Green Synthesis of Copper Oxide Nanoparticles Using Prosopis cineraria Extract: Evaluation of Cytotoxic Antioxidant and Antimicrobial Activity

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Research Article

Keywords: Copper nanoparticles (CuONp), Green synthesis, Prosopis cineraria, Antioxidant activity, Antibacterial activity, Cytotoxicity

Posted Date: July 15th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7092059/v1

Additional Declarations: No competing interests reported.

Abstract

Copper nanoparticles (CuONp) have garnered considerable attention due to their unique thermodynamic properties and broad bioactivities, including antioxidant, antibacterial, and cytotoxic effects. The green synthesis of CuONp, which employs natural plant extracts as reducing and stabilizing agents, is an eco-friendly and sustainable alternative to conventional methods. This study investigates the synthesis of CuONp using an aqueous extract of *Prosopis cineraria* and evaluates their biological activities. CuONp were synthesized by mixing *P. cineraria* extract with copper nitrate under magnetic stirring to promote nanoparticle formation. The synthesis was validated using UV-visible spectroscopy, revealing characteristic absorption peaks indicative of CuONp formation. Antioxidant activity was assessed using the DPPH assay, while antibacterial activity was tested against two bacterial strains. Cytotoxicity was evaluated via a brine shrimp lethality assay, measuring nauplii mortality at different CuONp concentrations. UV-visible spectroscopy confirmed the successful synthesis of CuONp. The DPPH assay showed dose-dependent free radical scavenging, with maximum activity at the highest concentration. Antibacterial testing demonstrated significant inhibition zones, indicating strong activity against both bacterial strains. The cytotoxicity assay revealed dose-dependent nauplii mortality, underscoring the cytotoxic potential of CuONp. Green-synthesized CuONp using *P. cineraria* exhibit strong antioxidant, antibacterial, and cytotoxic properties, highlighting their potential for clinical and therapeutic applications. This sustainable synthesis approach harnesses the bioactive potential of *P. cineraria* for nanoparticle production.

1. Introduction

Nanotechnology has revolutionized the field of science and technology by offering innovative solutions across multiple disciplines, including medicine, agriculture, and environmental sciences[1]. Among the various nanoparticles studied, copper nanoparticles (CuONp) have received considerable interest because of their unique qualities, such as high catalytic activity, conductivity, and antimicrobial efficacy [2]. Recently, the focus has shifted towards the green synthesis of nanoparticles, as conventional chemical and physical methods often involve toxic chemicals, high energy consumption, and expensive equipment [3]. Green synthesis, leveraging natural sources such as plant extracts, microorganisms, and biomolecules, provides an eco-friendly and sustainable approach [4, 5]. Plants, in particular, are an excellent resource for synthesizing nanoparticles, as they contain a diverse array of phytochemicals such as alkaloids, flavonoids, phenols, and tannins [6]. These compounds not only reduce metal ions to their nanoparticle form but also stabilize the nanoparticles, imparting additional bioactivity [7]. *Prosopsis cineraria* holds immense significance in traditional medicine and is renowned for its pharmacological characteristics, comprising anti-oxidants, antiviral, anti-fungal, and therapeutic activity[8, 9]. The phytochemical composition of *Prosopis cineraria* includes flavonoids, tannins, alkaloids, and phenolic acids, which make it an ideal candidate for the green synthesis of nanoparticles [10]. Utilizing this plant for synthesizing CuONp combines the inherent bioactivity of the plant with the enhanced properties of nanoparticles, paving the way for innovative applications in health and environmental sciences [11]. The biological activities of CuONp, such as antioxidant, antimicrobial, and cytotoxic effects, have been widely explored [12]. Antioxidant activity is an essential characteristic, because the inflammation induced by reactive oxygen species (ROS) is linked to ageing and the development of many illnesses, especially malignancy, neurological diseases, and circulatory maladies [13, 14]. CuONp, by their redox potential and the presence of surface-active groups, can effectively scavenge ROS and reduce oxidative damage [15]. Similarly, the antimicrobial properties of CuONp have demonstrated broad-spectrum efficacy against bacteria, fungi, and viruses [16]. Copper ions break membranes in microbes, impede cellular respiration, and produce reactive oxygen species, resulting in microbes' cell mortality [17]. These properties are especially relevant in combating antimicrobial resistance (AMR), which poses a global health challenge, and in food preservation, where microbial contamination is a major concern [18, 19]. Furthermore, the cytotoxic potential of CuONp against cancer cells offers promising applications in oncology [20]. CuONp induce cytotoxicity through mechanisms such as ROS generation, DNA damage, and disruption of mitochondrial function, selectively targeting cancer cells while sparing normal tissues [21]. This dual action of CuONp, combining therapeutic and diagnostic capabilities, highlights their potential as nanomedicine agents [11, 22]. The antioxidant activity was assessed using standard radical scavenging assays to determine their efficacy in neutralizing oxidative stress [23, 24]. The ability to inhibit microbes was evaluated towards several harmful microorganisms to investigate how they might help microbial resistance [25]. The cytotoxic activity was analyzed in vitro against specific cancer cell lines to evaluate their therapeutic applicability [14, 26]. The present investigation intends to add to the expanding body of research on green-synthesized nanoparticles in emphasising the beneficial effects of plant-mediated CuONp [27].

2. Materials and Methods

Extract preparation

In the current state investigation, 1gm of *Prosopsis cineraria* was dissolved in a hundred ml of purified water and boiled for ten to fifteen minutes at 70 degrees Celsius. After heating up, Whatman filtered the plant extract. No.1 filter paper [28]. In a 250 ml conical flask, 70 ml of 20 millimolar copper nitrate was prepared, and 30 ml of filtered vegetal extract was added and put into an iron magnetic stirrer to produce nanoparticles. After the synthesis of nanoparticles, a colour change was seen [29].

Antioxidant assay

DPPH method

The DPPH method was applied to study the protective characteristics of naturally occurring synthesised particles of copper. Copper nitrate nanomaterials have been added with *Prosopsis cineraria* herb extract (2–10 μg/ml) in 1 ml of 0.1 mm DPPH in methanol and 450 μl of 50 mm Tris HCl buffer (pH 7.4) for ten minutes. Afterwards, the drop of DPPH free radicals was measured using the intensity of absorption at 517 nm. BHT was used as control. [30]. The following equation determined the level of inhibition,

The percentage of inhibition = absorbance of control minus absorbance of test sample divided by 100.

H_2O_2 Assay

The potential of the biosynthesized tocopherol chitosan biocomposite to scavenge H_2O_2 was evaluated in 40 mM H_2O_2 solution in a pH 7.4 phosphate buffer. 0.6 mL of H_2O_2 solution was mixed with a solution of the test sample (-tocopherol chitosan biocomposite) and a standard sample of ascorbic acid at different concentrations (10–50μg/mL). Spectrophotometric measurements of the reaction solution absorbance at 532 nm were made following a 10-minute incubation period in a dark environment. The standard used was vitamin C. Using the following formula, the proportion of H_2O_2 scavenging activity was calculated

To calculate the percentage of inhibition, divide the absorbance of the control by the absorbance of the sample and multiply by 100.

Antimicrobial Assay

To manufacture Mueller-Hinton agar, 10 µL of fresh microbial cultures (*C. albicans, E. faeclalis, E. coli, Pseudomonas*, and *S. aureus*) were injected in a sterile Hi-veg media. A sanitized polystyrene tip was used to make 5 mm wells. Antimicrobial effect was assessed to determine the effectiveness of *Prosopsis cineraria*-mediated CuONp and biosynthesized copper Particle-mediated nanocomposite material. Wells were filled with varying amounts of three samples (25, 50, and 100 µL) and a conventional test. The specimens of Petri plates and zone inhibition were kept in an incubator at 37 degrees Celsius for 24 hours. The area was evaluated in order to contrast and examine the possible impact of *Prosopsis cineraria*-mediated copper nanoparticles [31].

Cytotoxicity assay

Brine shrimp eggs were placed in a hatching chamber filled with salty water. After 24 hours, precisely ten hatched larvae (nauplii) were floating in six wells, each holding 10 ml of saltwater. Nanoparticles were scattered in each well at varying quantities (5 µL, 10 µL, 20 µL, 40 µL, and 80 µL), with the very last well serving as a control (no nanoparticles). After 24 hours, the number of remaining nauplii was calculated and noted.

3. Results

Visual Observation and UV-Vis Studies for Copper Oxide NPs

The development of copper oxide NPs was tracked using a UV-visible spectrophotometer. *Prosopsis cineraria* leaf extract functions as a stabilizing and diminutive agent. Biological molecules in the leaf extract reduce copper nitrate nanoparticles to copper oxide nanoparticles. The color changes from reddish brown to dark reddish brown after adding leaf extract, and then progressively gets dark brown due to the synthesis of CuONp. (Fig. 1) depicts the color variations that occur during nanoparticle production with CuONp. UV-visible data were taken over a period of 0–84 hours. Copper nanoparticles' resonance with the surface exhibited the highest peak at 420 nm, as shown in Fig. 2).

FTIR Analysis

The FTIR spectrum of the sample labelled CuO + PC (Copper oxide nanoparticles synthesised using *Prosopsis cineraria* extract) offers useful insight into the functional compounds present in the sample, revealing the importance of plant phytochemicals in the green synthesis procedure. A large absorption peak at 3185.8 cm^1 corresponds to O-H stretching vibrations, which are indicative of hydroxyl groups from alcohols or phenolic compounds. This suggests the participation of plant metabolites like flavonoids or polyols in stabilising the nanoparticles. The signal at 2960.3 cm^1 is due to C-H stretching vibrations of aliphatic $-CH_2$ and $-CH_3$ groups, suggesting the presence of organic chemicals in the plant extract.

A peak at 1557.5 cm^1 may be due to N-H bending oscillations from amide connections or C = C stretching of aromatic rings, which are typically found in proteins or polyphenolic structures. The band at 1385.8 cm^1 may be attributed to C-N stretching or O-H bending, indicating the existence of biomolecules with amine or phenol groups. The absorption at 1034.7 cm-^{1} corresponds to C-O stretching vibrations of alcohols, ethers, or esters, suggesting the presence of plant-derived substances such as carbohydrates and flavonoids in the capping material.

The peaks at 813.58 cm^{-1} and 678.32 cm^{-1} show out-of-plane bending vibrations of aromatic C-H bonds, similar to those found in plant phenols. The high absorption bands at 613.3 cm^{-1} and 465.03 cm^{-1} show the production of copper oxide nanoparticles. These data combined show that *prosopsis cineraria* extract functions as a reducing and capping agent in the green production of CuONp, and the functional groups observed in the FTIR spectrum play an important role in nanoparticle stabilisation and bioactivity.

XRD Analysis

The X-ray diffraction (XRD) image depicts copper oxide nanoparticles (CuONp) synthesised utilising Prosopis cineraria. The prominent diffraction peaks at specific 2θ values can be indexed to crystalline planes such as (021), (121), (-213), (-204), (-214), (-245), (-107), (018), and (-261), which match the monoclinic phase of CuONP. These peaks are consistent with the JCPDS card No. 00-042-1959, demonstrating the creation of pure, crystalline monoclinic CuONp. The strong and powerful patterns show great crystallinity, and the lack of further patterns shows that the synthesised nanoparticles are pure, without no other copper phases or contaminants[32, 33].

SEM Analysis

A scanning electron microscope (SEM) image displaying the surface morphology of copper oxide nanoparticles (CuONp). The 30,000× magnification shows a granular structure with irregular particle shapes and distribution. These particles appear to be closely packed, generating clusters of high surface roughness. The shape indicates an enormous surface area, which is useful for catalytic, antibacterial, or cytotoxic applications. The dimensions of the bar represents a length of 300 nm, emphasising the nanoscale size of the particles. This shape is prevalent in biosynthesised nanoparticles and shows the effective generation of CuONp with improved reactivity and surface contact, which are important for biological and environmental purposes.

A energy dispersive x-ray spectroscopy (EDX or EDS) spectrum is utilised for identifying the constituent makeup of a material. This spectrum shows particular peaks for copper (Cu) and oxygen (O), demonstrating the existence of both of these substances in the sample. The strong peaks for copper indicate it is the major component, whereas the considerable peak at roughly 0.5 keV for oxygen verifies its relationship, proposing the creation of CuONp. The lack of any major peaks shows that the synthesised substance is highly pure, with negligible pollutants from other components. This EDX study confirms the effective creation of CuONp, which are predicted to contain the desired chemical and functional properties for applications in biomedicine, chemical reaction, and cleaning up the environment.

Antioxidant assay

DPPH method

CuONp synthesized from *Prosopsis cineraria* showed inhibition rates of 58.65% for 10μL, 69.63% for 20μL, 79.21% for 30μL, 82.5% for 40μL, and 84.1% for 50μL. The standard's inhibition percentages were 76.56% for 10μL, 78.52% for 20μL, 85.63% for 30μL, 88.68% for 40μL, and 90.15% for 50μL. The maximum inhibition was seen at 50μL, indicating a higher concentration. The nanoparticles of copper derived from Prosopis cineraria have significant antioxidant effects and are equivalent to the reference.

H_2O_2 Assay

Copper nanoparticles synthesized from *Prosopsis cineraria* showed inhibition rates of 45.6% at 10μL, 50.4% at 20μL, 60.5% at 30μL, 70.8% at 40μL, and 82.54% at 50μL. The standard had a percentage of inhibition ranging from 51.2% for 10μL to 88.6% for 50μL. Maximum inhibition was found at 50μL, indicating a higher concentration. Copper nanoparticles derived from *Prosopsis cineraria* exhibit high antioxidant activity and are similar to the standard.

Antimicrobial Assay

Copper nanocomposites antibacterial activity was assessed using an agar well diffusion assay. The antimicrobial effectiveness of *Prosopsis cineraria*-mediated CuONp nanocomposite was visualized against five clinical pathogens such as *C. albicans*, *E. faeclalis*, *E. coli*, *Pseudomonas*, and *S. aureus*, as represented in. A standard control was employed, which was *Prosopsis cineraria* leaf extract. *Staphylococcus aureus*, a gram-positive bacterium, and the opportunistic pathogenic yeast *C. albicans* showed a high inhibition zone. The gram-negative bacteria *E. faecalis* was shown to have a minimal inhibitory zone.

Cytotoxicity assay

Demonstrates the cytotoxicity at various conc (5–80 µL). Observations were taken over three days at concentrations of 5 µL, 10 µL, 20 µL, 40 µL, and 80 µL. Control Without CuONp, 10% cell death is seen. This reflects either baseline mortality from the experimental setting or spontaneous cell death. At 5µL, mortality reduces to 7%. Mortality stabilizes at 5% at doses ranging from 10µL to 40µL. At 80µL, the mortality rate drops to 4%. This might imply that CuONp do not cause considerable cytotoxicity at the studied doses, and may even reduce cell death when compared to the control.

4. Discussion

Multiple characterisation techniques were used to evaluate the green synthesis of copper oxide nanoparticles (CuONp) from Prosopis cineraria extract of the leaf. The UV-Vis examination showed nanoparticle creation by a prominent plasmon resonance surface peak at 420 nm, followed by visible colour modifications, showing gradual diminution in copper ions[34]. FTIR examination showed the existence of functional groups as O-H, N-H, and C-O, indicating the participation of plant-based compounds in both diminution and capping of CuONp[35]. XRD spectra revealed an amorphous character with minimal crystalline peaks, which is regular of biosynthesised nanoparticles[36]. SEM pictures proved irregular, nanosized particles with significant surface area, while EDX data validated elemental purity, demonstrating the effective synthesis of CuONp[37]. Antioxidant experiments (DPPH and H_2O_2) showed significant dose-related free radical scavenging activity, equivalent to conventional antioxidants. Antimicrobial investigations revealed substantial suppression of *S. aureus and C. albicans*, demonstrating excellent antibacterial and antifungal action[38]. Cytotoxicity experiments demonstrated low toxicity, with fewer fatalities in nauplii even at greater dosages. These results emphasise the variety of uses and biological safety of the synthesised CuONp[39]. Overall, the work confirms the eco-friendliness and biological potential of P. cineraria-mediated CuONp.

5. Conclusion

The work effectively demonstrated the green manufacturing of copper oxide nanoparticles utilising Prosopis cineraria leaf extract, an efficient and environmentally benign strategy. UV-Vis spectroscopy, FTIR, XRD, SEM, and EDX all verify the generation, shape, arrangements, and functionalisation of CuONp. The plant-derived compounds in the extract exert an important role in decreasing and stabilising the nanoparticles, leading to primarily amorphous, nanoscale particles with outstanding surface features. CuONp show significant antioxidant capacity in DPPH and H_2O_2 tests, showing their potential to neutralise free radicals. In addition, they exhibit promise antibacterial effectiveness, notably towards gram-positive bacteria and fungal diseases. The cytotoxicity investigation reveals that they are biocompatible at all investigated levels. These findings demonstrate that the synthesised nanoparticles are secure as well as successful for biological and environmental purposes. This green synthesis approach is a useful complement to established chemical pathways, delivering a low-cost, non-toxic, and sustainable choice for nanoparticle creation.

Declarations

Funding

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics approval and consent to participate

All the data was available from public databases and there is no need for ethics approval and consent.

Credit authorship contribution Statement:

RV: Writing-Review & editing, Writing Original Draft, Methodology, Data curation, Visualization, Drew the figures and pictures, Conceptualization. BP: Helped in writing the manuscript and revised it. RS: Validation and editing with review of the manuscript. SJ: Designed protocols for all the experiments, supervised, proofread, analyzed the data, and revised the reviewer's comments, investigation, and Conceptualization. All authors reviewed and discussed the results, provided comments on the manuscript, and approved its final published version.

Consent for publication

Not applicable.

Conflict of interest

The authors state no conflict of interest

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Figures

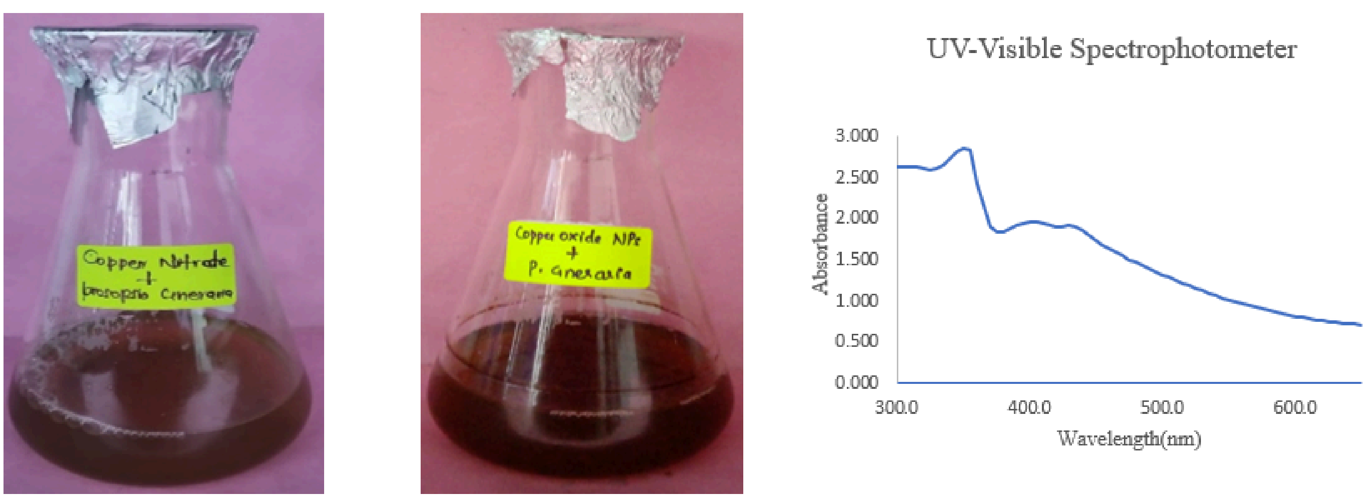

Figure 1

Copper oxide nanoparticles synthesis from copper nitrate with *prosopis cinerar*ia plant extractUV-Vis spectrum showing the absorbance of synthesized nanoparticles, with a distinct peak indicating successful synthesis.

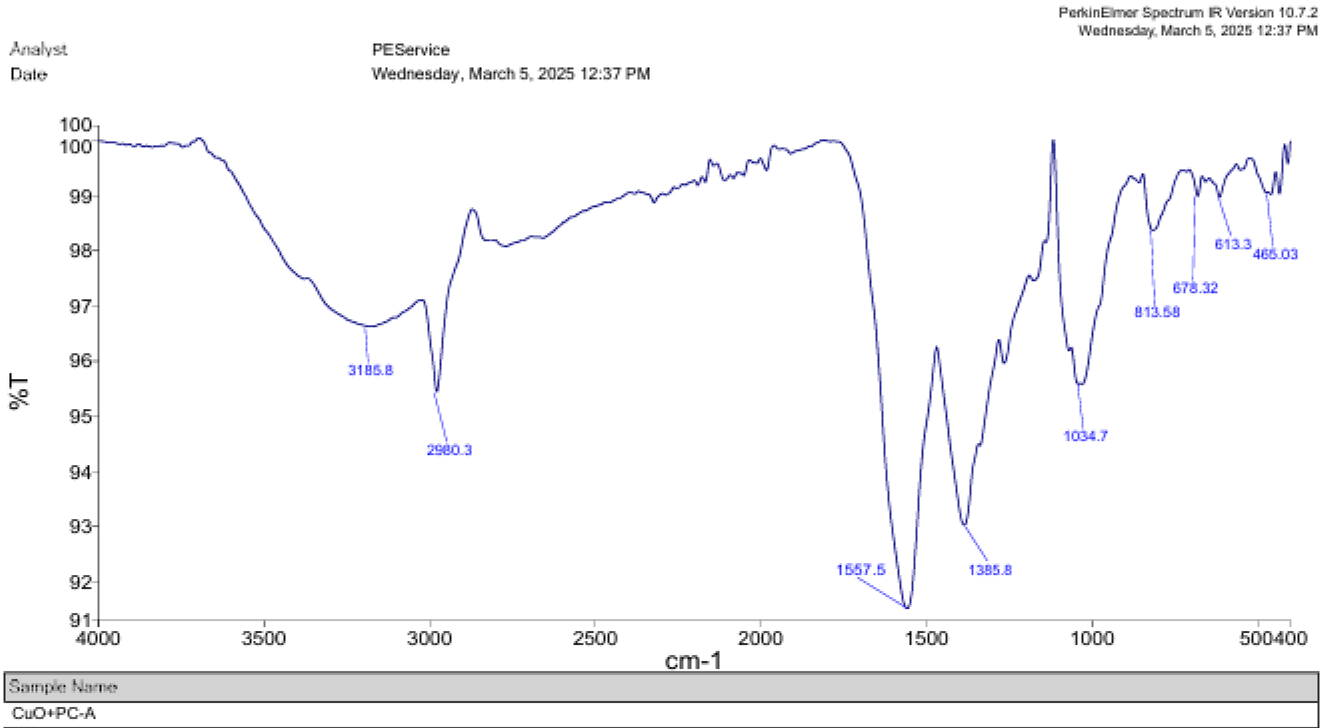

Figure 2

FTIR spectrum of NPS at different concentrations

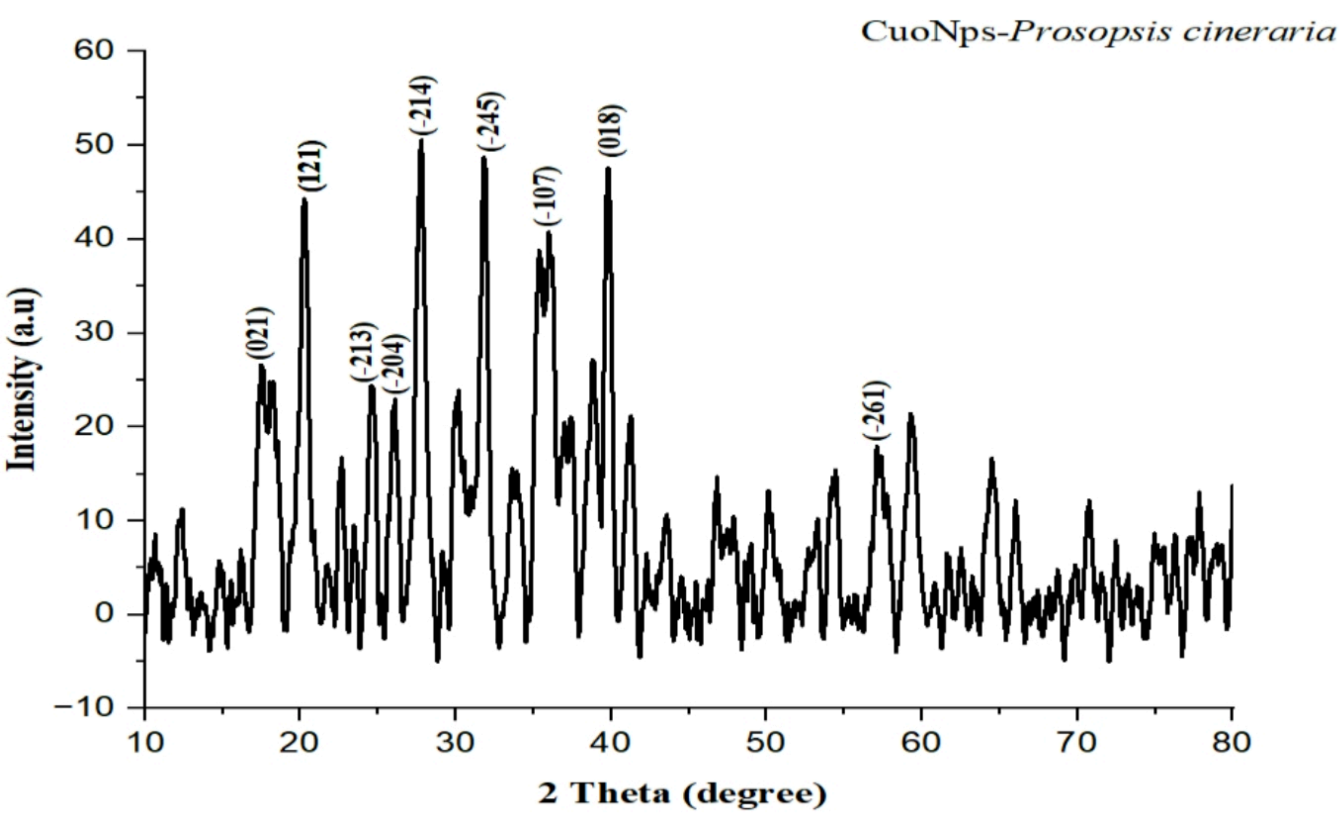

Figure 3

XRD pattern of copper oxide nanoparticles synthesized using *prosopsis cineraria*

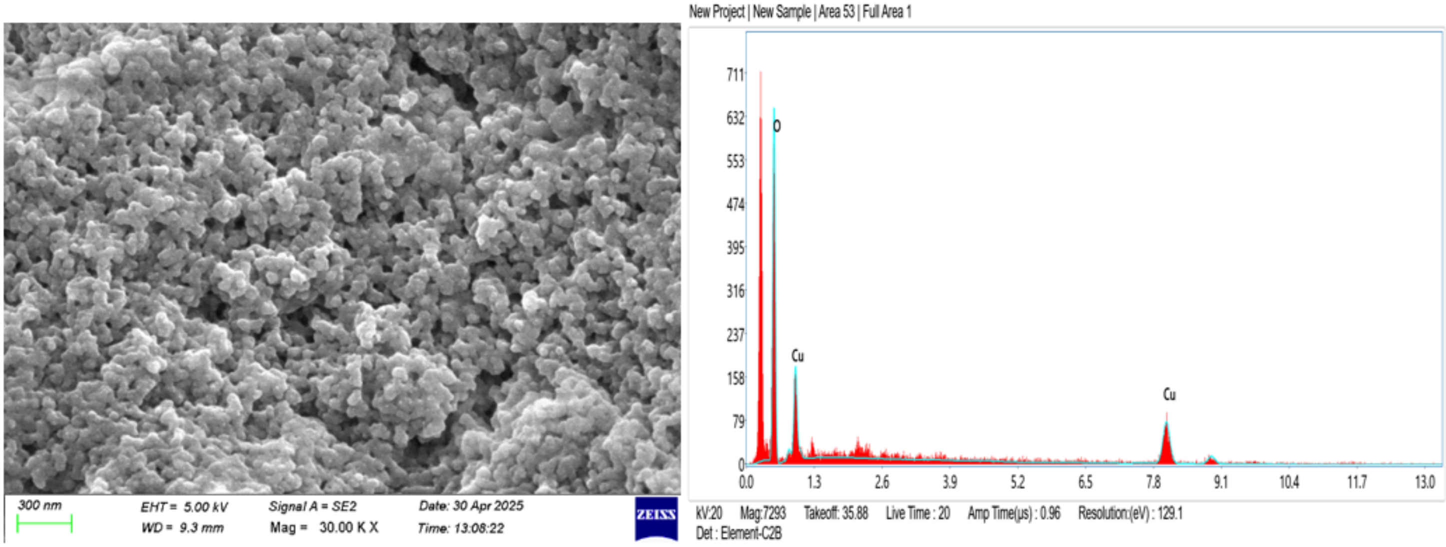

Figure 4

SEM images of NPs synthesized using *prosopsis cineraria*

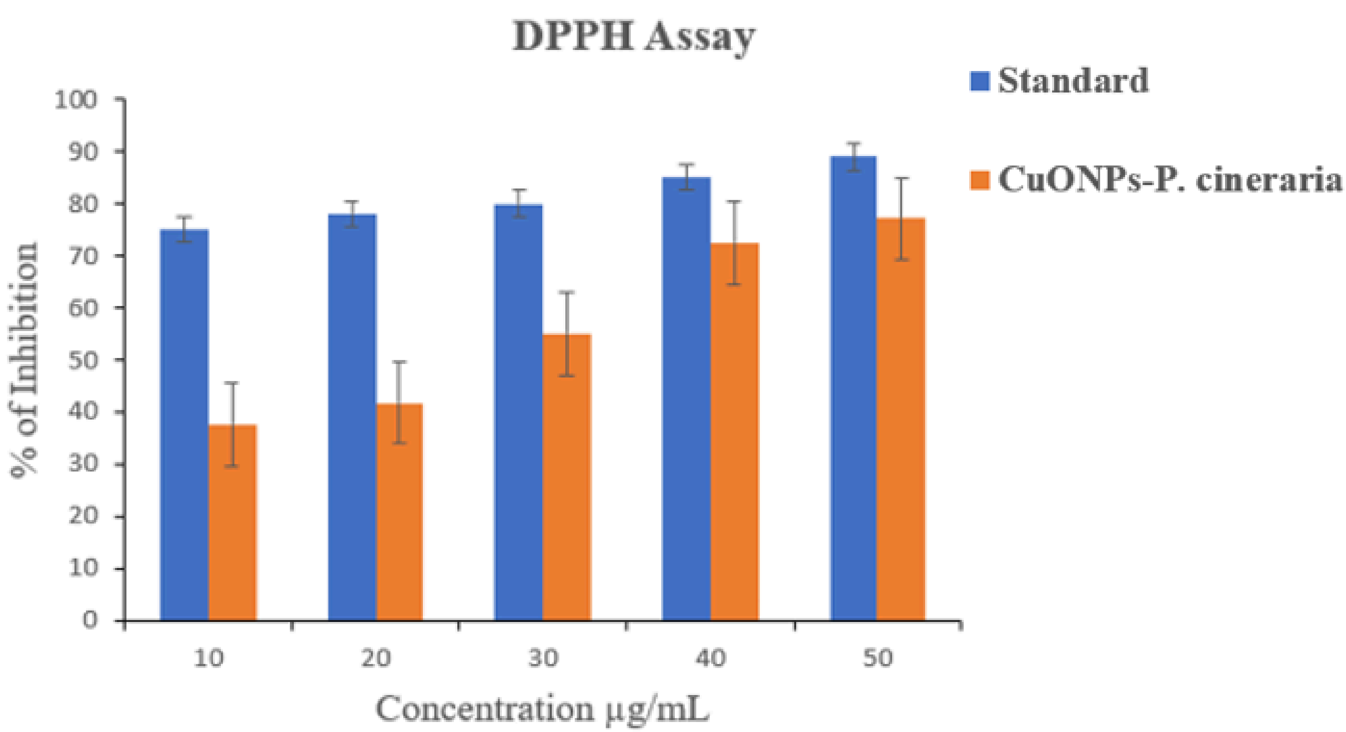

Figure 5

Antioxidant activity of copper oxide nanoparticles synthesized using *prosopsis cineraria*using the DPPH assay

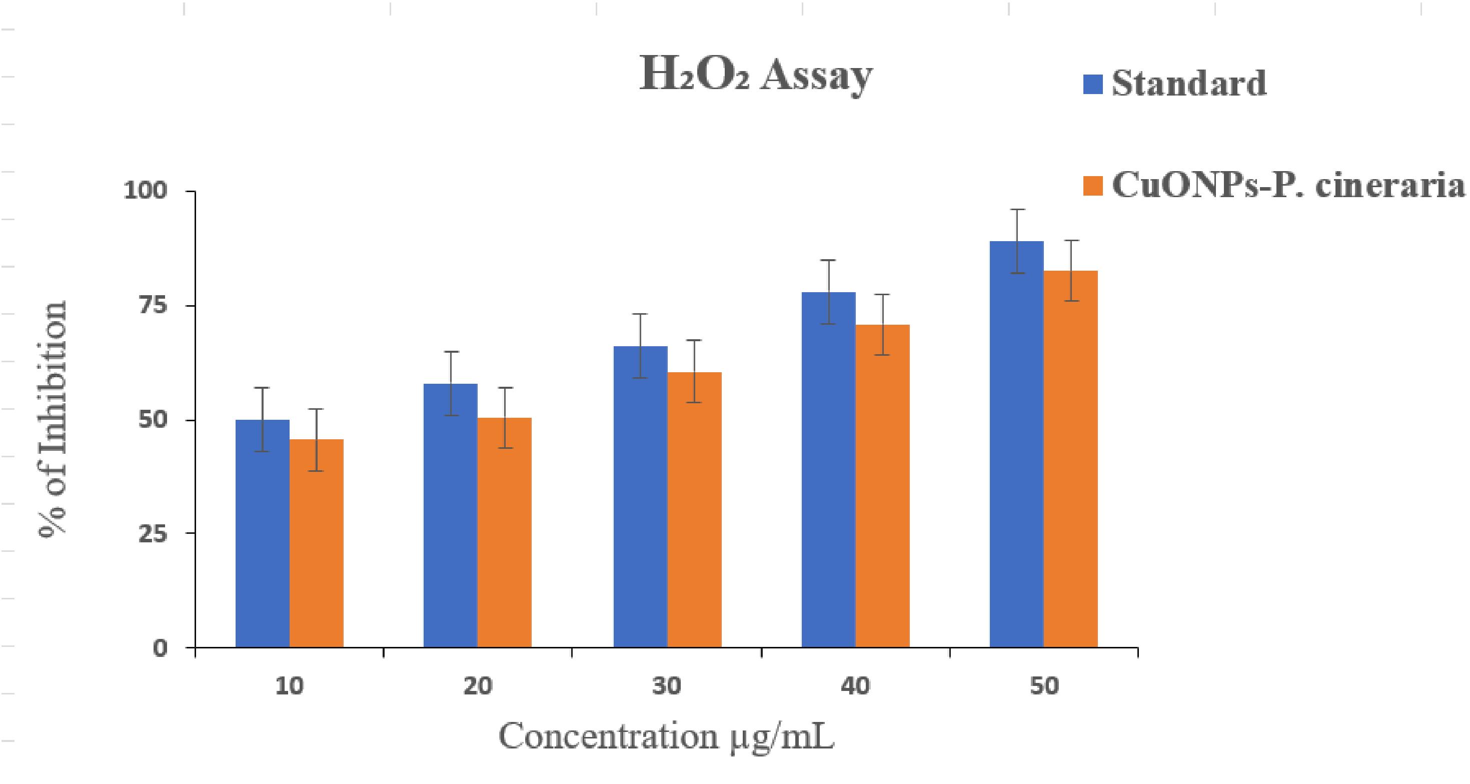

Figure 6

Antioxidant activity of copper oxide NPs synthesized using *prosopsis cineraria* using the Hydrogen peroxide assay

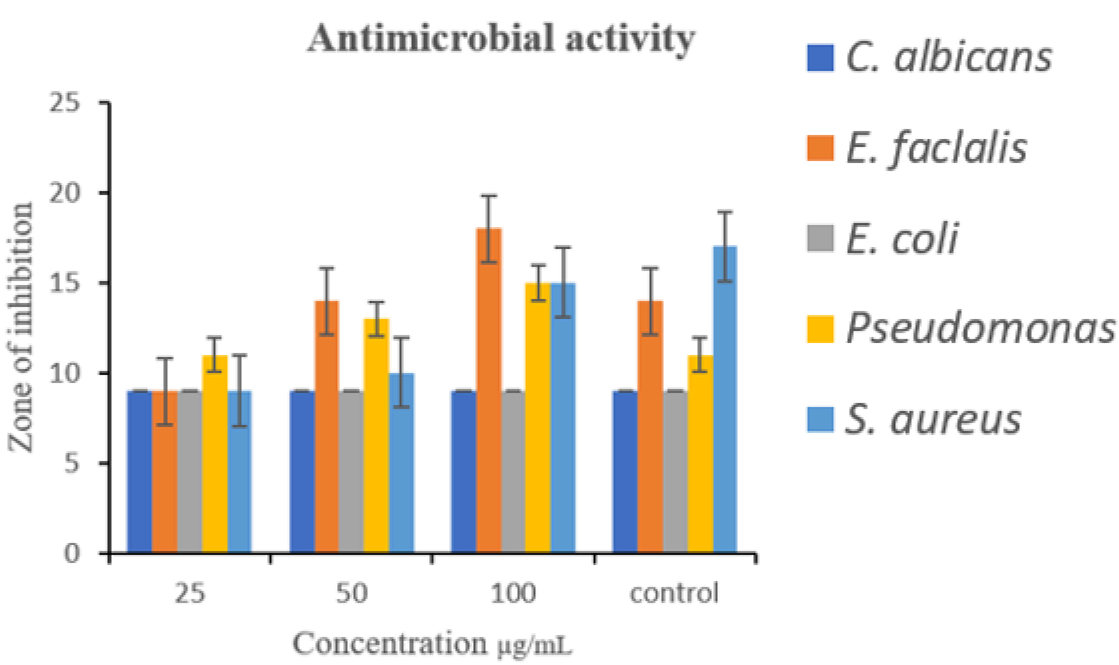

Figure 7

Anti-microbial activity of copper nanoparticles synthesized using agar well diffusion assay

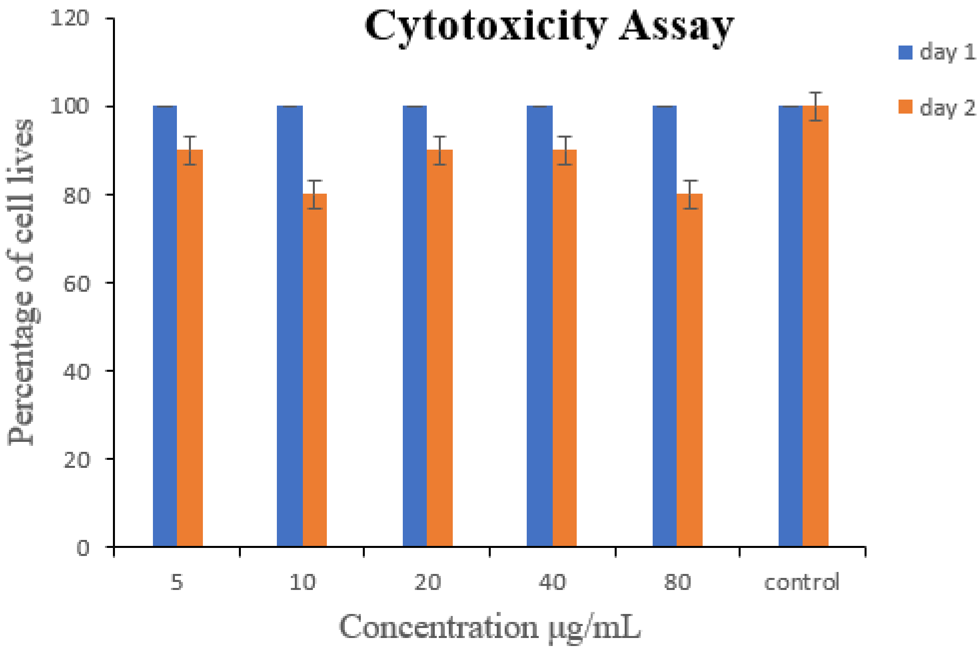

Figure 8

Cytotoxicity assay demonstrating the mortality of the nauplii at various concentrations.

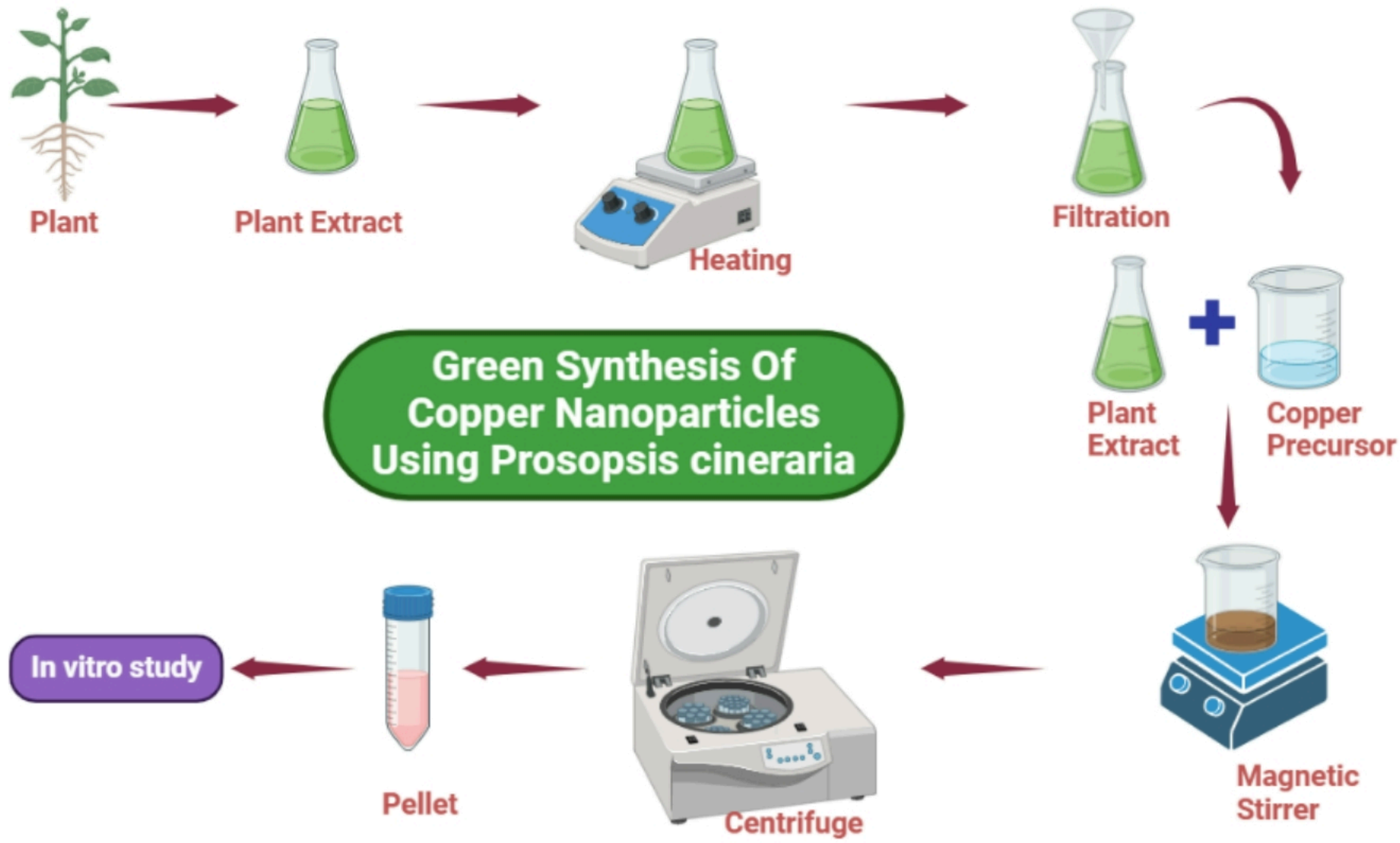

Figure 9

Unnumbered image in the Materials and Methods section.