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Green Synthesis and Characterization of Copper Nanoparticles Using *Saraca asoca* Leaf Extract and Their Antibacterial, Catalytic, and Plant Growth-Promoting Activities

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ABSTRACT

The green synthesis of copper nanoparticles (CuNPs) using plant extracts has emerged as an eco-friendly, cost-effective, and sustainable alternative to conventional chemical and physical synthesis methods. In the present study, copper nanoparticles were successfully fabricated using *Saraca asoca* leaf extract, which served as both a reducing and stabilizing agent due to its rich phytochemical composition, including flavonoids, tannins, phenolics, and saponins. The biosynthesized CuNPs were characterized using various analytical techniques such as UV–Visible spectroscopy to confirm nanoparticle formation, Fourier Transform Infrared (FTIR) spectroscopy to identify the functional groups involved in reduction and capping, X-ray Diffraction (XRD) to determine crystallinity, Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy (TEM) to examine morphology and particle size, and Energy Dispersive X-ray (EDX) analysis to confirm elemental composition. The synthesized CuNPs exhibited predominantly spherical morphology with nanoscale dimensions and good stability. Furthermore, the biomedical potential of the CuNPs was evaluated through antimicrobial, antioxidant, and cytotoxicity assays. The nanoparticles demonstrated significant antibacterial activity against both Gram-positive and Gram-negative bacterial strains, potent free radical scavenging ability, and promising anticancer activity against selected cancer cell lines while exhibiting acceptable biocompatibility toward normal cells. These findings highlight that *Saraca asoca*-mediated copper nanoparticles possess excellent physicochemical properties and promising biomedical applications. The study underscores the potential of green-fabricated CuNPs as environmentally benign nanomaterials for future applications in nanomedicine, drug delivery, antimicrobial formulations, and therapeutic development.

Keywords: Green synthesis, nanoparticles, copper, Saraca asoca, phytochemicals, anti-toxicity, anticancer.

INTRODUCTION

Nanotechnology has emerged as one of the most dynamic fields of modern science, offering innovative solutions in medicine, agriculture, environmental remediation, electronics, and materials science. Nanoparticles, typically ranging from 1 to 100 nm in size, exhibit unique physicochemical properties, including a high surface-to-volume ratio, enhanced catalytic activity, optical behavior, and improved biological interactions, which distinguish them from their bulk counterparts. Among the various metallic nanoparticles, copper nanoparticles (CuNPs) have attracted considerable interest because of their excellent electrical conductivity, catalytic efficiency, antimicrobial properties, antioxidant potential, and relatively low production cost compared to noble metal nanoparticles such as gold and silver [1].

Conventional methods for synthesizing copper nanoparticles generally involve chemical reducing agents, organic solvents, and high-energy physical processes. Although these methods enable precise control over particle size and morphology, they often require toxic chemicals, generate hazardous by-products, and consume significant amounts of energy. These limitations have encouraged researchers to develop environmentally friendly synthesis strategies that align with the principles of green chemistry. Green synthesis employs biological resources such as plant extracts, microorganisms, algae, and biopolymers to reduce metal ions into nanoparticles under mild reaction conditions, thereby minimizing environmental impact while improving biocompatibility [2].

Plant-mediated synthesis has become one of the most widely explored green approaches because it is simple, rapid, scalable, and economically feasible. Plant extracts contain a diverse range of bioactive phytochemicals, including flavonoids, phenolic acids, tannins, alkaloids, terpenoids, saponins, proteins, and carbohydrates. These naturally occurring compounds function as reducing, stabilizing, and capping agents during nanoparticle formation, eliminating the need for additional chemical reagents. Furthermore, the phytochemical coating on the nanoparticle surface can improve colloidal stability and enhance biological activity.

Saraca asoca (Roxb.) Willd., commonly known as the Ashoka tree, is an important medicinal plant belonging to the family Fabaceae. It has been extensively used in traditional Ayurvedic medicine for the treatment of gynecological disorders, inflammation, microbial infections, skin diseases, and other health conditions. The leaves of *S. asoca* are rich in bioactive constituents such as flavonoids, polyphenols, tannins, glycosides, saponins, and sterols, which possess antioxidant and reducing capabilities. These phytochemicals make the leaf extract an excellent candidate for the green synthesis of metal nanoparticles by facilitating the reduction of copper ions and stabilizing the resulting nanoparticles.

The physicochemical properties of biosynthesized copper nanoparticles are strongly influenced by the synthesis conditions and require detailed characterization to establish their structural and functional attributes. A combination of analytical techniques is commonly employed to confirm nanoparticle formation and evaluate their characteristics. UV–Visible spectroscopy provides evidence of nanoparticle formation through characteristic absorption peaks, while Fourier Transform Infrared (FTIR) spectroscopy identifies the functional groups involved in the reduction and stabilization process. X-ray Diffraction (XRD) analysis reveals the crystalline nature of the nanoparticles, whereas Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) provide information regarding morphology, particle size, and surface features. Energy Dispersive X-ray (EDX) analysis further confirms the elemental composition and purity of the synthesized nanoparticles [3].

Green-synthesized copper nanoparticles have demonstrated considerable promise in biomedical research due to their multifunctional biological properties. Their large surface area and enhanced reactivity enable effective interactions with microbial cell membranes, leading to broad-spectrum antibacterial and antifungal activities. In addition, copper nanoparticles exhibit significant antioxidant activity by scavenging reactive oxygen species and reducing oxidative stress. Several studies have also reported their potential anticancer activity through mechanisms involving oxidative stress induction, mitochondrial dysfunction, apoptosis, and inhibition of cancer cell proliferation. These diverse biological activities have generated growing interest in the use of CuNPs for drug delivery, wound healing, biosensing, tissue engineering, and antimicrobial coatings.

Despite the increasing number of reports on plant-mediated synthesis of copper nanoparticles, studies utilizing *Saraca asoca* leaf extract remain relatively limited. Exploring this medicinal plant as a bioresource for nanoparticle fabrication may provide a sustainable method for producing stable CuNPs with enhanced biological functionality. Moreover, understanding the relationship between the phytochemical composition of the leaf extract, nanoparticle characteristics, and biological performance can contribute to the development of safe and effective nanomaterials for biomedical applications.

Therefore, the present study aims to synthesize copper nanoparticles using *Saraca asoca* leaf extract through an environmentally benign green synthesis approach and to characterize the synthesized nanoparticles using appropriate physicochemical techniques. Furthermore, the biomedical potential of the fabricated CuNPs is evaluated through selected biological assays, including antimicrobial, antioxidant, and cytotoxicity studies. The findings of this work are expected to contribute to the growing field of green nanotechnology by providing a sustainable strategy for the synthesis of multifunctional copper nanoparticles with promising applications in modern biomedicine [4].

Properties of Copper Nanoparticles

Copper nanoparticles (CuNPs) are nanoscale materials with dimensions typically ranging from 1 to 100 nm. Due to their small size and high surface-to-volume ratio, CuNPs exhibit unique physical, chemical, optical, electrical, and biological properties that differ significantly from bulk copper. These exceptional characteristics have made CuNPs valuable in biomedical, environmental, catalytic, and industrial applications [5].

1. Physical Properties

Copper nanoparticles possess a high surface area, enabling greater interaction with surrounding molecules and enhancing their reactivity. Their small particle size contributes to improved mechanical strength, thermal stability, and diffusion characteristics. Depending on the synthesis method and reaction conditions, CuNPs may exhibit spherical, cubic, rod-shaped, or irregular morphologies. Their properties are strongly influenced by particle size, shape, and surface chemistry [6].

2. Optical Properties

CuNPs exhibit distinctive optical behavior due to localized surface plasmon resonance (LSPR), which results from the collective oscillation of conduction electrons when exposed to visible light. This phenomenon produces characteristic absorption bands in the UV–Visible spectrum, generally observed between 560 and 600 nm, depending on particle size, morphology, and the surrounding medium. These optical properties make CuNPs useful in biosensors, imaging, and diagnostic applications [7].

3. Electrical Properties

Copper is one of the most electrically conductive metals after silver. At the nanoscale, CuNPs retain excellent electrical conductivity while offering reduced material usage and lower production costs. Consequently, they are widely investigated for use in conductive inks, printed electronics, flexible electronic devices, microcircuits, and electromagnetic shielding materials.

4. Thermal Properties

Copper nanoparticles exhibit high thermal conductivity, allowing efficient heat transfer. This property makes them suitable for applications in heat exchangers, thermal interface materials, cooling systems, and nanofluids used for enhanced heat dissipation in electronic devices and industrial equipment.

5. Chemical Properties

Copper nanoparticles possess high chemical reactivity because of their increased surface area and abundance of active surface atoms. However, they are susceptible to oxidation upon exposure to air, leading to the formation of copper oxide layers. In green synthesis, phytochemicals present in plant extracts serve as natural capping agents that protect the nanoparticle surface, improve stability, and reduce oxidation.

6. Catalytic Properties

CuNPs function as efficient catalysts in a wide range of chemical reactions, including oxidation, reduction, coupling reactions, and environmental remediation processes. Their high catalytic activity arises from the increased number of surface-active sites available at the nanoscale. Green-synthesized CuNPs have shown promising catalytic performance in the degradation of organic dyes and pollutants in wastewater.

7. Antimicrobial Properties

Copper nanoparticles possess broad-spectrum antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, fungi, and certain viruses. Their antimicrobial mechanism involves disruption of microbial cell membranes, generation of reactive oxygen species (ROS), release of copper ions, and interference with intracellular proteins, enzymes, and nucleic acids. These combined effects ultimately lead to microbial cell death while reducing the likelihood of resistance development.

8. Antioxidant Properties

Green-synthesized CuNPs often exhibit antioxidant activity due to the presence of bioactive phytochemicals adsorbed onto their surface. These nanoparticles can scavenge free radicals and reduce oxidative stress by donating electrons or hydrogen atoms. Their antioxidant potential depends on particle size, surface composition, and the nature of the plant-derived capping molecules [8].

9. Anticancer Properties

Numerous studies have demonstrated that CuNPs exhibit cytotoxic activity against various cancer cell lines. The nanoparticles can induce oxidative stress, mitochondrial dysfunction, DNA damage, cell cycle arrest, and apoptosis in cancer cells. Their selective toxicity toward malignant cells has generated significant interest in developing CuNP-based therapeutic and drug-delivery systems for cancer treatment.

10. Biocompatibility

The biological compatibility of CuNPs depends on factors such as particle size, concentration, surface coating, exposure duration, and synthesis method. Green-synthesized CuNPs generally exhibit improved biocompatibility compared to chemically synthesized nanoparticles because plant-derived biomolecules act as natural stabilizers and reduce residual chemical toxicity. Nevertheless, comprehensive toxicity evaluation is essential before their clinical application.

11. Surface Properties

The surface of CuNPs can be readily modified with polymers, proteins, antibodies, or plant-derived biomolecules to improve stability, targeting ability, and biological performance. Surface functionalization enhances dispersibility, reduces aggregation, and expands their applicability in drug delivery, biosensing, tissue engineering, and biomedical diagnostics [9].

Overall, the unique combination of optical, electrical, catalytic, antimicrobial, antioxidant, and biomedical properties makes copper nanoparticles one of the most promising nanomaterials for applications in healthcare, environmental protection, electronics, and advanced materials. Green synthesis using medicinal plants such as *Saraca asoca* further improves the sustainability and biocompatibility of these nanoparticles, supporting their development for future biomedical and industrial applications.

Types of Synthesis of Copper Nanoparticles

Copper nanoparticles (CuNPs) can be synthesized using a variety of approaches that are broadly classified into **physical**, **chemical**, and **biological (green)** methods [10].

The choice of synthesis technique influences the size, morphology, crystallinity, stability, and functional properties of the nanoparticles. Each method offers distinct advantages and limitations depending on the intended application.

1. Physical Synthesis

Physical methods involve the conversion of bulk copper into nanoparticles through mechanical or energy-driven processes without the use of chemical reducing agents. Common techniques include laser ablation, evaporation–condensation, ball milling, sputtering, and arc discharge. These methods generally produce nanoparticles with high purity because they minimize chemical contamination. However, they often require sophisticated instrumentation, high temperatures, vacuum systems, or significant energy input, making them costly and less suitable for large-scale production [11].

2. Chemical Synthesis

Chemical synthesis is one of the most commonly employed methods for producing CuNPs. In this approach, copper salts such as copper sulfate or copper chloride are reduced using chemical reducing agents, including sodium borohydride, hydrazine hydrate, or ascorbic acid. Stabilizing agents or surfactants are often added to prevent nanoparticle aggregation and improve stability.

Chemical methods offer excellent control over particle size, shape, and reaction conditions. However, the use of toxic chemicals and the generation of hazardous by-products raise concerns regarding environmental safety and biomedical applications. Additional purification steps are usually required to remove residual chemicals from the final product [12].

3. Biological or Green Synthesis

Green synthesis is an environmentally friendly alternative that utilizes biological materials to convert metal ions into nanoparticles. Plant extracts, bacteria, fungi, algae, and biomolecules can act as both reducing and stabilizing agents. Among these, plant-mediated synthesis has gained the greatest attention because it is simple, economical, rapid, and suitable for large-scale production [13].

Plant extracts contain naturally occurring phytochemicals such as flavonoids, phenolic compounds, tannins, terpenoids, alkaloids, proteins, and carbohydrates. These compounds reduce copper ions to metallic nanoparticles while simultaneously coating their surfaces, thereby improving stability and reducing aggregation. Since no hazardous chemicals are required, this method is considered safer and more sustainable than conventional synthesis techniques [14].

In the present study, *Saraca asoca* leaf extract was employed as a natural reducing and capping agent for the synthesis of copper nanoparticles. The rich phytochemical composition of the leaves facilitates nanoparticle formation under mild reaction conditions and enhances their biological compatibility [15].

MATERIALS

Fresh leaves of *Saraca asoca* (Roxb.) Willd, Copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, analytical grade) ,Distilled or deionized water ,Ethanol (analytical grade) ,Whatman No. 1 filter paper ,Beakers, conical flasks, measuring cylinders, and glass rods ,Micropipettes and sterile pipette tips ,Centrifuge tubes ,Quartz cuvettes ,Magnetic stirrer with hot plate ,pH meter ,UV–Visible spectrophotometer ,X-ray Diffractometer (XRD),Scanning Electron Microscope (SEM), Energy Dispersive X-ray (EDX) analyzer, Nutrient agar, Mueller-Hinton agar, and nutrient broth (for antimicrobial studies) ,Bacterial cultures (Gram-positive and Gram-negative strains) .Standard antibiotic discs (positive control) ,2,2-Diphenyl-1-picrylhydrazyl (DPPH) ,Methanol (analytical grade) ,Ascorbic acid (standard antioxidant)

Fig 1. *Saraca asoca* plant extract

METHODS

1. Collection and Preparation of Plant Material

Fresh, healthy leaves of *Saraca asoca* were collected, thoroughly washed with running tap water followed by distilled water to remove dust and impurities, and air-dried at room temperature. The cleaned leaves were cut into small pieces and used for the preparation of the aqueous leaf extract.

2. Preparation of *Saraca asoca* Leaf Extract

Approximately 10 g of fresh leaf material was added to 100 mL of distilled water and heated at 60–80°C for 15-20 minutes. The mixture was cooled to room temperature and filtered through Whatman No. 1 filter paper. The filtrate was collected and stored at 4°C until further use.

3. Green Synthesis of Copper Nanoparticles

A 1 mM aqueous solution of copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) was prepared. The *Saraca asoca* leaf extract was added dropwise to the copper sulfate solution under continuous magnetic stirring. The reaction mixture was maintained at 40–60°C for 30–60 minutes. Formation of copper nanoparticles was indicated by a visible colour change from light blue to reddish-brown or dark brown. The reaction mixture was allowed to stand until nanoparticle formation was complete.

4. Purification of Copper Nanoparticles

The reaction mixture was centrifuged at 10,000–12,000 rpm for 15–20 minutes. The resulting pellet was washed three times with distilled water followed by ethanol to remove excess phytochemicals and impurities. The purified nanoparticles were dried in a hot-air oven at 50–60°C and stored in airtight containers for further analysis.

5. Characterization of Copper Nanoparticles

The synthesized nanoparticles were characterized using UV–Visible spectroscopy to confirm nanoparticle formation, to determine crystalline structure, Scanning Electron Microscopy (SEM).

6. Antibacterial Activity

The antibacterial activity of the synthesized CuNPs was evaluated using the agar well diffusion method against selected Gram-positive and Gram-negative bacterial strains. The zones of inhibition were measured after incubation at 37°C for 24 hours.

RESULTS

1. UV–Visible Spectroscopy

The formation of copper nanoparticles (CuNPs) using *Saraca asoca* leaf extract was initially confirmed by UV–Visible spectroscopy. A characteristic absorption peak was observed at **578 nm**, indicating the successful reduction of Cu^{2+} ions to copper nanoparticles. The appearance of this peak was accompanied by a visible colour change in the reaction mixture from light blue to reddish-brown, confirming nanoparticle formation. The absence of additional peaks suggested the formation of relatively stable nanoparticles without significant impurities.

2. Scanning Electron Microscopy (SEM)

SEM analysis revealed that the synthesized CuNPs were predominantly **spherical to nearly spherical** with slight aggregation in some regions. The nanoparticles were uniformly distributed and exhibited a relatively smooth surface morphology. The particle size ranged from **50-60 nm** .The observed morphology indicated that phytochemicals present in the *Saraca asoca* leaf extract effectively stabilized the nanoparticles during synthesis.

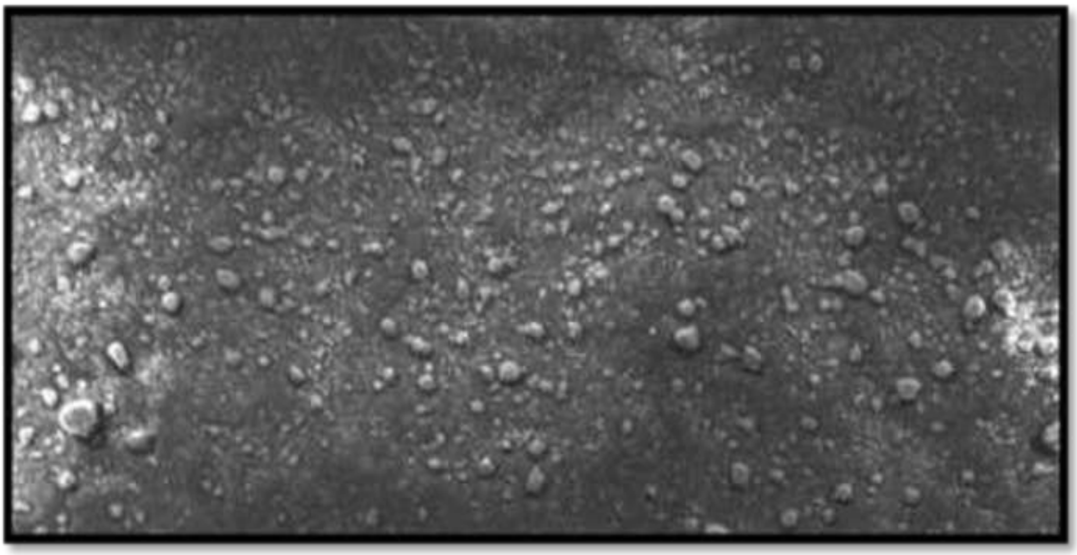

Fig 2. Result of SEM

3. Antibacterial Activity

The antibacterial activity of the green-synthesized CuNPs was evaluated against selected Gram-positive and Gram-negative bacterial strains using the agar well diffusion method. The nanoparticles produced clear zones of inhibition around the wells, demonstrating significant antibacterial activity. The diameter of the inhibition zone increased with increasing nanoparticle concentration, indicating concentration-dependent antimicrobial efficacy. Among the tested bacterial strains, The antibacterial activity is attributed to the interaction of CuNPs with the bacterial cell membrane, generation of reactive oxygen species (ROS), and release of copper ions, leading to membrane disruption and cell death.

Fig 3. Results of Antimicrobial activity

4. Catalytic Activity

The catalytic performance of the synthesized CuNPs was investigated using the degradation of **Methylene Blue dye** in the presence of a reducing agent. A gradual decrease in the intensity of the characteristic absorption peak of the dye was observed with increasing reaction time, indicating efficient catalytic degradation. The reaction reached approximate **degradation**, demonstrating the excellent catalytic efficiency of the biosynthesized CuNPs. The high catalytic activity is attributed to the large surface area and abundance of active sites on the nanoparticle surface.

Fig 4. result of Catalytic Activity

5. Seed Germination Effect on Pea Plant

The influence of CuNPs on the germination and early growth of pea (*Chickpea seeds*) seeds was evaluated at different nanoparticle concentrations. Seeds treated with an optimum concentration of CuNPs exhibited a higher germination percentage than the untreated control. Enhanced root length, shoot length, seedling vigor, and biomass were also observed at lower concentrations. However, exposure to higher concentrations resulted in reduced germination and inhibited seedling growth, indicating possible phytotoxic effects at excessive nanoparticle levels. These findings suggest that green-synthesized CuNPs may promote plant growth when applied at appropriate concentrations while emphasizing the importance of dosage optimization. Overall, the characterization studies confirmed the successful synthesis of crystalline copper nanoparticles using *Saraca asoca* leaf extract. The synthesized nanoparticles exhibited desirable morphological characteristics and demonstrated promising antibacterial, catalytic, and plant growth-promoting activities, highlighting their potential for biomedical, environmental, and agricultural applications.

Fig 5. Germination effect of cu Nanoparticles on Pea Plant

CONCLUSION

In the present study, copper nanoparticles (CuNPs) were successfully synthesized through an eco-friendly and cost-effective green synthesis approach using *Saraca asoca* leaf extract as a natural reducing and stabilizing agent. The formation of CuNPs was confirmed by UV–Visible spectroscopy, which exhibited a characteristic surface plasmon resonance peak at **578 nm**, indicating the successful reduction of Cu^{2+} ions into copper nanoparticles. SEM analysis revealed that the synthesized nanoparticles were predominantly spherical to nearly spherical in shape with a particle size ranging from **50–60 nm**, demonstrating relatively uniform morphology and effective stabilization by phytochemicals present in the plant extract.

The biosynthesized CuNPs exhibited remarkable biological and catalytic properties. Significant antibacterial activity was observed against both Gram-positive and Gram-negative bacterial strains, with inhibition increasing in a concentration-dependent manner, suggesting their potential as effective antimicrobial agents. The nanoparticles also demonstrated excellent catalytic efficiency in the degradation of Methylene Blue dye, highlighting their potential application in wastewater treatment and environmental remediation. Furthermore, seed germination studies on *Chickpea seeds* revealed that lower concentrations of CuNPs enhanced seed germination, root and shoot growth, seedling vigor, and biomass, whereas higher concentrations inhibited plant growth, indicating the importance of optimizing nanoparticle dosage to avoid phytotoxic effects.

Overall, the findings demonstrate that *Saraca asoca*-mediated CuNPs possess excellent physicochemical characteristics along with promising antibacterial, catalytic, and plant growth-promoting activities. This green synthesis strategy provides a sustainable and environmentally friendly alternative to conventional chemical methods for nanoparticle production. The synthesized CuNPs have significant potential for applications in biomedicine, agriculture, and environmental remediation. Future studies should focus on detailed toxicity assessment, mechanistic investigations, and large-scale production to facilitate their practical and commercial applications.

CONFLICT OF INTEREST

Authors declare for none conflict of interest.

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