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Review Article

From Crude Plant Extracts to Reproducible Nanomedicine: Phytochemical Standardization, Quality-by-Design and Functional Performance of Green-Synthesized Nanoparticles

Sonu Jacob[1*], Nehala M. N.[2], Fathimath Shahana[3], Aleena Paul[4], Devikrishna Vijayan[5], Vyshnavy Biju[6]

[1] *Associate Professor, Department of Pharmaceutical Chemistry and Analysis, Chemists College of Pharmaceutical Sciences and Research, Varikoli, Ernakulam, Keralam, India.*
[2,3,4,5,6] *Chemists College of Pharmaceutical Sciences and Research, Varikoli, Ernakulam, Keralam, India.*

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ABSTRACT

Green synthesis of nanoparticles using medicinal plant extracts has emerged as a promising alternative to conventional chemical and physical synthesis approaches. Plant-derived phytochemicals, including polyphenols, flavonoids, tannins, alkaloids, terpenoids and reducing sugars, act as natural reducing, stabilizing and capping agents during nanoparticle formation. Despite considerable progress in green nanotechnology, the transition from laboratory-scale synthesis to reproducible pharmaceutical nanomedicine remains challenging. Variations in plant species, geographical origin, harvesting conditions, extraction procedures and phytochemical composition can significantly influence nanoparticle characteristics and biological performance.This review examines the transformation of crude plant extracts into standardized, reproducible nanomedicinal systems, emphasizing phytochemical characterization, extract standardization, synthesis optimization and quality control. Particular attention is given to the application of Quality-by-Design (QbD), including identification of critical material attributes, critical process parameters and critical quality attributes. The relationship between phytochemical composition, nanoparticle physicochemical properties and functional performance is also discussed. Important characterization parameters such as particle size, polydispersity index, zeta potential, morphology, crystallinity, surface chemistry and stability are considered alongside biological evaluations, including antioxidant, antimicrobial, anti-inflammatory and cytotoxic activities.Furthermore, the

***Corresponding Author:** Sonu Jacob
Address: Associate Professor, Department of Pharmaceutical Chemistry and Analysis, Chemists College of Pharmaceutical Sciences and Research, Varikoli, Ernakulam, Keralam, India
Email ✉: chemistryadvsn2022@gmail.com
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review highlights challenges associated with batch-to-batch variability, scalability, biological safety, regulatory expectations and translational development. Integrating phytochemical fingerprinting, QbD-based optimization and systematic functional evaluation could establish a scientifically robust framework for converting plant-mediated nanoparticle synthesis into reproducible pharmaceutical nanomedicine.

INTRODUCTION

Nanotechnology has become an important area of pharmaceutical research because of its potential to improve drug delivery, therapeutic efficacy, biological availability and targeted treatment. Nanoparticles possess unique physicochemical characteristics arising from their small particle size, high surface-area-to-volume ratio and tunable surface properties. These characteristics have encouraged their investigation in drug delivery, antimicrobial therapy, cancer treatment, diagnostic imaging and tissue engineering.

Conventional nanoparticle synthesis generally involves physical and chemical methods. However, certain approaches require high energy consumption, expensive equipment and potentially hazardous chemicals. Residual reagents and toxic stabilizing agents may also create concerns regarding biological compatibility and environmental safety.

Green synthesis has emerged as an alternative approach that utilizes biological resources, particularly medicinal plant extracts, for nanoparticle production. Plants contain numerous secondary metabolites capable of reducing metal ions and stabilizing the resulting nanoparticles. These metabolites include flavonoids, phenolic acids, tannins, terpenoids, alkaloids and proteins.

Plant-mediated synthesis offers several potential advantages:

1. Utilization of renewable biological resources.
2. Reduction in the requirement for conventional chemical reducing agents.
3. Potential improvement in surface functionalization.
4. Relatively simple synthesis procedures.
5. Possibility of combining plant-derived bioactive compounds with nanoparticle-based delivery systems.

Despite these advantages, green synthesis faces an important scientific limitation: **the chemical composition of crude plant extracts is not constant.**

Two extracts prepared from the same plant may differ considerably because of geographical conditions, seasonal variations, plant maturity, drying procedures, extraction solvents and extraction temperatures. These variations may influence nanoparticle formation, particle size, stability and biological activity.

Consequently, obtaining nanoparticles with reproducible pharmaceutical properties requires more than demonstrating successful synthesis. It requires systematic standardization of the plant extract, controlled synthesis conditions, appropriate quality evaluation and reliable biological assessment.

This review therefore focuses on three interconnected requirements:

- Phytochemical standardization of plant extracts.
- Quality-by-Design-based optimization of nanoparticle synthesis.
- Evaluation of physicochemical properties and functional performance.

The integration of these approaches provides a pathway for transforming experimental green synthesis into reproducible nanomedicine.

2. Phytochemical Basis of Plant-Mediated Nanoparticle Synthesis

2.1 Role of plant secondary metabolites

Medicinal plants contain a complex mixture of primary and secondary metabolites. Several of these compounds participate directly or indirectly in nanoparticle formation.

The three major functions of plant phytochemicals during nanoparticle synthesis are:

- **Reduction:** Conversion of metal ions into their corresponding elemental or oxide nanoparticle forms.

- **Stabilization:** Prevention of particle aggregation through interactions with the nanoparticle surface.
- **Capping:** Formation of an organic coating around nanoparticles, which may influence surface charge, stability and biological interactions.

The contribution of individual phytochemical classes is summarized below.

Phytochemical class	Role in nanoparticle synthesis	Examples
Phenolic compounds	Electron donation and metal-ion reduction	Gallic acid, caffeic acid
Flavonoids	Reduction and surface stabilization	Quercetin, rutin
Tannins	Complex formation and capping	Tannic acid
Terpenoids	Surface interactions and stabilization	Limonene, ursolic acid
Alkaloids	Metal-ion interaction and surface modification	Berberine
Proteins and polysaccharides	Stabilization and surface coating	Plant-derived proteins, pectin

The actual contribution of these compounds depends on their concentration, molecular structure, extraction conditions and interactions with metal precursors.

2.2 Mechanism of green nanoparticle synthesis

Plant-mediated nanoparticle synthesis generally involves a sequence of chemical and physical processes.

Step 1: Preparation of plant extract

Plant materials are washed, dried, powdered and extracted using suitable solvents such as water, ethanol or hydroalcoholic mixtures.

Step 2: Metal-ion interaction

The prepared extract is mixed with a metal precursor, such as silver nitrate, zinc acetate or chloroauric acid.

Step 3: Reduction and nucleation

Phytochemicals interact with metal ions and facilitate reduction, followed by the formation of initial nanoparticle nuclei.

Step 4: Particle growth

The nuclei undergo further growth through the addition of reduced metal species.

Step 5: Stabilization

Phytochemicals adsorb onto the nanoparticle surface and may prevent excessive aggregation.

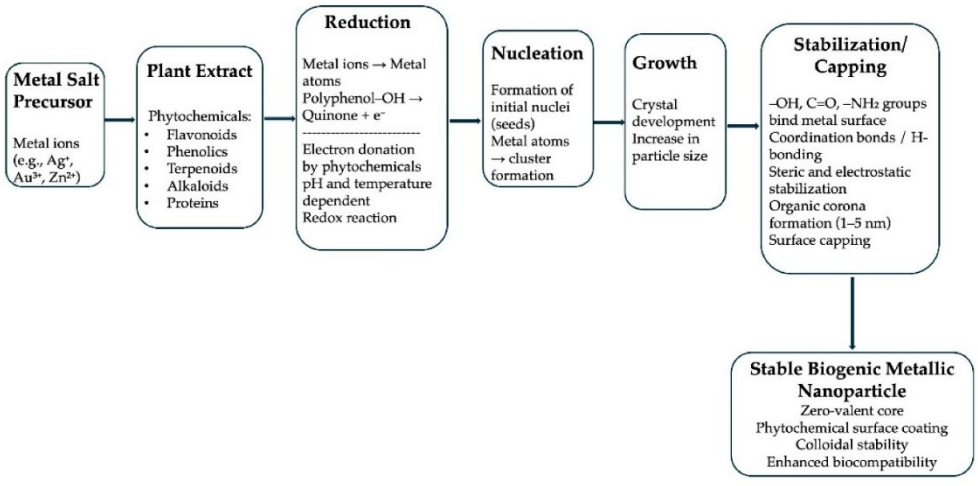

Figure 1: ***General mechanism of plant-mediated green nanoparticle synthesis.***

2.3 Influence of extraction solvents on phytochemical composition

The extraction solvent plays a significant role in determining the phytochemical profile of the resulting extract.

Solvent	Major compounds that may be extracted	Potential influence
Water	Polar phenolics, sugars, glycosides	Hydrophilic reducing and stabilizing components
Ethanol	Phenolics, flavonoids and some terpenoids	Broad phytochemical extraction
Methanol	Several polar and moderately polar metabolites	Useful for analytical profiling
Chloroform	Relatively nonpolar constituents	Lipophilic phytochemical fraction
Petroleum ether	Oils, waxes and nonpolar constituents	Nonpolar phytochemical fraction

Solvent selection should be based on the intended phytochemical composition and the final pharmaceutical application. Residual solvent levels must also be considered during product development.

2.4 The relationship between phytochemical composition and nanoparticle properties

One of the most important aspects of green nanotechnology is the relationship between plant extract composition and nanoparticle characteristics.

Changes in the concentration of phenolic compounds, flavonoids and other metabolites may affect:

- Rate of metal-ion reduction.
- Nucleation and particle growth.
- Particle size and morphology.
- Surface charge.
- Polydispersity.
- Colloidal stability.
- Biological activity.

For example, differences in reducing capacity between two plant extract batches may produce nanoparticles with different particle-size distributions even when the same metal precursor and reaction temperature are used.

Therefore, phytochemical profiling should be considered an essential part of nanoparticle manufacturing rather than an optional preliminary investigation.

3. Phytochemical Standardization of Crude Plant Extracts

3.1 Importance of botanical authentication

The first step in extract standardization is accurate identification of the selected medicinal plant.

Misidentification, substitution and adulteration may result in differences in phytochemical composition and biological activity.

The following information should be documented:

1. Scientific name and botanical authority.
2. Family and common name.
3. Plant part used.
4. Geographical location of collection.
5. Collection season and date.
6. Authentication details and voucher specimen number.
7. Processing and storage conditions.

Authentication through a qualified botanist or recognized herbarium improves the reliability of the starting material.

3.2 Standardization of extraction procedures

The extraction procedure must be standardized to obtain comparable phytochemical profiles between different batches.

Important extraction parameters include:

- Plant-to-solvent ratio.
- Solvent composition.
- Extraction temperature.
- Extraction duration.
- Extraction technique.
- Particle size of powdered plant material.
- Filtration and concentration procedures.
- Dry extract yield.

Maceration, Soxhlet extraction, ultrasonic-assisted extraction and microwave-assisted extraction can produce different phytochemical profiles.

Consequently, the selected extraction method should be maintained consistently during subsequent nanoparticle synthesis.

3.3 Preliminary phytochemical screening

Qualitative phytochemical screening provides preliminary information about the presence of major secondary metabolite classes.

Common tests include:

Phytochemical	Preliminary test
Alkaloids	Dragendorff's test
Flavonoids	Shinoda test
Tannins	Ferric chloride test
Phenols	Ferric chloride test
Saponins	Foam test
Terpenoids	Salkowski test
Glycosides	Appropriate class-specific chemical tests

Although useful for preliminary identification, qualitative tests are insufficient for establishing batch-to-batch consistency. Quantitative and chromatographic methods are also required.

3.4 Quantitative phytochemical estimation

Quantitative estimation provides numerical information about the major phytochemical groups.

A. Total phenolic content (TPC)

The Folin–Ciocalteu method is commonly employed to estimate total phenolic content.

Results are generally expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g).

B. Total flavonoid content (TFC)

The aluminium chloride colorimetric method is commonly used for flavonoid estimation.

Results are generally expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g).

C. Total tannin content

Colorimetric methods can be employed to estimate tannins, depending on the extract and analytical requirements.

D. Antioxidant capacity

DPPH, ABTS and FRAP assays can provide information about the reducing and radical-scavenging potential of plant extracts.

These assays can be useful supporting measurements, but they should not be treated as direct substitutes for chemical fingerprinting.

3.5 Advanced phytochemical fingerprinting techniques

Advanced analytical techniques help establish the chemical identity and consistency of plant extracts.

Analytical technique	Major application
TLC	Preliminary phytochemical profiling
HPTLC	Fingerprinting and batch comparison
HPLC	Separation and quantification of marker compounds
LC-MS/MS	Identification and quantification of metabolites
GC-MS	Analysis of volatile and suitable derivatized compounds
FTIR	Identification of characteristic functional groups
NMR	Structural characterization and metabolite profiling

Chromatographic fingerprinting is particularly important because crude plant extracts contain multiple compounds that may participate in nanoparticle synthesis.

For example, HPLC or LC-MS can help identify marker compounds associated with metal-ion reduction, whereas FTIR can provide information about functional groups involved in nanoparticle surface interactions.

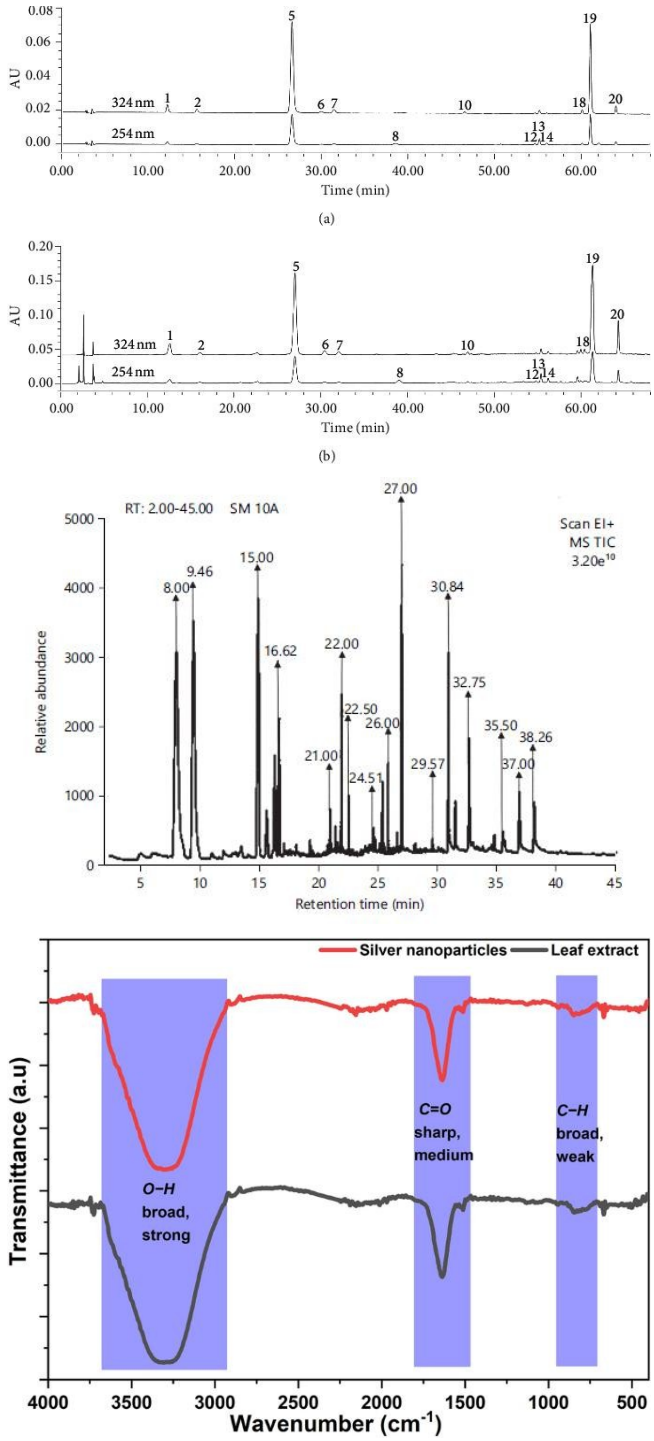

Figure 2: ***Examples of analytical approaches for phytochemical fingerprinting and nanoparticle characterization.***

3.6 Establishing extract acceptance criteria

A major advancement in extract standardization is the establishment of acceptance criteria before nanoparticle synthesis.

For example, a research laboratory may establish internal specifications for:

- Extractive yield.
- Moisture content.
- Total phenolic content.

- Total flavonoid content.
- Chromatographic fingerprint similarity.
- Marker-compound concentration.
- Microbial contamination, where relevant.

These specifications should be developed using experimental data and validated analytical methods rather than selecting arbitrary universal limits.

The Consensus-based Reporting Guidelines for Phytochemical Characterisation of Medicinal Plant Extracts (ConPhyMP) provide an important framework for improving the reporting and reproducibility of medicinal plant extract research [1].

4. Quality-by-Design Approach in Green Nanoparticle Synthesis

4.1 Introduction to Quality-by-Design

Quality-by-Design (QbD) is a systematic pharmaceutical development approach in which product quality is incorporated into the development process rather than evaluated only at the final stage.

The approach involves understanding the relationship between raw materials, manufacturing parameters and final product quality.

In conventional green nanoparticle synthesis, researchers frequently modify individual experimental parameters until nanoparticles with desirable characteristics are obtained.

However, this trial-and-error approach may not adequately explain the interactions between phytochemical composition, synthesis conditions and nanoparticle properties.

QbD offers a structured alternative by establishing predefined quality objectives and identifying the factors that influence them.

4.2 Quality Target Product Profile (QTPP)

The Quality Target Product Profile describes the desired characteristics of the final nanoparticle formulation.

For green-synthesized pharmaceutical nanoparticles, the QTPP may include:

1. Intended pharmaceutical application.
2. Route of administration.
3. Desired particle-size range.
4. Acceptable particle-size distribution.
5. Colloidal stability.
6. Biocompatibility.
7. Biological performance.
8. Storage stability.
9. Appropriate formulation characteristics.

The QTPP should be defined according to the intended product. For example, nanoparticles intended for topical antimicrobial applications may have different quality requirements from nanoparticles developed for intravenous drug delivery.

4.3 Critical Quality Attributes (CQAs)

Critical Quality Attributes are measurable physicochemical or biological characteristics that must be maintained within appropriate limits to ensure product quality.

Important CQAs for green-synthesized nanoparticles include:

Critical quality attribute	Pharmaceutical significance
Particle size	Influences distribution, interaction and cellular uptake
Polydispersity index	Indicates particle-size distribution
Zeta potential	Provides information about surface charge and colloidal stability
Morphology	Influences surface properties and particle interactions
Crystallinity	Provides information about material structure
Surface chemistry	Influences biological interactions
Nanoparticle yield	Indicates synthesis efficiency
Stability	Determines the suitability of storage and handling
Biological activity	Indicates functional performance
Residual impurities	Important for product safety

Not every attribute has equal importance for every nanoparticle system. CQAs must be selected according to the intended application.

4.4 Critical Material Attributes (CMAs)

Critical Material Attributes are properties of the starting materials that may influence final nanoparticle quality.

In plant-mediated synthesis, the major CMAs include:

- Botanical identity.
- Plant part.
- Extract concentration.
- Total phenolic content.
- Total flavonoid content.
- Marker-compound concentration.
- Extract pH.
- Moisture content.
- Metal precursor concentration.
- Solvent composition.

Among these, the phytochemical composition of the extract is particularly important because it determines the reducing and stabilizing capacity of the biological material.

4.5 Critical Process Parameters (CPPs)

Critical Process Parameters are manufacturing variables that can influence CQAs.

Important CPPs include:

1. Reaction temperature.
2. Reaction pH.
3. Reaction duration.
4. Metal precursor concentration.
5. Plant extract concentration.
6. Extract-to-metal precursor ratio.
7. Stirring speed.
8. Order of reagent addition.
9. Reaction volume.
10. Purification and drying conditions.

Changes in these parameters may affect nucleation, crystal growth, aggregation and nanoparticle stability.

4.6 Application of Design of Experiments (DoE)

Design of Experiments is an important statistical tool in QbD-based formulation development.

Instead of changing one factor at a time, DoE allows researchers to evaluate multiple variables simultaneously and identify interactions between them.

Common experimental designs include:

DoE method	Application
Factorial design	Evaluation of main effects and interactions
Plackett–Burman design	Screening of important variables
Box–Behnken design	Optimization of selected process parameters
Central Composite Design	Development of response-surface models
Mixture design	Optimization of extract or solvent mixtures

For example, the effects of reaction temperature, pH and extract concentration on nanoparticle size can be studied using a response-surface methodology.

The optimized conditions can then be verified experimentally.

4.7 Establishment of a design space

A design space represents the combination of input variables and process conditions that have been demonstrated to provide acceptable product quality.

In green nanoparticle synthesis, a design space may include:

- Acceptable extract concentration.
- Suitable precursor concentration.
- Controlled reaction pH.
- Temperature range.
- Reaction time.
- Mixing conditions.

Establishing a design space helps improve process understanding and supports reproducible manufacturing.

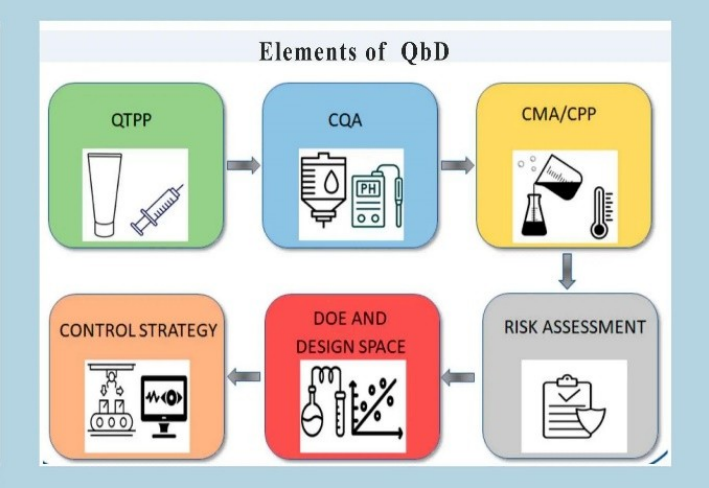

Figure 3: ***Proposed QbD framework for plant-mediated nanoparticle development***

5. Physicochemical Characterization and Quality Control of Green-Synthesized Nanoparticles

Successful nanoparticle synthesis does not necessarily indicate that the resulting material is suitable for pharmaceutical applications. Comprehensive physicochemical characterization is required to establish nanoparticle identity, purity, uniformity and stability.

5.1 UV–Visible spectroscopy

UV–Visible spectroscopy is commonly used for preliminary confirmation and monitoring of nanoparticle formation.

Metal nanoparticles exhibit characteristic optical absorption associated with surface plasmon resonance (SPR). For example, silver nanoparticles commonly show an absorption band in the visible region.

The position and intensity of the absorption band may change according to particle size, morphology, aggregation and surrounding medium.

However, UV–Visible spectroscopy alone cannot confirm nanoparticle purity or provide definitive particle-size measurements.

5.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy identifies functional groups present in plant extracts and nanoparticle samples.

It can help investigate the possible involvement of phytochemicals in nanoparticle reduction and surface stabilization.

Characteristic absorption regions may include:

- O–H stretching: hydroxyl-containing compounds.
- C–H stretching: aliphatic groups.
- C=O stretching: carbonyl-containing compounds.
- C=C stretching: aromatic structures.
- C–O stretching: alcohols, ethers and phenolic compounds.

Comparing the spectra of crude extract and synthesized nanoparticles can provide information about changes in functional-group environments.

5.3 Dynamic Light Scattering (DLS)

DLS is used to determine the hydrodynamic diameter and particle-size distribution of nanoparticles dispersed in a liquid medium.

It provides information about the apparent particle size, including the contribution of surface coatings and associated solvent layers.

The polydispersity index (PDI) indicates the breadth of the particle-size distribution.

A lower PDI generally indicates a narrower distribution, although its interpretation depends on the measurement method and nanoparticle system.

5.4 Zeta potential analysis

Zeta potential provides information about the electrokinetic potential near the slipping plane of dispersed nanoparticles.

It is useful for evaluating surface charge and predicting the tendency of particles to aggregate.

Both electrostatic and steric stabilization should be considered when interpreting nanoparticle stability. Therefore, zeta potential should not be used as the only stability indicator.

5.5 Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM)

SEM and TEM are used to investigate nanoparticle morphology and structure.

- SEM provides information about surface morphology and particle arrangement.
- TEM provides high-resolution images of individual nanoparticles and their dimensions.

These techniques are useful for determining whether the nanoparticles are spherical, rod-shaped, irregular or otherwise structured.

5.6 X-ray Diffraction (XRD)

XRD is used to determine the crystalline structure and phase composition of nanoparticles.

It can help identify crystalline phases, detect certain impurities and investigate crystallinity.

5.7 Stability studies

Stability is an essential requirement for pharmaceutical nanoparticle development.

Changes in storage conditions may cause aggregation, precipitation, oxidation or changes in surface chemistry.

Important stability parameters include:

- Particle size.
- PDI.
- Zeta potential.
- Visual appearance.
- pH.
- Aggregation.
- Chemical and structural integrity.

Stability should be evaluated under defined storage conditions over appropriate time intervals.

Major characterization methods and their applications

Technique	Main parameter evaluated	Importance
UV–Visible spectroscopy	Optical absorption	Preliminary formation monitoring
FTIR	Functional groups	Surface chemistry
DLS	Hydrodynamic diameter	Particle-size distribution
Zeta potential	Surface electrokinetic potential	Colloidal stability
SEM	Surface morphology	Structural examination
TEM	Particle morphology and dimensions	Nanoscale imaging
XRD	Crystal structure	Phase identification
EDX	Elemental composition	Elemental confirmation
TGA	Thermal behaviour	Thermal stability and organic coating assessment
ICP-MS or ICP-OES	Elemental concentration	Metal content and residual-ion analysis

The selection of characterization methods should depend on the type of nanoparticle, intended application and available analytical facilities.

6. Functional Performance of Green-Synthesized Nanoparticles

The pharmaceutical significance of green-synthesized nanoparticles depends not only on their physicochemical properties but also on their biological and functional performance.

A nanoparticle system may possess desirable particle size and stability but still demonstrate inadequate biological activity or unacceptable toxicity.

Therefore, functional evaluation should be integrated into the quality assessment process.

6.1 Antioxidant activity

Plant-mediated nanoparticles may demonstrate antioxidant activity due to the presence of surface-associated phytochemicals and other material-specific properties.

Common antioxidant assays include:

DPPH radical-scavenging assay: Evaluates the ability of a sample to reduce the stable DPPH radical.

ABTS assay: Measures the ability of a sample to neutralize ABTS radical cations.

FRAP assay: Estimates the ferric-reducing capacity of a sample.

For meaningful comparison, nanoparticle samples should be evaluated alongside the crude extract, precursor controls and appropriate reference antioxidants.

The contribution of the plant-derived surface coating should be distinguished from the intrinsic properties of the nanoparticle material.

6.2 Antimicrobial activity

Green-synthesized nanoparticles have been investigated against several Gram-positive and Gram-negative bacteria and certain fungal organisms.

Common evaluation methods include:

- Agar well diffusion.
- Disc diffusion.
- Minimum inhibitory concentration (MIC).
- Minimum bactericidal concentration (MBC).
- Time-kill studies.

Potential antimicrobial mechanisms include:

1. Interaction with microbial cell surfaces.
2. Disruption of membrane integrity.
3. Generation of reactive oxygen species.
4. Interference with cellular enzymes.
5. Release of biologically active metal ions.

The relative contribution of these mechanisms depends on the nanoparticle composition, surface chemistry and test organism.

6.3 Anti-inflammatory activity

Plant-mediated nanoparticles may demonstrate anti-inflammatory potential through interactions with inflammatory signalling pathways and oxidative stress mechanisms.

Common in vitro evaluation methods include:

- Protein denaturation inhibition assay.
- Proteinase inhibition assay.
- Membrane stabilization assay.

More advanced studies may investigate inflammatory mediators such as TNF-α, IL-6 and COX-related pathways.

These preliminary assays should not be considered sufficient evidence of therapeutic anti-inflammatory efficacy.

6.4 Anticancer activity

Metal and metal oxide nanoparticles synthesized using medicinal plants have been investigated for potential anticancer effects.

Possible mechanisms include:

- Induction of oxidative stress.
- Mitochondrial dysfunction.
- Cell-cycle alterations.
- Apoptosis.
- Modulation of intracellular signalling.

MTT, resazurin and other validated cell-viability assays may be used for preliminary evaluation.

However, the presence of cytotoxic activity does not automatically indicate selective anticancer activity. Comparisons with suitable non-cancerous cells are essential to establish selectivity.

6.5 Drug delivery applications

One of the important pharmaceutical applications of nanotechnology is the development of nanocarriers for drug delivery.

Green-synthesized nanoparticles may be investigated as:

- Drug-loading systems.

- Surface-functionalized carriers.
- Antimicrobial delivery platforms.
- Topical pharmaceutical systems.
- Controlled-release formulations.

Potential advantages include modification of surface properties and opportunities for combining natural phytochemicals with therapeutic agents.

Nevertheless, plant-mediated metal nanoparticles should not automatically be regarded as drug-delivery carriers. Their suitability depends on loading capacity, drug compatibility, release characteristics, biocompatibility and safety.

7. Relationship Between Phytochemical Composition, Nanoparticle Quality and Biological Performance

An important aspect of this review is the integration of three major parameters:

1. Phytochemical composition of the starting extract.
2. Physicochemical properties of the resulting nanoparticles.
3. Biological performance of the final nanoparticle system.

These parameters are interdependent.

For instance, a plant extract containing a higher concentration of certain reducing compounds may produce different nucleation and growth behaviour compared with an extract containing lower concentrations of those compounds.

Changes in nucleation and growth can influence particle size and morphology. These physicochemical changes may subsequently influence biological interactions.

The following table summarizes the proposed relationships.

Relationship between phytochemical attributes, nanoparticle characteristics and functional performance

Phytochemical attribute	Possible nanoparticle effect	Potential functional consequence
Total phenolic content	Influences reduction and surface interactions	Changes in antioxidant-associated activity
Flavonoid concentration	Influences reduction and surface coating	Changes in surface chemistry and biological interactions
Tannin concentration	Influences complexation and capping	Changes in aggregation and stability
Terpenoid composition	Influences surface interactions	Possible changes in biological compatibility
Protein content	May contribute to surface coating	Changes in colloidal behaviour
Polysaccharide content	May provide steric stabilization	Changes in dispersion stability

These relationships are mechanistic hypotheses that should be established experimentally for each plant–nanoparticle system.

7.1 Proposed integrated analytical approach

A systematic approach can be developed to investigate the relationships between extract composition and nanoparticle performance.

The workflow may include:

1. Preparation of multiple independently sourced plant extract batches.
2. Quantitative phytochemical estimation.
3. HPLC, GC-MS or LC-MS fingerprinting.
4. Standardized nanoparticle synthesis.
5. Physicochemical characterization.
6. Biological activity evaluation.
7. Statistical correlation analysis.
8. Multivariate data analysis.

Statistical methods such as principal component analysis (PCA), hierarchical clustering and partial least squares regression may help identify relationships between phytochemical profiles and nanoparticle characteristics.

Such an approach can help identify which extract characteristics are most strongly associated with desirable nanoparticle properties.

8. Batch-to-Batch Reproducibility and Standardization Challenges

Reproducibility is one of the major obstacles preventing the pharmaceutical translation of plant-mediated nanoparticle synthesis.

Unlike purified chemical reducing agents, crude plant extracts contain numerous compounds with variable concentrations.

These differences may result from:

- Geographical variations.
- Seasonal changes.
- Soil and climatic conditions.
- Plant maturity.
- Harvesting time.
- Extraction solvent.
- Extraction temperature.
- Storage duration.

Even when synthesis conditions are maintained, differences in extract composition may produce variations in particle size, morphology, surface properties and biological performance.

8.1 Strategies to improve reproducibility

Several approaches may improve batch-to-batch consistency.

Botanical standardization: Use authenticated plant material and document collection conditions.

Extraction standardization: Maintain consistent solvent composition, plant-to-solvent ratio and extraction conditions.

Phytochemical fingerprinting: Establish chromatographic profiles and quantify selected marker compounds.

Process control: Monitor pH, temperature, reaction time and precursor concentration.

Analytical validation: Use validated or appropriately qualified analytical methods.

Batch comparison: Compare independent nanoparticle batches using predefined acceptance criteria.

Stability monitoring: Evaluate changes in physicochemical properties during storage.

8.2 Role of reference materials and marker compounds

One promising approach is the use of selected marker compounds to normalize plant extracts.

For example, extracts may be standardized according to their gallic acid, quercetin or other suitable marker-compound concentrations.

Alternatively, reducing capacity or total phenolic content may be used as supporting indicators.

However, a single marker may not adequately represent the entire phytochemical composition. Combining marker quantification with chromatographic fingerprinting provides a more comprehensive approach.

9. Scale-Up and Pharmaceutical Manufacturing Considerations

Although plant-mediated nanoparticle synthesis is frequently demonstrated at laboratory scale, translating the process into large-scale pharmaceutical manufacturing presents several challenges.

9.1 Challenges during scale-up

1. Raw material availability

Large-scale production requires a consistent supply of authenticated plant material. Seasonal availability and geographical differences may affect raw-material quality.

2. Reaction uniformity

Maintaining uniform mixing, temperature and pH becomes more difficult when reaction volumes increase.

3. Particle-size control

Changes in heat transfer, mass transfer and mixing conditions may influence nucleation and particle growth.

4. Purification

Unreacted metal ions, residual phytochemicals and other impurities must be adequately removed or controlled.

5. Storage and transportation

Nanoparticles may undergo aggregation, oxidation or other physical and chemical changes during storage.

9.2 Process analytical technology (PAT)

Process Analytical Technology can support real-time monitoring and control of nanoparticle manufacturing.

Potential monitoring approaches include:

- UV–Visible spectroscopy for reaction monitoring.
- pH monitoring.
- Temperature monitoring.
- Turbidity measurements.
- Particle-size measurements.
- Spectroscopic monitoring of process changes.

Integrating process monitoring with QbD can improve manufacturing consistency.

9.3 Good Manufacturing Practice considerations

For pharmaceutical development, green-synthesized nanoparticles must undergo appropriate quality assessment and manufacturing controls.

Important considerations include:

- Raw-material qualification.
- Manufacturing documentation.
- Process validation.
- Analytical method validation.
- Impurity profiling.
- Microbiological quality.
- Stability testing.
- Batch-release specifications.

Importantly, the use of plant extracts does not automatically establish the safety or regulatory acceptability of a nanoparticle product.

10. Safety and Toxicological Evaluation

Biological synthesis is often described as environmentally friendly, but this does not necessarily mean that the resulting nanoparticles are safe for human use.

The toxicity of nanoparticles depends on several factors, including chemical composition, particle size, surface chemistry, aggregation behaviour, dissolution and exposure route.

10.1 In vitro cytotoxicity

Cell-based assays can provide preliminary information about the cytotoxic potential of nanoparticles.

Common methods include:

- MTT assay.
- MTS assay.
- Resazurin assay.
- LDH release assay.

Appropriate controls are important because nanoparticles may interfere with the optical signals or reagents used in certain assays.

10.2 Hemocompatibility

For nanoparticle systems intended for blood-contacting or systemic applications, evaluation may include:

- Hemolysis.
- Platelet interactions.
- Coagulation-related effects.
- Complement activation.

10.3 Genotoxicity

Genotoxicity testing may be required depending on the material, intended exposure and regulatory development pathway.

Potential evaluation methods include the comet assay and micronucleus assay.

10.4 In vivo toxicity

Where justified, animal studies may investigate:

- Acute toxicity.
- Repeated-dose toxicity.
- Biodistribution.
- Clearance.
- Organ accumulation.
- Histopathological changes.

Safety evaluation should be appropriate to the proposed route of administration and intended clinical use.

11. Regulatory Perspectives and Quality Frameworks

The pharmaceutical development of green-synthesized nanoparticles requires the integration of material characterization, manufacturing controls and biological safety assessment.

Several regulatory and scientific frameworks provide useful guidance.

11.1 ICH Q8 (R2): Pharmaceutical Development

ICH Q8 (R2) introduces the principles of pharmaceutical development and supports a systematic understanding of product and process variables.

Its concepts are applicable to the development of green nanoparticle formulations through:

- Definition of QTPP.
- Identification of CQAs.
- Understanding of material attributes.
- Process optimization.
- Establishment of design space.

11.2 ICH Q9 (R1): Quality Risk Management

ICH Q9 (R1) provides a framework for identifying, evaluating and controlling quality risks.

For green nanoparticle synthesis, risk assessment may include:

- Variability in plant material.
- Inconsistent extract composition.
- Incomplete nanoparticle formation.
- Particle aggregation.
- Residual metal ions.
- Microbial contamination.
- Product instability.

11.3 ICH Q10: Pharmaceutical Quality System

ICH Q10 provides a pharmaceutical quality-system framework covering the product lifecycle.

Its principles may support continuous improvement, process monitoring, deviation management and change control during nanoparticle development.

11.4 Nanomedicine-specific characterization

The characterization of nanomedicines requires particular attention because their behaviour may depend on particle size, surface characteristics, aggregation and interactions with biological systems.

Consequently, conventional pharmaceutical quality tests may need to be supplemented with nanoparticle-specific analytical procedures.

Regulatory development should also consider the intended product category and applicable national requirements. A green-synthesized nanoparticle intended as an active medicinal product may face different requirements from a nanoparticle used as an excipient or drug carrier.

12. Emerging Trends and Future Research Perspectives

The future of green nanomedicine will depend on the development of more predictable, standardized and scientifically validated synthesis approaches.

Several emerging strategies may contribute to this transition.

12.1 Metabolomics-assisted nanoparticle synthesis

Metabolomics can provide comprehensive information about the metabolites present in plant extracts.

LC-MS-based metabolomics may help identify compounds associated with nanoparticle formation and biological performance.

Combining metabolomic information with nanoparticle characterization could improve understanding of the relationships between extract composition and product quality.

12.2 Artificial intelligence and machine learning

Artificial intelligence and machine learning may help predict nanoparticle characteristics from extract composition and synthesis parameters.

Potential applications include:

- Prediction of particle size.

- Identification of important synthesis variables.
- Optimization of reaction conditions.
- Prediction of nanoparticle stability.
- Correlation of phytochemical profiles with biological activity.

However, reliable predictive models require sufficiently large, standardized and experimentally validated datasets.

12.3 AI-integrated Quality-by-Design

Combining AI with QbD may provide an advanced framework for controlling variability in plant-mediated synthesis.

A recent 2026 review specifically explores AI-QbD strategies for addressing natural-extract variability and improving the reproducibility of green nanomaterial production [3].

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12.4 Use of purified phytochemicals

An alternative to crude extracts is the use of purified plant-derived compounds.

Examples include:

- Quercetin.
- Gallic acid.
- Tannic acid.
- Rutin.
- Caffeic acid.

Purified compounds may offer better control over reducing and stabilizing activities. However, they may not reproduce the complex surface chemistry obtained using crude plant extracts.

A comparative approach involving crude extracts, standardized extracts and purified marker compounds could help establish the most suitable synthesis strategy.

12.5 Green synthesis using agricultural waste

Agricultural residues and food-processing waste may provide economical sources of phytochemicals.

Leaves, fruit peels, seed coats and other plant-derived residues may be investigated as reducing and stabilizing agents.

However, waste-derived extracts also require authentication of their composition, contaminant assessment and batch standardization.

12.6 Development of multifunctional nanomedicines

Future research may focus on multifunctional nanoparticles combining several properties, such as:

- Antimicrobial activity.
- Antioxidant functionality.
- Controlled drug release.
- Surface targeting.
- Imaging capability.

The development of multifunctional systems should be supported by separate evaluation of each claimed function and their combined effects.

13. Proposed Integrated Framework: From Crude Extract to Reproducible Nanomedicine

The central contribution of this review is the integration of phytochemical standardization, QbD and functional performance into a single pharmaceutical development framework.

Rather than treating plant extraction, nanoparticle synthesis and biological testing as separate activities, these processes should be considered interconnected stages of product development.

13.1 Stage I: Botanical and phytochemical standardization

- Authenticate the medicinal plant.
- Standardize plant collection and processing.
- Select appropriate extraction conditions.
- Determine extractive yield.
- Perform quantitative phytochemical analysis.
- Establish chromatographic fingerprints.
- Define extract acceptance criteria.

13.2 Stage II: QbD-based synthesis development

- Define the QTPP.
- Identify CMAs and CPPs.
- Select relevant CQAs.
- Conduct risk assessment.
- Apply DoE for optimization.
- Establish an appropriate design space.

13.3 Stage III: Nanoparticle characterization

- Confirm nanoparticle formation.
- Determine particle size and PDI.
- Evaluate zeta potential.
- Investigate morphology.
- Determine crystalline structure.
- Analyze surface functional groups.
- Assess residual impurities.

13.4 Stage IV: Functional performance evaluation

- Antioxidant activity.
- Antimicrobial activity.
- Anti-inflammatory activity.
- Cytotoxicity.
- Drug-loading and release studies, where applicable.

13.5 Stage V: Reproducibility and stability assessment

- Prepare independent batches.
- Compare phytochemical fingerprints.
- Evaluate physicochemical consistency.
- Compare biological performance.
- Conduct stability studies.
- Establish batch acceptance criteria.

This framework provides a practical route for improving the scientific reliability of green nanoparticle research and supporting its progression towards pharmaceutical development.

CONCLUSION

Green synthesis of nanoparticles using medicinal plant extracts represents a promising approach for the development of sustainable nanomaterials with potential pharmaceutical applications. However, the transition from laboratory-scale synthesis to reproducible nanomedicine requires a comprehensive understanding of the chemical composition of plant extracts and its influence on nanoparticle formation and biological performance.

Phytochemical standardization provides the foundation for controlling raw-material variability, while Quality-by-Design offers a systematic approach to identifying critical material attributes, critical process parameters and critical quality attributes. Advanced analytical characterization and functional evaluation further establish the quality, stability and biological relevance of the resulting nanoparticles.

Integrating phytochemical fingerprinting, QbD, design-of-experiments approaches, nanoparticle characterization and biological assessment may improve reproducibility and facilitate the development of more reliable green nanomedicinal systems.

Ultimately, the successful pharmaceutical translation of green-synthesized nanoparticles will depend not only on the sustainability of their preparation but also on their consistency, quality, safety and demonstrated therapeutic performance.

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