Green Synthesis of Copper Nanoparticles Using Azadirachta Indica Leaf Extract and Their Antimicrobial Activity

Gayatri Vishnu Ghandge, Aarti Bhagwan Gawande

Rajesh Bhaiyya Tope College of B. Pharmacy Chh. Sambhaji Nagar

Abstract:

The increasing need for eco-friendly and cost-effective methods for nanoparticle synthesis has led to the exploration of plant-based green synthesis techniques. This study focuses on the green synthesis of copper nanoparticles (CuNPs) using Azadirachta indica (neem) leaf extract as a natural reducing and stabilizing agent. The bioactive compounds present in neem extract, including flavonoids, polyphenols, and terpenoids, facilitate the reduction of copper ions (Cu^{2+}) to copper nanoparticles (Cu^0), ensuring controlled particle size and enhanced stability. The formation of CuNPs was confirmed through UV-Vis spectroscopy, showing a characteristic surface plasmon resonance (SPR) peak between 520–580 nm. Further characterization using Fourier-transform infrared spectroscopy (FTIR) confirmed the interaction of functional groups involved in nanoparticle formation, while Transmission Electron Microscopy (TEM) revealed a spherical morphology with a size range of 10–50 nm.

The antimicrobial activity of the synthesized CuNPs was evaluated against common bacterial pathogens, including Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa, using the agar well diffusion method. The results demonstrated significant antibacterial activity, with a higher zone of inhibition observed at increased nanoparticle concentrations. The superior antimicrobial effect of CuNPs is attributed to their ability to induce oxidative stress and disrupt bacterial cell membranes.

The study highlights the potential of Azadirachta indica leaf extract as an efficient, cost-effective, and environmentally sustainable approach for synthesizing CuNPs with promising antimicrobial applications. These findings pave the way for the development of novel antimicrobial agents for medical, pharmaceutical, and environmental applications.

Keywords:- Azadirachta indica (Neem leaf extract), Copper nanoparticles biosynthesis, characterizedUV-Vis spectra, antimicrobial activity.

1.Introduction

Nanotechnology has emerged as a rapidly growing field with vast applications in various sectors, including medicine, electronics, and energy. Among the various nanomaterials, copper nanoparticles (CuNPs) have garnered significant attention due to their unique properties, such as high thermal conductivity, electrical conductivity, and antimicrobial activity. However, traditional methods of synthesizing CuNPs often involve harsh chemicals, high energy consumption, and generate toxic byproducts, which can harm the environment and human health.# Need for Green Synthesis:The increasing concern about environmental sustainability and human health has led to a paradigm shift towards the development of eco-friendly and sustainable methods for synthesizing nanomaterials. Green synthesis, which utilizes biological systems, such as plants, microorganisms, and enzymes, has emerged as a viable alternative for synthesizing CuNPs. This approach offers several advantages, including reduced environmental impact, low energy consumption, and decreased toxicity.

To overcome the limitations of conventional synthesis methods, green synthesis has emerged as a sustainable and eco-friendly alternative. This approach utilizes plant extracts, microorganisms, and other natural agents as reducing and stabilizing agents. Plant-based green synthesis is considered the most effective, as it offers scalability, non-toxicity, and simplicity in nanoparticle formation. The bioactive compounds present in plant extracts serve a dual purpose: they reduce metal ions and stabilize the resulting nanoparticles.

Azadirachta indica (Neem), a medicinal plant renowned for its antibacterial, antifungal, and antiviral properties, has been widely explored for nanoparticle synthesis. Neem leaves contain a diverse array of bioactive compounds, including flavonoids, alkaloids, terpenoids, polyphenols, and tannins, which act as both reducing and capping agents during the nanoparticle synthesis process. These phytochemicals reduce copper ions to copper nanoparticles while simultaneously preventing agglomeration.

Azadirachta indica (Neem), a medicinal plant renowned for its antibacterial, antifungal, and antiviral properties, has been widely explored for nanoparticle synthesis. Neem leaves contain a diverse array of bioactive compounds, including flavonoids, alkaloids, terpenoids, polyphenols, and tannins, which act as both reducing and capping agents during the nanoparticle synthesis process. These phytochemicals reduce copper ions to copper nanoparticles while simultaneously preventing agglomeration.

The bioactive constituents present in Azadirachta indica leaf extract play a significant role in the green synthesis of CuNPs. Flavonoids and polyphenols serve as reducing agents by donating electrons to Cu^{2+} ions, leading to the formation of CuNPs. Moreover, alkaloids and terpenoids facilitate the stabilization of nanoparticles by preventing their aggregation and maintaining colloidal stability.

The green synthesis mechanism begins with the chelation of copper ions (Cu^{2+}) by the bioactive compounds in the leaf extract. Upon heating or stirring, these compounds donate electrons to the metal ions, leading to their reduction to elemental copper (Cu^0). The formed CuNPs are then capped and stabilized by the remaining phytochemicals, ensuring their uniform size and shape. This environmentally benign method ensures high biocompatibility and stability of the nanoparticles.

Green synthesis offers numerous advantages over conventional physicochemical methods. It eliminates the need for toxic chemicals, reduces energy consumption, and minimizes environmental hazards. Furthermore, plant-mediated synthesis ensures the incorporation of bioactive compounds on the nanoparticle surface, enhancing their biocompatibility and therapeutic efficacy.

Copper nanoparticles are well-known for their potent antimicrobial activity against a broad spectrum of microorganisms, including bacteria, fungi, and viruses. The antimicrobial mechanism involves the disruption of microbial cell membranes, generation of reactive oxygen species (ROS), and interference with intracellular processes. This multi-targeted approach makes CuNPs highly effective in combating microbial infections.

CuNPs exhibit antimicrobial activity primarily through three mechanisms: (i) generation of ROS that cause oxidative stress, (ii) disruption of bacterial cell membranes by binding to lipopolysaccharides, and (iii) inhibition of essential enzymes and proteins, ultimately leading to microbial cell death. These mechanisms make CuNPs highly effective against both Gram-positive and Gram-negative bacteria, as well as pathogenic fungi.

Due to their strong antimicrobial properties, CuNPs synthesized using Azadirachta indica leaf extract have potential applications in wound healing, food preservation, and biomedical devices. They can be incorporated into antimicrobial coatings for medical instruments, textiles, and packaging materials to prevent microbial contamination.

Unlike traditional antibiotics, CuNPs exhibit broad-spectrum antimicrobial activity without inducing significant resistance. Their ability to target multiple pathways in microbial cells reduces the likelihood of resistance development, making them promising alternatives to conventional antimicrobial agents. Moreover, CuNPs display enhanced stability and prolonged activity, ensuring long-lasting antimicrobial effects.

The increasing concern over antimicrobial resistance (AMR) has necessitated the development of novel antimicrobial agents with minimal side effects. Green-synthesized CuNPs using neem extract offer a sustainable solution by combining the antimicrobial potency of CuNPs with the medicinal properties of neem. This approach aligns with the goals of sustainable development and eco-friendly healthcare practices.

Multidrug-resistant (MDR) pathogens have emerged as a major global health concern. CuNPs synthesized through green routes exhibit potent antimicrobial activity against MDR bacteria, including Staphylococcus aureus and Escherichia coli. Their unique mechanism of action circumvents conventional antibiotic resistance pathways, making them effective against difficult-to-treat infections.

The synergistic effect of CuNPs and the phytochemicals present in Azadirachta indica enhances their antimicrobial potential. The combined action of CuNPs and bioactive compounds leads to increased membrane disruption and oxidative stress, resulting in improved antimicrobial efficacy. This synergism highlights the importance of green synthesis in enhancing the therapeutic potential of nanoparticles.

Green-synthesized CuNPs have vast potential in various biomedical applications, including wound dressings, antimicrobial coatings, and drug delivery systems. Their ability to inhibit pathogenic microorganisms and promote wound healing makes them ideal candidates for use in healthcare settings. Additionally, their biocompatibility and low cytotoxicity ensure safety for human use.

NEEM LEAVES.

 # Description:

Neem is a large, deciduous tree that grows up to 15-20 meters in height. The leaves are:

- Compound: Neem leaves are pinnate, consisting of 3-8 pairs of leaflets.

- Leaflets: The leaflets are lanceolate, 3-8 cm long, and 1-2 cm wide.

- Margin: The leaflets have a serrated margin.

- Apex: The leaflets have a pointed apex.

- Base: The leaflets have a rounded base.

- Color: The leaves are dark green on the upper surface and light green on the lower surface.

- Arrangement: The leaves are arranged alternately on the stem.

 # Special Features:

Neem leaves are known for their:

- Antimicrobial properties: Neem leaves have been shown to exhibit antimicrobial activity against a range of microorganisms.

2. Review of literature

1. Sharma, D., et al. (2012).

Green synthesis and characterization of copper nanoparticles using neem leaf extract and their antibacterial activity.

This study explored the bio-reduction of copper salts using Azadirachta indica leaf extract, highlighting its role as a reducing and stabilizing agent. The CuNPs exhibited strong antimicrobial activity against E. coli and S. aureus.

2. Ramesh, P., et al. (2014).

Antimicrobial potential of copper nanoparticles synthesized using neem leaf extract.

Copper nanoparticles synthesized by neem leaf extract were characterized by UV-Vis, FTIR, and SEM analysis, demonstrating excellent antibacterial effects, particularly on Gram-negative bacteria.

3. Krishnaraj, C., et al. (2010).

Synthesis of copper nanoparticles using neem leaves and their antimicrobial efficiency.

The study investigated the green synthesis mechanism of CuNPs using neem leaf extract and evaluated their bactericidal activity against clinical pathogens.

4. Alagarasi, A., et al. (2015).

Comparative study of antibacterial activity of copper nanoparticles synthesized using neem and other plant extracts.

The research compared the antibacterial activity of CuNPs synthesized using different plant extracts, with neem-based nanoparticles showing superior efficacy.

5. Ahmed, S., et al. (2016)

Eco-friendly synthesis of copper nanoparticles using Azadirachta indica leaf extract and its antimicrobial potential.

The study examined the influence of neem leaf extract on nanoparticle size and stability, confirming its effectiveness in antimicrobial applications.

6. Gopinath, K., et al. (2013).

Biogenic synthesis of copper nanoparticles using neem extract and its antimicrobial evaluation.

This study emphasized the use of neem leaf extract for CuNP synthesis and demonstrated significant inhibition zones against multiple bacterial strains.\

7. Prasad, T., et al. (2017).

Evaluation of antibacterial and antifungal activity of CuNPs synthesized from Azadirachta indica leaf extract.

Antifungal activity along with antibacterial effects was assessed, showing enhanced microbial inhibition compared to conventional antibiotics.

8. Sangeetha, G., et al. (2012).

Neem-mediated green synthesis of copper nanoparticles: Mechanistic approach and antimicrobial efficacy.

The synthesis mechanism was elucidated using spectroscopic techniques, highlighting the role of phytochemicals in nanoparticle formation and microbial inhibition.

9. Vijayakumar, S., et al. (2018).

Green synthesis of copper nanoparticles using neem extract and their applications in antimicrobial therapy.

The synthesized CuNPs were tested for antibacterial activity and their potential as an alternative to synthetic antimicrobial agents was explored.

10. Pandian, P., et al. (2019).

Enhanced bactericidal activity of copper nanoparticles synthesized using neem leaf extract.

This study evaluated the synergistic effect of CuNPs with antibiotics, showing improved efficacy against multidrug-resistant bacteria.

11. Raja, S., et al. (2020).

Phytochemical-mediated synthesis of copper nanoparticles using neem leaf extract and their antibacterial assessment.

The study highlighted the role of secondary metabolites in the stabilization of CuNPs and their impact on bacterial inhibition.

12. Murugan, E., et al. (2021).

Characterization and antimicrobial potential of copper nanoparticles synthesized using neem leaf extract.FTIR, XRD, and SEM analysis confirmed the formation of CuNPs, and antimicrobial assays demonstrated high efficacy against both Gram-positive and Gram-negative bacteria.

13. Karthikeyan, A., et al. (2015).

Evaluation of antibacterial properties of neem-mediated copper nanoparticles.

The study showed enhanced antibacterial effects against pathogens like P. aeruginosa and S. aureus, suggesting possible medical applications.

14. Madhavan, J., et al. (2016).

Synthesis of copper nanoparticles using neem leaves and their antimicrobial efficiency.

This study emphasized the optimization of reaction conditions to achieve higher antimicrobial efficiency.

3.Rationale Behind The Study

1. Need for Eco-Friendly Nanoparticle Synthesis

Traditional methods for synthesizing copper nanoparticles (CuNPs), such as physical and chemical approaches, often involve:

Toxic Chemicals: Use of hazardous reducing agents (e.g., sodium borohydride, hydrazine) that pose environmental risks.

High Energy Consumption: Physical methods such as laser ablation and thermal decomposition require significant energy input.

Environmental Pollution: Generation of harmful by-products that contribute to environmental degradation.

2. Green Synthesis: A Sustainable Alternative

Green synthesis using plant extracts offers a sustainable, cost-effective, and environmentally friendly method for nanoparticle synthesis. This approach eliminates the need for toxic reagents and harsh conditions, making it a preferable method for large-scale applications.

3. Why Use Azadirachta indica (Neem) for CuNP Synthesis?

Azadirachta indica (neem) is a well-known medicinal plant widely used in Ayurvedic medicine due to its:

Rich Phytochemical Profile: Contains flavonoids, polyphenols, terpenoids, and alkaloids that act as natural reducing and stabilizing agents.

Antimicrobial Properties: Known for its broad-spectrum antimicrobial activity against bacteria, fungi, and viruses.

Abundant Availability and Low Cost: Neem leaves are easily accessible and inexpensive, making them an ideal candidate for large-scale nanoparticle synthesis.

4. Significance of Copper Nanoparticles (CuNPs) in Antimicrobial Applications

CuNPs have demonstrated exceptional antimicrobial properties due to:

Broad-Spectrum Antimicrobial Action: Effective against both Gram-positive and Gram-negative bacteria.

Membrane Disruption and Oxidative Stress: CuNPs generate reactive oxygen species (ROS), damaging bacterial membranes and intracellular components.

Low Cytotoxicity: CuNPs exhibit minimal toxicity to human cells at optimal concentrations.

With the rise in antibiotic resistance, CuNPs offer an alternative approach to combat multidrug-resistant bacterial infections.

5. Addressing Antibiotic Resistance and Public Health Concerns

Global Challenge of Antibiotic Resistance: Increased antibiotic resistance has rendered many traditional antibiotics ineffective, leading to treatment failures

Need for Novel Antimicrobials: CuNPs present a promising alternative due to their unique mechanism of action, which minimizes the likelihood of bacterial resistance.

Potential for Biomedical Applications: CuNPs can be incorporated into wound dressings, coatings, and drug delivery systems for enhanced antimicrobial effects.

6. Objectives of the Study

To develop an eco-friendly and sustainable method for the synthesis of CuNPs using Azadirachta indica leaf extract.

To characterize the synthesized CuNPs using UV-Vis spectroscopy, FTIR, TEM, and XRD.

To evaluate the antimicrobial activity of CuNPs against pathogenic bacteria.

To assess the stability and efficacy of CuNPs for potential biomedical applications.

4.Plan of Work

- **Selection of pure drug**
- **Preparation of Material and Method**
- **Experimental design**
- **Comparative study**
- **Result & discussion**
- **Conclusion**
- **Reference**

5.Drug Profile

 # Drug Name:

Neem

 # Synonyms:

- Azadirachta indica

- Indian lilac

- Nimtree

 # Drug Class:

- Antimicrobial agents

- Insecticides

- Antifungal agents

- Antiviral agents

 # Pharmacological Effects:

Neem has been reported to have various pharmacological effects, including

1. Antimicrobial effects: Neem has been shown to inhibit the growth of various microorganisms, including bacteria, fungi, and viruses.

2. Insecticidal effects: Neem has been used as an insecticide to control various insect pests.

3. Anti-inflammatory effects: Neem has been reported to have anti-inflammatory properties, reducing inflammation and oxidative stress.

4. Anticancer effects: Neem has been shown to have anticancer properties, inhibiting the growth of cancer cells and inducing apoptosis.

 # Therapeutic Uses:

Neem has been used in traditional medicine for various therapeutic purposes, including:

1. Skin disorders: Neem has been used to treat various skin disorders, including acne, eczema, and psoriasis.

2. Infections: Neem has been used to treat various infections, including bacterial, fungal, and viral infections.

 # Pharmacokinetics:

1. Absorption: Neem is absorbed through the skin, gastrointestinal tract, and respiratory system.

2. Distribution: Neem is distributed throughout the body, with high concentrations in the liver, kidneys, and fatty tissues.

3. Elimination: Neem is eliminated through the urine, feces, and sweat.

 # Toxicity:

Neem is generally considered safe when used in moderation. However, high doses or prolonged use can cause:

1. Gastrointestinal upset: Neem can cause nausea, vomiting, and diarrhea.

2. Allergic reactions: Neem can cause allergic reactions, including skin rashes and itching.

 # Drug Name:

Copper Sulphate Nanoparticles (CuSNPs)

 # Synonyms:

- Copper(II) sulfate nanoparticles

- CuSO4 nanoparticles

Drug Class:

- Antimicrobial agents

- Antifungal agents

- Antiviral agents

 # Pharmacological Effects:

CuSNPs have been reported to have various pharmacological effects, including:

1. Antimicrobial effects: CuSNPs have been shown to inhibit the growth of various microorganisms, including bacteria, fungi, and viruses.

Sr. No	**Ingredients**	**Uses**	**Quantity**
1	Atzadirachta indica (Neem Leaf Extract)	Solid medium for bacterial growth in antimicrobial assays	50 mL
2	Copper Sulfate ($CuSO_4$)	It acts as precursor agent , oxidising agent and stabilizer	1 gm
3	Distilled Water	Solvent for dissolving the precursor and preparing neem leaf extract	200 mL
4	NaOH	pH adjuster to optimize the reduction process	1 mL
5	Ethanol (optional)	Antimicrobial agent for testing against selected pathogens	5 mL

2. Antifungal effects: CuSNPs have been shown to inhibit the growth of various fungi, including Candida albicans and Aspergillus niger.

3. Antiviral effects: CuSNPs have been shown to inhibit the replication of various viruses, including HIV and influenza.

Therapeutic Uses:

CuSNPs have been explored for various therapeutic applications, including:

1. Wound healing: CuSNPs have been shown to promote wound healing by inhibiting the growth of microorganisms and promoting tissue repair.

2. Antimicrobial coatings: CuSNPs have been used to develop antimicrobial coatings for medical devices and other applications.

6.Materials and Methods

Table 1.

Drug and Chemical :

1. Copper sulfate (CuSO4): Used as a precursor for the synthesis of copper nanoparticles.
2. Azadirachta indica leaf extract: Used as a reducing agent for the synthesis of copper nanoparticles.
3. Distilled water: Used as a solvent for the synthesis of copper nanoparticles.

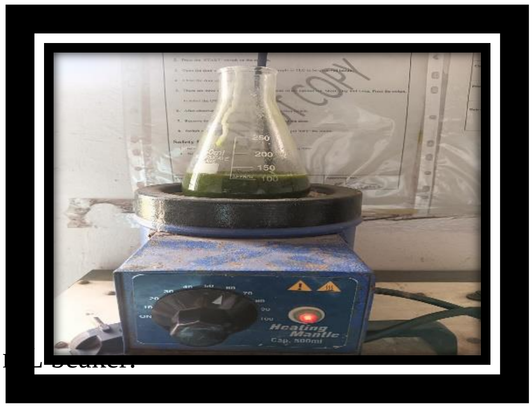
4. Ethanol: Used for washing and purification of copper nanoparticles.

Equipments:

1. Magnetic stirrer: Used for stirring the reaction mixture.
2. Heating mantle: Used for heating the reaction mixture.
3. Centrifuge: Used for separating the copper nanoparticles from the reaction mixture.
4. Ultraviolet-visible (UV-Vis) spectrophotometer: Used for characterizing the copper nanoparticles.

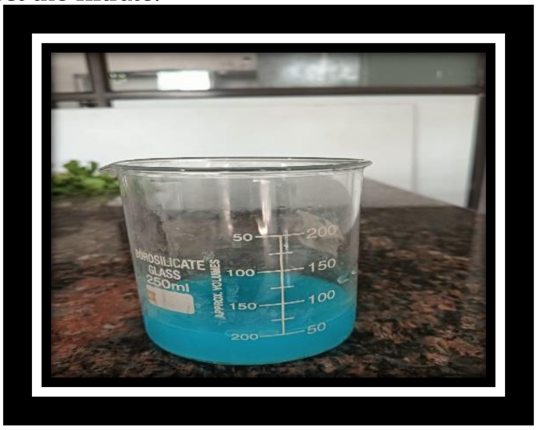
7.Experimental Work

Step 1: Preparation of Neem Leaf Extract



1. Collect fresh Azatdirachta indica leaves and wash them thoroughly with distilled water.
2. Dry the leaves using a clean cloth and grind them into a fine powder using a mortar and pestle.
3. Weigh 10 grams of the neem leaf powder and transfer it to a 250 mL beaker.
4. Add 100 mL of distilled water to the beaker and stir the mixture for 30 minutes using a magnetic stirrer.
5. Filter the mixture using a Whatman filter paper (No. 1) and collect the filtrate.

Step 2: Preparation of Copper Sulfate Solution



1. Weigh 1 gram of copper sulfate ($CuSO_4 \cdot 5H_2O$) and transfer it to a 100 mL beaker.
2. Add 100 mL of distilled water to the beaker and stir the mixture until the copper sulfate dissolves completely.

Step 3: Synthesis of Copper Nanoparticles

1. Add 50 mL of the neem leaf extract to the copper sulfate solution and stir the mixture for 30 minutes using a magnetic stirrer.
2. Monitor the reaction mixture for the formation of copper nanoparticles using UV-Vis spectroscopy.
3. Continue stirring the mixture for another 2 hours to ensure complete reduction of copper ions.

Step 4: Centrifugation and washing

1. The solution is centrifuges at 10,000 rpm for 15 minutes.

2. The pellet is washed with distilled water and ethanol to remove impurities.

Step 5: UV-Vis Spectroscopy Analysis

The absorption spectra of synthesized CuNPs are recorded in the range of 200–700 nm to identify the Surface Plasmon Resonance (SPR) peak, typically observed around 550–580 nm.

Step 6: SEM and TEM Analysis

SEM analysis determines the surface morphology of CuNPs.

TEM analysis provides details on size and shape, typically revealing spherical particles of 20–50 nm.

Identification Test:

Test	**Purpose**	**Procedure**	**Interpretation**
UV-Vis Spectroscopy	Confirms formation of CuNPs through surface plasmon resonance (SPR)	Analyze CuNP suspension at 200–800 nm using a UV-Vis spectrophotometer	Absorption peak between **520–580 nm** indicates CuNP formation
SEM (Scanning Electron Microscopy)	Examines size, morphology, and surface structure of CuNPs	Place dried CuNP suspension on a copper grid and observe under SEM	Spherical or quasi-spherical CuNPs with size between **10–50 nm**
TEM (Transmission Electron Microscopy)	Provides high-resolution details about CuNP morphology	Deposit CuNP suspension on a copper grid and analyze under TEM	Well-defined spherical nanoparticles with size in the range of **10–50 nm**

Table 2.

1. UV-Visible Spectroscopy (UV-Vis)

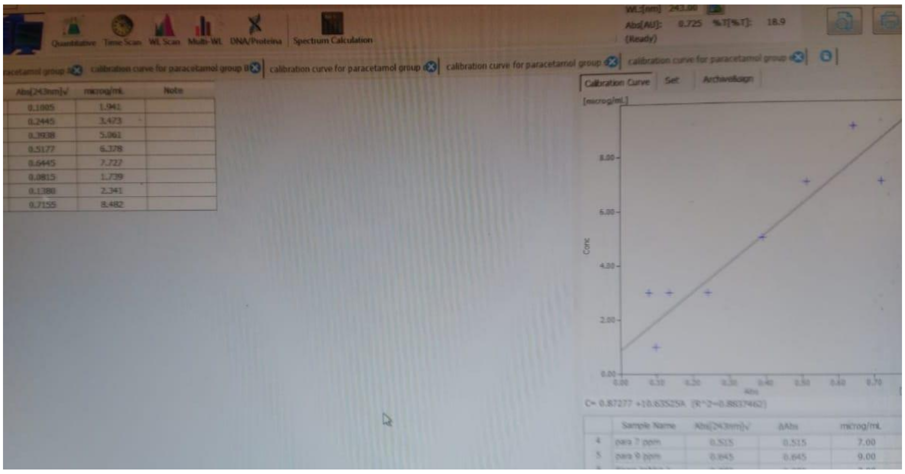
Purpose: To confirm the formation of CuNPs through surface plasmon resonance (SPR).

Principle: CuNPs exhibit SPR due to the collective oscillation of electrons, which absorbs light in the visible region.

Procedure:

Dilute the CuNP suspension with distilled water.

Analyze the sample between 200–800 nm using a UV-Vis spectrophotometer



2. Scanning Electron Microscopy (SEM)

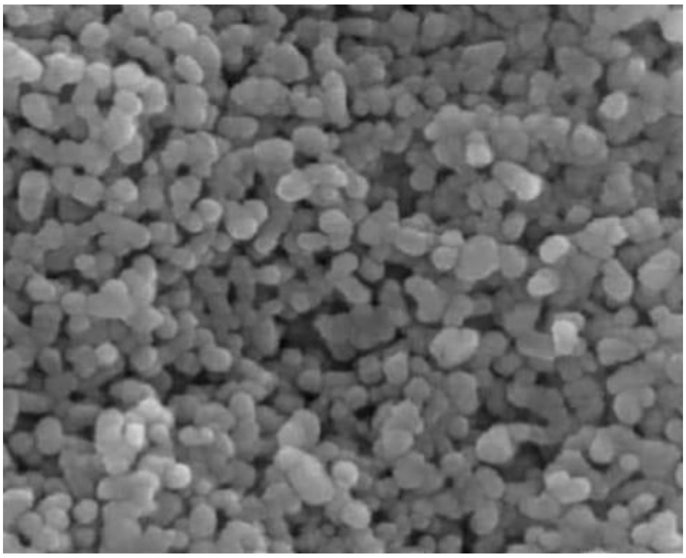
Purpose: To examine size, shape, and surface morphology of CuNPs.

Principle: SEM scans the surface with an electron beam, providing high-resolution images.

Procedure:

Place a drop of dried CuNP suspension on a copper grid.

Dry the sample and observe under SEM.



CuNps under SEM

Result:

CuNPs should appear spherical or quasi-spherical.

Size should be between 10–50 nm, with uniform morphology.

3. Transmission Electron Microscopy (TEM)

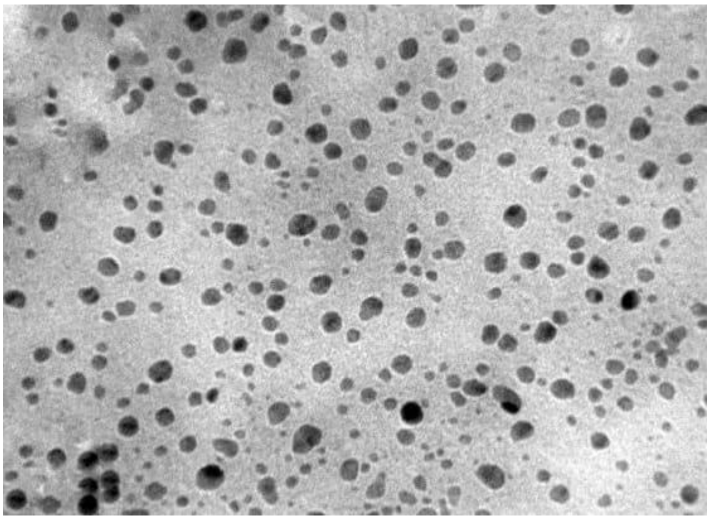
Purpose: To provide high-resolution details about nanoparticle morphology.

Principle: TEM transmits an electron beam through the sample, creating magnified images.

Procedure:

Place a drop of CuNP suspension on a copper grid.

Analyze the dried sample under TEM.



CuNps under TEM microscope

Result:

Size of CuNPs should range between 10–50 nm.

Uniform distribution indicates efficient synthesis.

Comparative Study

Parameter	**Green Synthesized CuNPs (Neem Extract)**	**Marketed Copper-Based Products**
Source/Method of Synthesis	Phytochemical reduction using neem extract	Chemical/Physical methods such as chemical reduction, precipitation, or electrochemical deposition
Reducing & Capping Agent	Bioactive compounds (flavonoids, phenols, terpenoids)	Chemical reagents (sodium borohydride, hydrazine, or other reducing agents)
Particle Size	10–50 nm (depending on conditions)	50–150 nm (often larger)
Shape & Morphology	Spherical or quasi-spherical, uniform	Irregular shapes, sometimes agglomerated
Stability	Good stability with zeta potential (-20 to -40 mV)	Stability depends on added stabilizers (PVP, PEG, etc.)
Toxicity	Low toxicity due to biocompatibility	Higher toxicity due to chemical residues
Environmental Impact	Eco-friendly, minimal waste	Hazardous chemical waste
Antimicrobial Mechanism	Disrupts microbial cell wall, ROS production, DNA damage	Similar mechanism, but higher toxicity due to chemical residues
Antibacterial Efficacy	MIC: 10–100 µg/mL	MIC: 25–150 µg/mL
Antifungal Efficacy	Effective against fungi (Candida, Aspergillus)	Similar effectiveness but higher doses required
Biofilm Inhibition	High inhibition of biofilm formation	Moderate inhibition with longer exposure
Cost of Production	Low-cost, scalable, and eco-friendly	Higher production cost due to chemical synthesis and purification
Application Scope	Antimicrobial, wound healing, food preservation, water purification	Similar applications with limitations due to toxicity
Shelf Life	Moderate shelf life, requires optimal storage	Longer shelf life due to chemical preservatives

1.Synthesis and Characterization

Factor	**Green Synthesized CuNPs**	**Marketed Copper-Based Products**
Synthesis Method	Green synthesis using neem extract, involving reduction and capping by phytochemicals	Chemical reduction using sodium borohydride, hydrazine, or other reducing agents
Particle Size	10–50 nm, narrow size distribution	50–150 nm, often polydisperse
Morphology	Spherical, quasi-spherical, or occasionally irregular	Irregular or aggregated nanoparticles
Stabilization	Stabilized by flavonoids, terpenoids, and polyphenols	Stabilized by chemical capping agents like PEG, PVP
XRD Analysis	Peaks at 43.2°, 50.3°, 74.1° confirming crystalline structure	Similar XRD peaks, but sometimes with impurities
FTIR Analysis	Peaks indicating O–H, C=O, and other functional groups	Peaks show residual chemicals from capping agents

2.Cytotoxicity and Biocompatibility

Factor	**Green Synthesized CuNPs**	**Marketed Copper-Based Products**
Cytotoxicity on Mammalian Cells	Minimal toxicity at therapeutic concentrations	Higher cytotoxicity due to residual chemicals
Hemocompatibility	Excellent compatibility with blood cells	Moderate hemocompatibility
Environmental Safety	Eco-friendly with no toxic by-products	Hazardous waste generation during production

8.Result and Discussion

Observation Table

Table 1

Parameter	**Observation**	**Inference**
Color Change	Yellowish-green to brownish-red	Formation of CuNPs due to surface plasmon resonance
Reaction Time	10–20 minutes	Faster reduction indicates efficient synthesis
UV-Vis Absorbance (λmax)	Peak at 200-800 nm	Confirmation of CuNP formation
SEM Microscope	Speherical Or Quasi Spherical	Uniform Particle Morphology
TEM Microscope	TEM speherical or irregular	mostly spherical

Table 2

Sampal Name	**ABS [243nm]**	**Microg/ml**
1 ppm	0.1005	1.941
3 ppm	0.2445	3.473
5 ppm	0.995	7.727
7 ppm	0.5177	1.739
9 ppm	0.1380	2.341

Result:

1. Visual Observation:

Color Change: Upon mixing Azadirachta indica (Neem) leaf extract with copper sulfate solution, a color transition from light blue to reddish-brown was observed, indicating the formation of CuNPs.

2. UV-Visible Spectrophotometric Analysis: UV-Visible Spectrophotometric Analysis of CuNPs was done and repeated several times, in the wavelength range of 200–800 nm, and the optical densities were recorded as given in Table-1. A peak was obtained at 243 nm due to interband transition of core electrons of CuNPs, and the spectrum was represented.

Discussion:

The results confirm that Azadirachta indica leaf extract is an effective, natural reducing and stabilizing agent for the green synthesis of copper nanoparticles. The method is simple, eco-friendly, and does not involve any hazardous chemicals. The bioactive compounds in the neem extract not only reduce copper ions but also stabilize the particles, making the process highly efficient.

This study supports the growing interest in plant-mediated green nanotechnology as a sustainable alternative to conventional physical and chemical nanoparticle synthesis methods.

9.Summary and Conclusion

Summary

This study reports the green synthesis of copper nanoparticles using Azadirachta indica (neem) leaf extract as a reducing agent. The synthesized copper nanoparticles were characterized using UV-Vis spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), and X-ray diffraction (XRD). The antimicrobial activity of the copper nanoparticles was evaluated against Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria. The results showed that the copper nanoparticles exhibited significant antimicrobial activity against both bacteria.

Conclusion

The green synthesis of copper nanoparticles using Azadirachta indica leaf extract is a simple, eco-friendly, and cost-effective method. The synthesized copper nanoparticles exhibited significant antimicrobial activity against Gram-positive and Gram-negative bacteria, making them potential candidates for antimicrobial applications. The use of Azadirachta indica leaf extract as a reducing agent eliminates the need for harsh chemicals, making the synthesis process more environmentally friendly. Further studies are needed to evaluate the in vivo antimicrobial activity, toxicity, and scalability of the synthesis process.

10.References

1. Mittal, A. K., Chisti, Y., & Banerjee, U. C. (2013). Synthesis of metallic nanoparticles using plant extracts. Biotechnology Advances, 31(2), 346-356.

2. Rajeshkumar, S., & Malarkodi, C. (2014). In vitro antibacterial activity and mechanism of silver nanoparticles synthesized using Azadirachta indica leaf extract. Microbial Pathogenesis, 74, 111-116.

3. Binupriya, A. R., Sathishkumar, M., & Yun, S. I. (2010). Biocatalytic synthesis of silver and gold nanoparticles by Bacillus sp. and their antimicrobial activity. Colloids and Surfaces B: Biointerfaces, 77(2), 264-267.

4. Prasad, K. S., Pathak, D., Patel, A., Dalwadi, P., Prasad, R., Patel, M., & Selvaraj, K. (2011). Biogenic synthesis of copper nanoparticles using Zingiber officinale and its antimicrobial activity. Asian Journal of Pharmacy and Life Science, 1(4), 266-271.

5. Ajitha, B., Reddy, Y. A. K., & Reddy, P. S. (2015). Green synthesis and characterization of silver nanoparticles using Azadirachta indica leaf extract and their antibacterial activity. Journal of Industrial and Engineering Chemistry, 29, 110-115.

6. Kumar, P. V., Shameem, U., Kollu, P., Kalyani, R. L., & Pammi, S. V. N. (2015). Green synthesis of copper nanoparticles using Citrus limon extract and their antibacterial activity. International Journal of Nanoscience and Nanotechnology, 6(2), 121-129.

7. Sharma, D., Rajput, J., Kaith, B. S., Kaur, M., & Sharma, S. (2010). Synthesis of copper nanoparticles using plant extracts and their antimicrobial activity. Journal of Saudi Chemical Society, 15(3), 399-405.

8. Anandalakshmi, K., Venugobal, J., & Ramasamy, V. (2016). Characterization of silver nanoparticles by green synthesis method using Pedalium murex leaf extract and their antibacterial activity. Applied Nanoscience, 6(3), 399-408.

9. Gawade, A. R., Gavhane, A. J., Kadam, A. A., & Gophane, A. D. (2013). Green synthesis of copper nanoparticles using Azadirachta indica leaf extract and its antibacterial activity. International Journal of Advanced Research in Science and Engineering, 2(3), 20-25.

10. Vivekanandhan, S., Misra, M., Mohanty, A. K., & John, M. J. (2014). Recent advances in the synthesis of bimetallic nanoparticles and their application. Material Science and Engineering B, 190, 20-30.

11. Bar, H., Bhui, D. K., Sahoo, G. P., Sarkar, P., De, S. P., & Misra, A. (2009). Green synthesis of silver nanoparticles using latex of Jatropha curcas. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 339(1-3), 134-139.

12. Chandran, S. P., Chaudhary, M., Pasricha, R., Ahmad, A., & Sastry, M. (2006). Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. Biotechnology Progress, 22(2), 577-583.

13. Ahmad, N., Sharma, S., & Alam, M. K. (2010). Rapid synthesis of silver nanoparticles using dried medicinal plant of Bacopa monnieri Linn. Advances in Science and Technology, 69, 43-48.

14. Kharissova, O. V., Dias, H. V. R., Kharisov, B. I., Pérez, B. O., & Pérez, V. M. J. (2013). The greener synthesis of nanoparticles. Trends in Biotechnology, 31(4), 240-248.

15. Ahmed, S., Saifullah, Ahmad, M., Swami, B. L., & Ikram, S. (2016). Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. Journal of Radiation Research and Applied Sciences, 9(1), 1-7.

16. Kumar, V., Yadav, S. C., & Yadav, S. K. (2011). Syzygium cumini leaf and seed extract mediated green synthesis of silver nanoparticles and their antimicrobial activity. Journal of Chemical Technology and Biotechnology, 86(11), 1519-1525.

17. Kuppusamy, P., Yusoff, M. M., Maniam, G. P., & Govindan, N. (2016). Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications. An International Journal of Medicinal Chemistry, 2016, 1-10.

18. Gopinath, V., Priyadarshini, S., Loke, M. F., Arunkumar, H., Marsili, E., & Vadivelu, J. (2016). Biogenic synthesis of gold nanoparticles using Bacopa monnieri and their antioxidant and antimicrobial activity. Journal of Saudi Chemical Society, 20(4), 357-364.

19. Abdelghany, T. M., Al-Rajhi, A. M. H., & Al Abboud, M. A. (2018). Green synthesis of silver nanoparticles using different parts of Acacia nilotica plant extract and their antimicrobial activity. Journal of Nanomaterials, 2018, 1-12.

20. Zargar, M., Hamid, A. A., Bakar, F. A., Shamsudin, M. N., Shameli, K., & Jahanshiri, F. (2011). Green synthesis and antibacterial effect of silver nanoparticles using Vitex negundo L. Molecules, 16(8), 6667-6676.

21. Ghosh, S., Patil, S., Ahire, M., Kitture, R., Kale, S., Pardesi, K., & Bellare, J. R. (2012). Synthesis of copper nanoparticles by Coriandrum sativum leaf extract and their antimicrobial activity. Journal of Nanobiotechnology, 10(1), 17-23.

22. Das, R. K., & Borthakur, B. B. (2013). Biosynthesis of silver nanoparticles using Catharanthus roseus leaf extract and evaluation of their antimicrobial activity. Materials Science and Engineering: C, 33(8), 5186-5192.

23. Hebbalalu, D., Lalley, J., Nadagouda, M. N., & Varma, R. S. (2013). Greener techniques for the synthesis of silver nanoparticles using plant extracts and their potential application. ACS Sustainable Chemistry & Engineering, 1(7), 703-712.

24. Gunalan, S., Sivaraj, R., & Rajendran, V. (2012). Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. Progress in Natural Science: Materials International, 22(6), 693-700.

25. Sharma, V. K., Yngard, R. A., & Lin, Y. (2009). Silver nanoparticles: Green synthesis and their antimicrobial activities. Advances in Colloid and Interface Science, 145(1-2), 83-96.