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Biogenic Synthesis Copper Nanoparticles Using *Azadirachta indica* Extract Characterization Potential Applications

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ABSTRACT

Nanotechnology, which explores materials within the 1-100 nm size range, has attracted significant interest due to the unique physiochemical properties of nanoparticles compared to their bulk counterparts. Among different synthesis approaches, green synthesis provides an environmentally benign, sustainable, and cost-effective alternatives to conventional physical and chemical methods. In this study, copper nanoparticles (CuNPs) were synthesized at room temperature using *Azadirachta indica* (neem) leaf extract as a natural reducing and stabilizing agent, with copper sulfate (CuSO4) serving as the precursor. The phytochemicals present in neem extract facilitated nanoparticle formation while eliminating the need for hazardous chemicals, thus aligning with the principles of green chemistry. The synthesized CuNPs were characterized using UV-Vis. Spectroscopy to confirm their optical properties, and their antimicrobial activity was evaluated. The findings highlight that neem-mediated synthesis offers a simple, eco-friendly, and efficient route for producing copper nanoparticles, with promising applications in biomedical, environmental, and industrial domains.

Keywords: Copper nanoparticles; Green synthesis; *Azadirachta indica*; Neem; Antimicrobial activity; Nanotechnology; Eco-friendly synthesis.

INTRODUCTION

New technologies often create new challenges to science in addition to their benefits, raise concerns about health and various environmental problems. Recent nanotechnology holds a promise and a broad aspect towards wide applications of nanoparticles in a multiple way of emerging fields of science and technology [1]. The recent few years have observed an expanding growth of interest in different areas like nanomedicine, nanomaterials ,nanoscience and foremost important remarkable growth is observed in nanotechnology [2]. The vast applications of nanoparticles in medical sciences are drug delivery, imaging and diagnosis. Nanoparticles possess high surface to volume ratio due to its small size, which gives very distinctive features to nanoparticles The materials that are formed with the nanotechnology ranges between one to several hundreds of nanometres (1nm = 10-9m). Over the preceding years nanoparticles are key of interest of research because of their various characteristics such as physical, magnetic, chemical, electronic, electrical, optical, mechanical, thermal, dielectric, and biological properties [3].

Properties of nanoparticles

1. Electronic properties

The electronic structure of nanoparticles may be considered to bridge between the discreet levels of an atom and the continuous band structure of a bulk solid. When bulk metal is reduced in size to a few nanometers, the continuous density of states in the conduction band is replaced by a set of discreet energy levels which raises the band gap (the gap between valence band and conduction band. Eventually, a size is reached where the distance between the surfaces of the particles becomes of the order of the wavelength of the electrons. At this point, the energy levels of the nanoparticles may be treated similar to those of a particle in a box.

2. Optical properties

One of the most fascinating aspects of metal nanoparticles is that they depend on the optical properties for their size and shape. Optical properties affect shape, size and colour of the nanoparticles such as bulk gold looks yellowish in reflected light, but thin gold films look blue in transmission. Change in size of nanoparticles also affect the colour of nanoparticles, as size decreases change in colour of nanoparticles starts. For example: Characteristic blue colour steadily changes to orange, through several tones of purple and red, as the particle size is reduced down to ~3 nm. The colour which nanoparticles exhibit is due to the consistent excitation of the conduction band electrons induced by interaction with an electromagnetic field and the phenomena by which this change occur is known as Surface Plasmon Resonance [4].

3. Magnetic properties

Magnetic nanoparticles are desirables in this emerging field due to their hug domains of application, especially in high-density magnetic recording media or magnetically responsive fluids.

Importance of copper nanoparticles

- Copper nanoparticles possess specially characteristics as they antimicrobial activity against different strains of pathogens.
- Acts as an anti-inflammatory agent.
- Also used as copper diet supplements with efficient delivery characteristics.
- High strength metals and alloys.
- Heat sinks and highly thermal conductive materials
- Copper nanoparticles exhibited practical applications such as therapeutics, biosensors, and drug delivery agents.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents employed in the present study were of analytical reagent (AR) grade and were used as received without further purification. Copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$; Nice Chemicals Pvt. Ltd., India) served as the metal precursor for the green synthesis of copper nanoparticles (CuNPs). Nutrient Agar (NA) and Nutrient Broth (NB), procured from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), were used for the preparation of microbiological culture media. Distilled water, prepared in-house, was used as the solvent throughout all experimental procedures. All media were prepared according to the manufacturer's specifications and sterilised prior to use. [5]

Biological materials

Plant materials

Fresh, mature leaves of *Azadirachta indica A. Juss*. (Neem; Family: Meliaceae) were collected from the campus of KCMT College, [Bareilly, Uttar pradesh, India]. Leaves were thoroughly washed under running tap water, followed by rinsing with double-distilled water to remove surface contaminants, and shade-dried at ambient temperature prior to extraction.

Bacterial strain

Escherichia coli was obtained from the Microbiology Laboratory, KCMT College, [Bareilly, UP, India].

Table 1. Glassware and laboratory consumables

Glassware and consumables	Apparatus / Equipments	
Measuring cylinders	1.weighing machine	11. UV-Vis spectrophotometer
Beakers	2. Micropipette	12. Raman spectrophotometer
Conical flasks	3. centrifuge machine	13. Scanning Electron Microscope (SEM)
Glass funnels	4. Hot plate	14. Vortex mixer
Petri plates	5. Magnetic stirrer with	15. Water bath

	heating	
Whatman filter paper No. 1	6. Autoclave	
Eppendorf microcentrifuge tubes	7. Digital pH meter	
Microfuge tips	8. Laminar air flow cabinet (LAF)	
Quartz cuvettes	9. Refrigerator (4˚C)	
Glass slides and cover slips	10. Inoculation / streaking needle	

Method

The synthesis of copper nanoparticles was carried out using a green synthesis approach involving Azadirachta indica(neem) leaf extract. The procedure consisted of the following steps: [6]

Preparation of Plant extract

Azadirachta indica (neem) plant leaves were collected from KCMT College. The leaves were washed several times with water to remove the dust particles and then extract was prepared simply by boiling. 10 gm of fresh leaves were boiled in 100 ml distilled water at 1000C for 20 min. The extract was cooled to room temperature and filtered using filter paper (Whatman filter paper No. 1). Then the solution was incubated for 30 min. and then subjected to centrifuge for 10 min. at room temperature with 10000 rpm. The supernatant was separated from pellet and in a different flask supernatant was stored for further use, and then the solution was used for the reduction of Cu 2+ ions to copper nanoparticles.

Preparation of Copper sulphate solutions of difference concentrations

Different concentrations of copper sulphate solution were prepared (1mM, 3mM, 5mM) by dissolving 0.20 g,0.60 g, 1.0 g of $CuSO_4.5H_2O$ in 50 ml of distilled water. Weighed amount of copper salt was carefully transferred in a 50-ml volumetric flask and dissolved in a distilled water with the help of magnetic stirrer for thorough mixing. The solution was diluted as required and all the solutions were kept away from light and kept in dark.

Preparation of Copper nanoparticles by adding *Azadirachta Indica* leaf extract to copper sulphate (CuSO4) solutions

For the synthesis of the copper nanoparticles, equal volume of the *Azadirachta indica* leaf extract (5 ml) as well as5ml of copper sulphate solution was added together in a conical flask and the volume was adjusted to 10 ml. The reduction process of $cu^{2+}$to Cu^0 nanoparticles was followed by the colour change of the solution from green to light green followed by dark green after long incubation of time. The significant change in colour of solution indicating the synthesis of copper nanoparticles, which was further, reconfirmed by the initial characterization using UV-Vis spectroscopy in the range of 200-500 nm at different time intervals. In order to observe the formation and stability of copper nanoparticles, it is required to incubate in room temperature and then regularly examine the formation of copper nanoparticles and their stability. Incubate at room temperature to allow nanoparticles formation

Fig 1. Neem (Azadirachta indica) leaf extract and copper sulfate solution (left) and green-synthesized copper nanoparticles indicated by color change (right)

Optimization of Parameters for Green Synthesis of Copper Nanoparticles

The green synthesis of copper nanoparticles (CuNPs) using *Azadirachta indica* leaf extract was systematically optimized by varying key physicochemical parameters, namely, the concentration of the precursor salt ($CuSO_4$), the volume of leaf extract, and the reaction temperature. Each parameter was evaluated independently while keeping all other conditions constant. The formation of CuNPs was confirmed by visual observation of a characteristic colour change in the reaction mixture and by UV–Vis spectrophotometric analysis.

Effect of Concentration of Copper Sulphate Solution on Synthesis of CuNPs

The concentration of the metal precursor salt plays a pivotal role in determining the yield, size, and physicochemical properties of the synthesised nanoparticles. To investigate its influence, aqueous solutions of copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) were prepared at three different molar concentrations, namely 1 mM, 3 mM, and 5 mM. A fixed volume of A. indica leaf extract was added to each of these $CuSO_4$ solutions under identical experimental conditions (constant temperature, pH, and reaction time). The extent of nanoparticle formation at each concentration was monitored using UV–Vis spectrophotometry by recording the characteristic surface plasmon resonance (SPR) absorption peak of CuNPs in the wavelength range of 500–600 nm.The concentration that yielded the highest absorbance corresponding to maximum nanoparticle production was selected as the optimum for subsequent experiments. [8]

Effect of quantity of leaf extract on CuNPs Synthesis

The volume of plant extract used in the reaction mixture serves as both the reducing agent and the stabilising (capping) agent in green synthesis, and hence its quantity directly influences the nucleation, growth, and colloidal stability of the resulting nanoparticles. To determine the optimum volume of A. indica leaf extract required for efficient CuNPs synthesis, varying volumes of the extract (1, 2, 3, 4, 5, 6, 7, 8, and 9 mL) were added separately to a fixed volume of 5 mL copper sulphate solution at the previously optimized concentration. All reaction mixtures were incubated under identical conditions of temperature and time. The formation of CuNPs in each reaction was confirmed by UV–Vis spectrophotometric analysis, and the volume yielding the maximum absorbance at the SPR peak wavelength was considered optimal. [9]

Effect of Temperature on synthesis of CuNPs

Temperature is a critical physicochemical parameter that profoundly influences the kinetics of nanoparticle nucleation and growth, as well as the size, shape, and crystallinity of the resulting nanoparticles. The method of synthesis significantly determines the temperature requirements: physical methods typically necessitate temperatures exceeding 350°C, whereas chemical reduction methods operate below this threshold. Green synthesis routes employing plant extracts are advantageous in that they are generally conducted at temperatures below 100°C, making them energy-efficient and environmentally benign. [10]

Effect of pH on synthesis of copper nanoparticles

pH is an important factor that influences the synthesis of nanoparticles by green technology methods. Researchers have discovered that pH of the solution medium influences the size and texture of the synthesized nanoparticle (Armendariz et al, 2004)[23] Therefore, nanoparticle size can be controlled by altering the pH of the solution media. The influence of pH in the nanoparticles was evaluated under different pH of the reaction mixture by the leaf extract. The effect of pH on synthesis of CuNPs, pH is significant factor for biosynthesis of nanoparticles. The effect of pH on the synthesis of CuNPs was studied in the range of 4–9. To control the pH, Hcl and NaOH (0.01 M) solutions were used. The other factors like volume of leaf extract, concentration of Zinc acetate and temperature were kept constant. The effect of pH on the synthesis of copper nanoparticles was monitored using UV–Vis spectroscopy.(200-500nm). [11].

RESULT AND DISCUSSION

Visual observation and UV-Vis. Studies

The qualitative analysis of CuNPswere carried out by UV-Vis spectrophotometer. When the leaf extract of Azadirachta indica was addedinto the aqueous solution of copper sulphate colour change was observed instantly by adding the the extract into the solution from clear solution to light greenish and finally it becomes greenish that indicates the formation of copper nanoparticles.. In 1Mm concentration, the production of nanoparticles is less while in 3 mM the production of nanoparticles is more and in 5 mM the production of nanoparticles has decreased , thus indicating that the maximum synthesis of nanoparticles takes place in 3 mM . From the above discussion it is clear that in the present study, an optimum concentration of precursor for the synthesis of CuNPs by using Azadirachta indica leaf extract indicaleaf extract.

Effect of PH on synthesis of CuNPs

pH also play an important role in the nanoparticles synthesis. The influence of pH in the nanoparticles was evaluated under different pH of the reaction mixture by the leaf extract. Different ph was optimize to see the maximum production of nanoparticles. pH 5 was considered to be the best pHfor the synthesis of nanoparticles using leaf extract of Azadirachta indica (when pH was varied from 5 to 4 it was observed that the production of nanoprticles decreases, while increasing the pH from 5 to 6 it was observed that by increasing the pH from 5 to 6 the production of nanoparticles decreases. From the above discussion it is clear that pH 5 is the best pH for maximum production of nanoparticles after optimization.

SEM analysis of Copper Nanoparticles

The surface morphology and particle distribution of the synthesized copper nanoparticles were examined using scanning electron microscopy (SEM). The micrograph reveals that the nanoparticles are predominantly spherical in shape with varying particle sizes. The image also demonstrates a relatively uniform distribution of particles across the surface, although some degree of agglomeration is evident, which is a common feature in metallic nanoparticles due to their high surface energy and strong inter-particle interactions. The SEM image clearly shows nanoparticles in the nanometer range, with sizes appearing to range from a few tens of nanometers to over 100 nm. The presence of both smaller and larger particles suggests a polydisperse size distribution, which may be attributed to differences in nucleation and growth rates during synthesis. Smaller nanoparticles are visible as fine, bright dots scattered densely across the surface, whereas larger particles appear as distinct, brighter spherical clusters. Agglomeration in the SEM image may also indicate partial oxidation or surface interactions among particles. This phenomenon is commonly observed in copper nanoparticles, as copper readily oxidizes when exposed to air, leading to surface modifications and clustering effects.

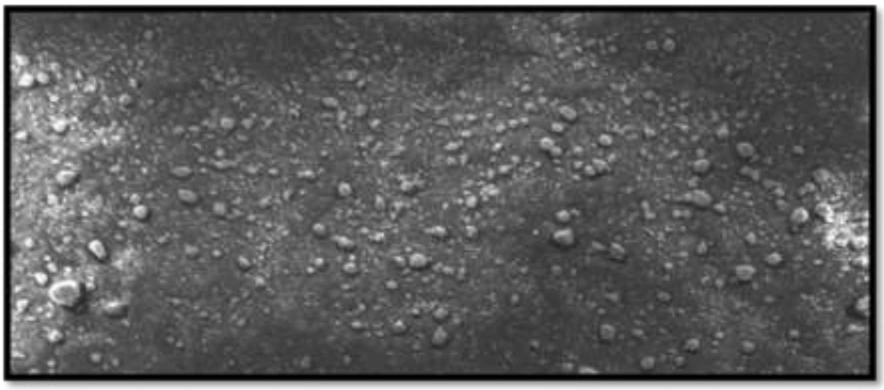

Fig 2. SEM micrograph showing the morphology of biosynthesized copper nanoparticles

Antimicrobial activity

The CuNPs shows excellent antimicrobial activity against E. Coli as well as Staphylococcus aureus. Clear zones of inhibition of bacterial growth were observed on nutrient agar plates around the wells loaded with test of CuNPs are shown in figure. Copper nanoparticles exhibit immensifying anti-microbial activity but in reference to extract wells and salt wells no inhibition zone was observed. Disordering of the helical structure by cross-linking within and between the nucleic acid strands is caused due to the copper ions subsequently released and binds with DNA. Copper ions disrupt the biochemical processes inside the bacterial cells. Copper nanoparticles show efficient antibacterial property due to their extremely large surface area, which provides better contact with microorganisms. In the present study *Azadirachta indica* was taken for synthesis of CuNPs because of its medicinal value. There are various reports which have been providing the evidences that copper nanoparticles were used as powerful tool against multidrug-resistant bacteria. In our experiment, when we compared the antibacterial activity of CuNPs and plant extract, it was found that copper nanoparticles have shown more antibacterial activity than plant extracts. Our results clearly shows that the plant extract showing no antibacterial activity as compared to leaf extract. The concentration of leaf extract has no effect on antimicrobial activity concentration increases no result as well as decreases result is same.

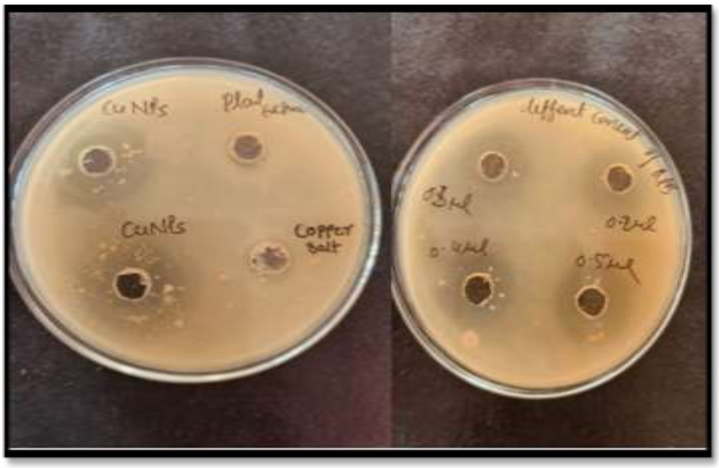

Fig 3. Antimicrobial activity result

CONCLUSION

The present study demonstrated a simple, cost-effective, and environmentally benign approach for the synthesis of copper nanoparticles (Cu NPs) using *Azadirachta indica* leaf extract as a natural reducing and stabilizing agent. This biosynthetic method eliminates the need for hazardous chemicals, making it an eco-friendly alternative to conventional chemical and physical synthesis techniques. The synthesized nanoparticles were systematically characterized using UV–Vis spectroscopy, XRD, and SEM analysis. UV–Vis confirmed the formation of Cu NPs, SEM micrographs showed that the Cu NPs exhibited predominantly spherical morphology with a polydisperse size distribution in the nanometre range. While the nanoparticles were relatively well dispersed, some degree of agglomeration was observed due to high surface energy and interparticle interactions. Biological evaluation revealed that the Cu NP exhibited significant antibacterial activity against Gram-negative (*Escherichia coli*) bacterial strains. This confirms their potential application in antimicrobial formulations and biomedical devices. The dual combination of green synthesis and effective antibacterial activity highlights their potential utility in pharmaceutical, environmental, and industrial fields. Overall, the study establishes that Azadirachta indica leaf extract is a suitable bio-reductant for the sustainable synthesis of Cu NP.

CONFLICT OF INTEREST

Authors declare for none conflict of interest.

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