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Green Chemistry-Mediated Nanoparticle Synthesis: Experimental Strategies and Application Perspectives

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Abstract
Green chemistry approaches have revolutionized the synthesis of nanoparticles by providing environmentally sustainable, cost-effective, and non-toxic alternatives to conventional physical and chemical methods. This study focuses on the development and application of green synthesis techniques utilizing biological entities such as plant extracts, microorganisms, and biopolymers as reducing and stabilizing agents. These eco-friendly methods not only minimize hazardous waste and energy consumption but also enhance the biocompatibility and functional efficiency of nanoparticles. The synthesized nanoparticles exhibit remarkable physicochemical properties, making them suitable for diverse applications in biomedical, environmental, agricultural, and industrial sectors. In particular, their roles in drug delivery, antimicrobial activity, wastewater treatment, and catalysis highlight their growing significance. Despite certain challenges such as scalability and standardization, green nanotechnology presents a promising pathway toward sustainable scientific and industrial advancements. Thus, the integration of green chemistry principles in nanoparticle synthesis contributes significantly to environmental protection and technological innovation.
Keywords: Green chemistry, Nanoparticle synthesis, Eco-friendly methods, Biomedical applications, Environmental remediation

Introduction

Green chemistry has emerged as a transformative paradigm in modern science, emphasizing the design of chemical processes that minimize environmental impact and enhance sustainability. In the field of nanotechnology, the synthesis of nanoparticles has gained immense attention due to their unique physicochemical properties such as high surface area, quantum effects, and tunable reactivity, which make them highly suitable for applications in medicine, agriculture, environmental remediation, and industrial catalysis. However, conventional methods of nanoparticle synthesis, including physical and chemical approaches, often involve hazardous reagents, high energy consumption, and the generation of toxic by-products, posing significant risks to both human health and the environment. In response to these challenges, green chemistry approaches have been increasingly adopted for the synthesis of nanoparticles, focusing on the use of eco-friendly, renewable, and non-toxic materials. These approaches utilize biological entities such as plant extracts, microorganisms (bacteria, fungi, algae), and biopolymers as reducing and stabilizing agents, thereby eliminating the need for harmful chemicals. The phytochemicals present in plant extracts, including flavonoids, phenolic compounds, and alkaloids, play a crucial role in the reduction of metal ions and stabilization of nanoparticles, enabling controlled synthesis with desired size and morphology. Moreover, green synthesis methods are cost-effective, energy-efficient, and scalable, making them attractive for large-scale production. The integration of green chemistry principles into nanoparticle synthesis not only enhances biocompatibility but also broadens the scope of applications, particularly in biomedical fields such as drug delivery, antimicrobial treatments, and cancer therapy, as well as in environmental applications like wastewater treatment and pollutant degradation. Therefore, the adoption of green chemistry approaches represents a sustainable and innovative pathway for advancing nanotechnology while addressing global environmental concerns and promoting safer industrial practices.

Concept of Nanotechnology and Nanoparticles

Nanotechnology is a multidisciplinary field of science that deals with the design, manipulation, and application of materials at the nanoscale, typically ranging from 1 to 100 nanometers. At this scale, materials exhibit unique physical, chemical, and biological properties that differ significantly from their bulk counterparts, primarily due to increased surface area and quantum effects. Nanoparticles are the fundamental building blocks of nanotechnology and can be composed of metals, metal oxides, polymers, or carbon-based materials. These particles possess enhanced reactivity, strength, electrical conductivity, and optical properties, making them highly valuable in diverse fields such as medicine, electronics, energy, and environmental science. For instance, nanoparticles are widely used in targeted drug delivery systems, biosensors, wastewater treatment, and catalytic processes. The ability to control their size, shape, and surface characteristics allows for tailored functionalities, thereby expanding their potential applications and making nanotechnology a rapidly advancing and impactful area of modern research.

Need for Sustainable Nanotechnology

The rapid advancement of nanotechnology has led to increased production and application of nanoparticles across various sectors, raising concerns about environmental safety, human health, and resource sustainability. Conventional nanoparticle synthesis methods often involve toxic chemicals, high energy consumption, and the generation of hazardous waste, which can negatively impact ecosystems and pose risks during manufacturing and disposal. Therefore, the need for sustainable nanotechnology has become crucial to ensure that technological progress does not compromise environmental integrity or public health. Sustainable nanotechnology emphasizes the adoption of green chemistry principles, including the use of renewable resources, eco-friendly solvents, and energy-efficient processes. It also focuses on minimizing toxicity, enhancing biodegradability, and promoting safe lifecycle management of nanomaterials. By integrating sustainability into nanotechnology, it is possible to develop safer, cost-effective, and environmentally responsible solutions that support long-term industrial growth while addressing global challenges such as pollution control, resource conservation, and climate change mitigation.

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Mechanism of Green Synthesis

1. Reduction of Metal Ions

The green synthesis of nanoparticles begins with the reduction of metal ions into their zero-valent state using natural reducing agents derived from biological sources such as plant extracts, microorganisms, or biopolymers. These biological systems contain a variety of bioactive molecules, including flavonoids, phenolics, proteins, enzymes, and sugars, which donate electrons to metal ions such as Ag^{+}, Au^{3+}, or Zn^{2+}. This reduction process converts the metal ions into neutral atoms, initiating nanoparticle formation. Unlike conventional chemical reducing agents such as sodium borohydride, these biomolecules are non-toxic and environmentally friendly. The efficiency of reduction depends on the type and concentration of phytochemicals or microbial metabolites present, which directly influence the rate of nanoparticle formation and their initial characteristics.

2. Nucleation and Growth Process

Following the reduction of metal ions, the process of nucleation begins, where reduced metal atoms aggregate to form small clusters or nuclei. These nuclei act as seeds for further growth, leading to the formation of nanoparticles. The nucleation phase is critical as it determines the number and size distribution of nanoparticles. Once nucleation occurs, the growth phase follows, during which additional metal atoms deposit onto the existing nuclei, increasing their size. The balance between nucleation and growth rates plays a crucial role in controlling the final morphology and uniformity of nanoparticles. A rapid nucleation process typically results in smaller and more uniform particles, while slower nucleation may lead to larger and less homogeneous structures.

3. Stabilization and Capping Mechanisms

After formation, nanoparticles tend to aggregate due to their high surface energy. To prevent this, stabilization and capping mechanisms are essential in green synthesis. Biological molecules present in the reaction medium act as natural capping agents, binding to the surface of nanoparticles and providing steric or electrostatic stabilization. Compounds such as proteins, polysaccharides, and polyphenols form a protective layer around the nanoparticles, maintaining their stability and preventing agglomeration. This capping not only enhances the stability of nanoparticles but also influences their surface properties, functionality, and biocompatibility. The presence of these natural stabilizers makes green-synthesized nanoparticles particularly suitable for biomedical and environmental applications.

4. Factors Affecting Synthesis

Several physicochemical parameters significantly influence the green synthesis of nanoparticles, determining their size, shape, and overall properties. These factors must be carefully controlled to achieve desired outcomes in nanoparticle production.

- **pH**

The pH of the reaction medium plays a crucial role in the reduction process and stability of nanoparticles. Changes in pH can alter the ionization state of biomolecules, affecting their reducing and capping abilities. Generally, alkaline conditions favor faster reduction and smaller particle sizes, while acidic conditions may slow down the process.

- **Temperature**

Temperature affects the kinetics of the reaction. Higher temperatures increase the rate of reduction and nucleation, leading to faster synthesis and often smaller nanoparticles. However, excessively high temperatures may destabilize biomolecules and affect the quality of nanoparticles.

- **Concentration**

The concentration of metal ions and biological reducing agents influences the rate of nanoparticle formation. Higher concentrations may lead to rapid nucleation and aggregation, while lower concentrations may produce well-dispersed nanoparticles with controlled size.

- **Time**

Reaction time is another critical factor that determines the extent of nanoparticle growth. Longer reaction times allow for complete reduction and growth, but excessive duration may result in aggregation or changes in particle morphology. Therefore, optimizing reaction time is essential for achieving stable and uniform nanoparticles.

The mechanism of green synthesis involves a coordinated interplay of reduction, nucleation, growth, and stabilization processes, all governed by environmentally benign biological systems, making it a sustainable alternative to conventional nanoparticle synthesis methods.

Applications of Green Synthesized Nanoparticles

1. Biomedical Applications

Green synthesized nanoparticles have gained significant importance in the biomedical field due to their enhanced biocompatibility, reduced toxicity, and functional efficiency. In drug delivery systems, these nanoparticles act as carriers that enable targeted delivery of therapeutic agents to specific sites in the body, thereby improving drug efficacy and minimizing side effects. Their small size and large surface area facilitate better cellular uptake and controlled release of drugs. Additionally, green nanoparticles exhibit strong antimicrobial activity against a wide range of pathogens, including bacteria, fungi, and viruses, making them valuable in wound healing and infection control. In cancer therapy, nanoparticles such as gold and silver synthesized through green methods are used in targeted treatment, photothermal therapy, and imaging, offering promising alternatives to conventional treatments with fewer adverse effects.

2. Environmental Applications

Green synthesized nanoparticles play a crucial role in environmental protection and remediation. In water purification, these nanoparticles are used to remove contaminants such as heavy metals, organic pollutants, and microorganisms from drinking water. Their high reactivity and surface area enhance adsorption and degradation processes. In wastewater treatment, nanoparticles such as metal oxides are employed to break down toxic substances and improve water quality before discharge into the environment. Furthermore, green nanoparticles are effective in pollutant degradation, including the removal of dyes, pesticides, and industrial chemicals through catalytic and photocatalytic processes. These applications contribute significantly to sustainable environmental management and pollution control.

3. Agricultural Applications

In agriculture, green synthesized nanoparticles are increasingly used to improve crop productivity and sustainability. Nano-fertilizers enhance nutrient availability and uptake by plants, reducing the need for excessive chemical fertilizers and minimizing environmental pollution. These fertilizers allow controlled and slow release of nutrients, ensuring efficient utilization by crops. Additionally, nanoparticles are used in pest control as nano-pesticides, providing targeted action against pests while reducing toxicity to non-target organisms. Green nanoparticles also help in improving soil health, plant growth, and resistance to diseases. Their application supports sustainable agricultural practices and contributes to food security.

4. Industrial Applications

Green synthesized nanoparticles have wide-ranging applications in various industrial sectors. In catalysis, they act as efficient catalysts due to their high surface area and reactivity, enhancing the rate of chemical reactions while reducing energy consumption. In sensor technology, nanoparticles are used in the development of highly sensitive and selective sensors for detecting chemicals, gases, and biological substances. These sensors are widely applied in healthcare, environmental monitoring, and industrial safety. Additionally, nanoparticles are used in energy storage systems such as batteries, supercapacitors, and fuel cells, improving energy efficiency and performance. Their role in renewable energy technologies further highlights their importance in sustainable industrial development.

Green synthesized nanoparticles offer versatile and sustainable solutions across biomedical, environmental, agricultural, and industrial fields, making them a cornerstone of modern nanotechnology and eco-friendly innovation.

Methodology

The methodology for green synthesis of nanoparticles was designed based on eco-friendly principles utilizing plant extracts, microbial systems, and controlled experimental conditions. All chemicals used in the study were of analytical grade and employed without further purification. Metal precursors such as silver nitrate ($AgNO_3$) and hydrogen tetrachloroaurate ($HAuCl_4 \cdot 3H_2O$) were used for the synthesis of silver and gold nanoparticles, respectively .

Preparation of Plant Extracts

Fresh plant materials, including leaves and rhizomes from selected species, were thoroughly washed with tap water followed by double-distilled water to remove impurities. The cleaned plant material was finely chopped and boiled in distilled water (typically 20 g in 100 mL) for approximately 20 minutes. The extract was then cooled, filtered using Whatman No. 1 filter paper, and stored at 4°C for further use.

Green Synthesis of Nanoparticles

Nanoparticle synthesis was carried out by mixing plant extract with aqueous metal salt solutions (1 mM concentration) in a typical ratio of 1:9 (extract:metal solution). The reaction mixture was subjected to microwave irradiation (800 W, 2450 MHz) to accelerate the reduction process. In some cases, synthesis was also performed at room temperature to compare reaction kinetics. The reduction of metal ions (Ag^{+}/Au^{3+}) to their corresponding nanoparticles was monitored through color change and confirmed using UV–visible spectroscopy in the range of 200–800 nm.

Characterization Techniques

The synthesized nanoparticles were characterized using multiple analytical techniques. UV–visible spectroscopy confirmed nanoparticle formation, while Fourier Transform Infrared (FTIR) spectroscopy identified functional groups responsible for reduction and stabilization. X-ray diffraction (XRD) analysis determined crystalline structure, and High-Resolution Transmission Electron Microscopy (HR-TEM) along with Energy Dispersive X-ray (EDX) analysis provided information on morphology, size distribution, and elemental composition .

Biological Evaluation

Microbial strains (both Gram-positive and Gram-negative bacteria, along with fungi) and cancer cell lines (A549) were used to evaluate antimicrobial and anticancer properties of the synthesized nanoparticles.

The methodology emphasizes a sustainable, efficient, and reproducible approach for nanoparticle synthesis using green chemistry principles.

Result and Discussion

Table 1: Green Synthesis Methods and Outcomes

Plant Source	Nanoparticle Type	Color Change Observed	SPR Peak (nm)	Inference
Indigofera tinctoria	AgNPs, AuNPs	Pale yellow → Brown (Ag), Ruby red (Au)	420–450 (Ag), 520–540 (Au)	Successful nanoparticle formation
Bauhinia purpurea	AgNPs, AuNPs	Light yellow → Dark brown	410–440 (Ag), 525–545 (Au)	Rapid reduction of metal ions
Myxopyrum serratulum	AgNPs, AuNPs	Yellow → Brownish-red	415–445 (Ag), 520–535 (Au)	Stable nanoparticle synthesis
Synedrella nodiflora	AgNPs, AuNPs	Colorless → Brown	420–450 (Ag), 525–540 (Au)	Efficient microwave-assisted synthesis
Costus speciosus	AgNPs, AuNPs	Yellow → Dark brown	410–430 (Ag), 520–530 (Au)	Fast nucleation and growth
Nervalia zeylanica	AgNPs	Pale yellow → Brown	~420	Time-dependent synthesis observed
Orthosiphon	AgNPs	Yellow → Brown	~415–425	Controlled

aristatus				synthesis under microwave

Table 1 summarizes the effectiveness of different plant-mediated green synthesis approaches for producing silver (AgNPs) and gold nanoparticles (AuNPs), highlighting key observable and spectroscopic indicators of nanoparticle formation. A distinct color change from pale yellow to brown (for AgNPs) and ruby red (for AuNPs) was consistently observed across plant extracts, confirming the reduction of metal ions (Ag^{+} and Au^{3+}) into their nanoscale metallic forms. This visual transformation is attributed to the excitation of surface plasmon resonance (SPR), a characteristic optical property of nanoparticles. The recorded SPR peaks further validate nanoparticle formation, with AgNPs typically appearing in the range of 410–450 nm and AuNPs between 520–545 nm. Slight variations in SPR values among different plant sources indicate differences in particle size, shape, and distribution, influenced by the type and concentration of phytochemicals present. For instance, *Bauhinia purpurea* demonstrated rapid reduction kinetics, while *Synedrella nodiflora* showed enhanced efficiency under microwave-assisted conditions, indicating faster nucleation. Plants such as *Myxopyrum serratulum* and *Costus speciosus* produced stable nanoparticles with controlled growth, whereas *Nervalia zeylanica* exhibited time-dependent synthesis behavior. The results confirm that plant extracts act as effective reducing and stabilizing agents, enabling efficient, eco-friendly nanoparticle synthesis with tunable properties.

Table 2. FTIR Analysis of Functional Groups Involved

Plant Extract	**Major Functional Groups Identified**	**Wavenumber Range (cm^{-1})**	**Role in Synthesis**
Indigofera tinctoria	–OH, –C=O, –NH	3200–3400, 1600–1700	Reduction and stabilization
Bauhinia purpurea	Phenolics, flavonoids	3300–3500, 1500–1650	Capping and electron donation
Myxopyrum serratulum	Alcohols, amides	3000–3400, 1400–1600	Stabilization of nanoparticles
Synedrella nodiflora	Proteins, polyphenols	3200–3500, 1600–1650	Reduction and binding
Costus speciosus	Terpenoids, alkaloids	2800–3000, 1400–1600	Structural stabilization
Nervalia zeylanica	Flavonoids, phenolics	3200–3400, 1500–1650	Reducing agents
Orthosiphon aristatus	Polyphenols, proteins	3000–3400, 1500–1700	Capping and stabilization

Table 2 presents the Fourier Transform Infrared (FTIR) spectroscopic analysis used to identify the functional groups in plant extracts responsible for the green synthesis of nanoparticles. The presence of characteristic absorption bands in the range of 2800–3500 cm^{-1} and 1400–1700 cm^{-1} confirms the involvement of various bioactive compounds such as phenolics, flavonoids, proteins, terpenoids, and alkaloids. These biomolecules play a dual role as both reducing and stabilizing (capping) agents during nanoparticle formation.

Functional groups such as hydroxyl (–OH), carbonyl (–C=O), and amine (–NH) are particularly significant, as they donate electrons to reduce metal ions (Ag^+, Au^{3+}) into their elemental nanoparticle form. For instance, *Indigofera tinctoria* and *Nervalia zeylanica* show strong –OH and phenolic signals, indicating their effectiveness in reduction processes. Similarly, plant extracts rich in proteins and polyphenols, such as *Synedrella nodiflora* and *Orthosiphon aristatus*, contribute to both reduction and stabilization through binding interactions. Additionally, compounds like terpenoids and alkaloids in *Costus speciosus* provide structural stability, preventing nanoparticle aggregation. The variation in functional groups across plant species explains differences in nanoparticle size, morphology, and stability. FTIR analysis confirms that phytochemicals are crucial in facilitating eco-friendly nanoparticle synthesis.

Synthesis of silver and gold nanoparticles and UV-vis. spectral analysis

Silver and gold nanoparticles were synthesized using plant-mediated green chemistry approaches by mixing aqueous plant extracts with metal salt solutions such as silver nitrate ($AgNO_3$) and hydrogen tetrachloroaurate ($HAuCl_4$). The phytochemicals present in the extracts acted as reducing and stabilizing agents, facilitating the conversion of Ag^+ and Au^{3+} ions into their respective nanoparticles under mild conditions. In several cases, microwave irradiation was employed to accelerate the reaction, leading to rapid nucleation and formation of nanoparticles. A visible color change—from pale yellow to brown for silver nanoparticles and to ruby red for gold nanoparticles—served as a preliminary confirmation of synthesis.

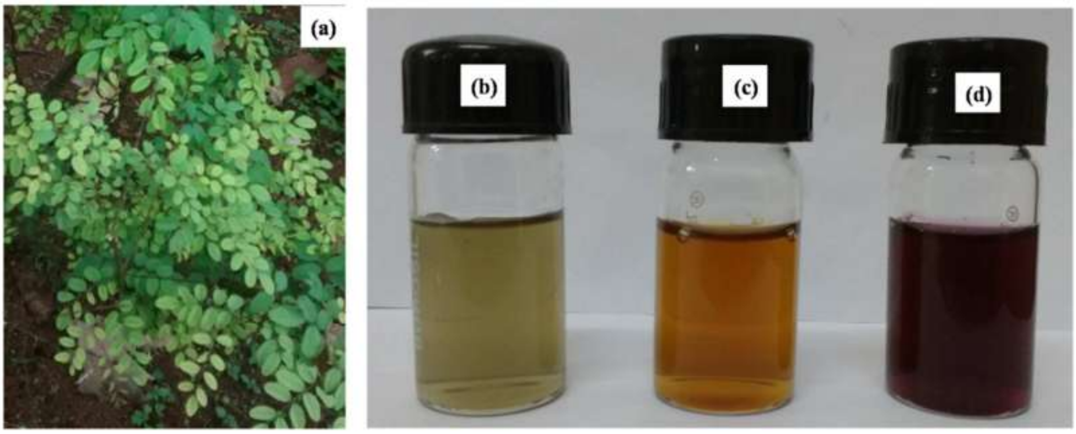

Fig. 1 Photographs of (a) Indigofera tinctoria plant, (b) Indigofera tinctoria leaf extract, (c) AgNP-tinctoria, and (d) AuNP-tinctoria

The formation and stability of nanoparticles were further confirmed using UV–visible spectroscopy in the range of 200–800 nm. Characteristic surface plasmon resonance (SPR) peaks were observed around 410–450 nm for AgNPs and 520–540 nm for AuNPs, indicating successful synthesis and providing insights into particle size and distribution.

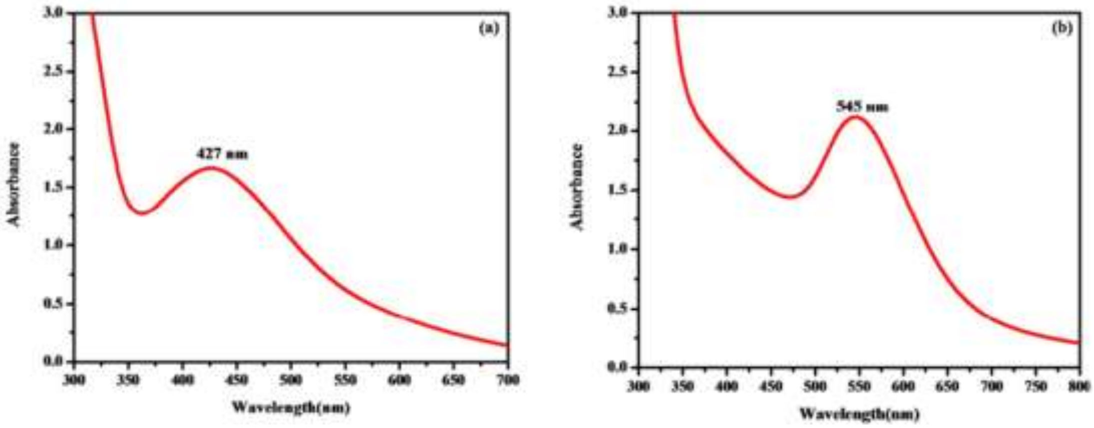

Fig. 2 UV-vis. absorption spectra of (a) AgNP-tinctoria, and (b) AuNPtinctoria

Figure 2 presents the UV–visible absorption spectra of biosynthesized (a) AgNP–*tinctoria* and (b) AuNP–*tinctoria*, confirming nanoparticle formation through characteristic surface plasmon resonance (SPR) bands. In the case of AgNP–*tinctoria*, a distinct absorption peak typically appears around 400–450 nm, which is attributed to the collective oscillation of conduction electrons in silver nanoparticles. This SPR band indicates successful reduction of Ag^{+} ions and suggests the formation of predominantly spherical and well-dispersed nanoparticles. For AuNP–*tinctoria*, the absorption peak is generally observed in the range of 520–550 nm, corresponding to the SPR of gold nanoparticles. The position and sharpness of the peak reflect particle size, shape, and uniformity. A narrow and intense peak suggests monodispersity and stability, while any broadening may indicate size variation or slight aggregation. The spectra validate the efficient green synthesis of both silver and gold nanoparticles using *tinctoria* extract.

In contrast, the UV–visible spectrum of AuNP-tinctoria (Fig. 4.2b) shows a prominent SPR band around 520–540 nm, confirming the formation of gold nanoparticles. The red shift compared to AgNPs is attributed to the larger size and different dielectric properties of gold nanoparticles. The relatively narrow peak indicates controlled nucleation and growth under the influence of plant-derived biomolecules. The stability of the peak position over time further suggests that the synthesized AuNPs are stable and well-capped. Overall, the UV–Vis analysis confirms the successful green synthesis of both silver and gold nanoparticles using *Indigofera tinctoria*.

Fourier transform infrared (FTIR) spectral analysis

Fourier Transform Infrared (FTIR) spectroscopy was employed to identify the functional groups present in plant extracts and to elucidate their role in the green synthesis of nanoparticles. The FTIR spectra of the synthesized nanoparticles typically exhibited prominent absorption bands in the regions of 3200–3400 cm^{-1}, 1600–1700 cm^{-1}, and 1400–1500 cm^{-1}. The broad peak around 3200–3400 cm^{-1} corresponds to O–H stretching vibrations of hydroxyl groups found in phenolics and flavonoids, indicating their involvement in the reduction of metal ions. Similarly, peaks observed in the range of 1600–1700 cm^{-1} are attributed to C=O stretching of carbonyl groups present in proteins and other biomolecules, suggesting their role in nanoparticle stabilization.

Additionally, absorption bands in the region of 1400–1500 cm^{-1} are associated with C–N stretching and amide linkages, confirming the presence of proteins that act as capping agents. Minor peaks corresponding to C–O and C–H vibrations further support the involvement of various phytochemicals such as terpenoids and alkaloids. The shift or reduction in intensity of these peaks after nanoparticle formation indicates their active participation in the synthesis process. FTIR analysis confirms that plant-derived biomolecules play a dual role in reducing metal ions and stabilizing the formed nanoparticles, ensuring efficient and eco-friendly synthesis.

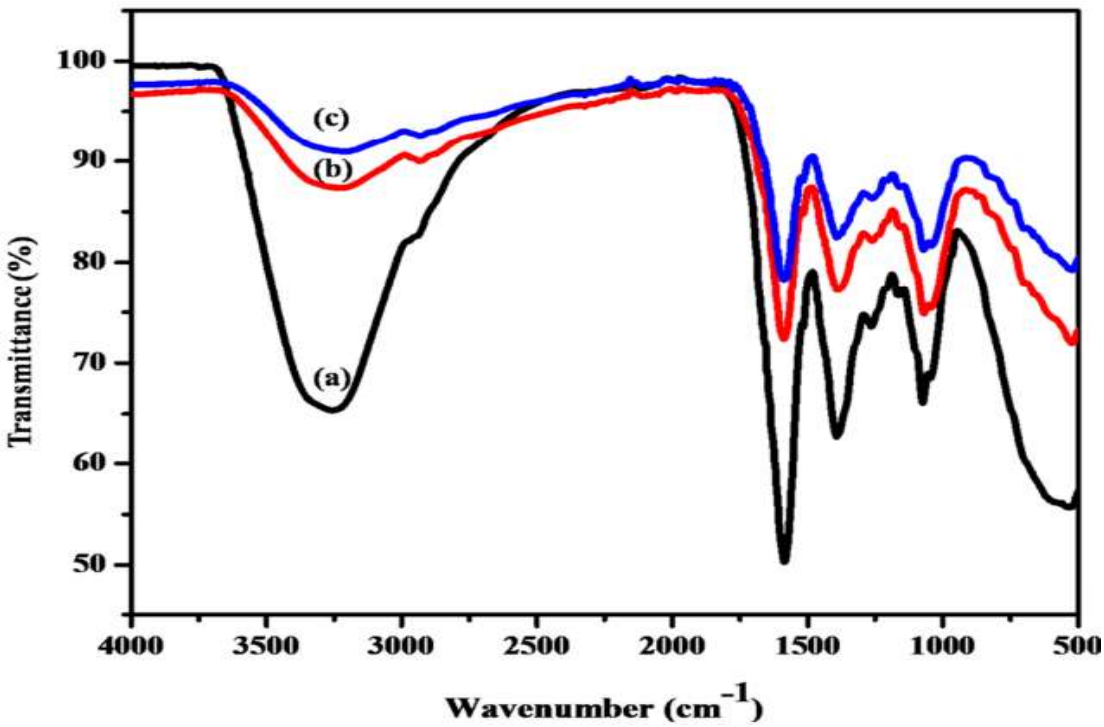

Fig. 3 FTIR spectra of (a) Indigofera tinctoria leaf extract, (b) AgNPtinctoria, and (c) AuNP-tinctoria

Figure 3 illustrates the FTIR spectra of (a) *Indigofera tinctoria* leaf extract, (b) AgNP–*tinctoria*, and (c) AuNP–*tinctoria*, highlighting the involvement of phytochemicals in nanoparticle synthesis. The leaf extract spectrum shows characteristic peaks corresponding to functional groups such as –OH (phenols/alcohols), –NH (amines), and C=O (carbonyl compounds), which act as reducing and stabilizing agents.

Upon formation of AgNPs and AuNPs, noticeable shifts in peak positions and changes in intensity are observed, indicating the interaction of these biomolecules with metal ions. These shifts confirm the reduction of Ag^{+} and Au^{3+} ions and subsequent capping of nanoparticles by plant-derived compounds. The presence of similar functional groups in nanoparticle spectra suggests effective stabilization, preventing aggregation. Overall, FTIR analysis verifies that *I.*

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tinctoria biomolecules play a dual role in both reduction and stabilization during green synthesis.

X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) analysis was performed to determine the crystalline structure, phase purity, and average crystallite size of the green-synthesized silver and gold nanoparticles. The XRD patterns exhibited distinct diffraction peaks corresponding to specific crystallographic planes, confirming the formation of highly crystalline nanoparticles. For silver nanoparticles, characteristic peaks were typically observed at 2θ values around 38°, 44°, 64°, and 77°, which are indexed to the (111), (200), (220), and (311) planes of a face-centered cubic (FCC) structure. Similarly, gold nanoparticles showed comparable diffraction patterns, indicating a similar FCC crystalline nature.

The sharp and intense peaks in the XRD spectra suggest high crystallinity and well-defined nanoparticle structures. The absence of additional impurity peaks indicates that the synthesized nanoparticles are of high purity and free from secondary phases. The average crystallite size of the nanoparticles was estimated using the Debye–Scherrer equation, which relates peak broadening to particle size. The calculated sizes were found to be in the nanometer range, consistent with results obtained from electron microscopy studies. Overall, XRD analysis confirms the successful formation of crystalline, phase-pure silver and gold nanoparticles through green synthesis methods.

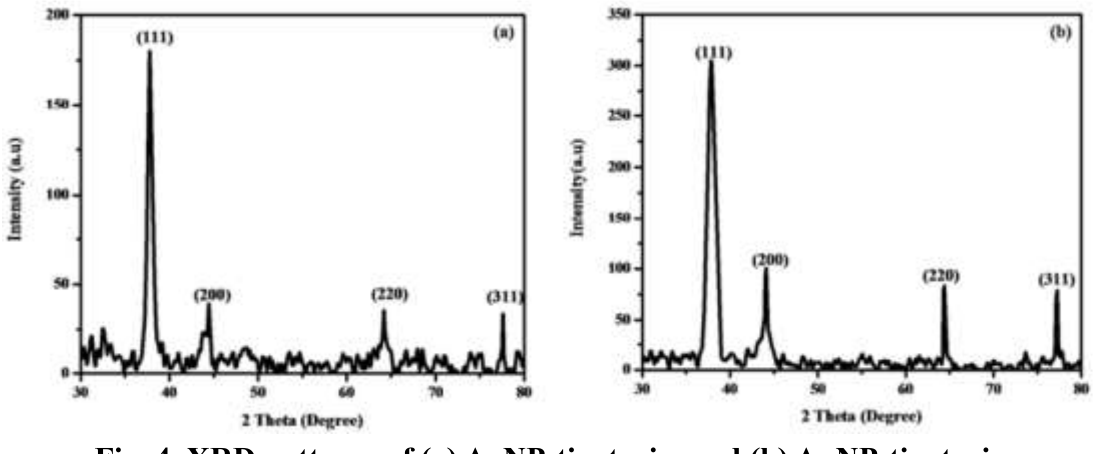

Fig. 4. XRD patterns of (a) AgNP-tinctoria, and (b) AuNP-tinctoria

Figure 4 presents the X-ray diffraction (XRD) patterns of (a) AgNP–*tinctoria* and (b) AuNP–*tinctoria*, confirming their crystalline nature. The diffraction peaks observed at specific 2θ values correspond to characteristic lattice planes such as (111), (200), (220), and (311), which are indexed to the face-centered cubic (fcc) structure of silver and gold nanoparticles.

The sharp and intense peaks indicate high crystallinity and purity of the synthesized nanoparticles. Minor unassigned peaks, if present, may arise from residual organic compounds from the *tinctoria* extract acting as capping agents. The dominance of the (111) plane suggests preferred orientation and stability of the nanoparticles. Overall, the XRD results validate the successful green synthesis of crystalline Ag and Au nanoparticles using *Indigofera tinctoria* leaf extract.

High resolution-transmission electron microscopy (HR-TEM) analysis

High Resolution-Transmission Electron Microscopy (HR-TEM) analysis was employed to investigate the morphology, size distribution, and structural details of the green-synthesized silver and gold nanoparticles. The HR-TEM images revealed that the nanoparticles were predominantly spherical in shape with a relatively uniform distribution, although slight variations in morphology were observed depending on the plant extract used. The particle size was found to be in the range of approximately 8–30 nm, confirming the nanoscale dimensions indicated by XRD analysis. The images also showed that the nanoparticles were well-dispersed with minimal aggregation, suggesting effective stabilization by phytochemicals present in the plant extracts. The clear contrast between particles and background further confirmed the formation of distinct nanoparticles.

At higher magnification, HR-TEM images exhibited well-resolved lattice fringes, indicating the crystalline nature of the synthesized nanoparticles. The measured interplanar spacing (d-spacing) corresponded to the characteristic planes of face-centered cubic (FCC) structures of silver and gold, supporting the XRD findings. Additionally, Selected Area Electron Diffraction (SAED) patterns displayed concentric rings with bright spots, confirming the polycrystalline nature of the nanoparticles. Energy Dispersive X-ray (EDX) analysis further verified the elemental composition, showing strong signals for silver or gold with minimal impurities. HR-TEM analysis confirms the successful synthesis of crystalline, uniformly distributed, and stable nanoparticles through green synthesis approaches.

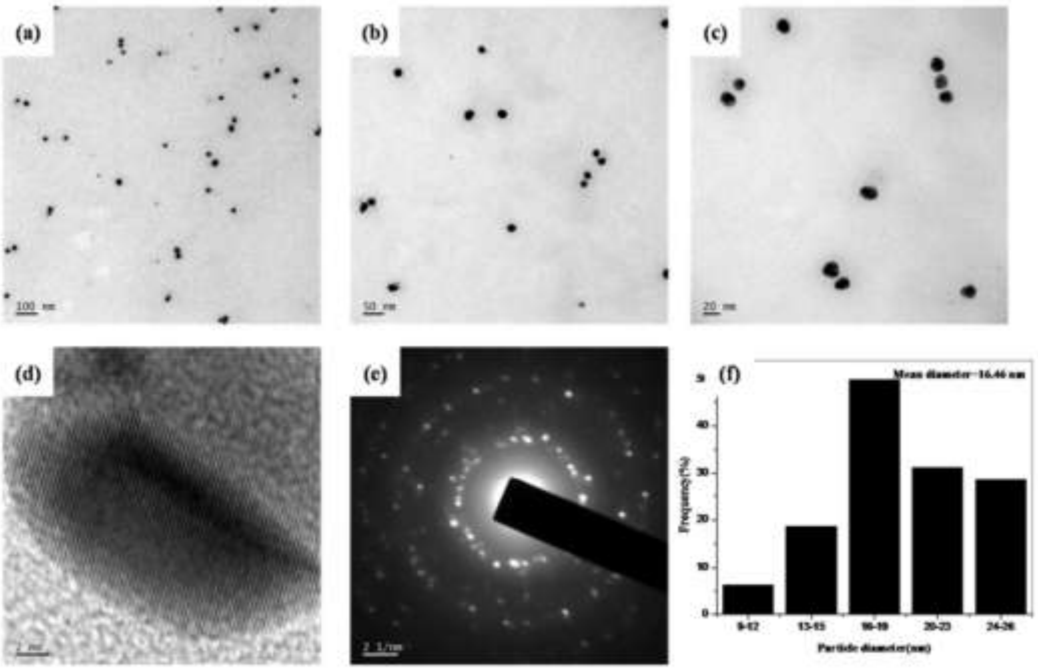

Fig. 5 TEM and Structural Characterization of AgNP–tinctoria

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Figure 5 illustrates the transmission electron microscopy (TEM) and structural characterization of AgNP–*tinctoria*. The TEM images reveal predominantly spherical nanoparticles with good dispersion and minimal aggregation, indicating effective stabilization by plant-derived biomolecules. The particle size appears to be within the nanoscale range with relatively uniform distribution.

The high-resolution TEM (HR-TEM) image shows distinct lattice fringes, confirming the crystalline nature of the nanoparticles. The selected area electron diffraction (SAED) pattern exhibits concentric rings corresponding to characteristic crystallographic planes, further validating the face-centered cubic (fcc) structure of silver. Additionally, the particle size distribution histogram demonstrates a narrow size range, suggesting monodispersity and controlled synthesis. These results confirm the successful formation of stable, crystalline AgNPs via green synthesis using *tinctoria* extract.

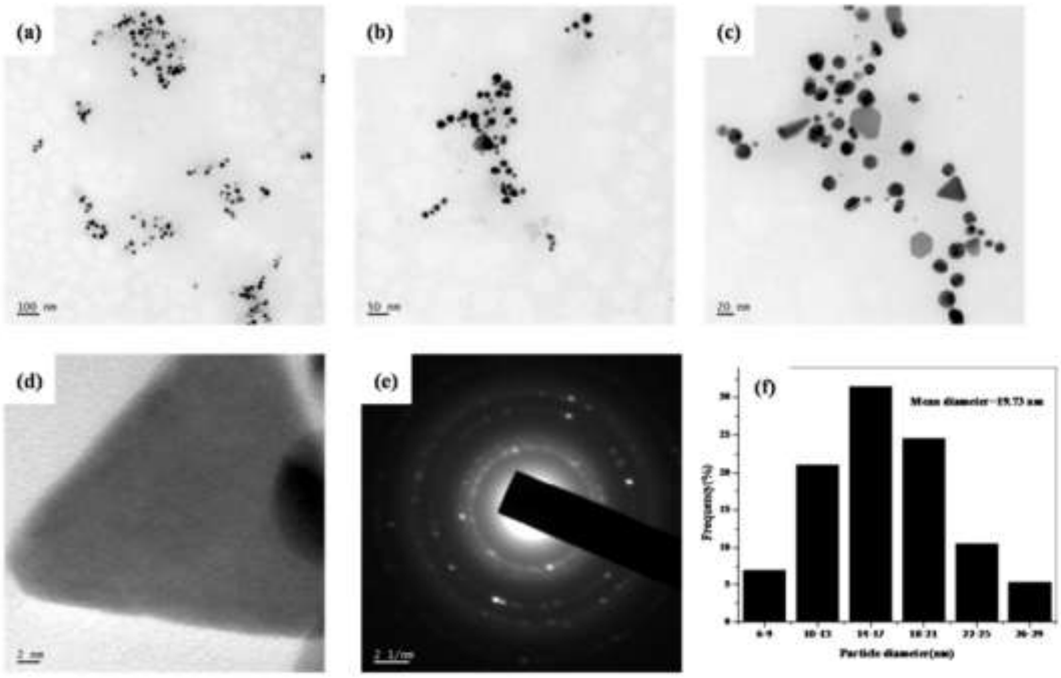

Fig. 6 TEM Analysis of AuNP–*tinctoria*

Figure 6 presents the TEM analysis of AuNP–*tinctoria*, demonstrating the morphology and structural features of the synthesized nanoparticles. The TEM images show predominantly spherical and well-dispersed gold nanoparticles with minimal aggregation, indicating effective capping and stabilization by phytochemicals present in the *tinctoria* extract. The particle size is observed within the nanoscale range with relatively uniform distribution.

The HR-TEM image reveals clear lattice fringes, confirming the crystalline structure of the nanoparticles. The SAED pattern exhibits distinct concentric rings indexed to characteristic planes of the face-centered cubic (fcc) structure of gold. Furthermore, the particle size distribution histogram indicates a narrow size range, suggesting monodispersity and controlled synthesis. The TEM analysis confirms the successful green synthesis of stable and crystalline AuNPs using *tinctoria* leaf extract.

Conclusion

Green chemistry approaches have significantly advanced the field of nanoparticle synthesis by providing sustainable, eco-friendly, and economically viable alternatives to conventional physicochemical methods. The use of biological resources such as plant extracts, microorganisms, and biomolecules enables the reduction and stabilization of metal ions without the need for toxic reagents or high energy input. Among these, plant-mediated synthesis has emerged as the most efficient and widely adopted strategy due to its simplicity, rapid reaction kinetics, and scalability. Characterization techniques including UV–Vis spectroscopy, FTIR, XRD, HR-TEM, and EDX consistently confirm the successful formation of stable, crystalline, and uniformly distributed nanoparticles. Furthermore, phytochemicals such as flavonoids, phenolics, and proteins play a crucial role in controlling nanoparticle size, morphology, and stability, ensuring functional efficiency.

The applications of green-synthesized nanoparticles span across diverse domains, including biomedical, environmental, agricultural, and industrial sectors. Their enhanced biocompatibility and reduced toxicity make them particularly suitable for antimicrobial, anticancer, and drug delivery applications, while their catalytic properties support environmental remediation processes such as dye degradation and water purification. Despite these advantages, challenges related to reproducibility, large-scale production, and incomplete mechanistic understanding remain critical barriers to commercialization. Future research should focus on standardizing synthesis protocols, improving scalability, and integrating advanced analytical and computational tools for process optimization. Overall, green chemistry-driven nanoparticle synthesis represents a promising pathway toward sustainable nanotechnology and environmentally responsible innovation.

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