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STUDY ON GREEN SYNTHESIS OF COPPER NANOPARTICLES BY USING DIFFERENT PLANT EXTRACTS & ITS CHARACTERIZATION, ANTIMICROBIAL AND ANTIOXIDANT STUDIES

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ABSTRACT

The report presents a cost-effective method for synthesizing Copper nanoparticles (Cu NPs) using various plant extracts. The NPs have a size range of 5-20 nm and show antibacterial activity against gram-positive and gram-negative microorganisms. Thestudy suggests that green synthesis of metallic nanoparticles could be an alternative tohazardous compounds and bitter reaction conditions. The NPs' unique structural properties and biological effects make them suitable for applications like antimicrobial, antifungal, and anticancer activity. The study investigates the synergistic influence of phytoconstituents in green copper nanoparticles (g-Cu NPs) using Hagenia abyssinica leaf extract from Ethiopia. The g-Cu NPs were characterized using various techniques, including UV-visible, UV-DRS, FT-IR, XRD, SEM, EDXA, TEM, HRTEM, and SAED. The g-Cu NPs showed a mix of spherical, hexagonal, triangular, cylindrical, and irregularly shaped Cu particles, with an average particle size of 34.76 nm. The antibacterial tests on E. coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis showed good zone of inhibitions, indicating potential as a remedy for infectious diseases.[2] Researchers are exploring eco-friendly and low-cost methods for biosynthesis of nanoparticles (NPs). A study involving copper oxide (CuO) NPs was conducted using a copper nitrate trihydrate precursor[3] and Catha edulis leaves extract. The NPs were characterized using various techniques, including X-ray diffractometer, SEM-EDS, TEM, UV-Vis, and FTIR. The study also tested the antimicrobial activities of different concentrations of CuO NPs using Catha edulis extract, with the highest zone of inhibition observed for S. aureus, S. pyogenes, E. coli, and K. pneumonia. This study synthesized copper nanoparticles (CuNPs) using mint extract. Various spectroscopy, X-ray diffraction, and Fourier transform infrared spectrometry were used to characterize the NPs. The NPs were found to have a spherical shape and showed a condition- dependent effect on wheat growth.[4] The maximum germination and growth rate were observed at 50 mg CuNPs/L, while growth declined beyond this concentration. The application of CuNPs improved wheat growth. This review provides an overview of the classification, characterization, preparation, and application of nanoparticles, focusing on their properties, synthesis, and applications. Ithighlights the importance of controlling shape, size, and composition to enhance nanoparticles' properties, including enhanced surface area, unique optical, electronic, and magnetic characteristics, and improved mechanical properties.[5] Nanoparticles have applications in catalysis, environmental remediation, and antimicrobial coatings, contributing to sustainable development and environmental protection. The review serves as a reference for understanding the field's latest research and developments.

KEYWORDS: Nanoparticles, Electron microscopy, Green synthesis, C.longa, Cu nanoparticles, Pathogenic activity.[1]

INTRODUCTION

The discovery of nanomaterials, particularly nanoparticles, has revolutionized various applications, including antimicrobial, antifungal, and anticancer activities. Various synthesis methods, including sono chemical reduction, thermal deposition, chemical reduction, and microwave methods, involve hazardous compounds and bitter reaction conditions. Green synthesis offers advantages such as avoiding hazardous chemicals, a clean process, nontoxicity, environmental friendliness, easy preparation, cost-effectiveness, and control oversize and shape.[6]

Nanoscience is a rapidly growing field that studies matter at the nanoscale (1-100 nm). Nanomaterials, such as metal and metal oxide nanoparticles, have made significant contributions to biomedical sensing, imaging, diagnosis, and treatment. Copper is a low-cost, highyielding material that can be used in biomedical and environmental remediation applications. Green methods for synthesizing nanomaterials have emerged, using organic and natural resources like bacteria, algae, plants, actinomycetes, and fungi. These methods are cost-effective, sustainable, reliable, and energy-efficient.

Microbial diseases are becoming more resistant to antimicrobials, and green synthesis methods based on plant extracts are preferred due to their availability, ease of handling, non- toxic, cost-effectiveness, and environmental acceptability. The natural antibacterial capabilities of plant extracts increase the qualities of green nanomaterials generated using plants.

Developing a diverse array of affordable and readily available antimicrobials is crucial for the development of new pandemic diseases. Plants are abundant and can be used to synthesize nanomaterials, such as Cu/CuO-based nanomaterials.[7]

Nanotechnology has been a growing field since the 1970s, with nanoparticles (NPs) being a significant topic. NPs are microscopic particles with dimensions less than 100 nm, and can be categorized based on morphology, size, physicochemical properties, and precursor type. Metal nanoparticles like gold and copper are created using synthetic or chemical methods, which can be environmentally unfriendly. Scientists are now using safer synthesis methods using biological reductants like bacterial, fungal, and plant material. Gold nanoparticles (AuNPs) are used in various fields[8], including gene therapy, protein delivery, cancer diagnosis, and DNA detection. Plant extracts have gained attention as reducing agents for AuNP synthesis due to their low toxicity, eco-friendliness, and simplicity of production. Copper nanoparticles (CuNPs) are gaining attention due to their ease of availability and economic feasibility. CuNPs are a stable substitute for gold and may be a low-cost replacement for unattainable precious metals.[8]

Nanotechnology has become a crucial technology with numerous applications in various industries. Recently, the focus has shifted to environmentally friendly green production of nanomaterials due to the challenges associated with chemical and physical techniques. The plant-mediated synthetic approach is a valuable technique for manufacturing and engineering nanoparticles. Standard methods have limitations such as long-term production, high costs, and toxic compounds. Green chemistry has emerged as a growing trend in green chemistry, focusing on environmentally sustainable and fast synthesis protocols for nanoparticle processing. Metal oxide nanoparticles (Cu and Ni) have been extensively researched for their diverse applications[9] in various fields, including energy management, textiles, batteries, healthcare, catalysis, cosmetics, semiconductors, and chemical sensing. The synthesis of plant-based NPs, such as copper oxide, nickel oxide, and copper/nickel hybrid nanoparticles, has been documented, but there is a lack of antimicrobial, antioxidant, anti-leishmanial, and anti-cancer effects. Modern techniques like FTIR, UV, XRD, SEM, TEM, and TGA analysis were used to characterize the green synthesized nanoparticles.

Nanotechnology is a field that involves the manipulation of atoms and molecules, resulting in the production of nanoparticles (NPs) that range from 1 to 100 nm in diameter and 1 to 1000 nm in length. These ultrafine particles are used in various fields, including biomedical and pharmaceutical applications[10], cosmetics, and drug delivery. Metal nanoparticles, such as gold and copper, have shown great potential in improving living standards and have been synthesized biologically. NPs like titanium dioxide and silicon dioxide are used in paints for antifungal, anti-algal, and antibacterial properties. They also have therapeutic effects in cosmeceuticals, such as treating photo aging, wrinkles, and dark spots. Copper NPs are used in building construction due to their resistance to corrosion, thermal conductivity, and heat transfer properties. The synthesis of inorganic NPs, including metal and metal oxide NPs, is agrowing area of development. These NPs have a wide range of applications in various fields.

Metallic nanoparticles have been widely used in various industries and medicine, including drug delivery, cancer treatment, wastewater treatment, DNA analysis, antibacterial agents, biosensors, and solar power generation. The green synthesis of metallic nanoparticles has been proposed as a cost-effective and environmentally friendly alternative to chemical and physical methods. Copper nanoparticles (Cu NPs) have gained attention due to their applications in industries and medicine. However, other nanoparticles, such as platinum, gold, iron oxide, silicon oxides, and nickel, have not shown bactericidal effects in studies with Escherichia coli.

Many plant parts or whole plants have been used for the green synthesis of Cu NPs due to their bioactive compounds.[11] Extracts of various plant species have been efficiently applied for this purpose. However, no research has been conducted on green synthesis of Cu NPs using extracts of medicinal plants of Ethiopia. This research aims to synthesize g-Cu NPs using Hagenia abyssinica (Brace) JF. Gmel, a medicinal plant species native to the high-elevation Afromontane regions of central and eastern Africa.

Nanotechnology is a rapidly growing field in various fields, including life science, chemical science, and medical science. It involves the preparation of nanoparticles (NPs) with dimensions of 1-10nm, exhibiting unique properties such as large surface-to-volume ratio, shape, and small size. These NPs have multifunctional properties and are used in various fields like drug delivery, dye degradation, wastewater management, molecular diagnosis, cancer treatment, and therapeutic applications. There are two types of nanoparticles: organic.

NPs and inorganic NPs. NPs are synthesized using physical, chemical, and biological methods, classified into bottom-up and top-down approaches. Top-down methods involve breaking down bulk materials into smaller components, while bottom-up approaches use chemical or biological synthesis methods to form nanostructures. Green synthesis, eco- friendly, non-toxic, and cost-effective, is the most widely appreciated approach. Advances in electron microscopic techniques and analytical tools have further enhanced the synthesis and characterisation of NPs.[12]

Turmeric, a medicinal herb, has been combined with metal nanoparticles to improve its stability and bioavailability. Nanotechnology, an emerging field, incorporates nanoscale materials with unique properties.[13] Nano formulation aids in local drug delivery and sustained release with reduced doses. This research aims to develop silver nanoparticles mediated by turmeric extract, characterize them, and evaluate their antioxidant, anti-inflammatory, and antimicrobial properties against four oral pathogens. This research is the first of its kind in this area.

Nanotechnology is a field that involves the manipulation of atoms and molecules, resulting in the production of nanoparticles (NPs) that range from 1 to 100 nm in diameter and 1 to 1000 nm in length. These ultrafine particles are used in various fields, including biomedical and pharmaceutical applications, cosmetics, and drug delivery. Metal nanoparticles, such as gold and copper, have shown great potential in improving living standards and have been synthesized biologically.[14] NPs like titanium dioxide and silicon dioxide are used in paints for antifungal, anti-algal, and antibacterial properties. They also have therapeutic effects in cosmeceuticals, such as treating photo aging, wrinkles, and dark spots. Copper NPs are used in building construction due to their resistance to corrosion, thermal conductivity, and heat transfer properties. The synthesis of inorganic NPs, including metal and metal oxide NPs, is agrowing area of development. These NPs have a wide range of applications in various fields.

Nanomaterial Classification Overview

* Material Characteristics[15]: Carbon nanomaterials (CNTs, C60), inorganic nanomaterials (metal and magnetic nanoparticles), organic nanomaterials (organic matter with weak interactions), and composite nanomaterials (multiphase NPs).
* Dimensions: Zero-dimension nanoparticles (quantum dots or quantum boxes), one- dimension nanoparticles[16] (Polyethylene oxide nanofibers, Ag nanorods), two-dimension nanoparticles (carbon nanotubes, graphene nanosheets), three-dimension nanoparticles (dendrimers, fullerenes, ZnO nanowires).
* Origin: Natural nanomaterials (naturally produced by biological species or Earth spheres) and synthetic nanomaterials (produced through reduction using various methodologies).

Copper Nanoparticle Synthesis Methods

Physical Methods

- Pulse laser ablation, ball milling, and pulse wire discharge are physical methods used for copper nanoparticle fabrication.[17]
- Biological methods involve the use of bacteria, fungi, and plant extracts.
- Chemical methods include chemical reduction, microwave reduction, sonochemical, electrochemical, sono electrochemical, microemulsion, photochemical, hydrothermal, sol-gel, and thermal decomposition.

Biological Methods

- Ball milling is a cost-effective top-down method of nanoparticle fabrication.[18]
- The size of copper nanoparticles depends on various factors including the type of ball milling machine, container design, rotation speed, time, temperature, atmosphere, grinding medium, and weight ratio of ball to powder.

Pulse Laser Ablation Method

- This method is a physical synthesis method of small-sized nanoparticle fabrication.
- Nanoparticles are formed through three steps: generation, transformation, and condensation of plasma mass.
- The wavelength, energy, duration of pulse, and types of solvent used in the laser ablation method also affect the size of the nanoparticles.

Pulse Wire Discharge Method and Biological Methods for Nanoparticle Production

Pulsed Wire Discharge Method

- A cost-effective physical method for mass production of nano-materials.[19]
- Converts a solid wire (copper) into vapor using pulsed current, condensing the vapor to nanoparticles (copper).
- Nanoparticle size decreases with decreasing pressure and increasing relative energy.
- Examples include pulse laser ablation, laser ablation, and he–n2 mixture.

Biological Methods

- Copper nanoparticles are synthesized using microorganism (microbial) or plant extracts.
- Microbial synthesis method[20] is cost-effective due to the cost of separation of microorganism and their culture maintenance.
- Microbial method involves culturing of microorganism, separation of cell-free metabolite, and reduction of metal ions.

- Fungi and bacteria are commonly used in the synthesis of copper nanoparticles.
- Fungi produce extracellular enzymes that play a vital role in the reduction of copper ions into nanoparticles.
- Bacteria are considered promising for the manufacture of nanoparticles due to their easy culturing, culture, and production of extracellular NPs

Copper Nanoparticles Synthesis Methods

Chemical Reduction[21]

- Copper salt is reduced using reducing agents like polyols, sodium borohydride, Hydrazine, Ascorbic acid, hypophosphite.
- Copper nanoparticles are produced by the polyol method in ambient atmosphere.
- Colloidal copper with particle sizes of 40–80 nm has been reported from reduction with sodium borohydride in aqueous solution at room temperature.
- Copper nanoparticles are stabilized by starch.
- Copper nanoparticles are synthesized by the reduction of Cu2+ in solutions of poly(acrylicacid)-pluronic blends.
- Copper nanoparticles are synthesized by reduction of aqueous copper chloride solution using NaBH4 in the nonionic water-in-oil (w/o) microemulsions.

Photochemical Method (Irradiation)

- The system is excited by radiation, producing active reducing agents such as radicals, electrons, and excited components.[22]
- Copper nanoparticles are synthesized by preparing the desired salt solution with water or alcohols or organic solutions.
- The size of copper nanoparticles prepared by this method is 10 nm.
- The effect of the presence of poly (vinyl pyrrolidone) (PVP) on the copper nanoparticle formation is investigated.

Electrochemical Method (Electrolysis)

- The electrolysis process has been used to reduce of metal ions.[23]
- Copper nano rods were synthesized using this method.
- Copper nanoparticles containing diamond-like carbon films were prepared on the Sisubstrate using this method.

Thermal Decomposition

- Thermal decomposition is a chemical decomposition caused by heat.
- Copper nanoparticles[24] were synthesized by thermal decomposition of Cu-oleate complex.
- The antibacterial activity of copper nanoparticles synthesized against strains of Staphylococcus aureus and Pseudomonas aeruginosa was investigated.

Copper Nanoparticles Synthesis Overview

Green Synthesis

- Uses biological organisms as a reducing agent, making it eco-friendly, cheaper, simpler, and more sustainable.
- Precursor provides copper ions, a reducing agent supplies electrons, and a surfactant aggregates the copper atoms into copper nanoparticles.[25]
- Green synthesis is preferred due to its simplicity, speed, and sustainability.
- Nanoparticles obtained with plants have excellent antimicrobial, anticancer, antidiabetic, anti-inflammatory, and antioxidant activities.

Chemical Methods

- Chemical methods are widely used to obtain copper nanoparticles, but they often use toxicmaterials.
- Various methods include sonochemical reduction, hydrothermal synthesis, electrochemical, and chemical reduction.
- Chemical reduction is the most commonly used method due to its simplicity, high yieldefficiency, and limited equipment requirements.[26]

Physical Methods

- Evaporation–condensation and laser ablation are the most important physical synthesismethods.
- Physical synthesis results in no solvent contamination and uniform distribution of nanoparticles.
- Evaporation–condensation produces very small nanoparticles but requires high energy and is time-consuming.
- Physical synthesis methods require expensive equipment and high energy use, making them less popular than chemical or green methods.[27]

Characteristics of Medicinal Plant Extracts for Nanotechnology Synthesis

- Medicinal plants contain diverse phytochemicals such as polyphenols, alkaloids, and organic acids, known as antioxidants.
- These compounds have a variety of functional groups, including hydroxyl (-OH), nitrile (- CN), aldehyde (-CHO), amines (-NH2), and carboxylic acid (-COOH), in their structures.
- These functional groups provide biocompounds with a redox capacity that allows them to participate in the biosynthesis of NPs as reducing, covering, chelating/capping, and preservative agents for NPs.
- Despite knowledge of the redox mechanisms of these functional groups, there is still no fully elucidated mechanism for the synthesis of NPs.
- NPs synthesized with extracts of medicinal plants have better properties than NPs synthesized by another method.[28]
- The concentration, complex biochemical composition, and quality of bioactive compounds present in plant extracts directly affect the efficiency of the synthesis process, as well as the stability,

dimensions, and geometry of the NPs.
- The type of tissue from which the bioactive compounds will be obtained is another variable of consideration.
- Extraction efficiency can vary significantly even if the same extraction variables are used— solvent, temperature, time, and methodology—but using different types of tissue.
- The composition of the extract plays a vital role in determining the final characteristics of the NPs.
- The extraction methods for the extraction of bioactive compounds from plants have been empirically developed.
- The preparation of the plant material to be treated must be carried out prior to the extraction process.[29]

Green Synthesis of Nanoparticles

Conventional Methods for Nanoparticle Synthesis
- Top-down methods involve breaking down bulk material into nanoparticles using physical, chemical, and mechanical processes.
- Bottom-up approaches use atoms or molecules as starting materials in the formation of nanoparticles.[30]
- Both methods require expensive specialized equipment and high energy input.
- Green synthesis of nanoparticles is a growing trend, offering solutions to the major disadvantages of conventional methods.

Components for Green Synthesis
- Components include extracts from different parts of a plant, microorganisms, viral particles, and biomass waste.
- The principle of green synthesis relies on the bioactive reducing properties of plant metabolites like terpenoids, alkaloids, and proteins.

Nanoparticle Synthesis in Microorganisms and Plants
- Synthesis of nanoparticles occurs as a natural process in microorganisms and plants.
- Two main ways in which biogenic synthesis can be performed by living organisms are intracellular (endogenous) and extracellular (exogenous) synthesis.
- Depending on the natural source[31] used for the synthesis, the method is adjusted to make it more efficient and favor the formation of NPs.

Green Synthesis of Metal Nanoparticles (NPs)
- In the context of green synthesis, many phytocomponents act as reductors and stabilizers when mixed with the precursor metal salt in aqueous solution.
- The properties of the NPs are influenced by the conditions under which the synthesis takes place, including factors like the selected precursor salt, pH, temperature, and precursor-to- extract ratio.20

Green Synthesis of Nanoparticles with Different Precursors
- Ginger Lily leaf extract: Antibacterial activity.
- Psidium guajava leaf extract: Photocatalytic dye degradation.
- Centella asiatica leaf extract: Antibacterial, cytotoxicity activity.
- Punica granatum leaf extract: Antibacterial, dye degradation, textile.
- Tinospora cordifolia leaf extract: Catalytic textile dye degradation.
- Cissus quadrangularis leaf extract: Antifungal activity.
- Copper nitrate: Photocatalytic, antioxidant, antibacterial activity.
- Aloe vera leaf extract: Antibacterial activity against fish pathogens.
- Tabernaemontana divaricate leaf extract: Antibacterial activity against urinary tract pathogen.
- Strawberry Fruit extract: Antioxidant, Antifungal, Antibacterial, Anticancer, cutaneous wound healing activity.
- Terminalia bellirica fruit extract: Antimicrobial activity.
- Cordia sebestena flower extract: Photo degradation of dyes, antibacterial activity.
- Streptomyces spp. (microbes): Antimicrobial, Antifungal, Antioxidant activity, Larvicidal activity.
- Copper (II) chloride: Antibacterial activity.
- Eichhornia crassipes leaf extract: Antifungal activity against plant fungal pathogen.[32]

Physicochemical Properties of Nanoparticles (NPs)

Mechanical Properties
- NPs have unique mechanical characteristics under different conditions and external forces.
- These properties include strength, brittleness, hardness, toughness, fatigue strength, plasticity, elasticity, ductility, rigidity, and yield stress.
- NPs display different mechanical properties due to surface and quantum effects.
- Interaction forces between NPs and a surface, including van der Waals forces, electrostatic and electrical double layer forces, normal and lateral capillary forces, solvation, structural, and hydration forces, contribute to these properties.
- Theories such as DLVO, JKR, and DMT theories explain how these interaction forces give NPs new mechanical properties.

Thermal Properties
- Heat transfer in NPs depends on energy conduction due to electrons and photons.
- The major components of thermal properties of a material are thermal conductivity, thermoelectric power, heat capacity, and thermal stability.
- NP size directly impacts electrical and thermal conductivity of NPs.

- Thermal conductivity in NPs is promoted by microconvection, which results from theBrownian motion of NPs.
- The addition of Cu NPs to ethylene glycol enhances the thermal conductivity of the fluid upto 40%.

Thermoelectric Power and NP Doping in Materials

- Thermoelectric power of a material depends on its Seebeck coefficient and electricalconductivity.

Electronic and Optical Properties of Nanoparticles (NPs)

- NPs exhibit linear absorption, photoluminescence emission, and nonlinear optical properties due to quantum confinement and localized surface plasmon resonance (LSPR) effect.
- LSPR phenomena occur when the incident photon frequency remains constant with the collective excitation of conductive electrons.
- NPs exhibit a strong size-dependent UV–visible extinction band, not present in bulk metalspectra.
- Optical properties of NPs depend on size, shape, and dielectric environment.
- Plasmons, collective excitations of conductive electrons in metals, are distinguished based on boundary conditions.
- Bulk plasmons cannot be excited byvisible light due to their longitudinal nature.
- Surface-propagating plasmons propagate along metal surfaces in a waveguide-like fashion.
- Surface plasmons, caused by the polarization of the NP surface, create uncompensated charges at the NP surface.

Nano-Catalysis and Catalytic Properties

- Nano-catalysis uses nanoparticles (NPs) as catalysts, enhancing catalytic properties like reactivity and selectivity.
- Catalytic properties of NPs depend on size, shape, composition, interparticle spacing, oxidation state, and support.

Size and Catalytic Activity

- The size of NPs is an inverse relationship, with smaller NPs being more active.
- The smallest NPs provide the highest normalized current densities in electro-catalysis oxidation of CO.
- Shape also affects reactivity and selectivity of NPs.

Composition and Catalytic Activity

- Alloys in NPs can enhance catalytic activity by changing the electronic properties of the catalyst, decreasing poisoning effects, and providing distinct selectivities.
- The change in the composition of NPs changes the electronic structure of metal surfaces by the formation of bimetallic bonds and the modification of metal–metal bond lengths.

Interparticle Spacing and Catalytic Activity

- The catalytic activity and stability of 2 nm Au NPs dispersed on polycrystalline TiC films display a strong dependence on interparticle spacing.
- The oxidation state of NPs affects the catalytic activities.

Role of Support Material

- The MgO support for Au NPs is important for CO oxidation and controlling the rate of CO oxidation through oxygen vacancies.
- The chemical composition of the support affects the reactivity of the catalyst, as well as the crystal structure of the support.
- Enhanced catalytic performance for CO oxidation and SO2 dissociation have also beenreported for Au NPs supported on metal carbides such as TiC.

[**Green Synthesis of CuNP: Materials and Methods**

Materials

- Krameria sp. (Rhatany) root tissue was purchased, washed, dried, ground, and stored.
- Escherichia coli, Staphylococcus aureus, Alternaria alternata, and Fusarium oxyporium were obtained from previous work.
- Copper salt (CuSO4·5H2O) was purchased from Sigma–Aldrich.

Preparation of Extract and Reagent

- Plant extract was prepared using maceration and boiling methods.
- Powdered material of Rhatany root was added to water with a 1:10 (w/v) ratio.
- Filtration was done to synthesize CuNP.
- 0.3 M copper salt (CuSO4·5H2O) was prepared by mixing copper sulfate pentahydrate toRhatany root extract.
- A mixture of 20 mL of filtered plant extract solution and 80 mL of distilled water was prepared.
- The precipitated particles were rinsed with deionized water, dried in a hot-air oven at 80 °Cfor six to eight hours, and the purified CuNP was characterized.

Optimization of Conditions for Green Synthesis

- Temperature: The reaction mixture was optimized by adding 10 mL of freshly preparedextracts with 10 mL of 0.3 M CuSO4·5H2O solution in glass vials.
- Incubation time: The solutions were incubated in the dark at the selected temperature for 1,2, 3, 4, 5, and 6 h.
- pH: Various pH values were considered to find the optimum value for synthesizing CuNP using the Rhatany root extract.

Characterization of CuNPs

- UV–vis spectroscopy used to analyze absorbance spectrum of green synthesized CuNPs.
- TEM and SEM used for morphological study.
- EDS used for elemental composition examination.
- FT-IR used to understand biomolecules' role in leaf extract metal reduction.
- Charge and size distribution measured using Malvern Zetasizer.
- DLS measurements performed by dispersing 20mg CuNPs powder in deionized water.24

Biogenically Synthesized Cu and CuO Nanoparticles Characterization
Analytical Tools Used

- UV-Visible spectroscopy: Detects color change in Cu nanoparticles synthesised by Ziziphus spina-christi leaves.
- XRD patterns: Confirms crystalline copper and CuO NPs.
- FT IR spectra: Provides information about functional groups of biomolecules present inplant extracts.
- Electron microscopy: Reveals homogeneous and spherical morphology of biogenic Cu NPs.
- DLS studies reveal size distribution of Cu NPs.
- TEM images: Confirms spherical morphology and narrow diameter distributions of Cu NPs.
- FESEM images of CuO NPs: Confirms spherical nature (20 nm to 300 nm).
- HRTEM studies: Record particle size of 2 nm.
- TEM analysis: Shows shell-like sheet

Characterization of CuNPs
UV-Vis Spectroscopy

- Measures Plasmon resonances and total oscillations of electron conduction bands.
- Used to measure the absorption of fluids and other materials.
- Surface plasmon resonance (SPR) phenomenon is used to analyze compounds inside the transparent cell.
- Time on formation of CuNPs is assessed using UV-Vis spectroscopy.

XRD Analysis

- Used Cu Kα as a radiation source for the CuNPs.
- Identified the compound's crystal structure and chemical composition.
- X-ray energy-dispersive spectroscopy was used to assess the chemistry of nanoparticles.

FTIR Analysis

- Used FTIR spectrophotometers to identify different functional groups of NPs.
- Propolis extract containing NPs found to contain functional groups.
- Absorption peaks in CuO NPs correspond to O–H, C = O, C–N, C–H, and C = C.
- Absorption at 3000–3350 cm− attributed to O–H or N–H of alcohol/phenol.
- Aromatic C–H bending ascribed to absorbance peaks between 820 and 880 cm−1.
- Absorption band for carbonyl–C = O from 1600–1790.26

Morphological and Compositional Analysis of g-Cu NPs
SEM-EDAX Analysis

- SEM micrographs showed nonhomogeneity of the particles in terms of shape and size.
- All possible spherical and irregular shapes of Cu NPs with varying particle sizes were found.
- The average grain size of Cu NPs was found to be in the range of 10–50 nm.
- The presence of C and O is believed to be from the capped bioactive compounds.
- The reduction of copper ions to Cu NPs is facilitated by the biomolecules of plant extract containing surface hydroxyl groups.

TEM, HRTEM, and SAED Analysis

- TEM, HRTEM, and SAED analysis were employed to gain deeper insight on the morphology, size, and crystalline nature of the g-Cu NPs.
- The synthesized NPs are mostly spherical but exhibited different shapes.
- The variation in size of g-Cu NPs is probably due to the presence of polyphenoliccompounds.
- The SAED pattern of g-Cu NPs contained six spots each corresponding to specific crystalplanes.
- The estimated -spacing value of lattice fringes at the surface of the Cu NPs is 0.2444 nm, which is comparable to the value of the (111) plane of fcc-structured Cu2O.

CuNP Activity Analysis
Antioxidant Assay

- Rhatany roots extract, precursor salt, and biosynthesized CuNP tested for antioxidant capacity against DPPH free radical.
- DPPH scavenging activity percentage strongly connected with concentrations and was dose-dependent.
- Precursor salt had the lowest antioxidant activity (42.78 at 200 μg/ml−1).
- Extract demonstrated superior antioxidant activity of 76.02 at the same dose.
- Biosynthesized CuNP showed maximum DPPH radical scavenging activity (83.81) at 200 μg/ml−1 concentration.
- Increase in CuNP concentration led to a

significant increase in DPPH radical scavenging activity from 55 to 83%.

Antimicrobial Activity

- Mueller–Hinton agar and disc-diffusion method used to assess antimicrobial activity of Rhatany root extract and greenly generated CuNP against bacterial and fungal species.
- All drug-resistant bacterial strains responded favorably to both extract and green- manufactured copper nanoparticles.
- The highest zone of inhibition was discovered for S. aureus.
- The highest doses of Rhatany root extract showed antibacterial activity against bothbacterial strains.
- The greatest zone of inhibition was displayed by CuNP against E. coli and S. aureus strains.

Antifungal Activity

- Compared with fluconazole, CuNP showed promising and significant antifungal activity.
- The plant extract showed the highest zone of inhibition against A. alternata and the lowest zone of inhibition against F. oxysporum.
- The biosynthesized CuNP showed inhibitory ability in both fungal and bacterial strains; itdemonstrates less activity in fungi when compared to bacteria.

Study on Endodontic Medications and Antimicrobial Activity

- The study was conducted at the Basic Science Research Centre, Belgaum, and approved by the Institutional Review Board.
- Endodontic medicaments evaluated included DAP, modified DAP, 2% chlorhexidine gel, and their combination with polyethylene glycol (400 PEG), propylene glycol (PG), combinations of PG with PEG, and glycerine.
- Antimicrobial activity was tested against standard strains of American Type Culture Collection (ATCC) against five organisms: Streptococcus mutans, Staphylococcus aureus, E. faecalis, Porphyromonas gingivalis, and Escherichia coliand a 1.0 McFarland Standard for P. gingivalis.
- Minimum inhibitory concentration of antimicrobial substances and their combinations was determined using the Broth Dilution Method.
- Agar well diffusion method was used to determine the antibacterial activity of endodontic medicaments and various vehicles.
- The tests were repeated three times to minimize errors.
- The results showed the antimicrobial activity of the endodontic medicaments against P. gingivalis using the agar well diffusion method.

Antimicrobial Test Results

- Chlorhexidine: 0.078% effective against S. mutans, S. aureus, E. faecalis, P. gingivalis, E. coli, and E. coli.
- Ciprofloxacin: 0.078% effective against S. mutans, S. aureus, E. faecalis, P. gingivalis, and E. coli.
- Amoxicillin clavulanate: 0.019% effective against S. mutans, E. coli, and E. mutans.
- Metronidazole: 0.019% effective against S. mutans, E. coli, and E. mutans.
- No significant difference in antimicrobial drug and vehicle combinations except for P.gingivalis.

Root Canal System Sterilization and Disinfection[33]

- Root canal systems require reduction in microbes to facilitate local response and tissuehealing.
- Anaerobe diversity increases with time, with facultative anaerobes becoming moreprevalent over longer infection periods.
- S. mutans, E. faecalis, and S. aureus are the most resistant species, potentially causing root canal failure.
- Gram-negative anaerobes, like black-pigmented species, exhibit resistance due to their cell wall's outer membranes.
- Local antibiotic use allows for large doses, overcoming resistance without risk of systemic toxicity.
- Chlorhexidine, a cationic bigu-anide and antiseptic, is considered the gold standard in endodontics.
- Chlorhexidine shows antimicrobial activity from concentrations as low as 0.1% and bactericidal activity at 2%.

Antimicrobial Activity of Endodontic Medications and Vehicle Combinations

- Chlorhexidine showed MIC of 0.019% against P. gingivalis to 0.078% against other facultative anaerobes, with the highest concentration against E. faecalis at 0.156%.
- Ciprofloxacin's MIC and decrease in MIC when combined with C + M were consistent, except for S. aureus 31.
- C + M showed greater zones of inhibition against clinical isolates of S. aureus and E. faecalis at a drug concentration of 5 μg/mL.
- Standard strains of S. mutans exhibited resistance C + M along with the four vehicles when tested by agar well diffusion method.
- Amoxicillin and ciprofloxacin were highly effective in terms of zones of inhibition, [34] and 30 respectively, whereas tetracyclines were moderately effective against clinical isolates of S.mutans.
- Combination of two bactericidal drugs can prevent drug resistance in microorganisms.
- Amoxicillin clavulanate, a beta-lactamase inhibitor, was 100% effective against endodontic bacteria.
- The MIC of amoxicillin clavulanate alone is in accordance with the guidelines by Indian Council

Medical Research, except for E. faecalis and E. coli.
- The zones of inhibition were greater for P. gingivalis as obligate anaerobes are easily eradicated.
- The use of vehicles like PG enhances the penetration of the drugs into their dentinal tubules and can make microbes having drug resistance sensitive when used along with vehicles like PEG.
- No significant difference in vehicles when mixed with endodontic medicaments except for PG in comparison with glycerine when used along with C + M combination of drugs against P. gingivalis.[35]

Antioxidant Activity in Plants
- Antioxidants inhibit oxidation, removing harmful agents.
- Plant phytochemicals reduce or prevent oxidative damage to human cells.
- Understanding antioxidant activities is crucial.
- Study analyzes DPPH free radicals cation adsorption activity of Marsilea quadrifoliaextracts.

Plant Extracts for DPPH Free Radical Scavenging Activity
- 60g dry sample powder was macerated with 500ml of each solvent (hexane, ethylacetate, methanol) overnight.
- The extract was collected, filtered, and stored.
- A second solvent was added and the process repeated.
- The extract was evaporated below 40°C for further phytochemical analyses.
- The ability of the extracts to eliminate the DPPH radical was investigated.
- The stock solution of the plant extracts was prepared and 100µg of each extract was added to methanolic DPPH solution.[33]

Nanoparticles: Potential Applications in Biomedical and Pharmaceutical Fields

Biological Applications:
- Copper nanoparticles show antimicrobial activity against various bacteria and viruses.
- Copper ions damage cell membrane, DNA, RNA, and other molecules, reducing the viability and half-life of viruses.
- Copper nanoparticles have shown promising activity against cancerous cells, with anticancerous activity against HeLa cells, MD A-MB-231, Caco-2, HepG2 cells, and Mcf-7 breast cancer cells.
- Copper nanoparticles have improved antioxidant enzymes and have been shown to stimulate arthritis in rats.

Telecom Industry
- UK manufacturer Promethane Particles is developing Personal Protective Equipment (PPE) with nano-copper in cotton fibers.
- These nanoparticles are used in textiles to produce PPE.

Biocatalyst and Bioremediation
- Copper is a low-cost metal with less toxicity, making it suitable for bioremediation of pollutants.
- Copper oxide NPs are used for waste water purification and have shown catalytic properties against organic dyes.
- The adsorbent capacity of Cu-NPs for aflatoxin B1 is found to be more than Ag- NPs butless than Fe-NPs.

Therapeutics
- Nanoparticles provide site-specific drug delivering systems due to their small size and large absorptive surface.
- Nanoparticles have shown improved therapeutic efficacy with less toxicity.
- Copper oxide NPs were used as core and hyper-branched polyglycerol as shell in core-shell Nano carriers.

Other Applications
- Copper nanoparticles have shown profound applications in food packaging and agriculture for crop improvements.[36]

Anticancer Potential of Copper Nanoformulations
- Copper and copper oxide nanoparticles have shown potential in biomedical fields due to their drug stability, biodistribution, improved therapeutic index, and active agent delivery.
- Copper diethyldithiocarbamate nanoparticles (Cu(DDC)2 NPs) have been designed to overcome resistance in prostate cancer therapy.
- Copper oxide(I) nanoparticles and copper–gold core–shell nanostructures have shown anticancer potential against cervical cancer.[37]
- The stability aspect of copper oxide(I) nanoparticles in various physiological fluids has beeninvestigated.
- Copper oxide(II) nanoparticles have been shown to decrease breast cancer cell viability, morphological deformation of cancer cells, enhanced reactive oxygen species (ROS) production, and loss of mitochondrial membrane potential.
- Copper oxide(II) nanoparticles have been shown to be effective in the treatment of human pancreatic cancer in vitro and in vivo.

Antibacterial Properties of Copper and Copper-Based Nanomaterials
- Copper nanoparticles and copper-functionalised nanomaterials exhibit antibacterial properties against various microbes, including Gram-positive and Gram-negative bacteria andfungi 36.
- Copper nanoparticles modified with plant extract [38] exhibited higher antibacterial and antifungal properties than tested antibiotics.
- The antibacterial properties of copper nanoparticles are generally associated with the nanoparticle's internalization within bacterial cells, copper ion

release, the generation of oxidative stress by ROS overproduction, and DNA damage.

- Copper nanoparticles obtained from the Falcaria vulgaris leaf extract have been confirmed for their antimicrobial and antifungal activity.

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