Pharmacogn. Res., 2025; 17(4):1235-1240.
https://www.phcogres.com

Original Article

Green Synthesis and Characterization of Copper Oxide Nanoparticles Using *Vitex negundo* Linn.: An *in vitro* study

Pravallika Kakada, Abilasha R*, Pratibha Ramani

Department of Oral Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, INDIA.

ABSTRACT

Background: Nanotechnology offers innovative approaches for biomedical applications, including antimicrobial agent development. Copper Oxide Nanoparticles (CuO NPs) exhibit strong antibacterial properties, but conventional synthesis methods rely on toxic chemicals and high energy consumption, raising environmental concerns. This study explores the green synthesis of CuO NPs using *Vitex negundo* Linn., a medicinal plant, as an eco-friendly alternative. **Objectives:** To synthesize and characterize CuO NPs using *Vitex negundo* Linn. leaf extract and evaluate their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. **Materials and Methods:** *Vitex negundo* leaf extract was used as a reducing and stabilizing agent for CuO NP synthesis. The nanoparticles were characterized using UV-visible Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM). Antibacterial activity was assessed using the agar well diffusion method against *E. coli* and *S. aureus*. **Results:** The synthesis of CuO NPs was confirmed by a UV-vis absorption peak at 277 nm. FTIR analysis indicated functional groups responsible for stabilization, and SEM imaging revealed spherical nanoparticles ranging from 50-100 nm. Antibacterial testing demonstrated concentration-dependent inhibition, with the largest zones of inhibition observed at 100 µL CuO NP concentration. **Conclusion:** Green synthesis of CuO NPs using *Vitex negundo* Linn is a sustainable and effective alternative to conventional methods. The nanoparticles exhibited significant antibacterial activity, highlighting their potential for biomedical applications. Further research should explore scalability, mechanisms of action, and *in vivo* efficacy.

Keywords: Antibacterial Activity, Copper Oxide Nanoparticles, Green Synthesis, Nanotechnology, Sustainable Synthesis, *Vitex negundo* Linn.

Correspondence:
Abilasha R
Department of Oral Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, INDIA.
Email: abilasha.ramasubramanian@gmail.com

Received: 09-05-2025;
Revised: 24-07-2025;
Accepted: 12-09-2025.

INTRODUCTION

Nanotechnology enables the manipulation of materials at the atomic and molecular level, producing nanoparticles with unique properties that make them suitable for a variety of applications. It is a rapidly evolving field, offering significant potential across various sectors, particularly in healthcare and pharmaceuticals.[1] Among the numerous applications, nanoparticles have emerged as key agents due to their unique physicochemical properties, including high reactivity, large surface area, and nanoscale dimensions.[2] These properties render nanoparticles highly suitable for applications such as drug delivery systems, medical imaging, and antimicrobial treatments. Copper Oxide Nanoparticles (CuO NPs) have gained particular interest in biomedicine due to their high reactivity, large surface area, and antimicrobial activity and are effective in combating a range of pathogens, positioning them as promising agents in the development of new antimicrobial treatments.[3]

Nanoparticles can be synthesised through various methods, including physical, chemical, biological, and hybrid techniques.[4] Conventional methods of synthesising metallic nanoparticles often involve hazardous chemicals and are energy-intensive, raising concerns regarding environmental sustainability.[5] However, the use of toxic substances in physical and chemical synthesis poses significant challenges, including environmental hazards and safety concerns.[6] The green synthesis methods can be used as eco-friendly alternatives that utilise biological materials for nanoparticle production.[7]

The biological methods, often termed biogenic synthesis, offer a sustainable and eco-friendly alternative. These methods utilise natural resources such as plant extracts, enzymes, or microorganisms, making the synthesis process safer and adaptable.[8] As a result, eco-friendly biogenic approaches have gained popularity for their effectiveness and reduced environmental impact in nanoparticle production.[9] These methods are cost-effective, sustainable, and reduce the generation

DOI: 10.5530/pres.20252186

of hazardous waste, aligning with the global shift towards greener technologies.[2]

Vitex negundo Linn., also known as the Chinese chaste tree, five-leaved chaste tree, horseshoe vitex, or nisinda, is a large aromatic shrub characterised by its quadrangular branches. It is extensively utilised in Ayurvedic medicine, especially in South and Southeast Asia, commonly known for its diverse therapeutic properties, including astringent, sedative, and antimicrobial effects.[10,11] Every part of the plant, from its roots to its fruits, contains numerous phytochemicals that function as secondary metabolites.[11] These compounds contribute to the plant's properties as an analgesic, antioxidant, and anti-inflammatory agent. While various phytochemicals from *Vitex negundo* have been isolated and studied for their pharmacological activities.[12] The bioactive compounds present in this plant make it source for the green synthesis of nanoparticles.[13]

This study aims to provide a comprehensive analysis of the green synthesis process of CuO NPs using *Vitex negundo* Linn. and evaluate their antibacterial efficacy against common bacterial pathogens such as *Escherichia coli* and *Staphylococcus aureus*. By doing so, the research underscores the potential of plant-based synthesis methods in producing effective and environmentally friendly nanoparticles, which could have far-reaching implications in the fields of medicine and materials science.

MATERIALS AND METHODS

The primary materials utilized in this study include the leaves of *Vitex negundo* Linn., a plant traditionally used in Ayurvedic medicine, known for its antimicrobial and therapeutic properties. Additionally, analytical-grade Chemicals such as Copper Nitrate ($Cu(NO_3)_2{\cdot}3H_2O$) were used as the precursor for the synthesis of Copper Oxide Nanoparticles (CuO NPs). Deionized water was employed throughout the experiment for the preparation of solutions and during the synthesis process.

Preparation of *Vitex negundo* Linn. Leaf Extract

Fresh leaves of *Vitex negundo* Linn. were collected and thoroughly washed with deionised water to remove dust and other contaminants. The cleaned leaves were then air-dried at room temperature until all moisture was removed. The dried leaves were finely ground into powder using a mechanical grinder. Approximately 10 g of this powdered leaf material was added to 100 mL of deionized water and heated at 60°C for 1 hr to extract the bioactive compounds. The extract was then filtered through Whatman No. 1 filter paper to remove particulate matter, yielding a clear filtrate that was used as the reducing agent in the synthesis of CuO NPs.

Synthesis of Copper Oxide Nanoparticles

Copper oxide nanoparticles were synthesised using the green method. Initially, 100 mL of 0.1 M copper nitrate solution was prepared and mixed with 50 mL of the *Vitex negundo* Linn. leaf extract. This mixture was stirred continuously at 60°C for 2 hr. The colour change from light blue to dark brown indicated the reduction of copper ions to copper oxide nanoparticles. After completion of the reaction, the solution was allowed to cool at room temperature. The resulting CuO NPs were then centrifuged at 10,000 rpm for 15 min to separate the nanoparticles from the aqueous phase. The collected nanoparticles were washed several times with demonised water to remove any unreacted components and were finally dried in an oven at 60°C for 24 hr. The dried nanoparticles were stored in airtight containers for further characterisation.

Characterization of Copper Oxide Nanoparticles

The synthesised CuO NPs were characterised using various analytical techniques. UV-visible Spectroscopy was employed to confirm the formation of nanoparticles by analyzing the optical properties of the CuO NPs. The absorbance spectra were recorded in the range of 200-600 nm. Fourier Transform Infrared Spectroscopy (FTIR) was conducted to identify the functional groups present in the synthesised nanoparticles. The FTIR spectra were obtained in the range of 4000-500 cm^{-1}. Scanning Electron Microscopy (SEM) was used to analyze the surface morphology and particle size of the CuO NPs. SEM images provided detailed insight into the shape and distribution of the nanoparticles.

Antibacterial Activity Testing

The antibacterial efficacy of the synthesised CuO NPs was evaluated using the agar well diffusion method against *Escherichia coli* and *Staphylococcus aureus*. Bacterial cultures were prepared

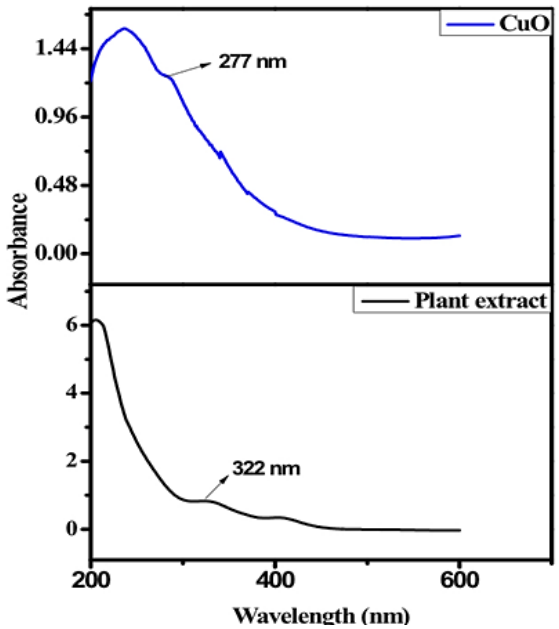

Figure 1: UV-vis Spectroscopy Analysis of CuO Nanoparticles and Plant Extract. UV-vis absorption spectra of biosynthesized CuO nanoparticles (top) showing a peak at 277 nm and plant extract (bottom) with a peak at 322 nm.

in nutrient broth and incubated at 37°C for 24 hr. Sterile nutrient agar plates were inoculated with the bacterial cultures, and wells were created on the agar surface. Different concentrations (50 µL and 100 µL) of CuO NPs were introduced into the wells. After incubation at 37°C for 24 hr, the zones of inhibition around each well were measured to assess the antibacterial activity of the nanoparticles.

RESULTS

The green synthesis of Copper Oxide Nanoparticles (CuO NPs) using *Vitex negundo* Linn. leaf extract as a reducing agent was successfully achieved. The change in colour of the reaction mixture from light blue to dark brown during the synthesis process visually indicated the reduction of copper ions to copper oxide nanoparticles.

UV-visible Spectroscopy

The UV-vis absorption spectra of the synthesised CuO NPs revealed a strong absorption peak at 277 nm, confirming the presence of copper oxide nanoparticles. This characteristic peak corresponds to the excitation of surface plasmon resonance in CuO NPs. The plant extract alone exhibited an absorption peak at 322 nm, indicating the presence of bioactive compounds in the *Vitex negundo* Linn. extract (Figure 1).

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the CuO NPs displayed prominent absorption bands corresponding to the metal-oxygen (Cu-O) bond, confirming the formation of copper oxide nanoparticles. Additionally, other absorption bands associated with the functional groups present in the plant extract were observed, indicating the involvement of bioactive compounds in the reduction process (Figure 2).

Scanning Electron Microscopy (SEM)

SEM analysis revealed that the synthesised CuO NPs were predominantly spherical in shape with a size range of approximately 50-100 nm. The nanoparticles were well-dispersed with minimal agglomeration, suggesting a stable synthesis process (Figure 3).

Antibacterial Activity

The antibacterial activity tests demonstrated that the CuO NPs exhibited significant inhibitory effects against both *Escherichia coli* and *Staphylococcus aureus*. The zone of inhibition was found to be concentration-dependent, with higher concentrations (100 µL) of CuO NPs showing larger zones of inhibition compared to lower concentrations (50 µL). This indicates that the synthesised CuO NPs possess potent antibacterial properties, making them a promising candidate for antimicrobial applications (Figure 4).

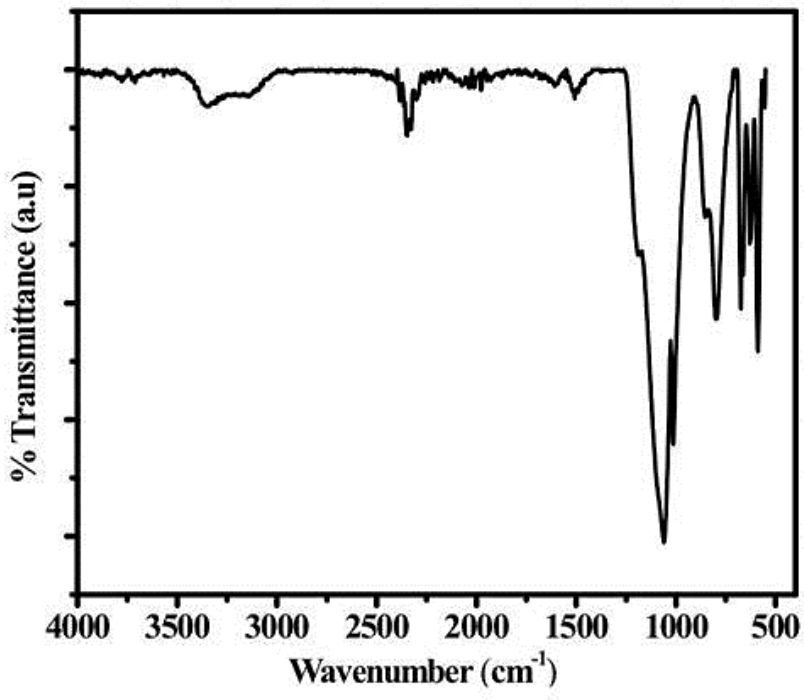

Figure 2: FTIR Spectrum of Biosynthesised CuO Nanoparticles. FTIR transmittance spectrum of CuO nanoparticles, indicating characteristic functional groups involved in nanoparticle synthesis.

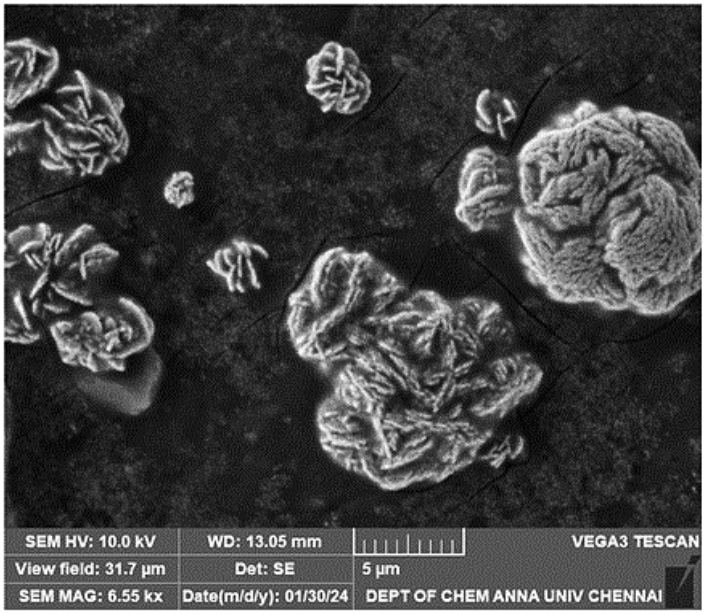

Figure 3: SEM Image of Biosynthesized CuO Nanoparticles. Scanning Electron Microscopy (SEM) image of CuO nanoparticles, showing their morphology at a magnification of 6.55 kx.

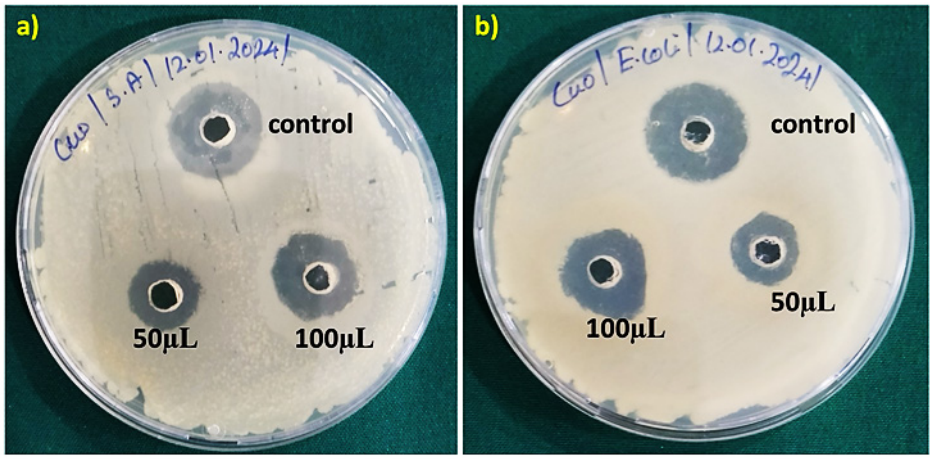

Figure 4: Antibacterial Activity of CuO Nanoparticles Against *S. aureus* and *E. coli*. (a) Zone of inhibition of CuO nanoparticles against *Staphylococcus aureus* at different concentrations (50 µL, 100 µL) compared to control. (b) Zone of inhibition of CuO nanoparticles against *Escherichia coli* at different concentrations (50 µL, 100 µL) compared to control.

DISCUSSION

The green synthesis of Copper Oxide Nanoparticles (CuO NPs) represents a significant advancement in the field of nanotechnology and green chemistry.[14] The use of plant extracts for the synthesis of nanoparticles offers a sustainable and environmentally friendly alternative to conventional chemical methods that often involve toxic reagents and generate hazardous waste.[15,16]

The present study demonstrated that *Vitex negundo* Linn., a plant with well-known medicinal properties, can effectively reduce copper ions to form CuO NPs. The UV-vis spectroscopy results, which showed a prominent absorption peak at 277 nm, confirm the formation of CuO NPs, while the FTIR analysis indicated the presence of functional groups associated with the bioactive compounds in the plant extract. These bioactive compounds likely played a dual role in the synthesis process: reducing agents and stabilizers for the nanoparticles.[17,18]

The morphological analysis using SEM revealed that the synthesised CuO NPs were spherical with a size range of 50-100 nm. Particle size and morphology are critical factors that influence the nanoparticles' properties and potential applications.[19] The small size and well-dispersed nature of the CuO NPs observed in this study suggests their suitability for various biomedical

applications, including drug delivery and antimicrobial treatments.[20,21]

The antibacterial activity tests further validated the potential of the synthesized CuO NPs. The observed concentration-dependent inhibition against *Escherichia coli* and *Staphylococcus aureus* indicates that these nanoparticles possess strong antibacterial properties, making them effective against common bacterial pathogens. The dose-dependent response also suggests that by modulating the concentration of CuO NPs, their antibacterial efficacy can be optimized for specific applications.[22]

The findings of this study highlight the advantages of green synthesis methods in producing nanoparticles with biological activity. The incorporation of plant-based synthesis methods not only contributes to environmental sustainability but also aids in the medicinal properties of the plants, thereby enhancing the functional aspects of the synthesized nanoparticles.[23]

Future studies should focus on scaling up the synthesis process and exploring the detailed mechanisms underlying the antibacterial activity of CuO NPs. Additionally, *in vivo* studies are necessary to evaluate the biocompatibility and therapeutic potential of these nanoparticles in clinical settings.

CONCLUSION

This study demonstrated the green synthesis of Copper Oxide Nanoparticles (CuO NPs) using *Vitex negundo* Linn. leaf extract, highlighting the potential of plant-based methods in producing environmentally friendly and biologically active nanoparticles.[24] The synthesis process was efficient, cost-effective, and aligned with the principles of green chemistry, offering a sustainable alternative to traditional chemical synthesis methods.[25]

The characterisation of the synthesised CuO NPs using UV-vis spectroscopy, FTIR, and SEM confirmed their formation, composition, and morphology. The nanoparticles exhibited a spherical shape with a size range of 50-100 nm, making them suitable for various biomedical applications.[26,27]

The antibacterial activity tests revealed that the CuO NPs synthesised in this study possess potent antibacterial properties against *Escherichia coli* and *Staphylococcus aureus*, two common bacterial pathogens. The observed dose-dependent inhibition suggests that these nanoparticles can be effectively utilised in antimicrobial treatments, with potential applications in healthcare, pharmaceuticals, and environmental protection.[28]

The study's findings underscore the importance of green synthesis approaches in nanotechnology for creating effective and environmentally sustainable solutions, with Vitex negundo Linn. enabling CuO nanoparticle production without toxic chemicals while enhancing biological activity, and future work focusing on expanding their applications through targeted research.

ACKNOWLEDGEMENT

We thank Dr. Deepak Nallaswamy, Director of Academics, Saveetha Institute of Medical and Technical Sciences, for initiating digital records in our institution, which facilitated data search for this study.

ABBREVIATIONS

CuO NPs: Copper Oxide Nanoparticles; **FTIR:** Fourier Transform Infrared Spectroscopy; **XRD:** X-ray Diffraction; **SEM:** Scanning Electron Microscopy; **EDX:** Energy Dispersive X-ray Spectroscopy; **DLS:** Dynamic Light Scattering; **UV-vis:** Ultraviolet-visible Spectroscopy.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Pravallika Kakada, Abilasha R, and Pratibha Ramani contributed to the study's conception, design, data acquisition, analysis, and interpretation. They drafted or revised the manuscript and approved the final version. All authors are accountable for the accuracy and integrity of the work.

SUMMARY

This study explores the green synthesis of Copper Oxide Nanoparticles (CuO NPs) using *Vitex negundo* Linn leaf extract as a natural reducing and stabilizing agent. Given the rising concerns over conventional synthesis methods involving toxic chemicals and high energy consumption, this eco-friendly approach offers a sustainable alternative. The synthesized CuO NPs were characterized using various analytical techniques, including UV-visible Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDX), and Dynamic Light Scattering (DLS). These techniques confirmed the successful formation, crystalline nature, and morphological properties of the nanoparticles. The antibacterial efficacy of CuO NPs was evaluated against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion method, demonstrating significant inhibitory effects. The findings suggest that the synthesized CuO NPs possess potent antimicrobial properties, making them promising candidates for biomedical and pharmaceutical applications. This study highlights an environmentally sustainable method for nanoparticle synthesis while maintaining their biological effectiveness.

REFERENCES

1. Khan L, Saccd K, Khan I. Nanoparticles: properties, applications and toxicities. Arab J Chem. 2019; 12(7): 908-31. doi: 10.1016/j.arabjc.2017.05.011, doi: 10.1016/j.arabjc.2017.05.011(2019).
2. Nzilu DM, Madivoli ES, Makhanu DS, Wanakai SI, Kiprono GK, Kareru PG. Green synthesis of copper oxide nanoparticles and its efficiency in degradation of

rifampicin antibiotic. Sci Rep. 2023; 13(1): 14030. doi: 10.1038/s41598-023-41119-z , PMID 37640783.

3. Nasrollahzadeh M, Sajjadi M, Sajadi SM, Issaabadi Z. Green nanotechnology. Interface Sci Technol. 2019; 28: 145-98. doi: 10.1016/B978-0-12-813586-0.00005-5.
4. Ying S, Guan Z, Ofoegbu PC, Clubb P, Rico C, He F, *et al.* Green synthesis of nanoparticles: current developments and limitations. Environ Technol Innov. 2022; 26: 102336. doi: 10.1016/j.eti.2022.102336.
5. Labaran AN, Zango ZU, Tailor G, Alsadig A, Usman F, Mukhtar MT, *et al.* Biosynthesis of copper nanoparticles using *Alstonia scholaris* leaves and its antimicrobial studies. Sci Rep. 2024; 14(1): 5589. doi: 10.1038/s41598-024-56052-y, PMID 38453990.
6. Vijayaram S, *et al.* Applications of green synthesized metal nanoparticles-a review. Biol Trace Elem Res. 2023. doi:10.1007/s12011-023-03645-9. doi: 10.1007/s12011-023-03645-9(2023).
7. Aswathi VP, Meera S, Maria CG, Nidhin M. Green synthesis of nanoparticles from biodegradable waste extracts and their applications: a critical review. Nanotechnol Environ Eng. 2023; 8(2): 377-97, doi: 10.1007/s41204-022-00276-8.
8. Ahmed SF, Mofijur M, Rafa N, Chowdhury AT, Chowdhury S, Nahrin M, *et al.* Green approaches in synthesising nanomaterials for environmental nanobioremediation: technological advancements, applications, benefits and challenges. Environ Res. 2022; 204(A):111967. doi: 10.1016/j.envres.2021.111967, PMID 34450159.
9. Nath D, Banerjee P. Green nanotechnology-A new hope for medical biology. Environ Toxicol Pharmacol. 2013; 36(3): 997-1014. doi: 10.1016/j.etap.2013.09.002, PMID 24095717.
10. Rajeshkumar S, Nandhini NT, Manjunath K, Sivaperumal P, Krishna Prasad G, Alotaibi SS, *et al.* Environment-friendly synthesis of copper oxide nanoparticles and its antioxidant, antibacterial activities using seaweed (*Sargassum longifolium*) extract. J Mol Struct. 2021; 1242: 130724. doi: 10.1016/j.molstruc.2021.130724.
11. Dharmasiri MG, Jayakody JR, Galhena G, Liyanage SS, Ratnasooriya WD. Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*. J Ethnopharmacol. 2003; 87(2-3): 199-206. doi: 10.1016/s0378-8741(03)00159-4, PMID 12860308.
12. Priya M, Venkatesan R, Deepa S, Sana SS, Arumugam S, Karami AM, *et al.* Green synthesis, characterization, antibacterial, and antifungal activity of copper oxide nanoparticles derived from *Morinda citrifolia* leaf extract. Sci Rep. 2023; 13(1): 18838. doi: 10.1038/s41598-023-46002-5, PMID 37914791.
13. Nyamweya B, Rukshala D, Fernando N, de Silva R, Premawansa S, Handunnetti S. Cardioprotective effects of *Vitex negundo*: a review of bioactive extracts and compounds. J Evid Based Integr Med. 2023; 28: 2515690X231176622. doi: 10.1177/2515690X231176622, PMID 37279951.
14. Ali S, Sudha KG, Karunakaran G, Kowsalya M, Kolesnikov E, Gorshenkov MV, *et al.* Novel *Leea grandifolia* leaves-mediated synthesis of ZnO nanorods for photocatalytic and anticancer applications. Appl Organomet Chem. 2021; 35(7). doi: 10.1002/aoc.6239.
15. Aabideen Z, Ahmad I, Ali S, *et al.* Antioxidant and anticancer activities of *Taraxacum officinale* (Dandelion). Antioxidants. 2020; 9(9): 909.
16. Ali S, Govindaraj Sudha KG, Karunakaran G, Kowsalya M, Kolesnikov E, Gorshenkov MV, *et al.* Anticancer and photocatalytic activities of zinc oxide nanorods synthesized from *Manilkara littoralis* leaf extract. Mater Chem Phys. 2022; 277: 125541. doi: 10.1016/j.matchemphys.2021.125541.
17. Rajeshkumar S, Sekar D, Ezhilarasan D, Lakshmi T. The role of diverse nanoparticles in oxidative stress: *in vitro* and *in vivo* studies. In: Maurya PK, Dua K, editors. Role of oxidative stress in pathophysiology of diseases. Singapore: Springer Singapore; 2020. p. 27-48. doi: 10.1007/978-981-15-1568-2_3.
18. Ezhilarasan D, Rajeshkumar S, Lakshmi T. The role of oxidative stress in chronic liver diseases. In: Maurya PK, Dua K, editors. Role of oxidative stress in pathophysiology of diseases. Singapore: Springer Singapore; 2020. p. 13-25. doi: 10.1007/978-981-15-1568-2_2.
19. Bala N, Saha S, Chakraborty M, Maiti M, Das S, Basu R, *et al.* Green synthesis of zinc oxide nanoparticles using *Hibiscus subdariffa* leaf extract: effect of temperature on synthesis, antibacterial activity, and anti-diabetic activity. RSC Adv. 2015; 5(7): 4993-5003. doi: 10.1039/C4RA12784F.
20. Barma MD, Raj SS, Indiran MA, Rajeshkumar S. Synthesis of Triphala-incorporated zinc oxide nanoparticles and assessment of its antimicrobial activity against oral pathogens: an *in vitro* study. Biosci Biotechnol Res Commun. 2020; 13(7): 74-8.
21. Rajakumar G, Thiruvengadam M, Mydhili G, Gomathi T, Chung IM. Green approach for synthesis of zinc oxide nanoparticles from *Andrographis paniculata* leaf extract and evaluation of their antioxidant, anti-diabetic, and anti-inflammatory activities. Bioprocess Biosyst Eng. 2018; 41(1): 21-30. doi: 10.1007/s00449-017-1840-9, PMID 28916855.
22. Lin FL, Wu SJ, Lee SC, Ng LT. Antioxidant, anti-oedema, and analgesic activities of *Andrographis paniculata* extracts and their active constituent andrographolide. Phytother Res. 2009; 23(7): 958-64. doi: 10.1002/ptr.2701, PMID 19142986.
23. Rajeshkumar S, Malarkodi C, Vanaja M, Annadurai G. Anticancer and enhanced antimicrobial activity of biosynthesized silver nanoparticles against clinical pathogens. J Mol Struct. 2016; 1116: 165-73. doi: 10.1016/j.molstruc.2016.03.044.
24. Ramsridhar S, Rajkumar C, Veeraraghavan VP, Francis AP, Balasubramaniam M, Bharkavi I. From cell lines to animal models: "plant- derived chemotherapeutics unlocking new frontiers against oral squamous cell carcinoma"-a comprehensive systematic review. Discov Oncol. 2025;16(1):340. doi: 10.1007/s12672-025-02057-6. PMID: 40097871; PMCID: PMC11914638.
25. Senthil R. Bevacizumab-Conjugated Curcumin Nanoparticles Promote Cytotoxicity and Apoptosis in Human Malignant Oral Keratinocytes *In Vitro*. J Oral Maxillofac Surg. 2025:S0278-2391(25)00319-2. doi: 10.1016/j.joms.2025.05.014. Epub ahead of print. PMID: 40514017.
26. Gupta M D, Gosala R, Ramadoss R. Enhanced surface properties and wettability of zirconia-hydroxyapatite-poly(acrylic acid) nanocomposites for dental applications. J Oral Biol Craniofac Res. 2025;15(5):1097-1102. doi: 10.1016/j.jobcr.2025.07.014. Epub 2025 Jul 24. PMID: 40746750; PMCID: PMC12311598.
27. Nitya Krishnasamy, Ramya Ramadoss,Functional evaluation of Desmostachya bipinnata-enhanced silver nanoparticles in mucoadhesive patches for enhanced oral drug delivery and tissue regeneration,Next Materials,Volume 8, 2025, 100821, ISSN 2949-8228, https://doi.org/10.1016/j.nxmate.2025.100821.
28. Sundar S, Pandiar D, Yuwanati M. Biological copper levels in oral squamous cell carcinoma: A meta-analysis with insights into disease progression and prognosis. J Oral Biol Craniofac Res. 2025;15(5):1010-1020. doi: 10.1016/j.jobcr.2025.07.003. Epub 2025 Jul 15. PMID: 40697752; PMCID: PMC12281403.

Cite this article: Kakada P, Abilasha R, Ramani P. Green Synthesis and Characterization of Copper Oxide Nanoparticles Using *Vitex negundo* Linn.: An *in vitro* study. Pharmacog Res. 2025;17(4):1235-40.