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GREEN SYNTHESIS AND CHARACTERIZATION OF COPPER NANOPARTICLES USING *AZADIRACHTA INDICA* LEAF EXTRACT

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ABSTRACT

The present study investigates the green synthesis, characterization, and biological activities of copper nanoparticles (CuNPs) synthesized using *Azadirachta indica* (neem) leaf extract. Green synthesis of nanoparticles has gained considerable attention as an eco-friendly, economical, simple, and sustainable alternative to conventional chemical and physical methods that may involve toxic reagents, high energy consumption, and hazardous by-products. Fresh *A. indica* leaves were collected, washed, dried, and used to prepare an aqueous leaf extract. The phytochemicals present in the extract acted as natural reducing and stabilizing agents during the synthesis of CuNPs. The formation of nanoparticles was initially indicated by a visible colour change in the reaction mixture. The synthesized CuNPs were subsequently purified, dried, and characterized using UV–Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and microscopic techniques such as Scanning Electron Microscopy (SEM) and/or Transmission Electron Microscopy (TEM). These analyses were used to determine the optical properties, functional groups, crystalline

structure, morphology, and particle size of the synthesized nanoparticles. Furthermore, the biological activities of the CuNPs were evaluated using appropriate in-vitro antimicrobial and antioxidant assays. The antimicrobial activity was assessed against selected bacterial and/or fungal strains, while antioxidant potential was determined using suitable free-radical-scavenging assays. Appropriate controls were included for comparison. The study highlights the potential of *A. indica* leaf extract as an effective natural source for the synthesis and stabilization of CuNPs. The synthesized nanoparticles may possess promising biological activities and potential applications in pharmaceutical, biomedical, agricultural, and environmental fields, supporting the development of safer and more sustainable nanomaterial synthesis approaches.

KEYWORDS: Green synthesis; Copper nanoparticles; *Azadirachta indica*; Nanotechnology; Antimicrobial activity; Antioxidant activity.

1 INTRODUCTION

1.1 Nanotechnology

Nanotechnology is a rapidly expanding multidisciplinary field of science that deals with the design, synthesis, characterization, and application of materials with dimensions ranging from 1 to 100 nanometres (nm). At the nanoscale, materials exhibit unique physical, chemical, mechanical, optical, electrical, and biological properties that differ significantly from those of their bulk counterparts due to their high surface area-to-volume ratio and quantum effects. These distinctive characteristics have opened new avenues for scientific research and technological innovation across various disciplines, including chemistry, physics, biology, medicine, agriculture, environmental science, and engineering.

The concept of nanotechnology was first introduced by the renowned physicist Richard P. Feynman in his famous 1959 lecture, "There's Plenty of Room at the Bottom," where he envisioned manipulating matter at the atomic and molecular levels. Later, in 1974, the term "nanotechnology" was coined by Norio Taniguchi to describe precision engineering at the nanometre scale. Since then, nanotechnology has evolved into one of the most important areas of scientific research, with widespread applications in both academic and industrial sectors.

Nanomaterials are generally classified based on their dimensions into zero-dimensional (nanoparticles and quantum dots), one-dimensional (nanowires and nanotubes), twodimensional (graphene and nanosheets), and three-dimensional nanostructured materials. Among these, metallic nanoparticles have attracted significant attention because of their exceptional physicochemical properties and broad range of applications. Metals such as silver, gold, copper, platinum, zinc, and iron are extensively used for nanoparticle synthesis owing to their catalytic, antimicrobial, optical, and electronic properties.

Copper nanoparticles (CuNPs) have emerged as one of the most promising metallic nanoparticles due to their excellent conductivity, catalytic activity, antioxidant potential, and broad-spectrum antimicrobial properties. In addition to being relatively inexpensive and abundant compared to noble metals such as gold and silver, copper nanoparticles possess remarkable biological activities that make them suitable for applications in pharmaceuticals, biomedical engineering, drug delivery, biosensors, food packaging, water purification, agriculture, and environmental remediation.

The synthesis of nanoparticles can be achieved through physical, chemical, and biological methods. Conventional physical and chemical methods often involve expensive instrumentation, high energy consumption, and hazardous chemicals that may pose environmental and health risks. These limitations have encouraged researchers to develop eco-friendly and sustainable approaches for nanoparticle production. Green synthesis, which utilizes biological resources such as plants, microorganisms, fungi, and algae, has emerged as an environmentally benign alternative that aligns with the principles of green chemistry.

Nanotechnology has revolutionized biomedical research by enabling the development of advanced diagnostic tools, targeted drug delivery systems, antimicrobial agents, tissue engineering scaffolds, biosensors, and imaging techniques. The unique properties of nanoparticles, including their small size, enhanced surface reactivity, and ability to interact with biological molecules, contribute significantly to improved therapeutic efficacy and reduced side effects. Furthermore, nanoparticles synthesized using medicinal plant extracts often exhibit enhanced biological activities because phytochemicals present in the extracts act as reducing, stabilizing, and capping agents.

In recent years, increasing attention has been focused on the green synthesis of copper nanoparticles using medicinal plants such as Azadirachta indica (Neem). Neem leaves are rich in bioactive phytochemicals, including flavonoids, terpenoids, alkaloids, tannins, saponins, and polyphenols, which facilitate the reduction of copper ions into stable nanoparticles. These phytochemical-coated nanoparticles have demonstrated significant antimicrobial, antioxidant, anti-inflammatory, and anticancer activities, highlighting their potential in biomedical and pharmaceutical applications.

Overall, nanotechnology has transformed the field of material science by providing innovative solutions for healthcare, agriculture, environmental protection, and industrial development. The integration of nanotechnology with green synthesis approaches has paved the way for the development of sustainable nanomaterials that are environmentally friendly, cost-effective, and biologically active. Consequently, green-synthesized copper nanoparticles have become an important area of research due to their promising applications and potential to address global challenges in medicine and environmental sustainability.[1-5]

1.2 Metal Nanoparticles

Metal nanoparticles are particles composed of metallic elements with dimensions ranging from 1 to 100 nm. Due to their extremely small size and high surface area-to-volume ratio, these nanoparticles exhibit unique physicochemical, optical, electrical, magnetic, catalytic, and biological properties that are significantly different from those of their bulk materials. These distinctive characteristics have made metal nanoparticles one of the most extensively studied nanomaterials in the fields of nanotechnology, biotechnology, medicine, agriculture, environmental science, and materials engineering.

The remarkable properties of metal nanoparticles arise primarily from quantum size effects and surface plasmon resonance (SPR), phenomena that occur when the particle size is reduced to the manometer scale. Their high surface energy and enhanced reactivity enable them to interact efficiently with biological molecules and cellular components, making them highly effective in various biomedical and industrial applications. The size, shape, morphology, and surface chemistry of metal nanoparticles greatly influence their functional properties and determine their suitability for specific applications.

Metal nanoparticles can be synthesized using physical, chemical, and biological methods. Physical methods such as laser ablation, evaporation–condensation, and ball milling generally produce high-purity nanoparticles but require sophisticated instrumentation and substantial energy consumption. Chemical methods, including chemical reduction, sol-gel processes, and microemulsion techniques, are widely employed because they offer better control over particle size and morphology. However, these methods often involve hazardous reducing agents, toxic solvents, and stabilizing chemicals that may pose environmental and health risks. Consequently, biological or green synthesis methods have gained considerable importance as environmentally friendly alternatives. Green synthesis utilizes plant extracts, microorganisms, algae, and other natural resources to reduce metal ions into nanoparticles while simultaneously stabilizing them through naturally occurring biomolecules.

Several types of metal nanoparticles have been developed for diverse applications. Silver nanoparticles are widely recognized for their potent antimicrobial properties and are used in wound dressings, textiles, coatings, and water purification systems. Gold nanoparticles possess excellent optical and biocompatible properties, making them suitable for drug delivery, biosensing, bioimaging, and cancer therapy. Copper nanoparticles have attracted increasing attention because of their excellent antimicrobial, antioxidant, catalytic, electrical, and thermal properties, combined with their low cost and high natural abundance. Similarly, platinum, palladium, zinc, iron, and nickel nanoparticles are extensively utilized in catalysis, environmental remediation, energy storage, and biomedical research.

Among these, copper nanoparticles have emerged as promising nanomaterials due to their broad-spectrum biological activities and economic advantages over noble metal nanoparticles such as silver and gold. Copper nanoparticles exhibit strong antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, and anticancer activities. Their antimicrobial action is primarily attributed to the generation of reactive oxygen species (ROS), disruption of microbial cell membranes, protein denaturation, and interference with nucleic acid synthesis. These mechanisms contribute to their effectiveness against both Gram-positive and Gram-negative bacteria as well as various pathogenic fungi.

The physicochemical properties of metal nanoparticles are commonly characterized using advanced analytical techniques. Ultraviolet-visible (UV–Vis) spectroscopy is used to confirm nanoparticle formation through surface plasmon resonance absorption. Fourier Transform Infrared (FTIR) spectroscopy identifies functional groups responsible for reduction and stabilization. X-ray diffraction (XRD) determines crystalline structure and phase purity, while scanning electron microscopy (SEM) and transmission electron microscopy (TEM) provide detailed information regarding particle size, morphology, and surface characteristics. Dynamic light scattering (DLS) and zeta potential analysis are also employed to assess particle size distribution and colloidal stability.

The biological activities of metal nanoparticles have become a major focus of current research due to their potential therapeutic applications. Green-synthesized nanoparticles often exhibit enhanced biological efficacy because phytochemicals adsorbed on their surfaces contribute synergistically to their antimicrobial, antioxidant, anti-inflammatory, and cytotoxic effects. Such nanoparticles have shown promising results in wound healing, targeted drug delivery, biosensing, cancer treatment, tissue engineering, and environmental applications.

In recent years, increasing emphasis has been placed on developing sustainable methods for producing metal nanoparticles with minimal environmental impact. Plant-mediated synthesis not only eliminates the need for toxic chemicals but also improves the biocompatibility and stability of the nanoparticles. Consequently, metal nanoparticles synthesized using medicinal plants such as Azadirachta indica have gained considerable scientific interest due to their enhanced biological activities and potential applications in pharmaceutical and biomedical research. Therefore, the synthesis and characterization of copper nanoparticles using Azadirachta indica leaf extract represent an important area of investigation for the development of safe, effective, and environmentally sustainable nanomaterials.[6-10]

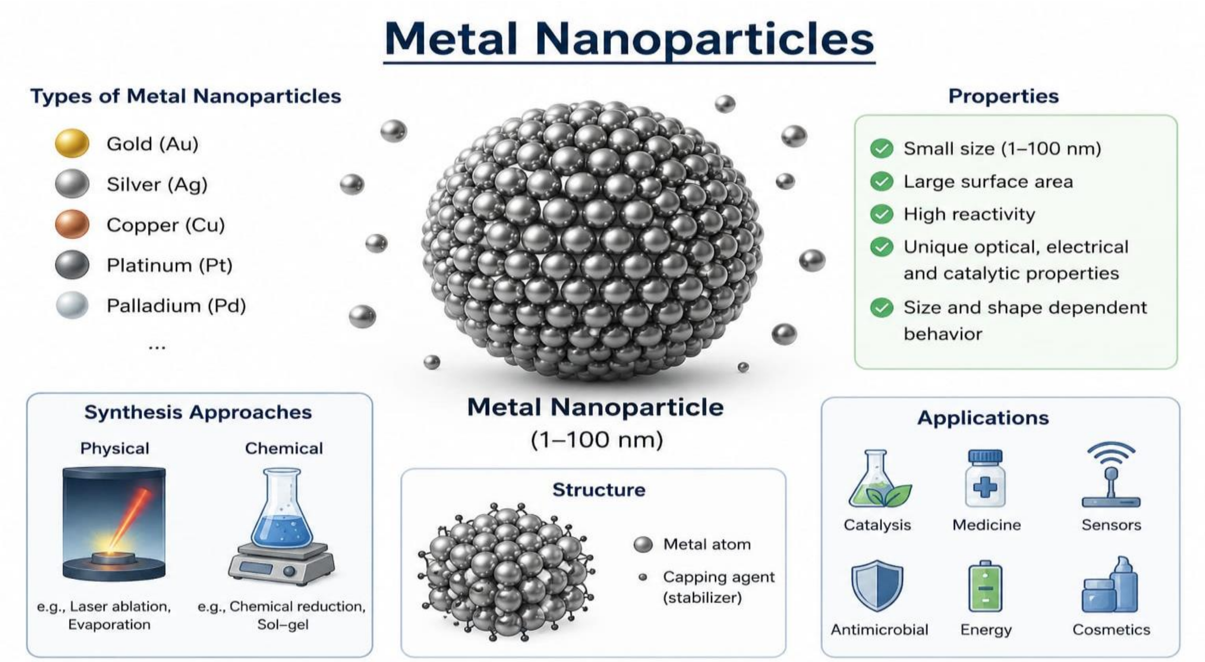


Figure 1: Schematic representation of metal nanoparticles.

1.3 Copper Nanoparticles

Copper nanoparticles (CuNPs) are metallic nanomaterials with particle sizes typically ranging from 1 to 100 nm. Due to their nanoscale dimensions, CuNPs exhibit unique physicochemical, optical, electrical, thermal, catalytic, and biological properties that differ significantly from those of bulk copper. These distinctive characteristics arise from their high surface area-to-volume ratio, quantum size effects, and increased surface reactivity, making copper nanoparticles valuable for a wide range of scientific and industrial applications.

Among various metal nanoparticles, copper nanoparticles have gained considerable attention because copper is abundant, inexpensive, and possesses excellent electrical conductivity and catalytic activity. Compared with noble metal nanoparticles such as gold and silver, CuNPs offer a cost-effective alternative while maintaining comparable functional properties. Consequently, they are increasingly utilized in biomedical research, environmental science, agriculture, electronics, catalysis, energy storage, and wastewater treatment.

Copper nanoparticles can be synthesized using physical, chemical, and biological methods. Physical methods include laser ablation, evaporation-condensation, and ball milling, whereas chemical methods involve chemical reduction using reducing agents such as sodium borohydride or hydrazine. Although these conventional methods produce nanoparticles with controlled size and morphology, they often require toxic chemicals, high temperatures, sophisticated instrumentation, and significant energy consumption. Furthermore, the use of hazardous reagents may generate environmentally harmful by-products and limit the biomedical applicability of the synthesized nanoparticles.

To overcome these limitations, green synthesis has emerged as a sustainable and environmentally friendly alternative for the production of copper nanoparticles. Plant-mediated synthesis has become particularly popular because plant extracts contain a wide range of bioactive compounds, including flavonoids, phenolic acids, tannins, alkaloids, terpenoids, proteins, sugars, and amino acids. These phytochemicals act as natural reducing agents by converting copper ions into copper nanoparticles while simultaneously functioning as stabilizing and capping agents that enhance nanoparticle stability and prevent aggregation.

The physicochemical properties of copper nanoparticles, such as particle size, shape, crystallinity, surface charge, and stability, are influenced by various synthesis parameters, including precursor concentration, plant extract composition, pH, temperature, reaction time, and the ratio of metal salt to extract. These characteristics play a crucial role in determining the biological activities and practical applications of CuNPs. Therefore, detailed characterization using analytical techniques such as UV–Visible spectroscopy, Fourier

Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS), and Energy Dispersive X-ray Spectroscopy (EDX) is essential to confirm nanoparticle formation and evaluate their structural and morphological properties.

Copper nanoparticles possess remarkable biological activities owing to their ability to generate reactive oxygen species (ROS), disrupt microbial cell membranes, interfere with intracellular metabolic processes, and induce oxidative stress in pathogenic microorganisms. These mechanisms contribute to their broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, and certain viruses. In addition, CuNPs exhibit significant antioxidant, anti-inflammatory, anticancer, antidiabetic, and wound-healing properties, making them promising candidates for biomedical and pharmaceutical applications.

In agriculture, copper nanoparticles have been investigated as nano-fertilizers, nano-pesticides, and antimicrobial agents for crop protection. Their controlled release properties and enhanced bioavailability improve nutrient utilization and reduce environmental pollution associated with conventional agrochemicals. In environmental applications, CuNPs are employed as catalysts for the degradation of organic pollutants, dye removal, wastewater purification, and environmental remediation due to their high catalytic efficiency and redox activity.

Despite their numerous advantages, the toxicity and environmental impact of copper nanoparticles remain important considerations. Factors such as particle size, concentration, surface chemistry, exposure duration, and method of synthesis significantly influence their biocompatibility and ecological safety. Green synthesized CuNPs are generally considered less toxic than chemically synthesized nanoparticles because the phytochemicals adsorbed on their surface improve stability, reduce toxicity, and enhance compatibility with biological systems.

Overall, copper nanoparticles represent one of the most promising classes of metallic nanomaterials due to their exceptional physicochemical properties, cost-effectiveness, and diverse biological activities. The development of eco-friendly synthesis strategies, particularly using medicinal plant extracts such as Azadirachta indica, offers an efficient approach for producing stable, biocompatible, and biologically active CuNPs. Such nanoparticles have immense potential for applications in medicine, agriculture, environmental protection, and various industrial sectors, highlighting their significance in modern nanotechnology research.[11-15]

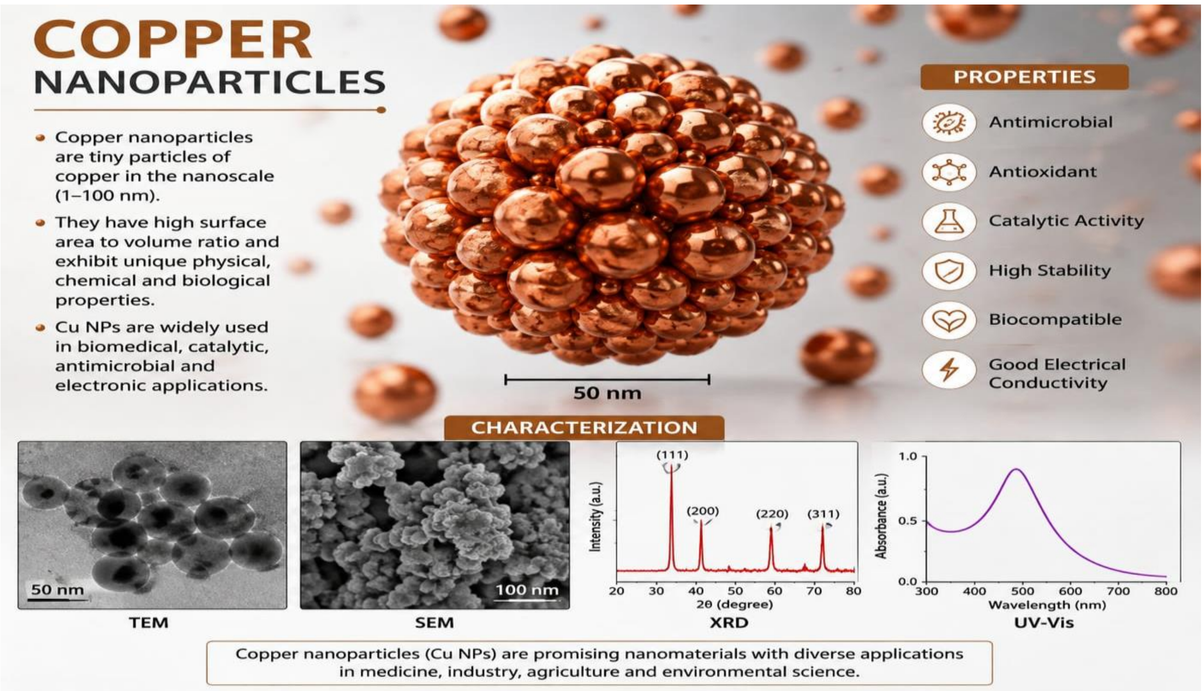


Figure 2: Schematic Diagram of Copper nanoparticles.

1.4 Green Synthesis of Nanoparticles

Nanotechnology has emerged as one of the most significant interdisciplinary fields of modern science, with nanoparticles exhibiting unique physical, chemical, optical, electrical, and biological properties that differ markedly from their bulk counterparts. Among various metallic nanoparticles, copper nanoparticles (CuNPs) have attracted considerable attention due to their excellent antimicrobial, antioxidant, catalytic, and electrical properties, making them suitable for applications in medicine, agriculture, environmental remediation, and electronics.

Conventional methods for synthesizing nanoparticles, including physical and chemical techniques, often require high energy input, expensive equipment, and hazardous chemicals that may generate toxic by-products. These limitations have prompted researchers to develop environmentally friendly, cost-effective, and sustainable alternatives for nanoparticle synthesis.

Green synthesis of nanoparticles is an eco-friendly approach that utilizes biological materials such as plants, bacteria, fungi, algae, and other natural resources as reducing and stabilizing agents. This method aligns with the principles of green chemistry by minimizing the use of toxic chemicals, reducing energy consumption, and generating fewer environmental pollutants. The biomolecules present in these biological sources, including phenolics, flavonoids, terpenoids, alkaloids, proteins, carbohydrates, and vitamins, facilitate the reduction of metal ions into nanoparticles while simultaneously stabilizing them against aggregation.

Among the various biological resources, plant-mediated synthesis has gained considerable importance due to its simplicity, rapidity, scalability, and economic feasibility. Plant extracts contain a diverse range of phytochemicals capable of reducing metal salts into nanoparticles under mild reaction conditions without the need for additional chemical reducing agents. Furthermore, plant-based synthesis is generally considered safer than microbial methods because it eliminates the need for maintaining sterile culture conditions and minimizes the risk of microbial contamination.

The synthesis of metallic nanoparticles using plant extracts has been successfully demonstrated for metals such as silver, gold, copper, zinc oxide, and iron oxide. The characteristics of the synthesized nanoparticles, including their size, shape, morphology, and stability, depend on several factors such as the type of plant extract, concentration of metal ions, pH, temperature, reaction time, and the nature of phytochemical constituents. These parameters significantly influence the biological and physicochemical properties of the nanoparticles.

Green synthesized nanoparticles have demonstrated enhanced biological activities, including antimicrobial, antioxidant, anti-inflammatory, anticancer, and wound-healing properties. The presence of phytochemical residues on the nanoparticle surface often enhances their biocompatibility and therapeutic potential. Consequently, green synthesis has become an attractive strategy for developing nanoparticles intended for biomedical applications while reducing environmental impact.[16-20]

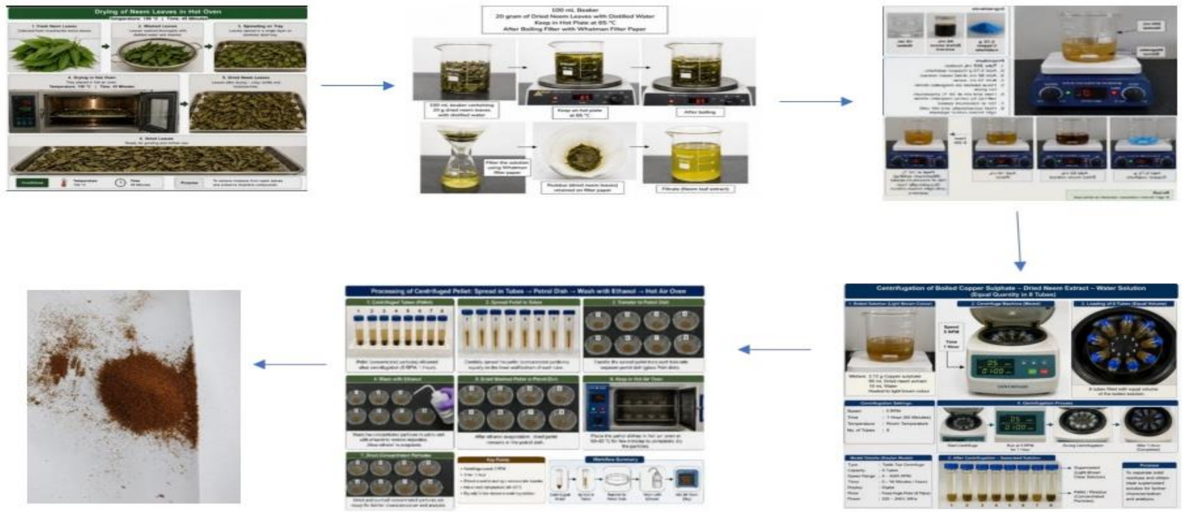


Figure 3: Green synthesis process of metal nanoparticles using plant extract.

1.5 Azadirachta indica Leaves

Azadirachta indica A. Juss., commonly known as Neem, belongs to the family Meliaceae and is one of the most valuable medicinal plants widely distributed in tropical and subtropical regions, particularly in India, Bangladesh, Pakistan, and Sri Lanka. Neem has been recognized for centuries in traditional systems of medicine such as Ayurveda, Siddha, and Unani because of its diverse therapeutic properties. Every part of the neem tree, including the leaves, bark, seeds, flowers, fruits, and roots, possesses significant medicinal value. Among these, the leaves are extensively investigated due to their rich phytochemical composition and broad spectrum of biological activities.

Neem leaves contain a variety of bioactive constituents, including flavonoids, alkaloids, terpenoids, tannins, saponins, glycosides, steroids, polyphenols, reducing sugars, proteins, amino acids, and essential oils. Major phytochemicals such as azadirachtin, nimbin, nimbidin, nimbolide, quercetin, salannin, gedunin, and sodium nimbinate are responsible for the plant's pharmacological activities. These compounds exhibit potent antioxidant, antimicrobial, anti-inflammatory, antiviral, antifungal, antiparasitic, anticancer, hepatoprotective, immunomodulatory, and wound-healing properties.

The high content of phenolic compounds and flavonoids present in neem leaves makes them excellent reducing and stabilizing agents for the biosynthesis of metal nanoparticles. During green synthesis, these phytochemicals reduce copper ions (Cu^{2+}) into copper nanoparticles while simultaneously acting as capping agents that prevent aggregation and enhance nanoparticle stability. Therefore, neem leaf extract serves as an eco-friendly alternative to hazardous chemical reducing agents.

Green synthesis using neem leaves has gained considerable attention because it offers several advantages over conventional physical and chemical methods. Traditional methods require toxic reducing agents, high energy consumption, expensive instrumentation, and harsh reaction conditions. In contrast, plant-mediated synthesis is simple, rapid, cost-effective, environmentally benign, and suitable for large-scale production. Furthermore, nanoparticles synthesized using plant extracts often exhibit improved biocompatibility and enhanced biological activities due to the presence of phytochemical coatings on their surfaces.

Several researchers have reported the successful synthesis of copper nanoparticles using neem leaf extract. The synthesized nanoparticles are generally spherical or quasi-spherical, with particle sizes ranging from 10–100 nm, depending on synthesis conditions such as pH, temperature, extract concentration, and precursor concentration. Characterization studies using UV–Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Energy Dispersive X-ray Analysis (EDX), and Dynamic Light Scattering (DLS) confirm nanoparticle formation, crystalline nature, morphology, elemental composition, and particle size distribution.

Figure 4: Azadirachta indica leaf extract.

The phytochemicals adsorbed on the nanoparticle surface enhance stability by providing steric and electrostatic protection against oxidation and aggregation. These surface biomolecules also contribute significantly to the biological performance of the nanoparticles, particularly their antimicrobial and antioxidant activities.

Copper nanoparticles synthesized from neem leaves possess unique physicochemical properties resulting from their nanoscale dimensions. Their high surface-area-to-volume ratio increases surface reactivity and interaction with microbial cells. In addition, phytochemical functionalization enhances biological compatibility and therapeutic efficacy.

Neem-mediated copper nanoparticles have attracted considerable interest in biomedical research because of their remarkable antimicrobial efficacy against various Gram-positive and Gram-negative bacteria, fungi, and drug-resistant microorganisms. Besides antimicrobial applications, these nanoparticles demonstrate antioxidant, anticancer, catalytic, larvicidal, anti-inflammatory, wound-healing, and environmental remediation properties.

The green synthesis of copper nanoparticles using Azadirachta indica leaves therefore represents an ideal combination of nanotechnology and herbal medicine, providing sustainable, economical, and biologically active nanomaterials suitable for pharmaceutical and biomedical applications.

1.6. Antibacterial Activity

The emergence of multidrug-resistant bacterial pathogens has become one of the greatest global public health challenges. The extensive and inappropriate use of antibiotics has resulted in bacterial strains that are resistant to multiple classes of antimicrobial agents, thereby reducing the effectiveness of conventional therapies. Consequently, there is an urgent need to develop alternative antimicrobial agents that are effective, safe, economical, and environmentally sustainable. Green synthesized copper nanoparticles have emerged as promising antibacterial agents due to their unique physicochemical characteristics and multiple mechanisms of action.

Copper has been recognized as a broad-spectrum antimicrobial metal since ancient times. At the nanoscale, copper exhibits significantly enhanced antibacterial activity because of its increased surface area and higher reactivity. Copper nanoparticles synthesized using Azadirachta indica leaf extract demonstrate superior antimicrobial activity owing to the combined effects of copper ions and bioactive phytochemicals present on the nanoparticle surface.

The antibacterial activity of copper nanoparticles depends on several physicochemical properties, including particle size, morphology, surface charge, crystallinity, concentration, and stability. Smaller nanoparticles possess a larger surface-area-to-volume ratio, enabling greater interaction with bacterial cell membranes and enhanced penetration into bacterial cells.

Several mechanisms have been proposed to explain the antibacterial activity of copper nanoparticles. Initially, nanoparticles adsorb onto the bacterial cell surface through electrostatic interactions, causing disruption of membrane integrity and increased membrane permeability. The damaged membrane results in leakage of intracellular components such as proteins, nucleic acids, enzymes, and electrolytes, ultimately leading to bacterial cell death.

Copper nanoparticles also generate reactive oxygen species (ROS) including hydroxyl radicals, superoxide radicals, and hydrogen peroxide. Excessive ROS production induces oxidative stress, resulting in lipid peroxidation, protein oxidation, DNA damage, enzyme inactivation, and mitochondrial dysfunction. These oxidative processes interfere with essential cellular functions and inhibit bacterial growth.

Another important antibacterial mechanism involves the gradual release of Cu^{2+} ions from copper nanoparticles. These ions bind strongly to proteins containing sulfhydryl groups, causing enzyme denaturation and disruption of metabolic pathways. Copper ions also interfere with DNA replication and protein synthesis, thereby preventing bacterial proliferation.

Copper nanoparticles exhibit antibacterial activity against both Gram-positive bacteria, such as Staphylococcus aureus and Bacillus subtilis, and Gram-negative bacteria, including Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, and Klebsiella pneumoniae. Generally, Gram-negative bacteria are more susceptible because of their thinner peptidoglycan layer, which facilitates nanoparticle penetration.

The phytochemicals present in neem leaf extract further enhance antibacterial activity. Flavonoids, tannins, alkaloids, terpenoids, and phenolic compounds possess intrinsic antimicrobial properties and act synergistically with copper nanoparticles. This synergistic interaction improves bacterial inhibition compared to chemically synthesized nanoparticles.

The antibacterial efficacy of green synthesized copper nanoparticles is commonly evaluated using standard microbiological methods such as the agar well diffusion method, disc diffusion assay, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), broth dilution method, and time-kill assay. Larger inhibition zones and lower MIC values indicate stronger antibacterial potential.

Recent studies have demonstrated that neem-mediated copper nanoparticles possess excellent antibacterial activity against antibiotic-resistant bacterial strains, suggesting their potential application in pharmaceutical formulations, wound dressings, medical device coatings, water purification systems, food packaging materials, and antimicrobial textiles.

The antibacterial effectiveness of copper nanoparticles is influenced by synthesis parameters such as precursor concentration, extract concentration, pH, temperature, reaction time, and storage conditions. Optimization of these parameters is essential to obtain nanoparticles with desirable size, stability, and biological performance.

Overall, green synthesized copper nanoparticles using Azadirachta indica leaf extract represent an effective and environmentally friendly antimicrobial nanomaterial. Their broad-spectrum antibacterial activity, multiple mechanisms of bacterial killing, reduced likelihood of resistance development, and enhanced biocompatibility make them highly promising candidates for future biomedical and pharmaceutical applications.[21-25]

2. METHODS AND MATERIALS

Copper sulphate, Neem leaf extract, ethanol, other chemicals were obtained from Sigma-Aldrich., India. All other chemicals purchased were of analytical grade.

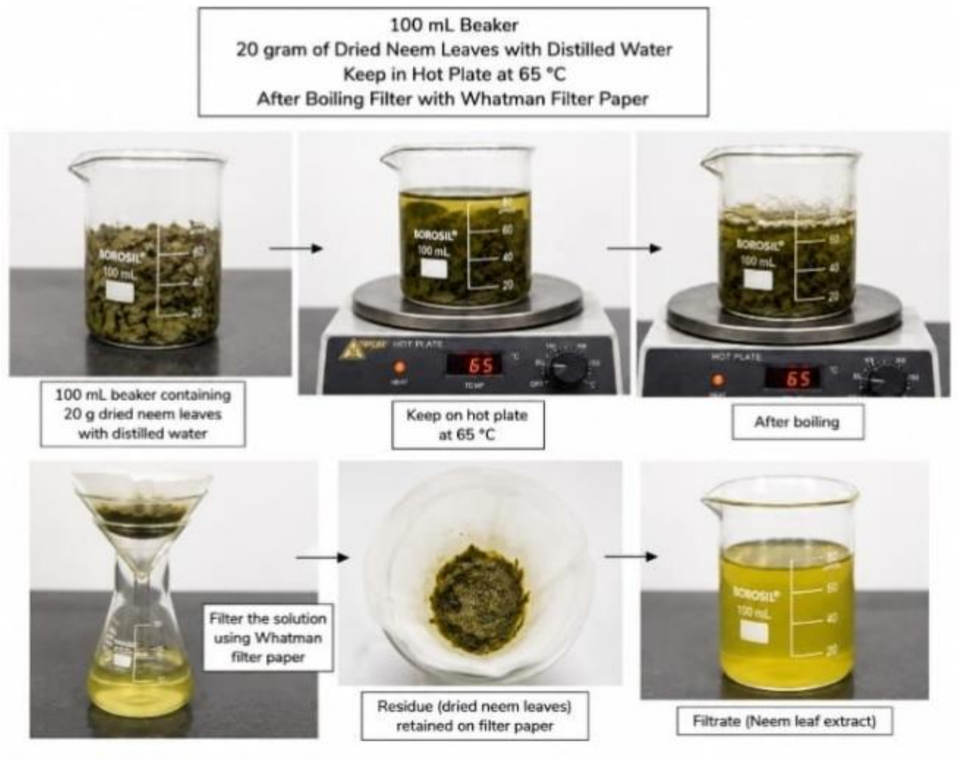
2.1 Preparation of Azadirachta indica Leaf Extract

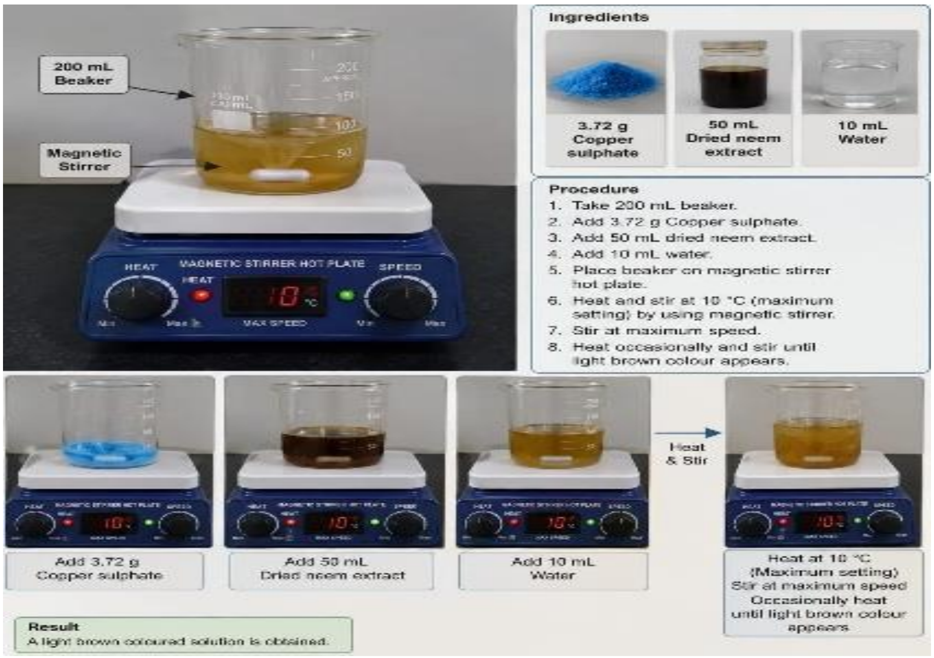
Fresh leaves of Azadirachta indica (neem) were collected and thoroughly washed with distilled water to remove dust and other impurities. The washed leaves were dried in a hot-air oven at 150°C for 25 minutes. The dried leaves were then cut into small **pieces** and crushed. Approximately 2 g of the dried neem leaves were transferred into a beaker containing 100 mL of distilled water. The mixture was heated on a hot plate at 65°C for 30 minutes with gentle stirring to facilitate the extraction of phytochemicals from the leaves. After heating, the mixture was allowed to cool and was filtered through Whatman filter paper to obtain a clear Azadirachta indica leaf extract. The obtained neem leaf extract was collected in a clean, labelled container and stored in a refrigerator at low temperature until further use for the green synthesis of copper nanoparticles.[26-30]

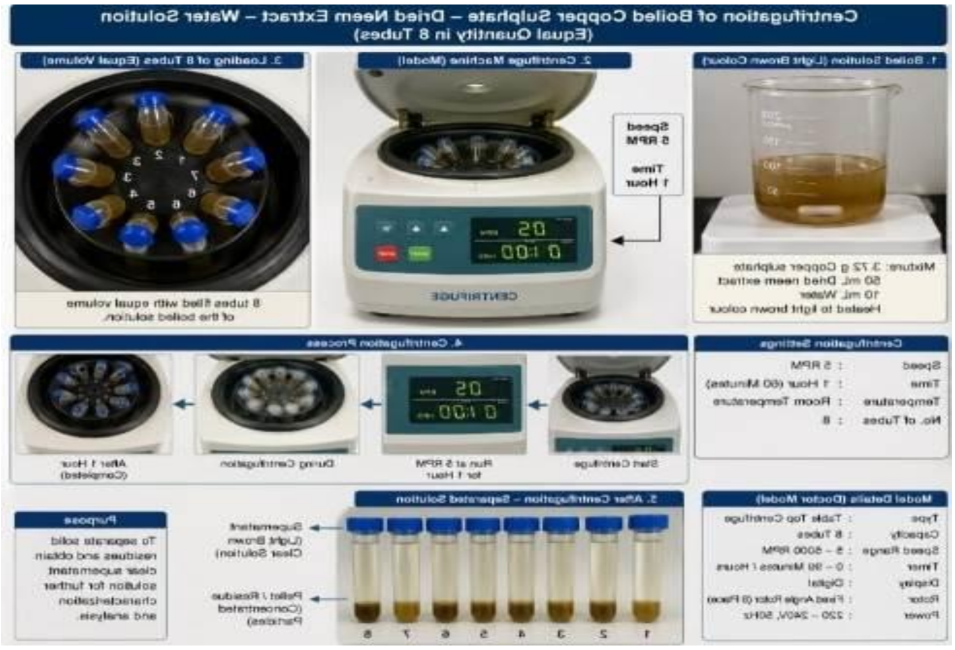
Figure 5: Drying of neem leaves in Hot Air oven.

2.2 Preparation and Purification of Copper Nanoparticles

A measured quantity of 3.72 g of copper sulphate was dissolved in 10 mL of distilled water to prepare the copper sulphate solution. The prepared copper sulphate solution was subsequently mixed with 50 mL of Azadirachta indica (neem) leaf extract. The reaction mixture was transferred to a magnetic stirrer hot plate and continuously stirred using a magnetic stirrer at maximum stirring speed. The mixture was maintained under the selected temperature conditions for an appropriate period until a light brown colour developed, indicating the formation of copper nanoparticles. The colour change was considered as a preliminary visual indication of the reduction of copper ions and formation of nanoparticles. The synthesized reaction mixture was equally distributed into eight centrifuge tubes to ensure balanced centrifugation. The tubes were placed in a centrifuge and centrifuged for approximately 1.5 hours under the selected experimental conditions. Following centrifugation, the concentrated nanoparticle pellet obtained at the bottom of the tubes was carefully collected. The pellet was washed with ethanol to remove residual plant constituents and other unreacted materials. The washed material was then filtered using Whatman filter paper and transferred into a clean Petri dish. The Petri dish containing the collected material was placed in a hot-air oven and maintained until the material was completely dry. The dried product was collected and stored appropriately for further characterization and evaluation of its biological activities.






Centrifugation of Boiled Copper Sulphate – Dried Neem Extract – Water Solution
(Equal Quantity in 8 Tubes)
1. Boiled Solution (Light Brown Colour)
2. Centrifuge Machine (Model)
3. Loading of 8 Tubes (Equal Volume)
4. Centrifugation Process
Centrifugation Settings
5. After Centrifugation – Separated Solution
Purpose
Model Details (Doctor Model)

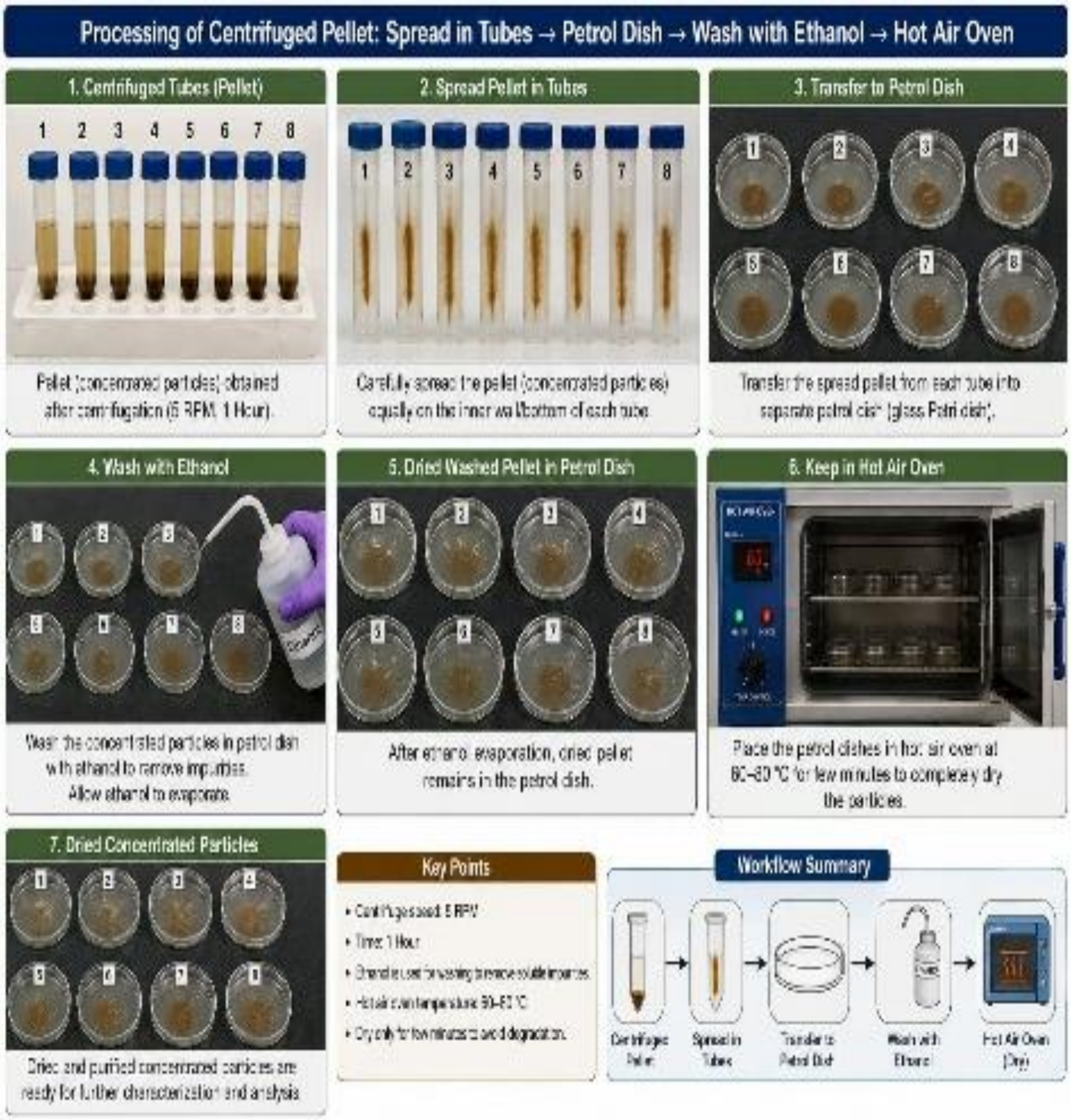

Processing of Centrifuged Pellet: Spread in Tubes → Petrol Dish → Wash with Ethanol → Hot Air Oven
1. Centrifuged Tubes (Pellet)
Pellet (concentrated particles) obtained after centrifugation (5 RPM, 1 Hour).
2. Spread Pellet in Tubes
Carefully spread the pellet (concentrated particles) equally on the inner wall/bottom of each tube.
3. Transfer to Petrol Dish
Transfer the spread pellet from each tube into separate petrol dish (glass Petri dish).
4. Wash with Ethanol
Wash the concentrated particles in petrol dish with ethanol to remove impurities. Allow ethanol to evaporate.
5. Dried Washed Pellet in Petrol Dish
After ethanol evaporation, dried pellet remains in the petrol dish.
6. Keep in Hot Air Oven
Place the petrol dishes in hot air oven at 60–80 °C for few minutes to completely dry the particles.
7. Dried Concentrated Particles
Dried and purified concentrated particles are ready for further characterization and analysis.
Key Points
Workflow Summary

Figure 6: Green synthesis of metal nanoparticles using *Azadirachta indica* (neem) leaf extract.

3. Characterization

3.1 Surface morphology by SEM

The surface morphology of the prepared samples was examined using scanning electron microscopy (SEM). The samples were appropriately dried and mounted on conductive carbon tape prior to analysis. The samples were coated with a thin layer of gold/platinum, where necessary, to improve surface conductivity. SEM images were recorded using a [instrument model, manufacturer] at an accelerating voltage of [X] kV and at suitable magnifications. The morphology and surface characteristics of the samples were subsequently analysed from the obtained micrographs.

3.2 X-ray diffraction (XRD)

The crystalline structure and phase composition of the prepared samples were analysed using X-ray diffraction (XRD). The XRD patterns were recorded using a [instrument model, manufacturer] with Cu Kα radiation ($\lambda = 1.5406$ Å) over a 2θ range of [X–Y]°, with a step size of [X]° and a scanning rate of [X]° min^{-1}. The obtained diffraction patterns were used to identify the crystalline phases and determine the structural characteristics of the prepared samples.

4. RESULTS AND DISCUSSION

4.1 Scanning electron microscopy

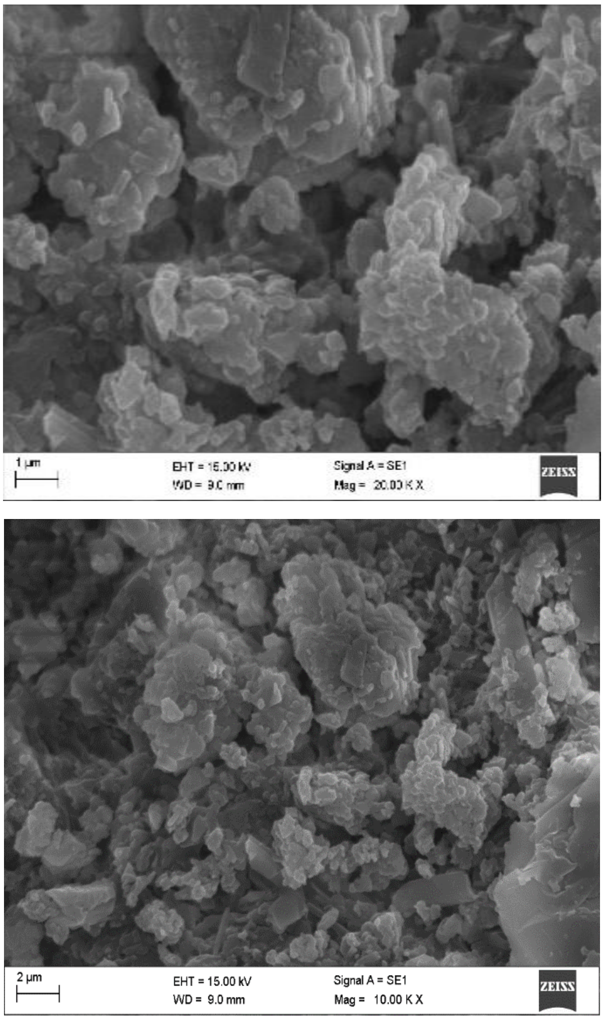
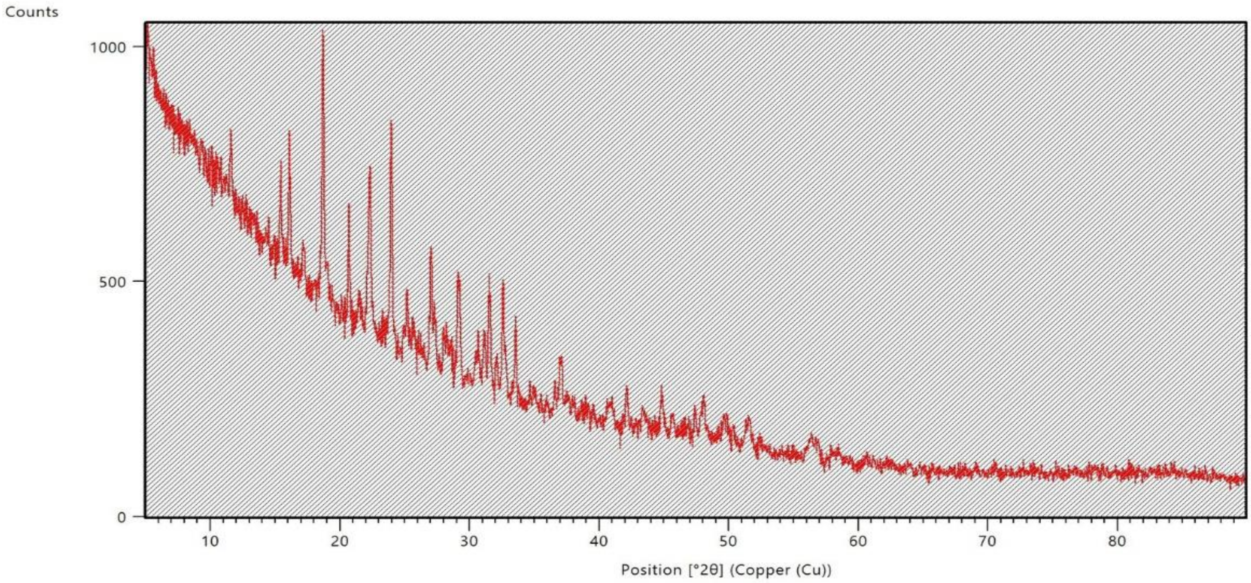


Figure 7: SEM micrograph of synthesized $CuSO_4$ Nanoparticles.

The SEM image of the synthesized copper sulfate ($CuSO_4$) sample, recorded at a magnification of 10.00 K×, provides information on its surface morphology and microstructural features. The sample displays a predominantly irregular morphology, with numerous fine primary particles gathered into larger aggregates. In addition to the clustered structures, some submicron flake- and plate-like features can be observed within the aggregated regions.

A pronounced degree of particle agglomeration is evident in the micrograph. Such aggregation may be associated with the high surface energy of fine particles and the attractive interactions acting between adjacent particles, including van der Waals forces and electrostatic interactions. Based on the 2 µm scale bar, the individual primary particles appear to fall within the nanoscale to submicron range, with estimated dimensions below approximately 200 nm. In contrast, the agglomerated structures exhibit considerably larger dimensions, extending approximately from 1 to 5 µm. Overall, the SEM observations indicate that the synthesized $CuSO_4$ sample possesses a heterogeneous, highly aggregated surface morphology with nanoscale primary features.



4.2 X-RAY DIFFRACTION

Figure 8: XRD pattern of synthesized $CuSO_4$ Nanoparticles.

Several distinct and well-defined diffraction peaks were observed, indicating the **crystalline nature of the synthesized material**. The maximum diffraction intensity was approximately 1000 counts. The observed reflections demonstrate that the sample possesses an ordered crystalline structure. However, definitive identification of the crystalline phase requires comparison of the experimentally observed 2θ values with standard reference data, such as JCPDS or ICDD databases. Similarly, the phase purity of the synthesized sample can only be confirmed after appropriate peak indexing and comparison with the corresponding standard diffraction pattern. The average crystallite size cannot be reliably determined from the XRD pattern alone and requires measurement of the full width at half maximum (FWHM) of a suitable diffraction peak followed by calculation using the Scherrer equation.

CONCLUSION

The present study entitled "Green Synthesis and Characterization of Copper Nanoparticles using Azadirachta indica Leaves and Evaluation of the Biological Activities" focuses on the development of an eco-friendly, simple, cost-effective, and sustainable method for the synthesis of copper nanoparticles using Azadirachta indica leaf extract. The use of plant-based materials provides an environmentally friendly alternative to conventional physical and chemical methods of nanoparticle synthesis, which may involve toxic chemicals and high energy requirements.

The phytochemicals naturally present in Azadirachta indica leaves, including phenolic compounds, flavonoids, terpenoids, alkaloids, tannins, proteins, and other bioactive constituents, can participate in the reduction of copper ions and stabilization of the synthesized nanoparticles. Thus, the plant extract can serve as a natural reducing and capping agent during the green synthesis process.

The successful formation of copper nanoparticles can be confirmed through suitable physicochemical characterization techniques such as UV–Visible spectroscopy, FTIR, XRD, SEM/TEM, and EDX. These techniques provide important information regarding the optical properties, functional groups, crystalline structure, elemental composition, particle size, and morphology of the synthesized nanoparticles. Characterization is essential for establishing the relationship between the structural properties of the nanoparticles and their biological activities.

The biological evaluation of the synthesized copper nanoparticles may demonstrate significant antimicrobial and antioxidant potential. Their biological activity can be attributed to their nanoscale size, high surface-area-to-volume ratio, surface-associated phytochemicals, and ability to interact with biological systems. The nanoparticles may exert antimicrobial effects through mechanisms such as disruption of microbial cell membranes, generation of reactive oxygen species, release of copper ions, and interference with essential cellular processes.

Overall, the study highlights the potential of Azadirachta indica leaves as a valuable biological resource for the green synthesis of copper nanoparticles. The approach combines the principles of nanotechnology and green chemistry and may provide a safer and more sustainable route for the development of biologically active nanomaterials. Green-synthesized CuNPs have potential applications in pharmaceutical, biomedical, agricultural, environmental, and other related fields.

However, further investigations are required to establish their long-term stability, toxicity, biocompatibility, mechanism of biological action, and safety profile. Advanced characterization and in vitro and in vivo studies may provide a better understanding of their therapeutic potential. Therefore, the present work provides a foundation for further research on Azadirachta indica-mediated copper nanoparticles and their possible applications as promising multifunctional nanomaterials.

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