RASĀYAN *J. Chem.*
Vol. 18 | No. 4 |2187-2194 October-December | 2025
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

BIOSYNTHESIS OF CuO NANOPARTICLES USING TRIANTHEMA PORTULACASTRUM: STRUCTURAL ANALYSIS AND ANTIMICROBIAL EVALUATION

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ABSTRACT

This research investigates the environmentally benign biosynthesis of copper oxide nanoparticles utilizing the leaf extract of *Trianthema portulacastrum* (TP), offering a sustainable substitute for conventional chemical and physical techniques. The synthesised CuO nanoparticles were thoroughly characterized using many techniques, where FTIR examination verified the existence of Cu–O bonds, signifying successful nanoparticle production, whilst UV-Vis spectroscopy displayed a prominent absorbance peak at 722.9 nm, typical of CuO nanoparticles. SEM investigation revealed primarily spherical particles measuring between 18 and 30 nm, corroborated by DLS observations. Antimicrobial activity was evaluated using well-diffusion experiments, demonstrating significant antibacterial efficacy. The CuO nanoparticles demonstrated greater zones of inhibition against *Escherichia coli* and *Salmonella* than ordinary gentamicin, highlighting their potential as effective antibacterial agents. Antifungal activity was moderate, with inhibition zones measuring 19 mm against *Candida albicans* and 21 mm against *Fusarium*.

Keywords: *Trianthema portulacastrum,* nanoparticles, characterization, antimicrobial assay, environmental.
RASĀYAN *J. Chem.*, Vol. 18, No.4, 2025

INTRODUCTION

Nanotechnology advances medicine, electronics, and environmental science by manipulating materials at the nanoscale. There are two primary methods for synthesizing nanoparticles: chemical and physical. The chemical method involves using hazardous chemicals to stabilize nanoparticles and reduce metal atoms, often leading to significant environmental pollution. On the other hand, the physical method is highly resource-intensive, requiring expensive equipment, high energy consumption, and extensive testing in controlled environments. Physical methods, such as laser ablation, evaporation-condensation, and ball milling, involve manipulating physical properties to produce NPs with precise control over size and shape, but they require expensive equipment and high energy input.[1] Precipitation, sol-gel, and hydrothermal synthesis are scalable nanoparticle production processes; however, they use hazardous chemicals and produce toxic byproducts, posing environmental and health issues.[2] Biological methods, or green synthesis, utilize plants, bacteria, fungi, and algae to reduce metal ions into NPs, providing an environmentally friendly, low-cost alternative with narrow size distributions and functionalized surfaces, although they may be slower and limited by organism growth rates.[3-5] In the past few decades,

http://doi.org/10.31788/RJC.2025.1849381

improvements in nanoparticle technology have greatly affected the medical, pharmaceutical, and textile businesses. Metal nanoparticles, such as silver, zinc, and gold, have been applied as therapeutic agents.[6,7] Transition metal oxides such as oxides of titanium, ferrites, magnesium, zinc, copper, nickel, gold, and silver are used in various advanced uses in energy, healthcare, and the environment because they can effectively attract and hold onto substances, improving their performance.[8] The production of metal and metal oxide nanoparticles is moving away from physical and chemical processes and towards biological methods, which are referred to as biosynthesis or green synthesis.[9,10] This method, which promotes biocompatibility and allows for large-scale production, makes use of reducing agents that are bacteria, fungi, algae, yeast, and plant extracts.[11,12] Researchers are becoming more interested in the biological features of metal nanoparticles, especially metal oxides such as copper oxide. CuO nanoparticles (CuONPs) show superior biological and photocatalytic activities compared to metal nanoparticles.[6-10] They can be used in gas monitors, waste treatment, catalysts, batteries, food preservation, superconductors, solar energy, solar panels, dye removal, field emission devices, and farming. CuONPs have strong anticancer, antimicrobial, and antioxidant qualities, which make them useful in medicine. Various physicochemical methods, such as microwave irradiation, thermal breakdown, sol-gel processes, colloidal synthesis, sonochemical techniques, hydrothermal methods[13], and quick precipitation, have been used to synthesise CuONPs with desired qualities.[14-15] The present study focuses on the synthesis of CuONPs from the leaves of the medicinal herb *Trianthema portulacastrum*. The obtained copper oxide nanoparticles were subjected to characterisation by FTIR, UV, Dynamic light scattering, Morphological study, diffraction study, and antibacterial studies.

Trianthema Portulacastrum

The Aizoaceae family includes the traditional and culinary plant *Trianthema portulacastrum* Linn. (TP), which originates in tropical Asia, Southeast Africa, and tropical America (Uttam et al and Kavitha et al). TP is a "weed" that grows rampant in areas that receive heavy rainfall and are well-irrigated, including parts of India and its neighbours Pakistan, Sri Lanka, and Bangladesh. It holds great importance in Ayurvedic procedures and other forms of traditional Indian medicine[16] (Uttam). The pharmacological effects of the plant are due in part to its rich content of bioactive chemical components, including alkaloids, glycosides, phenolic compounds, and flavonoids[17] (Patil-Patankar). TP has a wide range of medicinal uses, including as an antioxidant, hepatoprotective agent, antifungal, anti-inflammatory, and wound-healing agent[18]. *Trianthema portulacastrum* Linn. contains ecdysterone as its principal constituent, along with other phytochemicals such as trianthenol, leptorumol, 3-acetylaleuritolic acid, 5,2′-dihydroxy-7-methoxy-6,8-dimethylflavone, 3,4-dimethoxycinnamic acid, p-methoxybenzoic acid, 5-hydroxy-2-methoxybenzaldehyde, and beta cyanin.[19]

Fig.-1: *Trianthema portulacastrum L*eaves

EXPERIMENTAL

***Trianthema Portulacastrum* Leaf Extract Preparation**

The harvested *Trianthema portulacastrum* leaves were thoroughly washed with distilled water to remove any impurities. The leaves were then cut into small pieces to enhance the extraction process. Approximately 30 g of the prepared leaf material was used in the preparation of an aqueous plant extract. The extraction process involved boiling the leaf material in 100 mL of distilled water for 1 hour to release bioactive compounds. After boiling, the mixture underwent filtration using Whatman filter paper No. 44 to separate the solid residues from the liquid extract. The resulting filtrate was further purified by centrifugation, and the clear supernatant was carefully collected. The extract was then stored in a

refrigerator at 4 °C for future use. This extract served as a source of bioactive compounds for subsequent analysis or experimentation.

Synthesis of Copper Oxide Nanoparticles

CuO nanoparticles were synthesized by dissolving 0.1 N copper sulfate ($CuSO_4$) in a beaker, heating to 60°C, and stirring using a magnetic stirrer. The prepared leaf extract was added dropwise to the solution in a basic medium, resulting in a color change from blue to green over 45 minutes, indicating the reduction of Cu^{2+} to CuO. After the reaction, the mixture was centrifuged at 5000 rpm for 15 minutes to separate the nanoparticles. The resulting *TP*CuO NPs were calcined at 500°C to improve crystallinity and remove organic residues. The cooled CuO nanopowder was then packed for further characterization and experimental studies.[20]

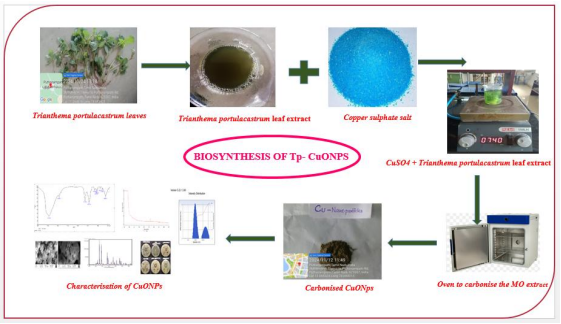

Fig.-2: Biosynthesis of *Trianthema portulacastrum*-CuONPs

Phytochemical Analysis

Phytochemical examination of primary and secondary metabolites in medicinal plants employs a variety of conventional techniques. Fehling's carbohydrate test was carried out by mixing equal amounts of leaf extract with Fehling's A and B reagents, then heating for 3-5 minutes until a red precipitate appeared, indicating the presence of reducing sugars.[21] The iodine test for starch entailed combining 2 mL of *Trianthema portulacastrum* leaf extract with iodine and potassium iodide, which resulted in a blue colour indicating starch. The Biuret test for proteins was performed by mixing 1 mL of leaf extract with 3% copper sulphate and 10% sodium hydroxide, resulting in the production of violet or red colour, demonstrating the presence of proteins or peptides.[22] Salkowski's test for steroids was used to detect secondary metabolites, in which *Trianthema portulacastrum* leaf extract was agitated with chloroform, and sulphuric acid was added, resulting in a crimson colour that indicated steroids.[23] Wagner's alkaloids test entailed combining the leaf extract with a solution of potassium iodide and iodine, which produced a precipitate that indicated the presence of alkaloids.[24]

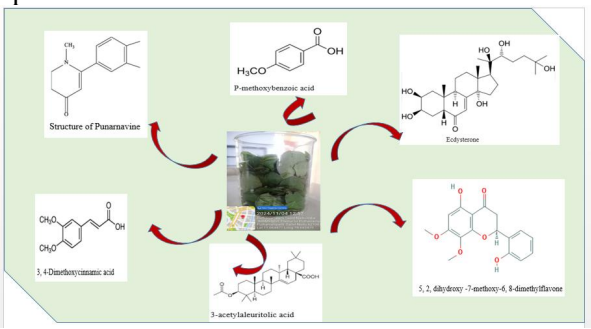

Fig.-3: Phytochemicals in *Trianthema portulacastrum*

RESULTS AND DISCUSSION

FTIR Spectrum

The FTIR spectrum of copper oxide nanoparticles displays (Fig.-4) distinct peaks that represent different functional groups. Indicating the presence of surface hydroxyl groups or water, the wide peak at 3437.46 cm^{-1} is correlated with O-H stretching. The stretching of C-H bonds and the bending of H-O-H bonds correspond to peaks at 2927.66 cm^{-1} and 1641.1 cm^{-1}, respectively, and suggest the presence of organic

residues and water, respectively. You may see C-O stretching at 1436.91 cm^{-1}, C-C vibrations at 1161.38 cm^{-1}, and C-C vibrations at 1028.88 cm^{-1}. Peaks at 778.25 cm^{-1} and 603.87 cm^{-1} validate the stretching of Cu-O bonds, which is a hallmark of copper oxide.

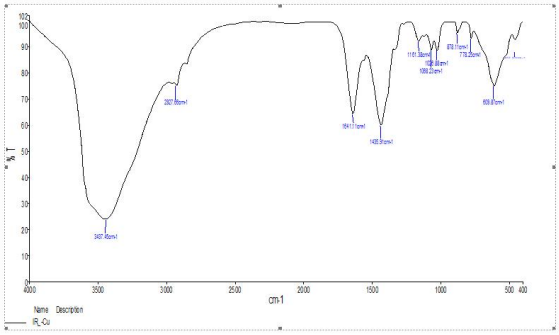

Fig.-4: FTIR Spectrum of *Tp*- CuO

UV-Vis Absorbance Spectrum

Copper oxide nanoparticles, specifically associated with the electronic transitions of Cu^{2+} ions, are indicated by the UV-Vis absorbance peak at 722.9 nm, which has an absorbance of 0.345 AU. Since copper oxide is a semiconductor, its surface plasmon resonance (SPR) or d-d transitions are what cause this peak to appear in the visible to near-infrared spectrum. Particle size, shape, and surface conditions can also affect the peak's intensity and location, which proves that the synthesis was effective and that the material is nanostructured (Fig.-5).

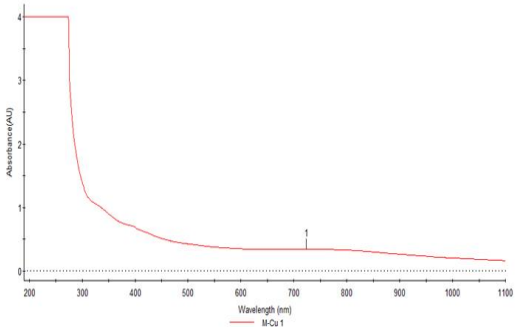

Fig.-5: UV-Visible Spectrum of *Tp*-CuONPs

Dynamic Light Scattering (DLS)

With a z-average diameter of 1059.8 nm and a high Poly Dispersion Index of 1.013, copper oxide nanoparticles aggregate and have a broad size dispersion under DLS analysis. The diffusion coefficient of 1.422×10^{-9} cm^2/s reflects the presence of large particles or aggregates (Fig.-6). The intensity distribution spans from 154.8 nm to 4,800 nm, with 52% cumulative intensity, showing that larger particles dominate the sample which highlights the significant aggregation.

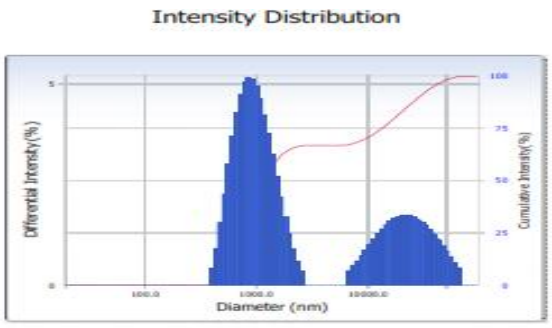

Fig.-6: DLS of *T*p-CuONPs

Morphological Features of CuO Nanoparticles

The surface morphology of *Tp*- CuO nanoparticles was analyzed using SEM and is shown in Fig.-7. SEM shows spherical nanoparticles with slight agglomeration.[25] Electrostatic and polarity interactions between CuO nanoparticles, which are affected by salt precursor concentration, may cause this aggregation. The particle size of the CuO nanoparticles, as observed in the SEM image, ranges from 18 to 30 nm, which is in good agreement with the XRD data.

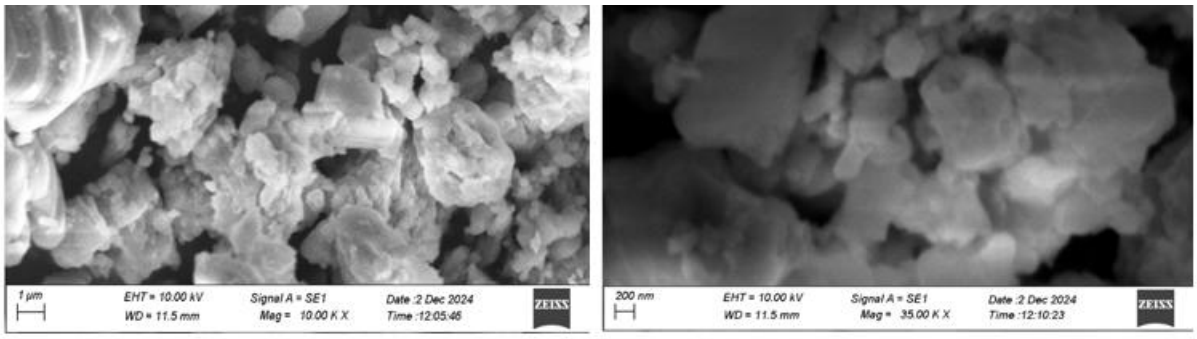

Fig.-7: SEM Images of *Tp*- CuO NPs at 1µm and 200 nm

XRD

The X-ray diffraction (XRD) data for copper nanoparticles shown in the Fig.-8 reveal the presence of multiple phases: metallic copper (Cu), copper (I) oxide (Cu_2O), and cuprous chloride. The peak at 43.27° (d ≈ 2.089 Å) corresponds to the (111) plane of face-centered cubic (fcc) metallic copper, as per JCPDS card No. 04-0836. The peak at 35.56° (d ≈ 2.52496 Å) is indicative of the (110) plane of cubic Cu_2O. The peak at 28.52° (d ≈ 3.1298 Å) may correspond to the (111) plane of CuCl, suggesting the presence of residual copper chloride in the sample.[26] These findings indicate successful synthesis of copper nanoparticles, with partial oxidation to Cu_2O and possible residual CuCl.

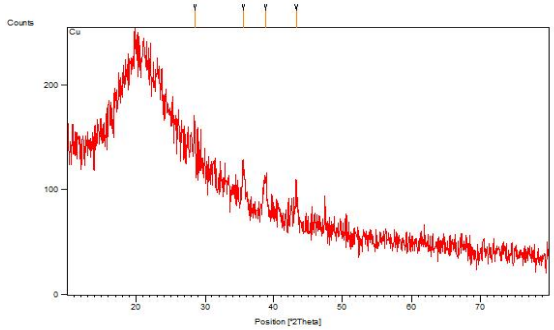

Fig.-8: Characterization by X-ray Diffraction

Antimicrobial Assay

Copper oxide nanoparticles (CuO NPs) were tested for their antibacterial and antifungal activities using the well-diffusion method, as shown in Fig.-9.

The CuO NPs were synthesised using *Trianthema portulacastrum* leaf extract. In the antibacterial test, CuO NPs were observed to be effective against gram-negative *Escherichia coli*, gram-positive *Pneumococci*, and *Mycobacterium tuberculosis*. Compared to the control gentamicin, CuO NPs displayed bigger zones of inhibition for *E. coli, Salmonella*, and *M. tuberculosis*, suggesting that these gram-negative and gram-positive bacteria were more effectively killed. The zone of inhibition is caused by the fact that CuO NPs can enter bacterial cells and interact with substances within them, leading to oxidative stress and a disruption of cellular processes. Gram-negative bacteria may be more susceptible to CuO NPs due to their distinct cell walls.[27]

This was especially true when testing against the gram-negative bacteria Escherichia coli and Salmonella, which showed higher inhibition. On the other hand, when it came to the antifungal test, CuO NPs showed modest activity against *Candida albicans* and *Fusarium*, with inhibition zones of 19 mm and 21 mm,

respectively. Gentamicin, on the other hand, showed much greater inhibition zones of 25 mm and 23 mm, respectively.

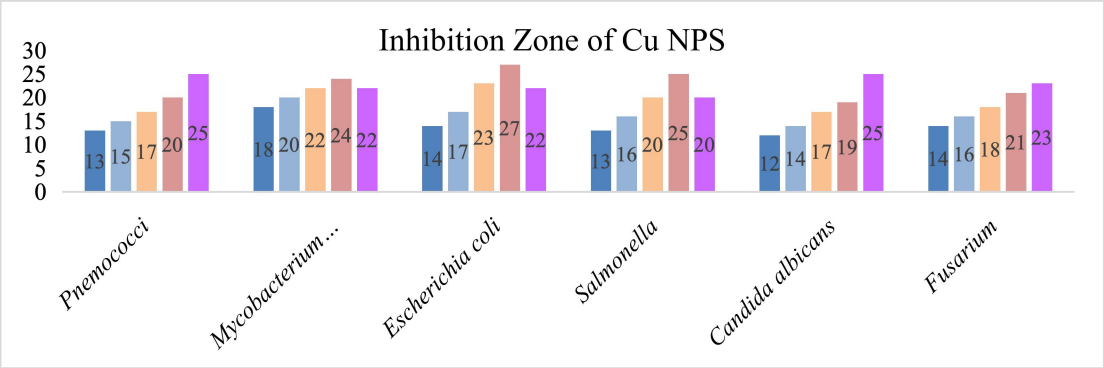

Fig.-9: Bar Chart of Inhibition Zone of Different Microbes at Various Concentrations against the Standard Gentamicin Antibiotic

These findings suggest that CuO NPs synthesized from *T. portulacastrum* leaf extract are more effective as antibacterial agents, particularly against gram-negative bacteria like *E. coli* and *Salmonella*, as well as gram-positive bacteria such as *M. tuberculosis*, while their antifungal activity is relatively less pronounced.

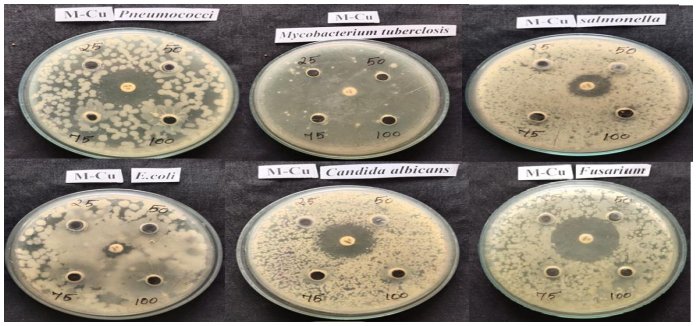

Fig.-10: Antibacterial Activity of the *Tp*- CuO at Different Concentrations

CONCLUSION

The biosynthesized CuO nanoparticles exhibited nanoscale features, as confirmed by FTIR and SEM analyses, which revealed characteristic Cu-O stretching and particle sizes between 18 and 30 nm. The UV-Vis spectrum verified successful synthesis with a peak at 722.9 nm, indicating a nanostructured material. Dynamic Light Scattering (DLS) results showed high polydispersity, suggesting nanoparticle aggregation, which may influence certain applications. Nanoparticles showed exceptional antibacterial action, notably against gram-negative bacteria like E. coli and Salmonella, with inhibition zones wider than traditional antibiotics, suggesting they could be a better antibacterial product. Conversely, their antifungal activity was modest, with inhibition zones of 19 mm and 21 mm for *Candida albicans* and *Fusarium*, respectively. These findings highlight the significant antibacterial potential of CuO NPs while suggesting the need for further investigation into their antifungal capabilities and broader applications in antimicrobial and environmental domains.

ACKNOWLEDGMENTS

Authors are grateful to the Management and the students of their institutions for their support.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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