Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.SE.25.01.Art019

Green synthesis and Characterization of Copper oxide nanoparticles using *Bauhinia tomentosa* leaf extract and evaluation of its antimicrobial activity against wound pathogens

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Abstract

The increasing problem of antibiotic resistance in the treatment of bacterial diseases has made the development of novel antimicrobial drugs crucial. In this study, we examine the environmentally friendly synthesis of copper oxide nanoparticles (CuONPs) using Bauhinia tomentosa (BT) leaf extract. The plant B. tomentosa, which has been used for centuries for its therapeutic uses, offers an eco-friendly and sustainable way to produce nanoparticles. Diverse techniques such as Transmission Electron Microscopy (TEM), UV-Vis spectroscopy, Scanning Electron Microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and Energy-Dispersive X-ray spectroscopy (EDX), were used to characterize these BT-CuONPs. The synthesis of CuO NPs is confirmed through UV-Vis spectroscopy, showing an absorption peak at 225nm. Morphological analysis via SEM and TEM reveals the presence of spherical and irregularly shaped nanoparticles, typically around 50 nm in size. FTIR analysis identifies characteristic absorption bands by indication of fuctional groups from leaf extract that may interact with nanoparticles. Additionally, EDX analysis confirms the elemental composition, predominantly revealing peaks for copper and oxygen, validation successful synthesis of BT-CuONPs. BT-CuONPs were synthesized and evaluated for their antimicrobial effectiveness against wound pathogens, including multiple drug resistant organisms. The findings showed a notable antimicrobial efficacy with highest inhibition zone against P. aeruginosa at 40mm, suggesting that BT-CuONPs had strong antimicrobial potential. The promise of employing plant extracts more especially, B. tomentosa as a green synthesis route for CuONPs and their use in the fight against drug-resistant microbial infections is highlighted in this study. In the continuous fight against drug resistance, these successful production and characterization of nanoparticles highlight their potential as strong antimicrobial agents.

Keywords: *Antimicrobial Potential, Bauhinia Tomentosa, Copper Oxide Nanoparticles, Green Synthesis, Nanotechnology.*

Received: 17.10.2024 ***Accepted: 02.12.2024*** ***Published on: 31.01.2025***

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Introduction

A major hazard to global public health is the widespread rise in antibiotic resistance caused by persistently resistant microorganisms. To prevent and treat microbial diseases, it is becoming increasingly important to find alternatives to antibiotics [1]. One such strategy is the application of nanotechnology in conjunction with naturally occurring botanicals, which may be employed in place of or in addition to antibiotics [2]. Because of the unique chemical and physical properties of nanomaterials, which include their hardness, durability, electrical conductivity, high diffusivity, resistivity, also variety of biological and chemical activity, interest in this field of research has increased significantly. Due to its growing use in the industrial and medical sectors as opacifiers, fillers, antimicrobial agents, disinfectants, drug delivery materials, catalysts etc., metal oxide nanoparticle synthesis has drawn particular attention in nanotechnology research [3].

Copper oxide (CuO) nanoparticles have received a lot of attention for their biocidal and antibacterial properties and their potential to successfully treat a variety of diseases that are resistant to currently available antimicrobial treatments [4]. CuO nanomaterials are used to alter the surfaces of antimicrobial compounds, allowing for the advancement of smart drug release kinetics. This is because the CuO nanosystem is not only utilized to treat infections but also to detect pathogens [5]. Additionally, the inorganic and organic nanocompounds made using CuO metal oxides have unique characteristics like strong mechanical strength, durability at high temperatures, and high thermal and electrical conductivity [6]. CuO nanostructures can be developed using diverse chemical and physical techniques. Nevertheless, there are several disadvantages to these CuO nanoparticle synthesis processes, including high energy consumption, the use of dangerous chemicals, and high temperature treatment, which have an impact on the environment and also the field of research. Presently, scientists have a further approach to investigating metal oxide nanoparticles in different biological applications, by synthesizing them with a green method [7].

The most widely used sustainable approach to generate metal oxide nanoparticles is bio-reduction, which involves reacting physiologically active compounds that are derived from plants with metal oxides in reduced form [8]. The current investigation used extracts from the Fabaceae family plant *Bauhinia tomentosa* to synthesize copper oxide nanoparticles using a green approach method. The entire edge and elliptical shape of *B. tomentosa*'s leaves are divided in the middle by a deep line. The plant has several stems and thin branches that can reach a height of 4 meters. The bark is grey and has a smooth, hairy feel. The yellow petals of the blooms are grouped in the form of a bell with dark maroon spots at the base. The herb is abundantly available in Asian nations including Zimbabwe, Mozambique, Sri Lanka, and India and has amazing antimicrobial properties [9]. Medicinal phytocompounds and antimicrobials, such as phytohemagglutinins, catechuic acid, rutin, quercetin, and isoquercitin, have been discovered to be present in *B. tomentosa* [10].

In this study, we used *B. tomentosa* leaf extract to explore the green synthesis of CuO NPs. The biosynthesized CuO NPs were characterized using Scanning electron microscope (SEM), UV-Vis spectroscopy, Energy dispersive X-ray (EDX), Fourier transforms infra-red spectroscopy (FTIR) and Transmission electron microscope (TEM). Additionally, we examined the antimicrobial potential of the synthesized BT-CuO NPs against both fungal and bacterial strains. Therefore, the green synthesis of CuO NPs using *B. tomentosa* leaf extract not only shows an environmentally friendly method but also shows that the biosynthesised BT-CuO NPs have promising antimicrobial potential against a variety of bacterial and fungal strains, highlighting their potential uses in environmental and medical fields.

Materials and Methods

Chemicals

Nutrient Agar (NA), antibiotic discs, Mueller Hinton Agar (MHA), and Copper sulphate were obtained from Hi-Media, and every chemical employed was of analytical quality. Nanopure water was utilized throughout this research work.

Collection of Microbes

The microbes that were isolated from wound infections in an aseptical manner. The samples collected from wound sites were cultured in suitable conditions to promote growth. Standard microbiological methods, such as Gram staining and biochemical testing, were used to identify each bacteria isolate. Genomic DNA was isolated from the isolates, and particular genetic markers were amplified using polymerase chain reaction (PCR) to identify the isolated microbes.

Preparation of Plant Extract

We procured fresh *B. tomentosa* leaves from Rasipuram, Tamilnadu. The plant specimen was authenticated and identified as *Bauhinia tomentosa* from Fabaceae family. After being washed of dust with tap water, the leaves were dried and ground into a powder using a blender. The plant extract was developed using the following technique: 50 mL of distilled water were combined with 5g of powdered *B. tomentosa* leaves and carefully stirred. For 15 minutes, this mixture was kept at 60°C in a water bath. A magnetic stirrer was then used to properly agitate the liquid for 30 minutes. After passing the mixture through Whatman No. 1 filter paper, a clear extract was obtained. This extract was then stored at 4°C and utilized for the sustainable production of CuO NPs [11].

Synthesis of Copper Oxide Nanoparticles

The green synthesis of CuO NPs was accomplished by mixing 80 mL of 2 mM $CuSO_4$ with 20 mL of *B. tomentosa* extract and then allowing the mixture to sit at room temperature for 4 days. For 5 days, the solution was stored in a dark area until its green colour turned dark brown. Copper was reduced as a result of this colour shift, and copper oxide developed. To extract the biomolecule bonded to the metal nanoparticles, a centrifuge was used at 4000 rpm for 15 minutes. The produced nanoparticles have been processed for 12 hours at 70°C in an oven following three steps of water washing. After being thoroughly dried, the copper oxide nanoparticles were annealed for 2h at 500°C and then kept in an airtight bottle for further analysis [12].

Characterization of Synthesized Nanoparticles

The optical, morphological, and compositional characteristics of the green formulated nanoparticles were evaluated by using diverse techniques. Using a UV-visible spectrometer, the absorbance of the nanoparticle samples was determined, resulting in the determination of their optical properties and band gap. Scanning electron microscopy was employed to study the morphology of the NPs, yielding precise images of their size distribution and surface structure. Single drop of CuO was deposited on the copper grid, allowed to dry, and then examined using transmission electron microscopy (TEM) at an operating voltage of about 200 kV to observe shape and size of the nanoparticles. Fourier transform infrared spectroscopy (FTIR), which reveals distinctive absorption bands, was used to examine the functional groups and chemical bonds present over a wavenumber range of 4000 to 400 cm^{-1}. Energy dispersive X-ray spectroscopy was employed in conjunction with SEM and TEM investigations to confirm the effective synthesis of the CuO nanoparticles and offer quantitative information on the elemental composition [13].

Antibacterial Activity of Bt-Cuonps

B. tomentosa-mediated copper oxide nanoparticles (BT-CuO NPs) were evaluated against isolates of fungi and bacteria, both Gram-positive and Gram-negative. A well diffusion approach was used to assess the nanoparticles' antibacterial properties. Six wells were created on Muller Hinton agar plates using a cork borer, and BT-CuO NPs at varying concentrations (25, 50, 75, 100, and 150 µg/mL) were introduced to each well. Dimethyl sulfoxide, or DMSO, used as a control. The plates were incubated for 24 hours at 37°C to assess the effectiveness of the BT-CuO NPs. The zones of inhibition (ZOI) for each microbial strain were then determined [14].

Result

Identification of Microbes

The microbes isolated from infected wound sites were identified using gram staining and biochemical assays. The pathogens were identified as *Staphylococcus aurues* (gram positive), *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (gram negative) and *Candida albicans*, a fungal strain. After identification, genomic DNA was amplified using PCR, and the resulting sequences were submitted to the National Center for Biotechnology Information (NCBI) with accession codes PP917548 for *Staphylococcus*, PP917549 for *Escherichia*, PP917550 for *Pseudomonas*, PP917551 for *Klebsiella*, and PP917552 for *Candida albicans*.

Synthesize of BT-CuO NPs

During the formulation of BT-CuO NPs, the solution was initially in a vibrant green color, which is a characteristic of the plant extract. As the reaction progressed, this green colour transitioned to dark brown color, signifying the successful development of copper oxide nanoparticles. This color change is a visual marker for copper ion reduction and the subsequent generation of CuO NPs, confirming the synthesis process. The dark brown coloration is typically associated with the presence of copper oxide, further supporting the successful development of CuONPs using the *B. tomentosa* extract (Figure 1).

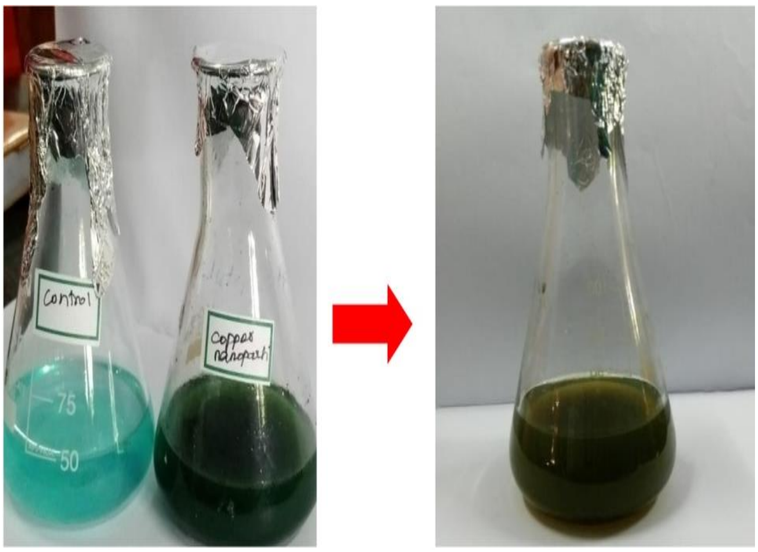

Figure 1. Synthesis of BT-CuONPs

Characterization of Synthesized BT-CuONPs

UV-Vis Spectroscopy

The production of CuO NPs by the bioreduction process using leaf extract from *B. tomentosa* was observed using UV-Vis spectrophotometry. The most crucial technique for identifying the surface plasmon resonance (SPR) characteristic of the biosynthesised CuO NPs is UV-Vis spectral analysis. The distinctive SPR peak at 225 nm, which indicates the green production of CuO NPs, is clearly visible in the UV-Vis spectrum displayed in Figure 2.

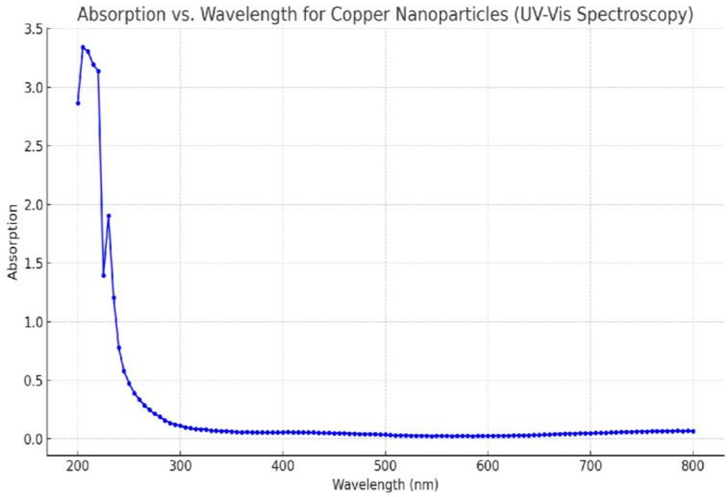

Figure 2. UV-Vis Absorption Spectrum of Synthesized BT-CuONPs

FTIR analysis

FTIR analysis was used to determine which functional groups were present in the biosynthesised BT-CuO NPs, and Figure 3 shows the spectrum. The Fourier-transform infrared (FTIR) spectrum of BT-CuONPs displayed several notable peaks, each corresponding to specific functional groups and molecular vibrations. The peak at 3432.31 cm^{-1} is associated with O-H stretching, indicating the presence of hydroxyl groups from the plant extract, which play a role in nanoparticle stabilization [15]. The peaks at 2921.95 cm^{-1} and 2853.34 cm^{-1} are attributed to C-H stretching vibrations from aliphatic compounds, suggesting the involvement of organic molecules in the synthesis process. The peak at 2060.19 cm^{-1} may indicate the presence of C≡C stretching, while the peak at 1631.84 cm^{-1} is likely due to C=O stretching vibrations, further confirming the presence of various organic constituents in the extract. Peaks at 1423.33 cm^{-1} and 1316.17 cm^{-1} can be linked to C-N stretching and C-O bending, respectively, suggesting the interaction of the CuO NPs with functional groups from the extract. The peaks at 1118.04 cm^{-1} and 986.90 cm^{-1} correspond to C-O stretching and C-C bending, respectively, indicating the stabilization of nanoparticles through the plant's organic compounds [16]. Lastly, the peaks at 931.30 cm^{-1}, 779.34 cm^{-1}, 720.50 cm^{-1}, and 618.63 cm^{-1} are attributed to metal-oxygen (Cu-O) vibrations, confirming the successful formation of CuO NPs [17]. The phytochemicals of *B. tomentosa* leaf extract are primarily responsible for these chemical groups, which are engaged in the bioreduction process. FTIR study results clarified the role of several *B. tomentosa* phytochemicals, including proteins, tannins, and phenolics, in the formation of CuO NPs.

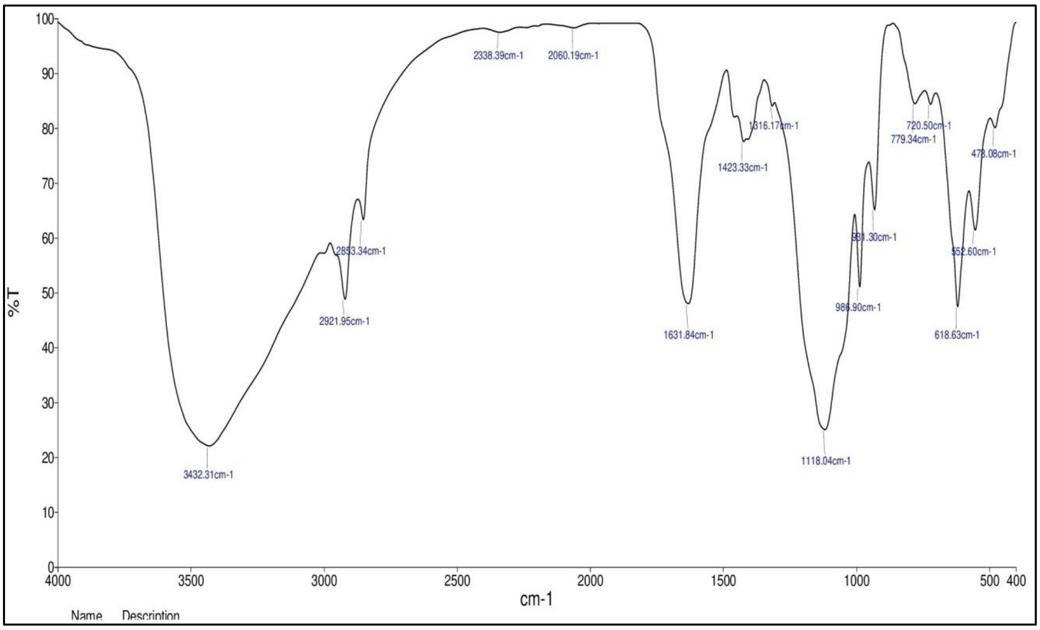

Figure 3. FTIR Spectrum of BT-CuONPs

SEM and EDX Analysis

Figure 4 displayed the surface morphology of the CuONPs generated with *B. tomentosa*. It indicates that uniformly shaped spherical nanoparticles are abundant. The EDX spectrum, which displays the largest peak for copper and oxygen, was used to analyse the purity. This indicates that the developed copper oxide nanoparticles are extremely pure.

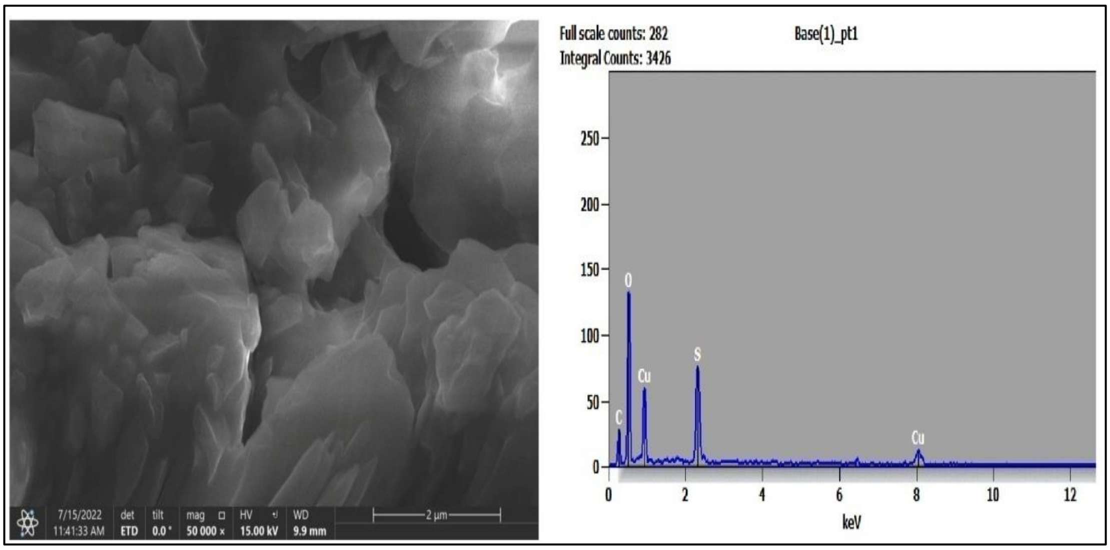

Figure 4. SEM with EDX Analysis of BT-CuONPs

TEM Analysis

A unique spherical particle shape was found by TEM examination of CuO NPS produced using *B. tomentosa* leaf extract. The average crystallite size, as determined by particle measurement, was roughly 50 nm, indicating that the nanoparticles fall within the desired size range (Figure 5). The efficiency of the *B. tomentosa* leaf extract in mediating the synthesis process is demonstrated by the spherical form and distinct size distribution of the CuO NPs.

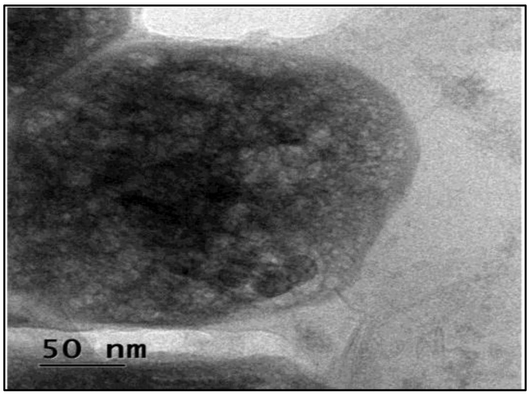

Figure 5. TEM Analysis of BT-CuONPs

Antimicrobial Activity

Using the agar well diffusion assay, the antibacterial activity of the biosynthesized BT-CuO NPs was evaluated against the Gram-negative bacteria *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and Gram-positive bacteria *S. aureus*, as well as the fungal strain *C. albicans*. The antibacterial capability of biosynthesized CuO NPs was evident from the zone of inhibition observed surrounding the wells in every petriplate. BT-CuONPs demonstrate significant antibacterial action against test strains of both bacteria and fungi. The BT-CuO NPs shown significant antibacterial activity against *P. aeruginosa* at a concentration of 150µg/mL, with an inhibition zone of 40 mm. *E. coli* and *C. albicans* followed at 38 mm, and *K. pneumoniae* and *S. aureus* at 37 mm (Figure 6 and Table 1). CuO NPs' ability to pass through microbial cell membranes and cause membrane disruption and eventual cell death is thought to be the mechanism behind their antibacterial action. CuO NPs' huge surface-to-volume ratio and nanosized shape improve their contact with the cell membrane. Due to variations in cell membrane structure, the inhibitory effect of CuO NPs on Gram-negative bacteria was shown to be higher in this study than that on Gram-positive bacterium. Therefore, it has been shown that CuO NPs made from *B. tomentosa* leaf extract are effective antibacterial agents against microorganisms responsible for wound infections.

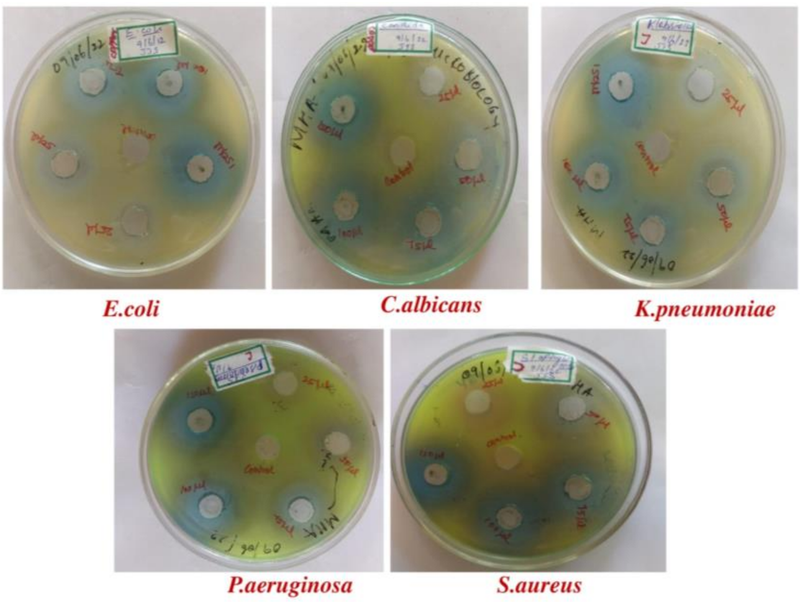

Figure 6. Antimicrobial Activity of Synthesized BT-CuONPs against Wound Pathogens

Table 1. ZOI in mm for BT-CuONPs Against Wound Pathogens

S.No	**Name of the organism**	**25µl**	**50µl**	**75µl**	**100µl**	**150µl**
1	*E. coli*	22mm	29mm	34mm	36mm	38mm
2	*C.albicans*	20mm	30mm	35mm	37mm	38mm
3	*K. pneumoniae*	32mm	35mm	37mm	35mm	37mm
4	*P.aeruginosa*	31mm	34mm	37mm	38mm	40mm
5	*S. aureus*	24mm	26mm	29mm	32mm	37mm

Discussion

The utilization of *Bauhinia tomentosa* leaf extract in the sustainable synthesis of CuO NPs represents a significant advancement in sustainable nanotechnology. The conventional approaches to synthesizing nanoparticles are less sustainable since they frequently entail hazardous chemicals and excessive energy usage. Conversely, the utilization of green extracts not only lessens the impact on the environment but also gives the synthesized nanoparticles biocompatible and biodegradable characteristics [18]. *B. tomentosa*, noted for its rich phytochemical compounds, functions as a stabilizing and reducing agent in the biosynthesis process. The leaf extract contains flavonoids, phenolic compounds, and other bioactive chemicals that help in reducing Cu^{+} ions to CuO NPs while also it caps the nanoparticles preventing them from agglomeration [19].

The characterization of the green synthesized CuO NPs is crucial to understanding their structural, morphological, and optical properties. Techniques such as UV-Vis spectroscopy, Transmission electron microscopy (TEM), scanning electron microscopy (SEM), Energy-dispersive X-ray spectroscopy (EDX) and Fourier-transform infrared spectroscopy (FTIR) provide insights into the shape, size and crystallinity of the NPs [20]. Typically, the absorption peak observed in UV-Vis spectra at 225 nm corresponds to the formation of CuO. SEM and TEM images further elucidate the morphology, often showcasing spherical or irregularly shaped nanoparticles with sizes typically ranging at 50 nm. Additionally, FTIR analysis shows characteristic absorption bands at 3432.31 cm^{-1} (O-H stretching), 2921.95 cm^{-1} and 2853.34 cm^{-1} (C-H stretching), and several others, indicating the presence of functional groups from the leaf extract that may interact with the nanoparticles. Furthermore, the primary elemental composition of the synthesised BT-CuO NPs is confirmed by EDX analysis, which predominantly shows peak for copper and oxygen, thus verifying the successful synthesis of copper oxide. The controlled size and morphology of CuO NPs are critical factors that influence their reactivity and, consequently, their antimicrobial properties.

The evaluation of antimicrobial activity is a vital aspect of this research, given the increasing prevalence of antibiotic-resistant pathogens [21]. The synthesized BT-CuO NPs exhibit potent antimicrobial activity against various bacterial strains, like Gram-positive and negative bacterial strains with highest ZOI against *P. aeruginosa*, with an inhibition zone of 40 mm. Previous studies have also demonstrated significant antimicrobial activity synthesized using *B. tomentosa* plant extract. Renganathan et al. [22] synthesized silver nanoparticles (AgNPs) employing the flower extract of *B. tomentosa Linn*. Significant antibacterial activity was seen against *E. coli* (6.75 mm) and *S. aureus* (9.25 mm). Plant-based gold nanoparticles (Au NPs) were developed by Gnanamoorthy et al. [23] using *B. tomentosa* Linn leaf extract. The antibacterial activity of the produced Au NPs was assessed against the Gram-positive bacterium S. aureus and the Gram-negative bacterium *E. coli*. Using leaf extract from *B. tomentosa*, Sharmila et al. [24] reported synthesizing copper oxide nanoparticles (CuO NPs). With a zone of inhibition of 22 mm against *E. coli* and 17 mm against *P. aeruginosa*, the generated CuO NPs showed strong antibacterial activity. Using the leaf extract of *B. tomentosa* Linn., Ramar et al. [25] reported the green synthesis of silver nanoparticles (Ag NPs), emphasizing their in vitro biological activities and photocatalytic properties. Effective antibacterial action against *S. aureus* and *E. coli* was demonstrated by the synthesized Ag NPs. In conclusion, the synthesis of nanoparticles using plant extracts from *B. tomentosa* shows that nanotechnology can be applied in a sustainable and promising manner. Significant antimicrobial properties against pathogenic microbes are demonstrated by these biogenic nanoparticles.

The production of reactive oxygen species (ROS) upon interaction with microbial cells, rupture of cellular membranes, and disruption of metabolic processes are some of the mechanisms responsible for this antimicrobial efficacy of BT-CuONPs. Because of their small size, the nanoparticles can more effectively penetrate microbial cells and increase their inhibitory effects [26]. Additionally, the bioavailability and durability of CuO NPs produced using sustainable processes indicate a viable substitute in producing antimicrobial agents for application in medical fields. It's also important to pay attention to the biocompatibility of CuO NPs made from *B. tomentosa*. Even if their antimicrobial properties are beneficial, it's crucial to evaluate their safety for both environmental and human health. Based on preliminary toxicity investigations, the synthesis mediated by plant extracts produces less impact nanoparticles than conventional approaches [27-29]. By lowering the potential cytotoxicity of the nanoparticles, the addition of phytochemicals from the leaf extract may also have beneficial effects.

In conclusion, the green formulation of CuO NPs utilizing *B. tomentosa* leaf extract, along with their successful characterization and demonstrated antimicrobial activity, opens new avenues for their application in pharmaceuticals, agriculture, and nanomedicine. This approach not only fosters innovation in nanotechnology but also promotes the responsible use of natural resources to address contemporary challenges in health and disease management [30-32].

Conclusion

In conclusion, the green formulation of copper oxide nanoparticles employing *Bauhinia tomentosa* leaf extract not only presents an eco-friendly approach to nanoparticle synthesis but also demonstrates effective antimicrobial properties, making them promising candidates for various biomedical applications. The phytochemicals found in the leaf extract demonstrate a crucial part in reducing copper ions to form nanoparticles while providing stabilization, which is vital for maintaining their unique properties. The characterization results confirm the successful synthesis, by revealing the shape, size, also crystalline nature of the synthesized BT-CuONPs. The potential of these BT-CuONPs as alternative antimicrobial agents is demonstrated by the observed antibacterial activity against a variety of pathogens, paving up the possibilities for further investigation and advancement in the field of nanotechnology and its use in biomedical and nanomedicine applications.

Acknowledgement

We would like to acknowledge the Department of Microbiology, J.J. College of Arts and Science (Autonomous) (Affiliated to Bharathidasan University, Tiruchirapalli) Pudukkottai-622422, Tamil Nadu, India for providing the necessary facilities.

Conflict of Interest

The authors hereby declare that there is no conflict of interest in this study.

References

[1]. Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., Alqumber, M. A., 2023, Antimicrobial resistance: a growing serious threat for global public health. In Healthcare, 11(13), 1946.

[2]. Kumari, P., Luqman, S., Meena, A., 2019, Application of the combinatorial approaches of medicinal and aromatic plants with nanotechnology and its impacts on healthcare. *DARU Journal of Pharmaceutical Sciences*, *27*, 475-489.

[3]. Alshammari, B. H., Lashin, M. M., Mahmood, M. A., Al-Mubaddel, F. S., Ilyas, N., Rahman, N., & Khan, R., 2023, Organic and inorganic nanomaterials: fabrication, properties and applications. *RSC Advances*, *13*(20), 13735-13785.

[4]. Bezza, F. A., Tichapondwa, S. M., Chirwa, E. M., 2020, Fabrication of monodispersed copper oxide nanoparticles with potential application as antimicrobial agents. *Scientific Reports*, 10(1), 16680.

[5]. Fazal, A., Ara, S., Ishaq, M. T., Sughra, K., 2022, Green fabrication of copper oxide nanoparticles: a comparative antibacterial study against gram-positive and gram-negative bacteria. *Arabian Journal for Science and Engineering*, 47(1), 523-533.

[6]. Majumdar, D., Ghosh, S., 2021, Recent advancements of copperoxide based nanomaterials for supercapacitor applications. *Journal of Energy Storage*, 34, 101995.

[7]. Nair, G. M., Sajini, T., Mathew, B., 2022, Advanced green approaches for metal and metal oxide nanoparticles synthesis and their environmental applications. *Talanta Open*, *5*, 100080.

[8]. Jeevanandam, J., Kiew, S. F., Boakye-Ansah, S., Lau, S. Y., Barhoum, A., Danquah, M. K., & Rodrigues, J., 2022, Green approaches for the synthesis of metal and metal oxide nanoparticles using microbial and plant extracts. *Nanoscale*, 14(7), 2534-2571.

[9]. Mukundan, D., Mohankumar, R., Vasanthakumari, R., 2015, Green synthesis of silver nanoparticles using leaves extract of Bauhinia tomentosa linn and its invitro anticancer potential. Materials Today: *Proceedings*, *2*(9), 4309-4316.

[10]. Mukundan, D., Mohankumar, R., Vasanthakumari, R., 2015, Green synthesis of silver nanoparticles using leaves extract of Bauhinia tomentosa linn and its invitro anticancer potential. Materials Today: *Proceedings*, *2*(9), 4309-4316.

[11]. Singh, A., Kaur, J., Kapoor, M., 2023, Phytochemical screening and antibacterial potential of Methanol, Ethanol and aqueous extracts from Seed, Bark and Leaf of Bauhinia tomentosa L. *Agricultural Science Digest-A Research Journal*, 43(1), 10-17.

[12]. Vakayil, R., Muruganantham, S., Kabeerdass, N., Rajendran, M., Ramasamy, S., Alahmadi, T. A., Mathanmohun, M., 2021, Acorus calamus-zinc oxide nanoparticle coated cotton fabrics shows antimicrobial and cytotoxic activities against skin cancer cells. *Process Biochemistry*, 111, 1-8.

[13]. Prabu, P., &Losetty, V., 2024, Green synthesis of copper oxide nanoparticles using MacroptiliumLathyroides (L) leaf extract and their spectroscopic characterization, biological activity and photocatalytic dye degradation study. *Journal of Molecular Structure*, 1301, 137404.

[14]. Balaji, T., Manushankar, C. M., Al-Ghanim, K. A., Kamaraj, C., Thirumurugan, D., Thanigaivel, S., Govindarajan, M., 2023, Padina boergesenii-mediated copper oxide nanoparticles

synthesis, with their antibacterial and anticancer potential. *Biomedicines*, 11(8), 2285.

[15]. Kabeerdass, N., Thangasamy, S., Murugesan, K., Arumugam, N., Almansour, A. I., Suresh Kumar, R., Mathanmohun, M., 2022, Embedding green synthesized zinc oxide nanoparticles in cotton fabrics and assessment of their antibacterial wound healing and cytotoxic properties: An eco-friendly approach. *Green Processing and Synthesis*, 11(1), 875-885.

[16]. Abdelbaky, A. S., Abd El-Mageed, T. A., Babalghith, A. O., Selim, S., Mohamed, A. M., 2022. Green synthesis and characterization of ZnO nanoparticles using Pelargonium odoratissimum (L.) aqueous leaf extract and their antioxidant, antibacterial and anti-inflammatory activities. *Antioxidants*, *11*(8), 1444.

[17]. Ali Alhaidrai, S. A., Al-Hadi, F. A., Al-Kaf, A. G., Taj, A. D., 2022, A. Phytochemical Screening by FTIR Spectroscopic Analysis in the Methanolic Extracts Coffee (C. Arabica. L) to Seeds and Peels (Unroasted and Roasted) Cultivars Grown in Yemen. *Bioequiv. Bioavailab. Int. J* 6, 000179.

[18]. Renuga, D., Jeyasundari, J., Athithan, A. S., Jacob, Y. B. A., 2020, Synthesis and characterization of copper oxide nanoparticles using Brassica oleracea var. italic extract for its antifungal application. *Materials Research Express*, 7(4), 045007.

[19]. Elango, B., Okram, G. S., Mathanmohun, M., 2023, Pharmacological Applications of Plant-Mediated Synthesized Nanomaterials. *Current Pharmacology Reports*, 9(6), 511-522.

[20]. Osman, A. I., Zhang, Y., Farghali, M., Rashwan, A. K., Eltaweil, A. S., Abd El-Monaem, E. M., Yap, P. S. 2024, Synthesis of green nanoparticles for energy, biomedical, environmental, agricultural, and food applications: A review. *Environmental Chemistry Letters*, 22(2), 841-887.

[21]. Andualem, W. W., Sabir, F. K., Mohammed, E. T., Belay, H. H., Gonfa, B. A., 2020, Synthesis of copper oxide nanoparticles using plant leaf extract of Catha edulis and its antibacterial activity. *Journal of Nanotechnology*, 2020(1), 2932434.

[22]. Muteeb, G., Rehman, M. T., Shahwan, M., Aatif, M., 2023, Origin of antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. *Pharmaceuticals*, 16(11), 1615.

[23]. Renganathan, S., Subramaniyan, S., Karunanithi, N., Vasanthakumar, P., Kutzner, A., Kim, P. S., Heese, K., 2021, antibacterial, antifungal, and antioxidant activities of silver nanoparticles biosynthesized from Bauhinia Tomentosa Linn. *Antioxidants*, *10*(12), 1959.Antioxidants 10, no. 12 (2021): 1959.

[24]. Gnanamoorthy, G., Ramar, K., Ali, D., Yadav, V. K., Kumar, G., 2022, Synthesis and effective performance of Photocatalytic and Antimicrobial activities of Bauhinia tomentosa Linn plants using of gold nanoparticles. *Optical Materials*, 123, 111945.

[25]. Sharmila, G., Pradeep, R. S., Sandiya, K., Santhiya, S., Muthukumaran, C., Jeyanthi, J., Thirumarimurugan, M., 2018, Biogenic synthesis of CuO nanoparticles using Bauhinia tomentosa leaves extract: characterization and its antibacterial application. *Journal of Molecular Structure*, 1165, 288-292.

[26]. Ramar, K., Vasanthakumar, V., Priyadharsan, A., Priya, P., Raj, V., Anbarasan, P. M., Jafar Ahamed, A., 2018, Green synthetic approach of silver nanoparticles from Bauhinia tomentosa Linn. leaves extract for potent photocatalytic and in vitro biological applications. *Journal of Materials Science: Materials in Electronics*, *29*, 11509-11520.

[27]. Devaraji, M., Thanikachalam, P. V., & Elumalai, K., 2024, The Potential of Copper Oxide Nanoparticles in Nanomedicine: A Comprehensive Review. *Biotechnology Notes*.

[28]. 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.

[29]. Velsankar, K., Suganya, S., Muthumari, P., Mohandoss, S., Sudhahar, S., 2021, Ecofriendly green synthesis, characterization and biomedical applications of CuO nanoparticles synthesized

using leaf extract of Capsicum frutescens. *Journal of Environmental Chemical Engineering*, *9*(5), 106299.

[30]. Govindharaj, S., Nizar, A. M., Akilaa, O. B., Shanmugam, R., Thangavelu, L., 2024, Green Synthesis of Tannic Acid-Mediated Chromium Oxide Nanocomposites using Acalypha Indica and Carica Papaya Leaf and its Biomedical Applications. *Nanotechnology Perceptions*, 304-326.

[31]. Gayathri, B., Shanmugam, R., Thangavelu, L., Muthukumaran, D., 2024, Antibacterial Activity of Selenium Nanoparticles Synthesized using Mimosa Pudica against Wound Pathogens. *Nanotechnology Perceptions*, 369-376.

[32]. PunnoseKovoor, A., Krishnan, A., Manigandan, P., Shanmugam, R., Thangavelu, L., 2024, Green Synthesis of Silver Nanoparticles from Aegle Marmelos and Cissus Quadrangularis Leaf Extract and Evaluation of Its Anti-Inflammatory and Antioxidant Effect. *Nanotechnology Perceptions,* 276-289.