Copper Oxide-Based Antibacterial Compound Synthesis and Characterization for Biomedical Applications

Hanish Manoj1, S.Roshini1,a)

1HANISH Health and Wellness, Thanjavur, Tamilnadu, India

Corresponding Author: a)[roshinisureshkumar2006@gmail.com](mailto:roshinisureshkumar2006@gmail.com)

**Abstract:** Copper oxide (Cu2O), a versatile metal oxide, exhibits unique chemical and physical properties at the nanoscale, making it a promising material for biomedical applications. Its antimicrobial activity, particularly against antibiotic-resistant bacteria, has garnered significant attention.This study aims to synthesize and characterize Cu2O nanoparticles and evaluate their antibacterial properties, biocompatibility, and potential applications in wound healing, tissue engineering, and dentistry.CuO nanoparticles were synthesized using a chemical precipitation method, followed by calcination at 800°C to obtain a monoclinic crystal structure. Characterization was performed using XRD, HR-TEM, and SAED techniques. Antibacterial activity was assessed against *E. coli* and *Enterococcus* through inhibition zone measurements.XRD analysis shows the single-phase cubic Cu2O with (110), (111) and (220) diffraction peaks [JCPDS file No. 05-0667]. FT-IR shows the presence of surface OH, and the bond between Cu–O it confirms formation Cu2O. HRTEM images show the regular particle shape with size of 50 nm. Synthesized compound shows significant antibacterial activity against *E. coli* and *Enterococcus.*Cu2O nanoparticles hold potential in biomedical applications due to their antibacterial properties. However, challenges such as cytotoxicity and stability need to be addressed. Advancements in synthesis and controlled release methods improve their safety and efficiency, making them a promising solution for infection control and regenerative medicine.

**Keywords** - copper oxide nanoparticles, Cytotoxicity, Anti microbial activity

# Introduction

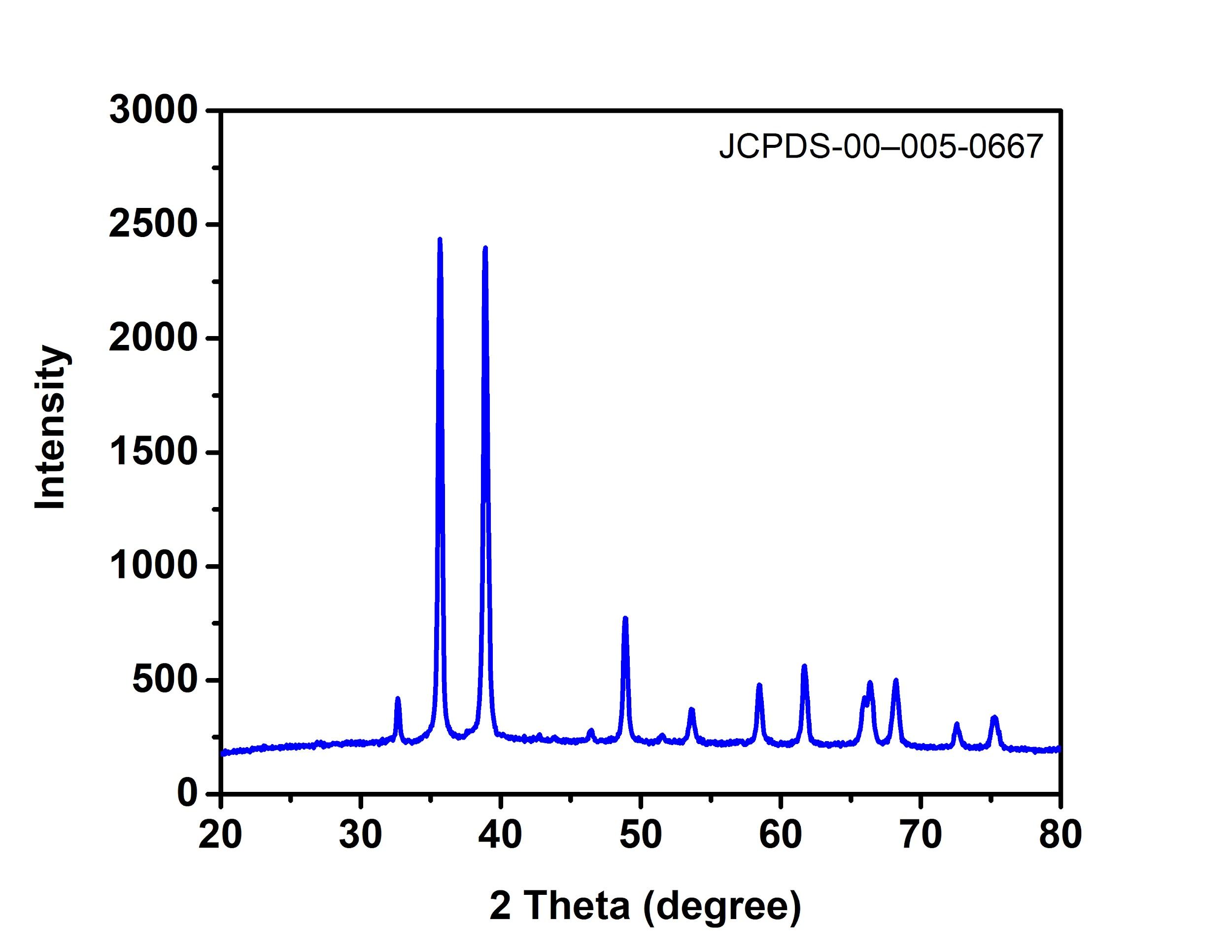
Copper oxide (CuO), one of the well known metal oxide available today, has been attracting significant attention because of its various applications in different fields. This compound has a very distinctive chemical and physical properties, which is due to their shape, structure, size etc. When confined to nano scaled particles, there are notable changes seen in copper oxide and other metal oxides such as a distinct change in surface energy [(Harsha & Subramanian, 2022)](https://paperpile.com/c/9FuCD9/eVrex)[(Deepika et al., 2022)](https://paperpile.com/c/9FuCD9/YhJ0Z)[(Solanki et al., 2022)](https://paperpile.com/c/9FuCD9/Vp8Gw). These changes result in inducing new chemical and physical properties, which cannot be seen when in its large scale structure[(Jillani et al., 2018)](https://paperpile.com/c/9FuCD9/bjCIX) A systemized and strategic synthesis in these types of nanoparticles involving change in size, shape etc paves way for various applications in the fields of nanotechnology and medicine[(Chidambaram et al., 2022)](https://paperpile.com/c/9FuCD9/21t7M).[(Ajay, Sasikala, et al., 2022)](https://paperpile.com/c/9FuCD9/hJvT2).A very simple and flexible method of synthesizing copper oxide nanoparticles is by hydrothermal method, in which the precursor material is copper sulfate (CuSO4.5H2O). CuO nanoparticle is about 23nm, and has a monoclinic crystal structure Hydrothermal process has many pros, one of them mainly focusing on establishing uniform size and shape of CuO nanoparticles [(*(PDF) Syntheses of Copper Oxide Nanoparticle by Hydrothermal Method and Its Structural & Surface Morphological Studies*, n.d.)](https://paperpile.com/c/9FuCD9/rS5F) In today's era, under medical and scientific fields, antibacterial materials and its systems are growing in importance among hospitals and other various healthcare environments, scientific laboratories, in major industrial factories etc. The main rationale behind the development of antibacterial materials and systems is for the prevention, spread and transfer of harmful bacterias, and also to disable their harmful activity [(*Website*, n.d.-a)](https://paperpile.com/c/9FuCD9/vdti). The contamination of microorganisms can severely affect the quality of scientific and medical instruments, which is a huge disadvantage, but, as we have antibacterial materials introduced in various fields, it helps us to encapsulate these problems [(González-Henríquez et al., 2019)](https://paperpile.com/c/9FuCD9/DTKv). Commonly, the nanoparticles' antimicrobial activity varies on the pathogens’ type and composition. They also show positive benefits such as the reduction in toxicity, delivering drugs to a distinct target [(González-Henríquez et al., 2019)](https://paperpile.com/c/9FuCD9/DTKv). Metallic copper is a very important antibacterial material, renowned for its easy synthesis and high effect against the antibiotic resistance shown by bacterias[(Ajay, Rakshagan, et al., 2022)](https://paperpile.com/c/9FuCD9/bYg9S). Copper oxide, under the influence of thermal energy, was seen to be very effective in contact killing, gaining the upper hand against pure copper [(Hans et al., 2013)](https://paperpile.com/c/9FuCD9/73zt). Copper oxide is also considered as one among the smart transitional metal oxides, having a narrow band gap (-2.0eV), and when taken at nanoscale level, shows efficient electrochemical activity, high redox potential etc [(*Website*, n.d.-b)](https://paperpile.com/c/9FuCD9/kuyR) . Due to these features on and off the paper, copper oxide nanoparticles show promises in area regarding to catalysis [(*Website*, n.d.-b)](https://paperpile.com/c/9FuCD9/kuyR), also shows rate of effectiveness in sensors/biosensors [(*Website*, n.d.-b)](https://paperpile.com/c/9FuCD9/kuyR). Nanoparticles of CuO have a significant impact in pharmacological activities, specifically targeting antitumor therapy [(10)](https://www.researchgate.net/publication/331080663_Synthesis_and_Biomedical_Applications_of_Copper_Oxide_Nanoparticles_An_Expanding_Horizon). The use of copper being utilized in dentistry has also grown in recent years. Many types of materials used in dental restorations contain few amounts of copper in them. For instance, high copper amalgam contains copper, which is present to mainly prevent corrosiveness after restoration [(Tiwari & Jain, 2023)](https://paperpile.com/c/9FuCD9/Seata)[(Graf et al., 2023)](https://paperpile.com/c/9FuCD9/9h6iH). It is also used in fixing implants and in fixing partial denture attachments [(Ma et al., 2022)](https://paperpile.com/c/9FuCD9/vgkN). Copper metal plays a vital role in bone regeneration, or popularly called angiogenesis[(Ajay, Suma, et al., 2022)](https://paperpile.com/c/9FuCD9/NwOLo) [(Katyal et al., 2021)](https://paperpile.com/c/9FuCD9/wOlEs). Copper nanoparticles promote bone regeneration, where few amounts of CuO nanoparticles were added to PLGA/CuTCP and were electrospun and resulted in nanocomposite. CuO also reduces bacterial adhesion with high efficiency [(Ribatti et al., 2021)](https://paperpile.com/c/9FuCD9/k849). Under biomedical fields, copper nanoparticles are widely regarded as efficient bio sensors, sensing DNA, cholesterol levels, lactate level etc, which can be used for diagnostic purposes and early treatment of disorders. Copper nanoparticles play a vital role in terms of antitumor agents, for the treatment of prostate cancer, kidney cancer etc, and are unnoticeably necessary. They are also helpful as effective nano carriers [(Naz et al., 2023)](https://paperpile.com/c/9FuCD9/w9iH). The major reason for this study is to extract all possible/ beneficial uses of CuO based antibacterial materials, and use it to increase the effectiveness of treatment plan, diagnostics etc [(Jabin et al., 2021)](https://paperpile.com/c/9FuCD9/w1pVC)[(Balaji Ganesh S & Sugumar, 2021)](https://paperpile.com/c/9FuCD9/WEHAh) [(Govindaraj & Dinesh, 2021)](https://paperpile.com/c/9FuCD9/WxxUr)

# Materials and Methods

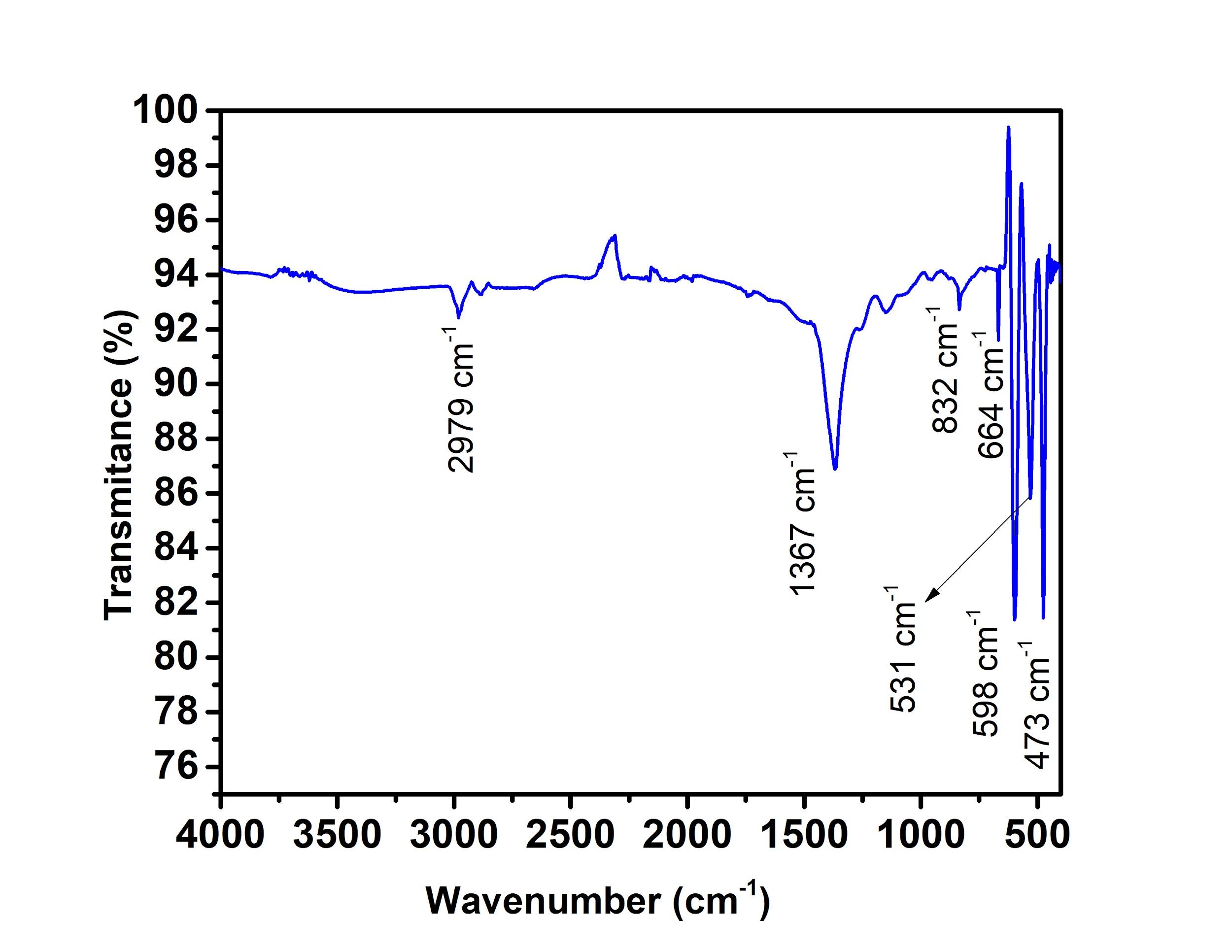
The synthesis of pure copper oxide (CuO) nanoparticles was carried out using a chemical precipitation method. The procedure began with the preparation of a copper acetoacetate solution. Specifically, 0.5 grams of copper acetoacetate (Cu(CH3COO)2) were dissolved in 30 milliliters of boiling ethanol. This solution was stirred continuously to ensure complete dissolution of the copper acetoacetate. Once fully dissolved, the solution was then cooled to a temperature of 4°C. In a separate beaker, a solution of sodium hydroxide (NaOH) was prepared. For this, 10 millimoles of NaOH were dissolved in ethanol. This NaOH solution was then added to the copper acetoacetate solution. The addition was carried out dropwise using a burette to control the rate of addition, ensuring a gradual and controlled mixing process. The beaker containing the combined solutions was placed on a magnetic stirrer and stirred continuously for 24 hours to allow for the complete reaction and formation of the copper oxide nanoparticles. After the 24-hour stirring period, the resulting mixture was subjected to centrifugation. The centrifugation process was performed at a speed of 3000 rpm for 10 minutes. This step was crucial for separating the solid nanoparticles from the liquid phase. Following centrifugation, the solid nanoparticles were collected and washed thoroughly with distilled water, ethanol, and acetoacetate to remove any impurities and unreacted precursors. The washed nanoparticles were obtained in a moist form. To remove the moisture, the sample was dried in a hot oven. The drying process was conducted at a temperature of 80°C for a duration of 10 hours. Once dried, the next step involved calcination of the dried nanoparticles. Calcination was performed in a muffle furnace at a temperature of 800°C for 2 hours. This high-temperature treatment was essential for obtaining the pure CuO nanoparticles in a well-defined powdered form.

# Results

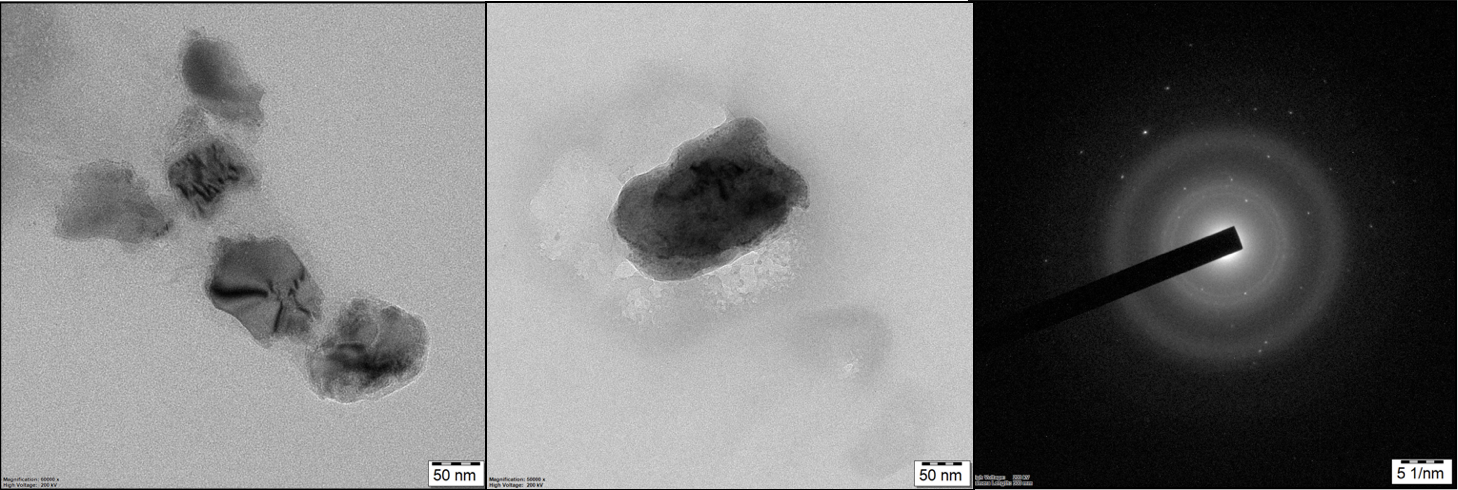
The copper oxide electrode films formed with 20% and 30% oxygen flow rates during reactive magnetron sputtering of a pure copper target are depicted in Figure 1's X-ray diffraction patterns. Strong (111) and (200) diffraction peaks for Cu and a weak (111) diffraction peak for Cu2O were observed in the film formed at a 10% oxygen flow rate (See Supplementary Information Fig. S1). The deposited film is then transformed into single-phase cubic Cu2O with (110), (111), and (220) diffraction peaks when the oxygen flow rate rises from 10% to 20% [JCPDS file No. 05-0667]. Cu2O is transformed into single-phase monoclinic CuO with a (−111) orientation in the films deposited at 30% oxygen flow rates, displaying a crystalline structure [JCPDS file No. 45–0937]. The monoclinic CuO (−111) phase does not alter as the oxygen flow rate rises above 30% (See Supplementary Information, Fig. S1). The XRD data validate that the oxygen flow rate has a significant impact on the film's crystalline structure. Subsequently, Cu2O and CuO will be used to refer to copper oxide films that were formed at 20% and 30% oxygen flow rates, respectively.



**Fig 1** X Ray Diffraction Pattern of Cu2O nanoparticles

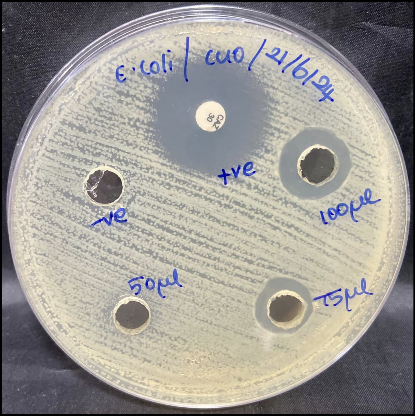


**Fig 2** FTIR Spectrum of Cu2O nanoparticle



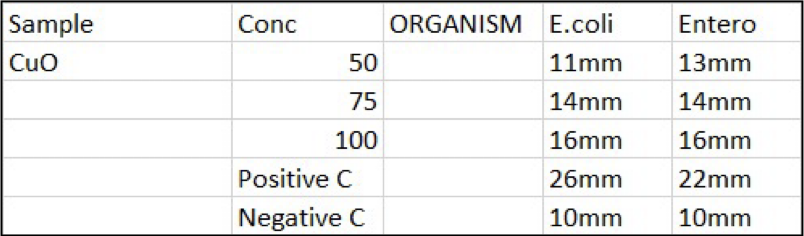
1. (b) (c)

**Fig 3** (a) (b) (c) shows us the HRTEM images of the obtained copper oxide nanoparticle crystals SAED pattern of copper oxide nanoparticles.



1. (b)

**Figure 4** (a)(b) shows the antibacterial and anti-microbial effects of CuO nanoparticles against E.coli bacteria and Enterococcus bacteria.



**Fig 5** Zone of inhibition values by the action of copperoxide nanoparticles at various concentrations.

# Discussion

The use of copper oxide nanoparticles in the medical field has proven to be advantageous due to its antibacterial properties, against various microorganisms [(Woźniak-Budych et al., 2023)](https://paperpile.com/c/9FuCD9/t5Gu). All the microbes used in this research showed us the they were in fact sensitive to the action of copper oxide nanoparticles, and the effect of nanoparticles through its antibacterial and antifungal properties were also tested, which are generally associated with the copper oxide nanoparticles internalization inside the bacterial cell, where ions of copper gets released which thereby generates oxidative stress through the production of reactive oxygen species (ROS), which damages the bacteria’s DNA [(Raffi et al., 2010)](https://paperpile.com/c/9FuCD9/llFS). The inhibition of E. Coli’s growth was also looked into by the action of copper oxide nanoparticles [(Sabarathinam & Madhulaxmi, 2021)](https://paperpile.com/c/9FuCD9/wGWlT)[(Sushanthi et al., 2021)](https://paperpile.com/c/9FuCD9/72LXX)[(Harsha et al., 2022)](https://paperpile.com/c/9FuCD9/gtA4P). It can be seen that the increase in concentration of copper oxide nanoparticles has more action on the microorganism compared to the initial standard concentration . [(Bocarando-Chacón et al., 2020)](https://paperpile.com/c/9FuCD9/IjX6)It was also identified that copper oxide nanoparticles can also be effective in cutaneous wound healing because of its involvement in blood clot formation, its anti-inflammatory properties, which overall helps is the formation of new epithelium over the wound [(Neha et al., 2021)](https://paperpile.com/c/9FuCD9/3aDks)[(Maliael et al., 2021)](https://paperpile.com/c/9FuCD9/Sp63h)[(Lakshmi, 2021)](https://paperpile.com/c/9FuCD9/S45sS). Based on the action of copper oxide nanoparticles effects on both gram negative and gram positive bacteria, with regards to their antibacterial and antifungal properties, the nanoparticles, preferably of size 20 nm, can be used to provide disinfection to the wound and also catalyzes the wound healing process[(Dharman 2021)](https://paperpile.com/c/9FuCD9/gMm13). It also stimulates the increased production of fibrocytes, which is required for facilitating the wound counter action process, as fibrocytes are one of the sources for the production of collagen, which helps the wound healing process by providing tensile strength, in the wound bed [(Hans et al., 2013)](https://paperpile.com/c/9FuCD9/73zt). The process of angiogenesis (process of formation of new blood vessels) in wound healing is seen to be accelerated when copper oxide nanoparticles are introduced at the particular site of interest. In previous researches done based on this topic, the amintotransferase (ALT) and adenosine triphosphate (ALP) in different blood samples did not show and changes, while being influenced with copper oxide nanoparticles, which proves the low cytotoxic activity of copper oxide nanoparticles [(“Copper Nanoparticles Promote Rapid Wound Healing in Acute Full Thickness Defect via Acceleration of Skin Cell Migration, Proliferation, and Neovascularization,” 2019)](https://paperpile.com/c/9FuCD9/tvnH). The diverse properties of copper oxide nanoparticles opens up its applications to various other medical fields such as dentistry [(“Copper Nanoparticles Promote Rapid Wound Healing in Acute Full Thickness Defect via Acceleration of Skin Cell Migration, Proliferation, and Neovascularization,” 2019)](https://paperpile.com/c/9FuCD9/tvnH). The nanoparticles can be altered in such a way that it can be used in dental adhesives, implant coatings, filling materials, denture materials etc . PMMA, or poly methacrylate, is one of the most used biological materials in the field of medicine as a type of bone cements [(“Bone Cement,” 2013)](https://paperpile.com/c/9FuCD9/6vFB). This PMMA bone cement, when loaded with antibiotic materials, can be used in a continuous treatment post surgery, but most antibiotic materials used previously have not been very effective, when seen in the long term run. But the use of copper, along with PMMA has proved to improve the effects of the antibacterial activity post surgery in patients, the main reason being the release of free copper ions and by also releasing reactive oxygen species (ROS) which has the ability to take down or damage the cell membrane of the foreign cells [(“Copper-Polymer Nanocomposites: An Excellent and Cost-Effective Biocide for Use on Antibacterial Surfaces,” 2016)](https://paperpile.com/c/9FuCD9/MXne). The use of hydrogels in post wound surgeries is one of the most common insoluble polymeric materials used, because of its water retention property, which is used to hydrate the wound [(Kopeček & Yang, 2007)](https://paperpile.com/c/9FuCD9/o225). There is one limitation in using alginate saline gel, which is not having any biological activity/antibacterial properties. The introduction of copper nanoparticles induced alginate saline gel, as microbeads, during post surgical treatment has shown to decrease the rate of scar formation and prevention of infections [(*Radware Bot Manager Captcha*, n.d.)](https://paperpile.com/c/9FuCD9/uoLL). Scaffolds biomaterials, a very useful method in tissue engineering can be used for the treatment of bone infections, defects, providing framework for new bone to grow in defected areas and more.the main advantage of this technique is that the material I used will gradually degrade, in time, and will be replaced by new tissue(Chehelgerdi et al., 2023). In this treatment, materials containing antibacterial properties are also added to catalyze the procedure and also to prevent infections. Copper, and its ions play a vital role in bone growth and regeneration, and low levels of copper can hinder the process and also cause abnormalities in the affected area post treatment. The use of scaffolds doped with copper oxide nanoparticles has been found to be the best combination for tissue engineering, mainly for bone tissue. A research study done based on this shows copper containing bioactive glass scaffold was prepared with interconnected macropores and a mesoporous structure was obtained. This particular combination of copper oxide nanoparticles and the scaffolds was seen to promote the process of angiogenesis, and was also seen to continuously release copper ions which helped in the inhibition of bacterial growth. This was also found to be biocompatible to the tissue [(“Copper-Containing Mesoporous Bioactive Glass Scaffolds with Multifunctional Properties of Angiogenesis Capacity, Osteostimulation and Antibacterial Activity,” 2013)](https://paperpile.com/c/9FuCD9/jSAG)

## Limitations

Copper oxide-based antibacterial compounds for biomedical applications have various drawbacks. One significant difficulty is their possible toxicity to human cells, necessitating a careful balance between antibacterial activity and biocompatibility. Stability difficulties emerge because copper oxide nanoparticles can change chemically over time, lowering their effectiveness and affecting their biocompatibility. Controlled and sustained release of copper ions is difficult to achieve since quick release might be harmful, but slow release may not offer adequate antibacterial effect (Saadh et al., 2024). The ability of these nanoparticles to agglomerate decreases their surface area and antibacterial effectiveness, necessitating effective dispersion strategies. The regulatory hurdles are high, with comprehensive safety and efficacy studies required for approval. High production costs are another impediment, emphasizing the necessity for cost-effective synthesis techniques. The environmental impact of creating and disposing of these nanoparticles is poorly understood, necessitating extensive ecological risk evaluations. To avoid unfavorable interactions, biomedical device materials must be compatible with one another. There are few long-term research on the effects of copper oxide exposure on human health, raising worries about potential chronic impacts. Finally, the absence of established techniques for synthesis, characterisation, and testing produces variable results and impedes comparison investigations.

# Conclusion

To summarize, advances in copper oxide-based antibacterial materials for biomedical applications represent a very promising future in medical science. Recent investigations show that these materials have tremendous potential for fighting a wide spectrum of bacterial illnesses, providing a unique answer to the rising problem of antibiotic resistance. Copper oxide nanoparticles have been proven in studies to have excellent antimicrobial capabilities, which can be used to develop more effective and long-lasting antibacterial coatings for medical devices and implants. Articles highlight successful attempts to improve the biocompatibility of certain materials, allowing them to be utilized safely in a variety of biomedical applications. Synthesis process advancements have addressed past problems about stability and aggregation, resulting in more consistent and effective antibacterial efficacy. Furthermore, advances in controlled release methods have enabled persistent antibacterial action with low toxicity, improving the overall safety profile of these compounds. Economic and environmental concerns are also being addressed, with recent advancements emphasizing cost-effective manufacturing techniques and sustainable practices. Long-term research have begun to provide light on the biocompatibility and safety of copper oxide nanoparticles, laying a solid foundation for their future use in healthcare. Thus, ongoing research and collaboration in this field, aided by standardized protocols, will help to unlock the full potential of copper oxide-based antibacterial materials, revolutionizing infection control in biomedical applications and contributing to better patient outcomes and healthcare solutions.

# References

1. [Ajay, R., Rakshagan, V., Queenalice, A., Vinothkumar, S., Ravivarman, C., & Saravanadinesh, P. (2022). Effect of triazine comonomer substitution on the structure and glass transition temperature of monomethacrylate-based resin polymer: An in vitro study. *The Journal of Contemporary Dental Practice*, *23*(2), 202–207. https://doi.org/](http://paperpile.com/b/9FuCD9/bYg9S)[10.5005/jp-journals-10024-3260](http://dx.doi.org/10.5005/jp-journals-10024-3260)
2. [Ajay, R., Sasikala, R., Rakshagan, V., Raghunathan, J., LalithaManohari, V., & Baburajan, K. (2022). Evaluation of cytocompatibility of thermopolymerized denture base copolymer containing a novel ring-opening oxaspiro comonomer. *World Journal of Dentistry*, *13*(2), 127–132. https://doi.org/](http://paperpile.com/b/9FuCD9/hJvT2)[10.5005/jp-journals-10015-1901](http://dx.doi.org/10.5005/jp-journals-10015-1901)
3. [Ajay, R., Suma, K., Sasikala, R., Rakshagan, V., Baburajan, K., & Kalarani, G. (2022). Evaluation of linear dimensional stability of monomethacrylate-based dental polymer containing a novel tricyclic diacrylate cross-linker using a novel surface-level index technique. *World Journal of Dentistry*, *13*(6), 568–573. https://doi.org/](http://paperpile.com/b/9FuCD9/NwOLo)[10.5005/jp-journals-10015-2106](http://dx.doi.org/10.5005/jp-journals-10015-2106)
4. [Balaji Ganesh S, & Sugumar, K. (2021). Internet of Things—A novel innovation in dentistry. *Journal of Advanced Oral Research*, *12*(1), 42–48. https://doi.org/](http://paperpile.com/b/9FuCD9/WEHAh)[10.1177/2320206820980248](http://dx.doi.org/10.1177/2320206820980248)
5. [Bocarando-Chacón, J., Vargas-Vazquez, D., Martinez-Suarez, F., Flores-Juárez, C., & Cortez-Valadez, M. (2020). Surface-enhanced Raman scattering and antibacterial properties from copper nanoparticles obtained by green chemistry. *Applied Physics A*, *126*(7), 1–9. https://doi.org/](http://paperpile.com/b/9FuCD9/IjX6)[10.1007/s00339-020-03704-1](http://dx.doi.org/10.1007/s00339-020-03704-1)
6. [Bone cement. (2013). *Journal of Clinical Orthopaedics and Trauma*, *4*(4), 157–163. https://doi.org/](http://paperpile.com/b/9FuCD9/6vFB)[10.1016/j.jcot.2013.11.005](http://dx.doi.org/10.1016/j.jcot.2013.11.005)
7. Chehelgerdi M., Chehelgerdi, M., Allela, O. Q. B., Pecho, R. D. C., Jayasankar, N., Rao, D. P. & Akhavan-Sigari, R. (2023). Progressing nanotechnology to improve targeted cancer treatment: overcoming hurdles in its clinical implementation. Molecular cancer, 22(1), 169.
8. [Chidambaram, S. R., George, A. M., Muralidharan, N. P., Prasanna Arvind, T. R., Subramanian, A., & Rahaman, F. (2022). Current overview for chemical disinfection of dental impressions and models based on its criteria of usage: A microbiological study. *Indian Journal of Dental Research : Official Publication of Indian Society for Dental Research*, *33*(1), 30–36. https://doi.org/](http://paperpile.com/b/9FuCD9/21t7M)[10.4103/ijdr.IJDR\_623\_20](http://dx.doi.org/10.4103/ijdr.IJDR_623_20)
9. [Copper-containing mesoporous bioactive glass scaffolds with multifunctional properties of angiogenesis capacity, osteostimulation and antibacterial activity. (2013). *Biomaterials*, *34*(2), 422–433. https://doi.org/](http://paperpile.com/b/9FuCD9/jSAG)[10.1016/j.biomaterials.2012.09.066](http://dx.doi.org/10.1016/j.biomaterials.2012.09.066)
10. [Copper nanoparticles promote rapid wound healing in acute full thickness defect via acceleration of skin cell migration, proliferation, and neovascularization. (2019). *Biochemical and Biophysical Research Communications*, *517*(4), 684–690. https://doi.org/](http://paperpile.com/b/9FuCD9/tvnH)[10.1016/j.bbrc.2019.07.110](http://dx.doi.org/10.1016/j.bbrc.2019.07.110)
11. [Copper-polymer nanocomposites: An excellent and cost-effective biocide for use on antibacterial surfaces. (2016). *Materials Science and Engineering: C*, *69*, 1391–1409. https://doi.org/](http://paperpile.com/b/9FuCD9/MXne)[10.1016/j.msec.2016.08.041](http://dx.doi.org/10.1016/j.msec.2016.08.041)
12. [Deepika, B. A., Ramamurthy, J., Girija, S., & Jayakumar, N. D. (2022). Evaluation of the antimicrobial effect of Ocimum sanctum L. oral gel against anaerobic oral microbes: An in vitro study. *World Journal of Dentistry*, *13*(S1), S23–S27. https://doi.org/](http://paperpile.com/b/9FuCD9/YhJ0Z)[10.5005/jp-journals-10015-2140](http://dx.doi.org/10.5005/jp-journals-10015-2140)
13. [Dharman, S (2021). Ecofriendly Synthesis, Characterisation and Antibacterial Activity Of Curcumin Mediated Silver Nanoparticles. *International Journal of Dentistry and Oral Science*, 2314–2318. https://doi.org/](http://paperpile.com/b/9FuCD9/gMm13)[10.19070/2377-8075-21000457](http://dx.doi.org/10.19070/2377-8075-21000457)
14. [González-Henríquez, C. M., Sarabia-Vallejos, M. A., & Rodríguez, H. J. (2019). Antimicrobial Polymers for Additive Manufacturing. *International Journal of Molecular Sciences*, *20*(5). https://doi.org/](http://paperpile.com/b/9FuCD9/DTKv)[10.3390/ijms20051210](http://dx.doi.org/10.3390/ijms20051210)
15. [Govindaraj, A., & Dinesh, S. P. S. (2021). Effect of chlorhexidine varnish and fluoride varnish on White Spot Lesions in orthodontic patients- a systematic review. *The Open Dentistry Journal*, *15*(1), 151–159. https://doi.org/](http://paperpile.com/b/9FuCD9/WxxUr)[10.2174/1874210602115010151](http://dx.doi.org/10.2174/1874210602115010151)
16. [Graf, S., Thakkar, D., Hansa, I., Pandian, S. M., & Adel, S. M. (2023). 3D metal printing in orthodontics current trends, biomaterials, workflows and clinical implications. *Seminars in Orthodontics*. https://doi.org/](http://paperpile.com/b/9FuCD9/9h6iH)[10.1053/j.sodo.2023.01.001](http://dx.doi.org/10.1053/j.sodo.2023.01.001)
17. [Hans, M., Erbe, A., Mathews, S., Chen, Y., Solioz, M., & Mücklich, F. (2013). *Role of Copper Oxides in Contact Killing of Bacteria*. https://doi.org/](http://paperpile.com/b/9FuCD9/73zt)[10.1021/la404091z](http://dx.doi.org/10.1021/la404091z)
18. [Harsha, L., Navaneethan, R., Acid, T., & Acid, C. A.-A. (2022). CITRIC ACID-AN VITRO STUDY. *International Journal Clinical Dentistry*, *15*(3), 413–419.](http://paperpile.com/b/9FuCD9/gtA4P)
19. [Harsha, L., & Subramanian, A. K. (2022). Comparative assessment of pH and degree of surface roughness of enamel when etched with five commercially available etchants: An in vitro study. *The Journal of Contemporary Dental Practice*, *23*(2), 181–185. https://doi.org/](http://paperpile.com/b/9FuCD9/eVrex)[10.5005/jp-journals-10024-3252](http://dx.doi.org/10.5005/jp-journals-10024-3252)
20. [Jabin, Z., Nasim, I., Vishnu Priya, V., & Agarwal, N. (2021). Quantitative Analysis and Effect of SDF, APF, NaF on Demineralized Human Primary Enamel Using SEM, XRD, and FTIR. *International Journal of Clinical Pediatric Dentistry*, *14*(4), 537–541. https://doi.org/](http://paperpile.com/b/9FuCD9/w1pVC)[10.5005/jp-journals-10005-1988](http://dx.doi.org/10.5005/jp-journals-10005-1988)
21. [Jillani, S., Jelani, M., Hassan, N. U., Ahmad, S., & Hafeez, M. (2018). Synthesis, characterization and biological studies of copper oxide nanostructures. *Materials Research Express*, *5*(4), 045006. https://doi.org/](http://paperpile.com/b/9FuCD9/bjCIX)[10.1088/2053-1591/aab864](http://dx.doi.org/10.1088/2053-1591/aab864)
22. [Katyal, D., Subramanian, A. K., Venugopal, A., & Marya, A. (2021). Assessment of Wettability and Contact Angle of Bonding Agent with Enamel Surface Etched by Five Commercially Available Etchants: An In Vitro Study. *International Journal of Dentistry*, *2021*, 9457553. https://doi.org/](http://paperpile.com/b/9FuCD9/wOlEs)[10.1155/2021/9457553](http://dx.doi.org/10.1155/2021/9457553)
23. [Kopeček, J., & Yang, J. (2007). Hydrogels as smart biomaterials. *Polymer International*, *56*(9), 1078–1098. https://doi.org/](http://paperpile.com/b/9FuCD9/o225)[10.1002/pi.2253](http://dx.doi.org/10.1002/pi.2253)
24. [Lakshmi, T. (2021). Medicinal value oral health aspects acacia catechu-an update. *International Journal Dentistry Oral ScienceVolume*, *8*, 1399–1401J.](http://paperpile.com/b/9FuCD9/S45sS)
25. [Maliael, M. T., Subramanian, A. K., & Srirengalakshmi. (2021). Effectiveness of a fluoride-releasing orthodontic primer in reducing demineralization around brackets – a systematic review. *Orthodontic Waves (English Ed.)*, *80*(4), 218–223. https://doi.org/](http://paperpile.com/b/9FuCD9/Sp63h)[10.1080/13440241.2021.2007678](http://dx.doi.org/10.1080/13440241.2021.2007678)
26. [Ma, X., Zhou, S., Xu, X., & Du, Q. (2022). Copper-containing nanoparticles: Mechanism of antimicrobial effect and application in dentistry-a narrative review. *Frontiers in Surgery*, *9*, 905892. https://doi.org/](http://paperpile.com/b/9FuCD9/vgkN)[10.3389/fsurg.2022.905892](http://dx.doi.org/10.3389/fsurg.2022.905892)
27. [Naz, S., Gul, A., Zia, M., & Javed, R. (2023). Synthesis, biomedical applications, and toxicity of CuO nanoparticles. *Applied Microbiology and Biotechnology*, *107*(4), 1039–1061. https://doi.org/](http://paperpile.com/b/9FuCD9/w9iH)[10.1007/s00253-023-12364-z](http://dx.doi.org/10.1007/s00253-023-12364-z)
28. [Neha, N., Maiti, S., & Jessy, P. (2021). Adhesion microflora role denitrifies colour stability provisional crowns: in-vitro study. *Int J Dentistry Oral Sci*, *8*(8), 3805–3809.](http://paperpile.com/b/9FuCD9/3aDks)
29. [*(PDF) Syntheses of Copper Oxide Nanoparticle by Hydrothermal Method and Its Structural & Surface Morphological Studies*. (n.d.). ResearchGate. Retrieved March 25, 2025, from](http://paperpile.com/b/9FuCD9/rS5F) <https://www.researchgate.net/publication/369236141_Syntheses_of_Copper_Oxide_Nanoparticle_by_Hydrothermal_Method_and_Its_Structural_Surface_Morphological_Studies>
30. [*Radware Bot Manager Captcha*. (n.d.). Retrieved March 25, 2025, from](http://paperpile.com/b/9FuCD9/uoLL) <https://iopscience.iop.org/article/10.1088/1748-6041/11/3/035015/meta>
31. [Raffi, M., Mehrwan, S., Bhatti, T. M., Akhter, J. I., Hameed, A., Yawar, W., & ul Hasan, M. M. (2010). Investigations into the antibacterial behavior of copper nanoparticles against Escherichia coli. *Annals of Microbiology*, *60*(1), 75–80. https://doi.org/](http://paperpile.com/b/9FuCD9/llFS)[10.1007/s13213-010-0015-6](http://dx.doi.org/10.1007/s13213-010-0015-6)
32. [Ribatti, D., Tamma, R., & Annese, T. (2021). Chorioallantoic membrane vascularization. A meta-analysis. *Experimental Cell Research*, *405*(2). https://doi.org/](http://paperpile.com/b/9FuCD9/k849)[10.1016/j.yexcr.2021.112716](http://dx.doi.org/10.1016/j.yexcr.2021.112716)
33. Saadh, M. J., Rasulova, I., Almoyad, M. A. A., Kiasari, B. A., Ali, R. T., Rasheed, T. & Ciongradi, C. I. (2024). Recent progress and the emerging role of lncRNAs in cancer drug resistance; focusing on signaling pathways. Pathology-Research and Practice, 253, 154999.
34. [Sabarathinam, J., & Madhulaxmi, R. (2021). Development anti inflammatory antimicrobial silver nanoparticles coated suture materials. *Int J Dentistry Oral Sci*, *8*(3), 2006–2013.](http://paperpile.com/b/9FuCD9/wGWlT)
35. [Solanki, L., Shantha Sundari, K. K., Muralidharan, N. P., & Jain, R. (2022). Antimicrobial effect of novel gold nanoparticle oral rinse in subjects undergoing orthodontic treatment: An ex-vivo study. *Journal of International Oral Health: JIOH*, *14*(1), 47. https://doi.org/](http://paperpile.com/b/9FuCD9/Vp8Gw)[10.4103/jioh.jioh\_155\_21](http://dx.doi.org/10.4103/jioh.jioh_155_21)
36. [Sushanthi, S., Doraikannan, S., Indiran, M., & Rathinavelu, P. (2021). *Rajeshkumar S. Vernonia Amygdalina*. 3330–3334.](http://paperpile.com/b/9FuCD9/72LXX)
37. [Tiwari, A., & Jain, R. K. (2023). Comparative evaluation of White Spot lesion incidence between NovaMin, probiotic, and fluoride containing dentifrices during orthodontic treatment using laser fluorescence - A prospective randomized controlled clinical trial. *Clinical and Investigative Orthodontics*, 1–8. https://doi.org/](http://paperpile.com/b/9FuCD9/Seata)[10.1080/27705781.2023.2190950](http://dx.doi.org/10.1080/27705781.2023.2190950)
38. [*Website*. (n.d.-a).](http://paperpile.com/b/9FuCD9/vdti) <https://www.researchgate.net/publication/233709276_Antibacterial_materials>
39. [*Website*. (n.d.-b).](http://paperpile.com/b/9FuCD9/kuyR) <https://www.researchgate.net/publication/331080663_Synthesis_and_Biomedical_Applications_of_Copper_Oxide_Nanoparticles_An_Expanding_Horizon>
40. [Woźniak-Budych, M. J., Staszak, K., & Staszak, M. (2023). Copper and Copper-Based Nanoparticles in Medicine—Perspectives and Challenges. *Molecules*, *28*(18), 6687. https://doi.org/](http://paperpile.com/b/9FuCD9/t5Gu)[10.3390/molecules28186687](http://dx.doi.org/10.3390/molecules28186687)