (113), (311), (220), (311), and (004) hkl planes, respectively. This pattern aligns with standard values from JCPDS file No. 89–5895 (47), confirming the monoclinic crystal structure of the synthesized CuO nanoparticles (48,49). The observed peaks signify the high degree of crystallinity and structural consistency of the prepared nanoparticles.

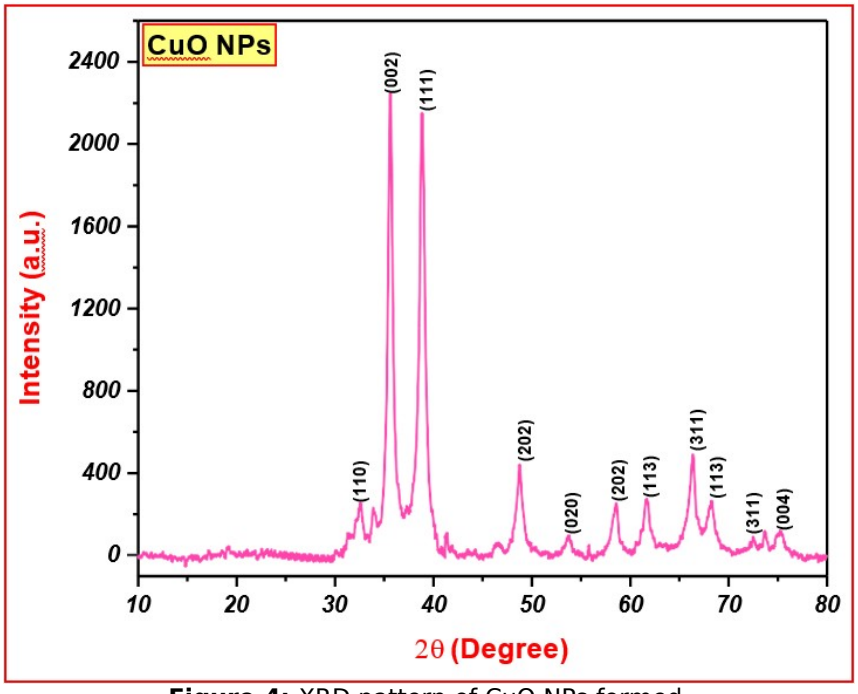

Figure 4: XRD pattern of CuO NPs formed.

Scanning electron microscopy (SEM) was employed to assess the surface morphology of CuO nanoparticles synthesized from Nicotiana plumbaginifolia. In Figure 5, SEM images reveal uniform shapes with varying sizes and occasional surface aggregation. The observed size variations are attributed to synthesis parameters influenced by phytochemical constituents in the plant (50). Surface aggregations suggest localized influences of diverse plant-derived species on nanoparticle assembly.

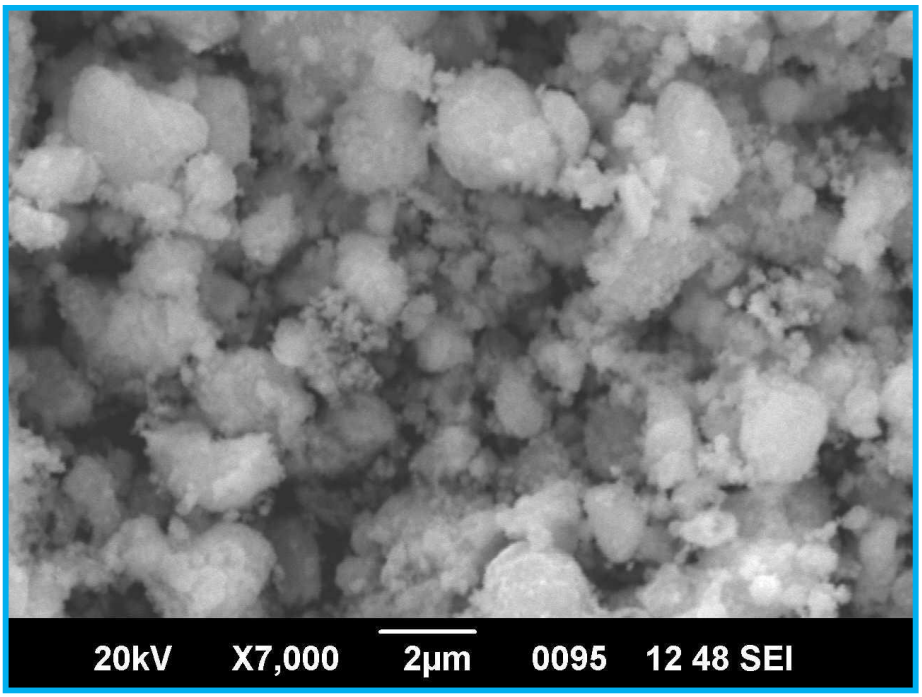

Figure 5: SEM figure of CuO NPs prepared.

Energy dispersive X-ray analysis (EDAX) stands as a highly valuable technique for elucidating the chemical composition and elemental constitution of nanomaterials. In Figure 6, the EDAX spectrum corresponding to the CuO nanoparticles synthesized through the utilization of *Nicotiana plumbaginifolia* Viv. is presented. The spectrum prominently exhibits distinctive signals indicative of copper and oxygen. These discernible signals unequivocally authenticate the existence of copper and oxygen in the CuO nanoparticle form, as visually represented in Figure 6.

The copper signals in the spectrum are notably characterized by a weight percentage of 53.62% and an atomic percentage of 28.5%. Concurrently, the oxygen signals manifest a weight percentage of 24.46% with an atomic percentage of 51.63%. This quantitative analysis attests to the precise elemental makeup of the synthesized CuO nanoparticles. The discerned weight and atomic percentages offer quantitative insights into the proportional contribution of each element, underscoring the composition of the nanomaterial.

Furthermore, the EDAX spectrum reveals additional peaks, which are attributed to photochemical constituents inherent in the *Nicotiana plumbaginifolia* Viv. plant. These supplementary peaks underscore the presence of diverse elements associated with the plant-derived synthesis process. The comprehensive analysis not only validates the synthesis of CuO nanoparticles but also provides a nuanced understanding of the elemental composition, thereby contributing to the in-depth characterization of the nanomaterial.

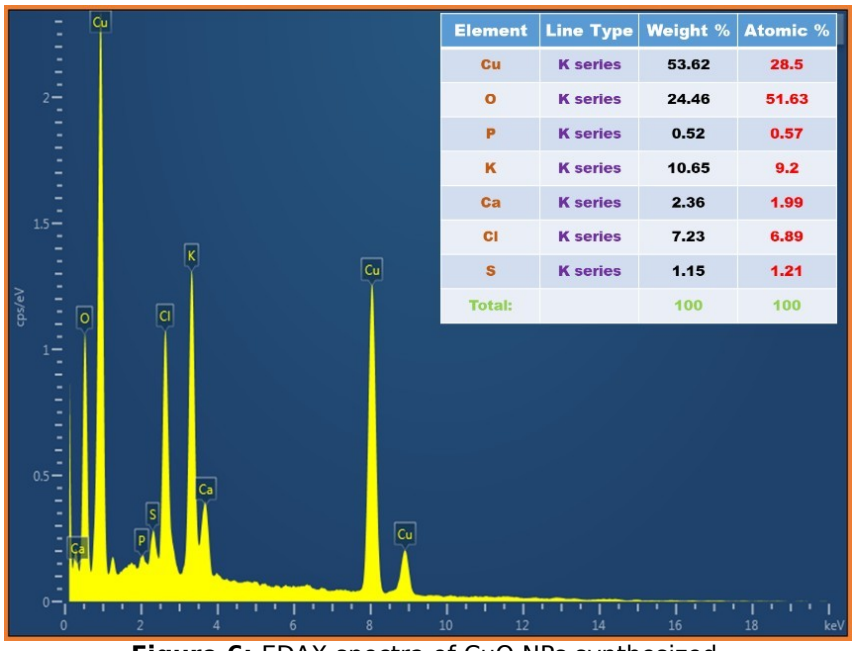

Figure 6: EDAX spectra of CuO NPs synthesized.

Transmission electron microscopy (TEM) stands as an indispensable tool for the detailed examination of materials, facilitating the elucidation of their morphology, size, and structural arrangement. In figure 7, distinctive images of the synthesized CuO nanoparticles are presented, offering valuable insights into their physical characteristics. The observations derived from these images reveal that the designed CuO nanoparticles exhibit quasi-spherical morphology, with an average size distribution spanning from 12 to 14 nm. The observed size variation is ascribed to surface agglomeration phenomena, influenced by the concentration of the plant extract employed during the nanoparticle synthesis process. The interplay of these parameters contributes to the nuanced size distribution of the CuO nanoparticles, reflecting the dynamic nature of their formation. The utilization of Selected Area Electron Diffraction (SAED) further substantiates the crystalline nature of the synthesized CuO nanoparticles derived from *Nicotiana plumbaginifolia* Viv. The SAED pattern, as depicted in figure 8, manifests a distinctive quasi-spherical pattern, affirming the ordered atomic arrangement within the nanoparticles. This crystalline pattern serves as compelling evidence of the high structural integrity and regularity of the CuO nanoparticles at the nanoscale.

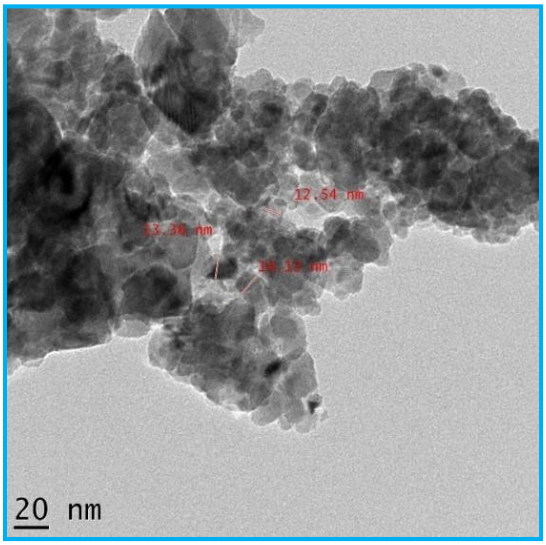

Figure 7: TEM image of CuO NPs formed.

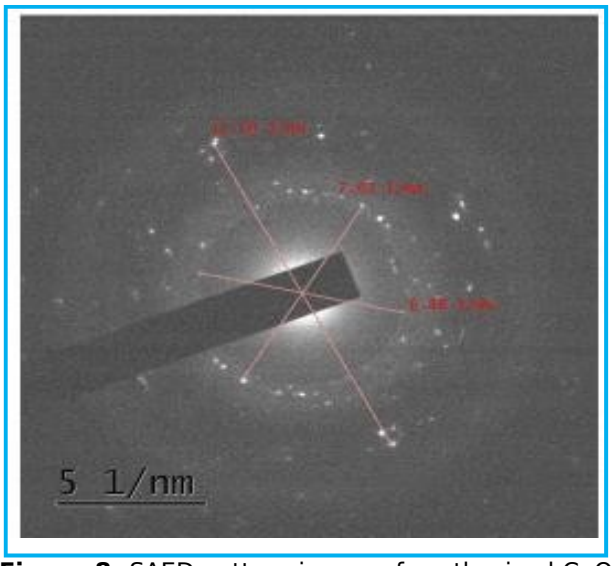

Figure 8: SAED pattern image of synthesized CuO NPs.

In figure 9, amikacin at 30 µg demonstrates sensitivity in *E. coli,* yielding a 19 mm inhibition zone. In contrast, CuO NPs exhibit no inhibition at 25 µL and 50 µL, with a 15 mm zone observed at 100 µl. For *S. aureus* and *P. aeruginosa,* amikacin (30 µg) elicits 25 mm and 10 mm inhibition zones, respectively. CuO NPs produce inhibition zones of 17 mm, 20 mm, and 22 mm for S. aureus (at 25 µL, 50 µL, and 100 µL) and show no inhibition for *P. aeruginosa* (25 µL, 50 µL, and 100 µL*). K. pneumoniae* displays sensitivity to amikacin (30 µg) with a 22 mm inhibition zone, while CuO NPs exhibit no inhibition at all concentrations. Remarkably, at a 100 µL concentration of CuO NPs, robust antimicrobial activity is evident only against *E. coli* and *S. aureus*, with *S. aureus,* demonstrating significant activity even at 25 µL and 50 µL concentrations (Table 1). In the past, many workers have carried out the antimicrobial activities from different plant-mediated extract-based synthesized copper oxide nanoparticles (51-55).

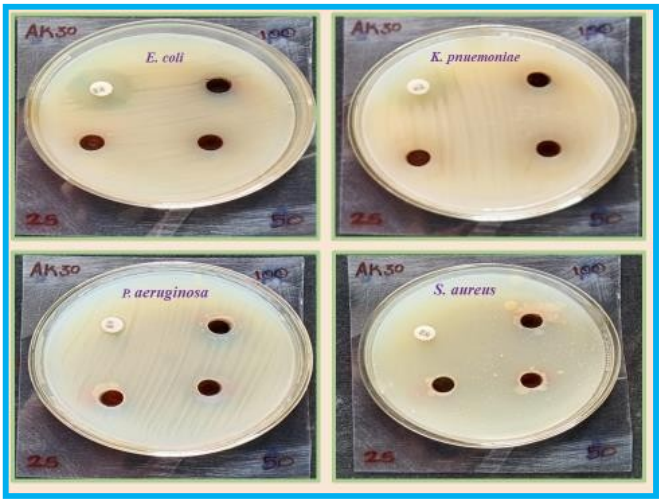

Figure 9: Synthesized CuO NPs exhibit antibacterial activity comparable to standard Amikacin (AK30 µg).

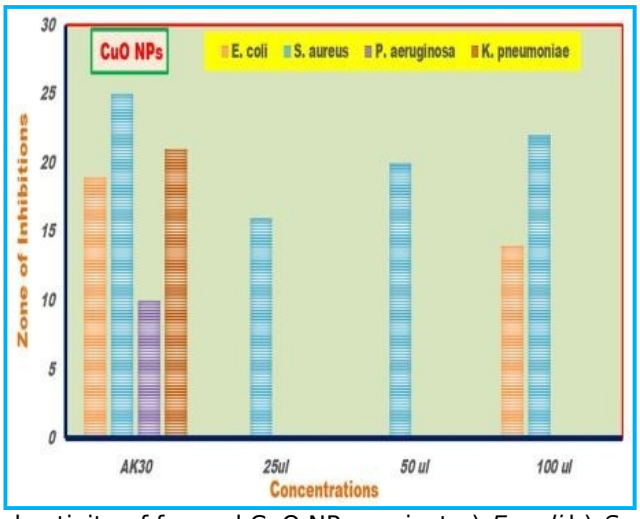

Figure 10: Antimicrobial activity of formed CuO NPs against a) *E. coli* b) *S. aureus* c) *P. aeruginosa* d) *K. Pneumoniae*.

Table 1: ZOI (Zone of inhibition) of organisms at different concentrations.

ZOI (mm) Organisms	**Concentrations**			
	AK30	**25 uL**	**50 uL**	**100 uL**
E. coli	19	NI	NI	15
K. pneumoniae	22	NI	NI	NI
S. aureus	25	17	20	22
P. aeruginosa	10	NI	NI	NI

Note*: E. coli =Escherichia coli, S. aureus= Staphylococcus aureus, P. aeruginosa= Pseudomonas aeruginosa, K. pnuemoniae = Klebsiella pnuemoniae*, NI= No Inhibition. AK30= Amikacin 30 mcg.

The antioxidant efficacy of CuO nanoparticles on 1,1-Diphenyl-2-picrylhydrazyl (DPPH) is depicted in figure 11. The findings reveal potent antioxidant activity, with escalating CuO nanoparticle concentrations correlating to increased percentage inhibition: 50 µg/mL – 1.68%, 100 µg/mL – 10.45%, 150 µg/mL – 18.54%, 200 µg/mL – 37.83%, and 250 µg/mL – 51.72%. The highest activity, at 51.72%, occurs at 250 µg/ml. This heightened antioxidative capability is attributed to bioactive compounds, including alkaloids, phenolic compounds, and flavonoids, in *Nicotiana plumbaginifolia*. Similar studies have also been carried out, showing enhancement of the antioxidant properties of the material (56-57). The synergistic interaction with nanomaterials enhances CuO nanoparticle antioxidant properties, showcasing potential for addressing diverse diseases.

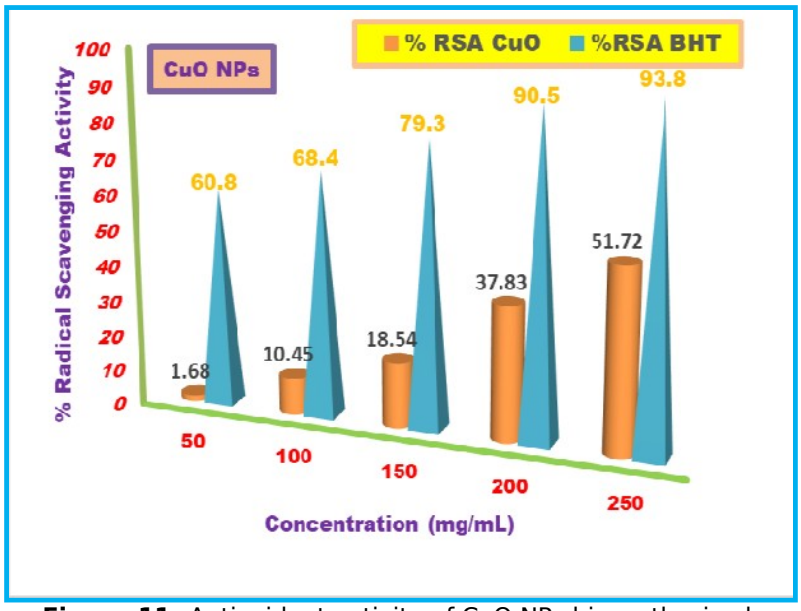

Figure 11: Antioxidant activity of CuO NPs biosynthesized.

4. CONCLUSION

This study presents a cost-effective and benign approach for the synthesis of copper oxide nanoparticles (CuO NPs) utilizing the entire *Nicotiana plumbaginifolia* Viv. plant. The plant's abundance of diverse phytochemicals serves a dual purpose by reducing metal ions and stabilizing the resulting nanoparticles. Structural characteristics of the CuO NPs were systematically analyzed using spectroscopic techniques. Furthermore, the antimicrobial and antioxidant properties of the synthesized nanoparticles were investigated. The band gap of the biogenically synthesized CuO NPs was quantified at 5.16 eV, demonstrating notable antimicrobial efficacy against pathogenic bacteria and significant antioxidant activity.

5. CONFLICT OF INTEREST

All authors declare no conflicts of interest.

6. ACKNOWLEDGMENTS

We extend heartfelt appreciation to the principal of Nevjabai Hitkarini College, Bramhapuri, Maharashtra (India), for steadfast support, encouragement, and providing research facilities. Our gratitude also extends to the Sophisticated Test and Instrumentation Centre (STIC) at Cochin University of Science and Technology, Cochin, Kerala, India, for their invaluable assistance in characterizing the samples.

7. REFERENCES

1. Akhtar MS, Panwar J, Yun YS. Biogenic Synthesis of Metallic Nanoparticles by Plant Extracts. ACS Sustain Chem Eng [Internet]. 2013 Jun 3;1(6):591–602. Available from: <URL>.

2. Roco MC. The long view of nanotechnology development: The National Nanotechnology Initiative at 10 years. J Nanoparticle Res [Internet]. 2011 Feb 12;13(2):427–45. Available from: <URL>.

3. Kim BS, Song JY. Biological synthesis of gold and silver nanoparticles using plant leaf extracts and antimicrobial applications. In: Hou CT, Shaw JF, editors. Biocatalysis and biomolecular engineering. USA: Wiley & Sons; 2010. p. 447–57.

4. Buzea C, Pacheco II, Robbie K. Nanomaterials and nanoparticles: Sources and toxicity. Biointerphases [Internet]. 2007 Dec 1;2(4):MR17–71. Available from: <URL>.

5. Singh P, Ali SW, Kale RD. Antimicrobial Nanomaterials as Advanced Coatings for Self-Sanitizing of Textile Clothing and Personal Protective Equipment. ACS Omega [Internet]. 2023 Mar 7;8(9):8159–71. Available from: <URL>.

6. Černík M, Thekkae Padil VV. Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application. Int J Nanomedicine [Internet]. 2013 Feb;8:889–98. Available from: <URL>.

7. Chaudhary S, Rohilla D, Umar A, Kaur N, Shanavas A. Synthesis and characterizations of luminescent copper oxide nanoparticles: Toxicological profiling and sensing applications. Ceram Int [Internet]. 2019 Aug;45(12):15025–35. Available from: <URL>.

8. Chang MH, Liu HS, Tai CY. Preparation of copper oxide nanoparticles and its application in nanofluid. Powder Technol [Internet]. 2011 Feb;207(1–3):378–86. Available from: <URL>.

9. Ikhioya IL, Onoh EU, Nkele AC, Abor BC, Obitte BCN, Maaza M, et al. The The Green Synthesis of Copper Oxide Nanoparticles Using the Moringa Oleifera Plant and its Subsequent Characterization for Use in Energy Storage Applications. East Eur J Phys [Internet]. 2023 Mar 2;(1):162–72. Available from: <URL>.

10. Ganesan K, Jothi VK, Natarajan A, Rajaram A, Ravichandran S, Ramalingam S. Green synthesis of Copper oxide nanoparticles decorated with graphene oxide for anticancer activity and catalytic applications. Arab J Chem [Internet]. 2020 Aug;13(8):6802–14. Available from: <URL>.

11. Rehana D, Mahendiran D, Kumar RS, Rahiman AK. Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts. Biomed Pharmacother [Internet]. 2017 May;89:1067–77. Available from: <URL>.

12. Singh A, Singh NB, Hussain I, Singh H. Effect of biologically synthesized copper oxide nanoparticles on metabolism and antioxidant activity to the crop plants Solanum lycopersicum and Brassica oleracea var. botrytis. J Biotechnol [Internet]. 2017 Nov;262:11–27. Available from: <URL>.

13. Khatoon UT, Mohan Mantravadi K, Nageswara Rao GVS. Strategies to synthesise copper oxide nanoparticles and their bio applications – a review. Mater Sci Technol [Internet]. 2018 Dec 1;34(18):2214–22. Available from: <URL>.

14. El Bialy BE, Hamouda RA, Abd Eldaim MA, El Ballal SS, Heikal HS, Khalifa HK, et al. Comparative Toxicological Effects of Biologically and Chemically Synthesized Copper Oxide Nanoparticles on Mice. Int J Nanomedicine [Internet]. 2020 May;15:3827–42. Available from: <URL>.

15. Chakraborty N, Banerjee J, Chakraborty P, Banerjee A, Chanda S, Ray K, et al. Green synthesis of copper/copper oxide nanoparticles and their applications: a review. Green Chem Lett Rev [Internet]. 2022 Jan 2;15(1):187–215. Available from: <URL>.

16. Vincent J, Lau KS, Evyan YCY, Chin SX, Sillanpää M, Chia CH. Biogenic Synthesis of Copper-Based Nanomaterials Using Plant Extracts and Their Applications: Current and Future Directions. Nanomaterials [Internet]. 2022 Sep 23;12(19):3312. Available from: <URL>.

17. Eid AM, Fouda A, Hassan SED, Hamza MF, Alharbi NK, Elkelish A, et al. Plant-Based Copper Oxide Nanoparticles; Biosynthesis, Characterization, Antibacterial Activity, Tanning Wastewater Treatment, and Heavy Metals Sorption. Catalysts [Internet]. 2023 Feb 3;13(2):348. Available from: <URL>.

18. Atri A, Echabaane M, Bouzidi A, Harabi I, Soucase BM, Ben Chaâbane R. Green synthesis of copper oxide nanoparticles using Ephedra Alata plant extract and a study of their antifungal, antibacterial activity and photocatalytic performance under sunlight. Heliyon [Internet]. 2023 Feb 1;9(2):e13484. Available from: <URL>.

19. Vinothkanna A, Mathivanan K, Ananth S, Ma Y, Sekar S. Biosynthesis of copper oxide nanoparticles using Rubia cordifolia bark extract: characterization, antibacterial, antioxidant, larvicidal and photocatalytic activities. Environ Sci Pollut Res [Internet]. 2022 Feb 17;30(15):42563–74. Available from: <URL>.

20. Thandapani G, Arthi K, Pazhanisamy P, John JJ, Vinothini C, Rekha V, et al. Green synthesis of copper oxide nanoparticles using Spinacia oleracea leaf extract and evaluation of biological applications: Antioxidant, antibacterial, larvicidal and biosafety assay. Mater Today Commun [Internet]. 2023 Mar;34:105248. Available from: <URL>.

21. Saligedo TS, Muleta GG, Tsega TW, Tadele KT. Green Synthesis of Copper Oxide Nanoparticles Using Eichhornia Crassipes Leaf Extract, its Antibacterial and Photocatalytic Activities. Curr Nanomater [Internet]. 2023 Apr;8(1):58–68. Available from: <URL>.

22. Javid-Naderi MJ, Sabouri Z, Jalili A, Zarrinfar H, Samarghandian S, Darroudi M. Green synthesis of copper oxide nanoparticles using okra (Abelmoschus esculentus) fruit extract and assessment of their cytotoxicity and photocatalytic applications. Environ Technol Innov [Internet]. 2023 Nov;32:103300. Available from: <URL>.

23. Derakhshani E, Asri M, Naghizadeh A. Plant-Based Green Synthesis of Copper Oxide Nanoparticles Using Berberis vulgaris Leaf Extract: an Update on Their Applications in Antibacterial Activity. Bionanoscience [Internet]. 2023 Mar 30;13(1):212–8. Available from: <URL>.

24. Ramasubbu K, Padmanabhan S, Al-Ghanim KA, Nicoletti M, Govindarajan M, Sachivkina N, et al. Green Synthesis of Copper Oxide Nanoparticles Using Sesbania grandiflora Leaf Extract and Their Evaluation of Anti-Diabetic, Cytotoxic, Anti-Microbial, and Anti-Inflammatory Properties in an In-Vitro Approach. Fermentation [Internet]. 2023 Mar 27;9(4):332. Available from: <URL>.

25. Arun S., Karthik B., Yatish K., Prashanth K., Balakrishna GR. Green synthesis of copper oxide nanoparticles using the Bombax ceiba plant: Biodiesel production and nano-additive to investigate diesel engine performance-emission characteristics. Energy [Internet]. 2023 Jul;274:127345. Available from: <URL>.

26. Abhimanyu P, Arvind M, Kishor N. Biosynthesis of CuO Nanoparticles Using Plant Extract as a Precursor: Characterization, Antibacterial, and Antioxidant Activity. Nano Biomed Eng [Internet]. 2023 Dec;15(4):369–77. Available from: <URL>.

27. Hano C, Abbasi BH. Plant-Based Green Synthesis of Nanoparticles: Production, Characterization and Applications. Biomolecules [Internet]. 2021 Dec 25;12(1):31. Available from: <URL>.

28. Shafey AM El. Green synthesis of metal and metal oxide nanoparticles from plant leaf extracts and their applications: A review. Green Process Synth [Internet]. 2020 Jun 18;9(1):304–39. Available from: <URL>.

29. Nande A, Raut S, Michalska-Domanska M, Dhoble SJ. Green Synthesis of Nanomaterials Using Plant Extract: A Review. Curr Pharm Biotechnol [Internet]. 2021 Sep 20;22(13):1794–811. Available from: <URL>.

30. Soni V, Raizada P, Singh P, Cuong HN, S R, Saini A, et al. Sustainable and green trends in using plant extracts for the synthesis of biogenic metal nanoparticles toward environmental and pharmaceutical advances: A review. Environ Res [Internet]. 2021 Nov;202:111622. Available from: <URL>.

31. Baek YW, An YJ. Microbial toxicity of metal oxide nanoparticles (CuO, NiO, ZnO, and Sb_2O_3) to Escherichia coli, Bacillus subtilis, and Streptococcus aureus. Sci Total Environ [Internet]. 2011 Mar;409(8):1603–8. Available from: <URL>.

32. Sharma JK, Srivastava P, Singh G, Akhtar MS, Ameen S. Catalytic thermal decomposition of ammonium perchlorate and combustion of composite solid propellants over green synthesized CuO nanoparticles. Thermochim Acta [Internet]. 2015 Aug;614:110–5. Available from: <URL>.

33. Akintelu SA, Folorunso AS, Folorunso FA, Oyebamiji AK. Green synthesis of copper oxide nanoparticles for biomedical application and environmental remediation. Heliyon [Internet]. 2020 Jul;6(7):e04508. Available from: <URL>.

34. Pawar AP, Naktode KS, Mungole AJ. Green synthesis of silver nanoparticles from whole plant extract analyzed for characterization, antioxidant, and antibacterial properties. Phys Chem Solid State [Internet]. 2023 Nov 21;24(4):640–9. Available from: <URL>.

35. Manasa DJ, Chandrashekar KR, Madhu Kumar DJ, Niranjana M, Navada KM. Mussaenda frondosa L. mediated facile green synthesis of Copper oxide nanoparticles – Characterization, photocatalytic and their biological investigations. Arab J Chem [Internet]. 2021 Jun;14(6):103184. Available from: <URL>.

36. Aziz WJ, Jassim HA. A new paradigm shift to prepare copper nanoparticles using biolog- ical synthesis and evaluation of antimicrobial activity. Plant Arch [Internet]. 2018;18(2):2020–4. Available from: <URL>.

37. Kuppusamy P, Ilavenil S, Srigopalram S, Maniam GP, Yusoff MM, Govindan N, et al. Treating of palm oil mill effluent using Commelina nudiflora mediated copper nanoparticles as a novel bio-control agent. J Clean Prod [Internet]. 2017 Jan;141:1023–9. Available from: <URL>.

38. Maruthupandy M, Zuo Y, Chen JS, Song JM, Niu HL, Mao CJ, et al. Synthesis of metal oxide nanoparticles (CuO and ZnO NPs) via biological template and their optical sensor applications. Appl Surf Sci [Internet]. 2017 Mar;397:167–74. Available from: <URL>.

39. Sriranjani R, Srinithya B, Vellingiri V, Brindha P, Anthony SP, Sivasubramanian A, et al. Silver nanoparticle synthesis using Clerodendrum phlomidis leaf extract and preliminary investigation of its antioxidant and anticancer activities. J Mol Liq [Internet]. 2016 Aug;220:926–30. Available from: <URL>.

40. Kasatikov S, Fantin A, Manzoni AM, Sakhonenkov S, Makarova A, Smirnov D, et al. Chemical interaction and electronic structure in a compositionally complex alloy: A case study by means of X-ray absorption and X-ray photoelectron spectroscopy. J Alloys Compd [Internet]. 2021 Mar;857:157597. Available from: <URL>.

41. Parthibavarman M, Sharmila V, Sathishkumar P, Gaikwad SA. A Rapid One-Pot Synthesis of CuO Rice-Like Nanostructure and Its Structural, Optical and Electrochemical Performance. J Electron Mater [Internet]. 2018 Sep 18;47(9):5443–51. Available from: <URL>.

42. Landi S, Segundo IR, Freitas E, Vasilevskiy M, Carneiro J, Tavares CJ. Use and misuse of the Kubelka-Munk function to obtain the band gap energy from diffuse reflectance measurements. Solid State Commun [Internet]. 2022 Jan 1;341:114573. Available from: <URL>.

43. Berger T, Trunschke A. Optical Properties: UV/Vis Diffuse Reflectance Spectroscopy and Photoluminescence. In: Metal Oxide Nanoparticles [Internet]. Wiley; 2021. p. 435–82. Available from: <URL>.

44. Renuga D, Jeyasundari J, Shakthi Athithan AS, Brightson Arul Jacob Y. Synthesis and characterization of copper oxide nanoparticles using Brassica oleracea var. italic extract for its antifungal application. Mater Res Express [Internet]. 2020 Apr 1;7(4):045007. Available from: <URL>.

45. Vidovix TB, Quesada HB, Januário EFD, Bergamasco R, Vieira AMS. Green synthesis of copper oxide nanoparticles using Punica granatum leaf extract applied to the removal of methylene blue. Mater Lett [Internet]. 2019 Dec;257:126685. Available from: <URL>.

46. Antonoglou O, Lafazanis K, Mourdikoudis S, Vourlias G, Lialiaris T, Pantazaki A, et al. Biological relevance of $CuFeO_2$ nanoparticles: Antibacterial and anti-inflammatory activity, genotoxicity, DNA and protein interactions. Mater Sci Eng C [Internet]. 2019 Jun;99:264–74. Available from: <URL>.

47. Anto Feradrick Samson V, Mohamed Racik K, Prathap S, Madhavan J, Victor Antony Raj M. Investigations of Structural, Optical and dielectric studies of Copper Oxide nanoparticles. Mater Today Proc [Internet]. 2019;8:386–92. Available from: <URL>.

48. Vetrimani A, Geetha K, Angel Jemima E, Arulnathan N, Kim HS, Kathalingam A. Effect of the green synthesis of CuO plate-like nanoparticles on their photodegradation and antibacterial activities. Phys Chem Chem Phys [Internet]. 2022;24(47):28923–33. Available from: <URL>.

49. Ganga BG, Varma MR, Santhosh PN. Evidence of reduced antiferromagnetic transition in mesocrystals of CuO synthesized by a surfactant-free solution phase method. CrystEngComm [Internet]. 2015;17(37):7086–93. Available from: <URL>.

50. Mittal AK, Chisti Y, Banerjee UC. Synthesis of metallic nanoparticles using plant extracts. Biotechnol Adv [Internet]. 2013 Mar 1;31(2):346–56. Available from: <URL>.

51. Singh P, Singh KR, Singh J, Das SN, Singh RP. Tunable electrochemistry and efficient antibacterial activity of plant-mediated copper oxide nanoparticles synthesized by Annona squamosa seed extract for agricultural utility. RSC Adv [Internet]. 2021;11(29):18050–60. Available from: <URL>.

52. Achamo T, Zereffa EA, Murthy HCA, Ramachandran VP, Balachandran R. Phyto-mediated synthesis of copper oxide nanoparticles using Artemisia abyssinica leaf extract and its antioxidant, antimicrobial and DNA binding activities. Green Chem Lett Rev [Internet]. 2022 Jul 3;15(3):598–614. Available from: <URL>.

53. Bhatia N, Kumari A, Chauhan N, Thakur N, Sharma R. Duchsnea indica plant extract mediated synthesis of copper oxide nanomaterials for antimicrobial activity and free-radical scavenging assay. Biocatal Agric Biotechnol [Internet]. 2023 Jan;47:102574. Available from: <URL>.

54. Maheo AR, B. SMV, T. AAP. Biosynthesis and characterization of Eupatorium adenophorum and chitosan mediated Copper oxide nanoparticles and their antibacterial activity. Results in Surfaces and Interfaces [Internet]. 2022 Feb;6:100048. Available from: <URL>.

55. Kamçı H, Taş R, Çelebioğlu HU. Antibacterial Activity of Copper Nanoparticles Synthesized by Using Peumus boldus Leaf Extract. Eur J Sci Technol [Internet]. 2022 May 2;5(36):139–42. Available from: <URL>.

56. Peddi P, PTSRK PR, Rani NU, Tulasi SL. Green synthesis, characterization, antioxidant, antibacterial, and photocatalytic activity of Suaeda maritima (L.) Dumort aqueous extract-mediated copper oxide nanoparticles. J Genet Eng Biotechnol [Internet]. 2021 Dec;19(1):131. Available from: <URL>.

57. Sarkar J, Chakraborty N, Chatterjee A, Bhattacharjee A, Dasgupta D, Acharya K. Green Synthesized Copper Oxide Nanoparticles Ameliorate Defence and Antioxidant Enzymes in Lens culinaris. Nanomaterials [Internet]. 2020 Feb 12;10(2):312. Available from: <URL>.