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# ANALYTICAL CHARACTERIZATION AND GREEN SYNTHESIS OF OROXYLUM INDICUM-MEDIATED METALLIC NANOPARTICLES

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**Abstract**
Plant-mediated synthesis of metallic nanoparticles has become an important interface between pharmacognosy, analytical chemistry and pharmaceutical nanotechnology because phytochemicals can function simultaneously as reducing, surface-capping and colloid-stabilizing agents. Oroxylum indicum (L.) Kurz is a medicinal tree rich in flavonoids, particularly baicalein, baicalin, chrysin, oroxylin A and related glycosides, making it a chemically plausible bio-reductant for metallic nanoparticle fabrication. This analytical study critically integrates phytochemical profiling of O. indicum with green synthesis, process optimization, physicochemical characterization and stability assessment of plant-mediated metallic nanoparticles, using silver nanoparticles (AgNPs) as the best documented model system. The study follows a pharmaceutical-science format and distinguishes literature-reported observations from proposed analytical procedures. Recent O. indicum-specific work reports aqueous bark-mediated AgNP formation at 1 mM AgNO3, an optimized extract-to-precursor ratio of 1:9, alkaline reaction conditions, a surface-plasmon-resonance maximum near 403 nm, face-centered-cubic silver reflections by X-ray diffraction, and predominantly spherical particles with limited agglomeration. The same evidence indicates participation of hydroxyl, carbonyl and related phytochemical functionalities in reduction and capping. A quality-by-design-oriented optimization strategy is proposed in which pH, precursor concentration, extract ratio, temperature and reaction time are treated as critical process parameters, while SPR position/intensity, hydrodynamic diameter, polydispersity, zeta potential, crystallite size and yield are treated as critical quality attributes. Stability is assessed through time-dependent UV-visible spectra, DLS/zeta potential, visual sedimentation, pH, and solid-state characterization, with pharmaceutical stability principles used as a framework rather than direct regulatory equivalence.

**Keywords:** Oroxylum indicum; green synthesis; silver nanoparticles; phytochemical profiling; baicalein; chrysin; oroxylin A; UV-visible spectroscopy; FTIR; XRD; zeta potential; stability; pharmaceutical nanotechnology

## 1. Introduction

Metallic nanoparticles occupy a distinctive position in pharmaceutical research because nanoscale size changes surface area, optical response, catalytic behavior and biological interactions. Silver, gold and selected metal-oxide nanoparticles have therefore been explored in antimicrobial systems, wound-care materials, diagnostics, biosensors, drug-delivery interfaces and analytical platforms. Conventional nanoparticle synthesis can require strong reducing agents, organic solvents or high-energy processing. Green synthesis replaces part of that chemical burden with biological reducing systems derived from plants, microorganisms or biomolecules. Among these routes, plant extracts are operationally attractive because they can be prepared rapidly and contain chemically diverse metabolites capable of electron donation and surface coordination [1–4]. Oroxylum indicum (L.) Kurz (Bignoniaceae), commonly known as the Indian trumpet tree, has a long history of medicinal use in South and Southeast Asia. Phytochemical reviews describe more than one hundred constituents across different plant parts, with flavonoids as a dominant class [5,6]. Reported compounds include baicalein, baicalin, chrysin, oroxylin A, oroxin A, oroxin B, scutellarein derivatives and other phenolic constituents. These molecules contain hydroxyl, carbonyl and aromatic systems that can participate in redox reactions and adsorb on nascent metallic surfaces. Consequently, O. indicum is not merely a botanical source but a chemically meaningful reaction matrix for phytogenic nanoparticle formation.

Two recent O. indicum-specific studies strengthen this rationale. A 2024 report used leaf extract to synthesize AgNPs and characterized the products by UV-visible spectroscopy, FTIR, TEM, SEM and XRD, while also examining nanoparticle elicitation of secondary metabolites in tissue culture [7]. A open-access study using aqueous bark extract systematically optimized synthesis variables and reported a distinct SPR maximum around 403 nm, face-centered-cubic silver by XRD, predominantly spherical morphology and short-term colloidal stability [8]. These studies

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establish direct evidence for O. indicum-mediated AgNP synthesis, but they also reveal a broader analytical need: plant identity, extract chemistry, synthesis parameters, nanoparticle attributes and stability must be linked within one reproducible pharmaceutical workflow.

1.1 Aim and analytical scope

The objective of this study is to construct that integrated workflow. It

(i) summarizes the phytochemical basis of O. indicum-mediated reduction and capping;
(ii) defines an analytical profiling strategy for the plant extract;
(iii) evaluates green-synthesis variables and proposes a design-of-experiments approach for optimization;
(iv) interprets orthogonal characterization methods for identity, size, morphology, crystallinity and surface chemistry;
(v) develops a stability-assessment plan suitable for pharmaceutical research; and
(vi) identifies quality, safety and translational gaps. Because no new laboratory dataset was supplied for this manuscript, numerical values attributed to O. indicum nanoparticles are explicitly literature-derived, while optimization matrices and acceptance criteria are presented as proposed methodological tools rather than fabricated experimental findings.

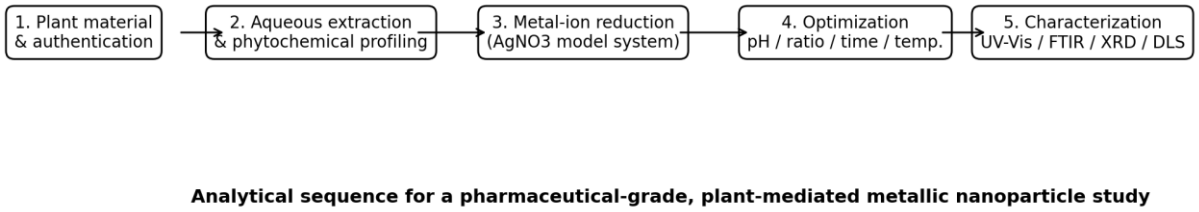


***Figure 1. Integrated analytical workflow proposed for O. indicum-mediated metallic nanoparticle development.***

## 2. Oroxylum indicum as a Phytochemical Platform

2.1 Botanical and pharmaceutical relevance

O. indicum is a medicinal tree whose bark, roots, fruits, seeds and leaves have been used in traditional systems of medicine. Pharmaceutical interest is driven less by any single ethnomedical claim than by the reproducible presence of flavonoid-rich chemical fractions. Reviews identify flavonoids, naphthalenoids and cyclohexylethanoids as major chemical groups, and emphasize baicalein, chrysin and oroxylin A among prominent stem-bark constituents [5]. For nanoparticle synthesis, this chemical diversity is advantageous because reduction of a metal ion and stabilization of the newly formed surface need not be performed by the same molecule. Polyphenols can donate electrons after oxidation, carbonyl-containing compounds can coordinate metal surfaces, and sugars/proteins/polysaccharides can contribute steric stabilization.

2.2 Marker flavonoids and expected analytical behavior

| Constituent/marker | Chemical feature | Relevance to nanoparticle synthesis |
|---|---|---|
| Baicalein | Flavone; multiple phenolic –OH groups | Strong redox/chelating plausibility; HPLC/UV marker |
| Baicalin | Baicalein glucuronide | Polar marker; major constituent in several fruit extracts |
| Chrysin | 5,7-dihydroxyflavone | Phenolic reducing/capping candidate; lipophilic marker |

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| | | |
|---|---|---|
| Oroxylin A | Methoxylated flavone | Chemotaxonomic/pharmacological marker; surface-interaction candidate |
| Oroxin A / Oroxin B | Flavonoid glycosides | Hydrophilic markers useful for extract standardization |
| Phenolics/tannins (class) | Polyhydroxylated constituents | Total phenolic content and broad reducing capacity |

***Table 1. Representative O. indicum phytochemical markers and their analytical relevance.***

HPLC-DAD/QAMS analysis of O. indicum fruits has demonstrated that constituent abundance varies substantially with developmental stage and plant part; baicalin was reported as the major flavone across the investigated extracts, ranging from approximately 0.4% to 11% w/w of extract [9]. This variability is critical for green synthesis. An extract prepared only by mass of crude plant powder can have different reducing capacity from batch to batch even when the extraction procedure is unchanged. For pharmaceutical reproducibility, the extract should therefore be standardized by at least one quantitative marker and one global chemical index, such as total phenolic content (TPC) or total flavonoid content (TFC).

### 2.3 Mechanistic role of phytochemicals

A simplified mechanistic sequence comprises complexation of metal ions by oxygen-containing phytochemicals, electron transfer from oxidizable phenolic groups, nucleation of zero-valent metal clusters, growth by further reduction, and adsorption of extract constituents on the particle surface. The capping layer decreases interparticle contact and can impart a negative surface charge. FTIR peak shifts between crude extract and nanoparticles do not prove the identity of an individual capping molecule, but they can support participation of functional-group classes. Definitive assignment requires chromatographic or mass-spectrometric analysis of the corona or comparison with purified phytochemical standards.

## 3. Phytochemical Profiling and Extract Standardization

### 3.1 Raw-material controls

Analytical reliability begins before nanoparticle synthesis. Plant material should be authenticated botanically, voucher-documented, cleaned under controlled conditions, dried away from excessive heat and direct sunlight, milled to a defined particle-size range, and stored in moisture-protective containers. The plant part must be stated because bark, leaf, root and fruit extracts are chemically non-equivalent. Moisture content, total ash, extractive value and, where relevant, microbial quality provide basic pharmacognostic controls. Collection season and geographical origin should be recorded because they can influence flavonoid abundance.

### 3.2 Extraction and preliminary screening

For a green nanoparticle process, water is the most defensible first-line solvent because it minimizes solvent residues and directly supports aqueous metal-ion chemistry. A practical screening extraction is 2 g powdered bark in 100 mL purified water, stirred for 30 min and filtered; this mirrors the O. indicum AgNP study [8]. However, the extraction ratio should be treated as a controlled manufacturing variable rather than copied uncritically. Extraction temperature, time and plant-to-solvent ratio can be optimized against TPC/TFC and nanoparticle response. Qualitative tests for phenolics, flavonoids, tannins, carbohydrates and proteins can provide preliminary information, but chromatographic fingerprinting is needed for batch standardization.

### 3.3 Proposed HPLC-DAD profiling method

A stability-indicating botanical fingerprint may use a reversed-phase C18 column with acidified water and acetonitrile or methanol in gradient mode, DAD acquisition across the UV range, and targeted quantification of baicalin, baicalein, chrysin and oroxylin A where standards are available. System suitability should include retention-time repeatability, peak-area precision and resolution of critical marker peaks. Method validation for intended quantitative use should address specificity, linearity, range, precision, accuracy/recovery, detection/quantitation limits and robustness. LC-MS can be used as an orthogonal identification tool when marker identity is uncertain.

| **Attribute** | **Method** | **Purpose/reporting** |
|---|---|---|

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| | | |
|---|---|---|
| Identity | Macroscopy/microscopy + voucher | Confirmed O. indicum and specified plant part |
| Moisture | Loss on drying/Karl Fischer | Batch-specific controlled range |
| TPC | Folin-Ciocalteu | mg gallic-acid equivalents/g extract |
| TFC | AlCl3 colorimetry | mg quercetin equivalents/g extract |
| Marker assay | HPLC-DAD | Baicalin/baicalein/chrysin/oroxylin A as applicable |
| Fingerprint | HPLC-DAD/LC-MS | Similarity profile against qualified reference batch |
| Reducing capacity | FRAP or related assay | Supportive process-performance indicator |

***Table 2. Proposed extract-standardization panel before nanoparticle synthesis.***

A useful pharmaceutical concept is to qualify an extract by a chemical window rather than by a single marker alone. A batch may contain an acceptable baicalin concentration yet differ markedly in total reducing capacity because other phenolics vary. Pairing marker assay with a fingerprint and TPC/TFC provides a more robust link between botanical chemistry and nanoparticle process performance.

## 4. Green Synthesis of O. indicum-Mediated Metallic Nanoparticles

### 4.1 Silver nanoparticles as the evidence-rich model

Although the title encompasses metallic nanoparticles broadly, direct O. indicum evidence is currently strongest for silver. Accordingly, AgNPs are used here as the primary model, while the analytical logic can be extended cautiously to gold or bimetallic systems after metal-specific optimization. In the bark-extract study, 1 mM AgNO3 was combined with extract at an optimized 1:9 extract-to-metal precursor volume ratio, stirred at 25 °C for 30 min and incubated in the dark for 24 h. The visual transition from pale yellow to deep brown accompanied nanoparticle formation [8].

### 4.2 Reaction chemistry

The core reaction is reduction of Ag+ to Ag0, followed by nucleation and growth. The rate of electron transfer determines how many nuclei form before growth dominates. Rapid nucleation followed by controlled growth tends to favor smaller, narrower particle distributions, whereas slow or heterogeneous reduction can generate broad size distributions and aggregation. Plant extract concentration therefore has a dual role: too little extract can leave unreduced precursor or insufficient capping, while too much extract can introduce excess organic matter, alter viscosity, obscure spectroscopic measurements and change the corona composition.

### 4.3 Purification and handling

Purification is analytically important because unbound phytochemicals can distort DLS, zeta potential, antioxidant assays and biological tests. The literature protocol centrifuged the AgNP suspension at 8500 rpm for 25 min and washed the pellet three times with distilled water and absolute ethanol before drying [8]. For pharmaceutical work, centrifugal force (×g), rotor radius, wash composition and number of cycles should be reported instead of rpm alone, because rpm is instrument-dependent. If nanoparticles are intended to remain as a colloid, dialysis, tangential-flow filtration or standardized centrifugal washing may be preferable to complete drying and redispersion.

### 4.4 Extension to Au and bimetallic nanoparticles

Plant-mediated synthesis principles also apply to Au3+ reduction and to mixed-metal systems, but surface-plasmon behavior, reduction potentials and nucleation kinetics differ. Simultaneous reduction of silver and gold precursors can produce alloy or core-shell structures depending on reaction sequence and relative reduction rates [10]. Therefore, an O. indicum extract that performs well for AgNPs cannot be assumed to produce equivalent AuNPs or Ag-Au nanoparticles. Metal identity must be confirmed by elemental analysis and phase-sensitive methods, and optimization must be repeated for each precursor system.

- Recommended starting AgNP screen: 0.5–2.0 mM AgNO3, extract:precursor ratios from 1:3 to 1:9, pH 5–11, and 20–50 °C.
- Use purified water, light-protected reaction vessels when appropriate, and matched extract/precursor blanks for UV-visible analysis.

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- Record reaction kinetics rather than relying only on an end-point color change.

## 5. Optimization Strategy: From One-Factor Screening to QbD

Green synthesis is highly sensitive to pH, precursor concentration, extract dose, temperature and reaction time. Reviews of plant-mediated AgNP synthesis consistently identify these variables as determinants of nucleation, size, morphology and colloidal stability [2,11,12]. The O. indicum bark study found its most pronounced and symmetric SPR response at pH 11 and selected a 1:9 extract-to-precursor ratio [8]. These conditions are valuable literature anchors, but pharmaceutical optimization should confirm them with independent batches because extract composition is variable.

### 5.1 Critical process parameters and quality attributes

| Critical process parameter | Primary mechanistic effect | Critical quality attribute |
|---|---|---|
| Reaction pH | Ionization of phenolics; reduction rate; surface charge | SPR λmax/width, size, zeta potential |
| AgNO3 concentration | Supersaturation and growth | Yield, residual Ag+, size/PDI |
| Extract:precursor ratio | Reducing/capping capacity | SPR intensity, organic corona, PDI |
| Temperature | Kinetics and phytochemical integrity | Formation time, size, aggregation |
| Reaction time | Extent of reduction and ripening | SPR plateau, size drift |
| Mixing/light exposure | Mass transfer/photoreduction | Batch uniformity, kinetics |

***Table 3. Proposed QbD linkage between synthesis variables and nanoparticle quality attributes.***

### 5.2 Design-of-experiments proposal

A sequential DoE is more efficient than exhaustive one-factor-at-a-time testing. First, a fractional factorial or Plackett-Burman design can identify dominant variables. Second, a Box-Behnken or central-composite design can model curvature and interactions among the most influential factors. A multi-response desirability function can then minimize particle size and PDI while maximizing absolute zeta potential, SPR intensity, conversion of Ag+ and process yield. Replicated center points provide an estimate of pure experimental error.

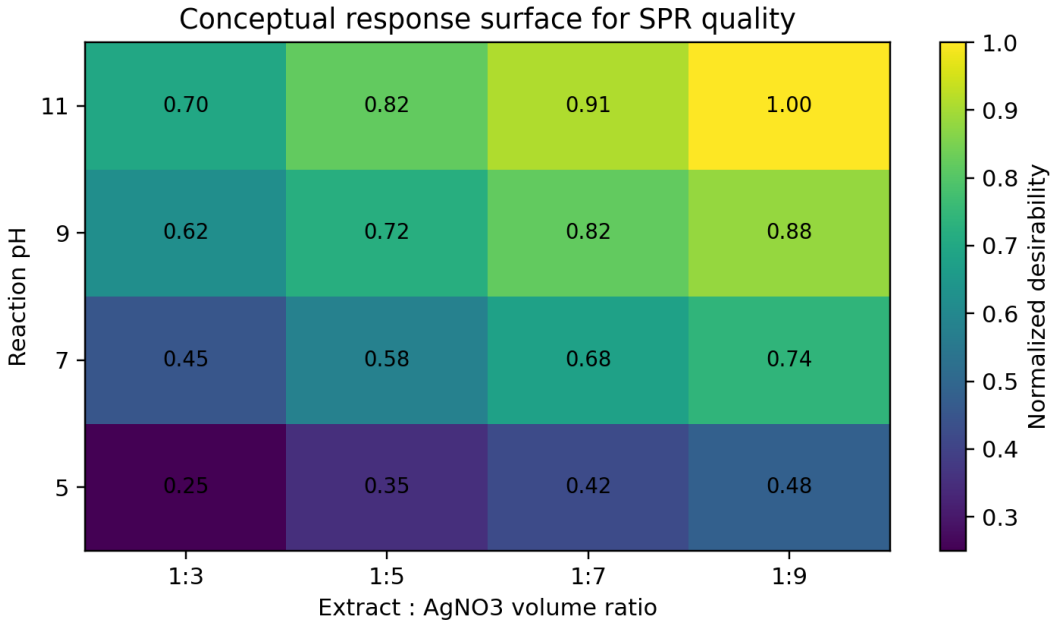


***Figure 2. Conceptual optimization map showing how pH and extract:precursor ratio can be combined in a desirability-based design.***

The optimization target should not be 'maximum absorbance' alone. A high SPR intensity can reflect greater

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nanoparticle concentration but does not guarantee monodispersity or long-term stability. A pharmaceutical-quality optimum is therefore multivariate: it must balance yield, particle-size distribution, surface charge, residual precursor, reproducibility and storage behavior.

## 6. Analytical Characterization of the Nanoparticles

### 6.1 UV-visible spectroscopy

UV-visible spectroscopy provides a rapid process analytical readout for noble-metal nanoparticles through localized surface plasmon resonance (SPR). O. indicum bark-mediated AgNPs showed a sharp peak at approximately 403 nm, while extract and AgNO3 controls lacked the corresponding nanoparticle SPR band [8]. Peak position, intensity and full width at half maximum should be recorded. A red shift or broadening during storage can indicate growth, aggregation or changes in the surrounding dielectric environment.

### 6.2 FTIR and surface-capping chemistry

FTIR comparison of the crude extract and purified AgNPs can reveal functional-group changes associated with reduction and adsorption. The O. indicum bark study reported an extract O–H band around 3360 cm−1, C–H near 2920 cm−1, C=O near 1635 cm−1, C–N/C–O-region bands, and shifts after nanoparticle formation; the nanoparticle spectrum showed a sharper O–H feature near 3490 cm−1 and a low-frequency feature assigned to Ag–O-related vibration [8]. Such changes support involvement of phenolic and carbonyl-containing biomolecules, but FTIR alone cannot identify the exact molecular species on the particle corona.

### 6.3 X-ray diffraction and crystallite size

XRD confirms crystalline phase and allows approximate crystallite-size estimation from peak broadening. Literature-reported O. indicum AgNP reflections occur near 2θ = 38.12°, 44.28°, 64.43° and 77.47°, corresponding to the (111), (200), (220) and (311) planes of face-centered-cubic silver. The mean Scherrer crystallite size was approximately 10.43 nm in the study [8]. Crystallite size should not be equated automatically with TEM diameter or DLS hydrodynamic diameter because these methods measure different physical quantities.

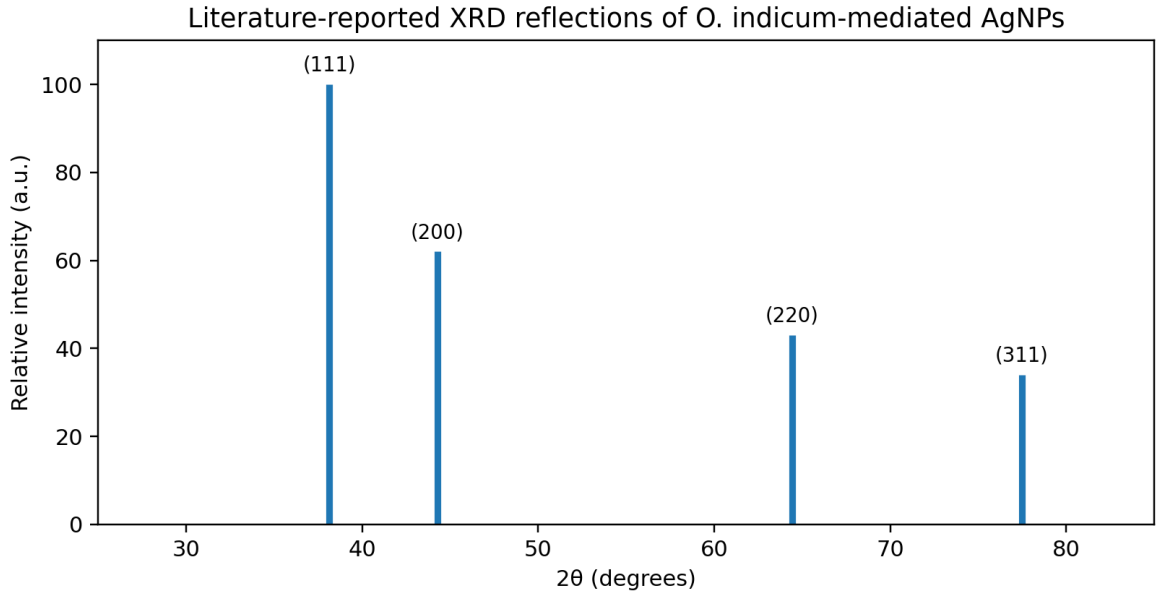


***Figure 3. Stick representation of literature-reported XRD reflections for O. indicum-mediated AgNPs [8].***

## 7. Stability Assessment

### 7.1 Why stability is a distinct nanoparticle problem

Nanoparticle stability includes chemical, physical and functional dimensions. Chemical instability may involve oxidation, dissolution or transformation of the metal surface and degradation of capping phytochemicals. Physical

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instability includes aggregation, agglomeration, sedimentation, Ostwald ripening and irreversible changes after freeze-drying or redispersion. Functional instability occurs when antimicrobial, catalytic, sensing or biological performance changes despite apparently acceptable particle size. Therefore, a single UV-visible spectrum at day 0 is insufficient to support shelf-life claims.

7.2 Short-term evidence for O. indicum AgNPs

The O. indicum bark study monitored UV-visible spectra over 15 days and reported minimal change in SPR position and absorbance intensity, interpreted as evidence of colloidal stability due to phytochemical capping [8]. This is useful proof-of-concept evidence, but it remains a short observation window. For a pharmaceutical research program, UV-visible stability should be paired with DLS/PDI, zeta potential, pH, visual inspection, sedimentation/redispersibility, microbial quality where relevant, and periodic elemental or solid-state testing.

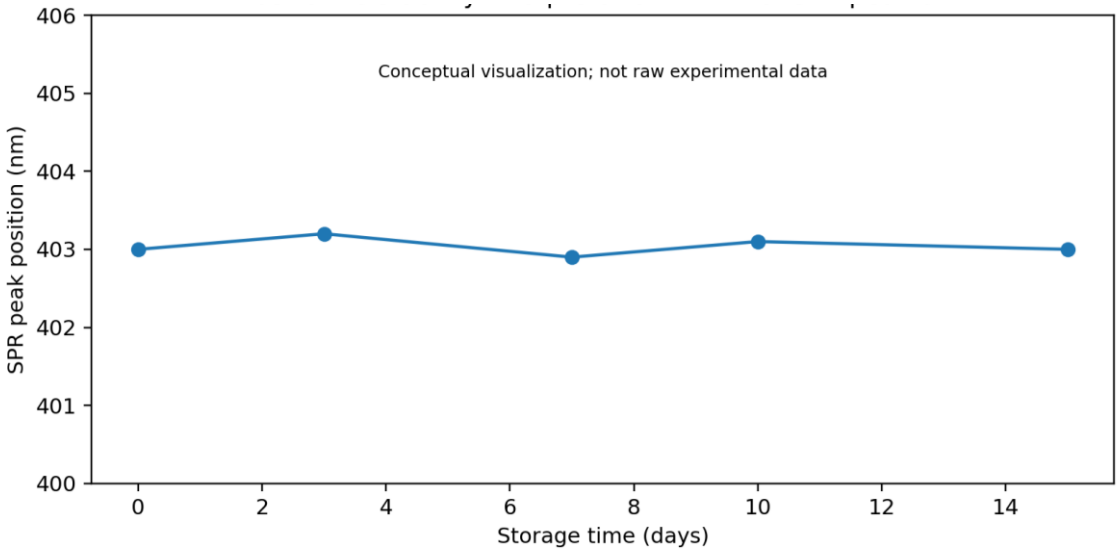


***Figure 4. Conceptual visualization of stable SPR position over a 15-day observation window; plotted points, while the reported O. indicum study described minimal spectral variation [8].***

7.3 Proposed stability matrix

| Condition | Nominal setting | Suggested points | Key tests |
|---|---|---|---|
| Refrigerated | 5 ± 3 °C | 0, 7, 15, 30, 60, 90 d | UV-Vis, DLS/PDI, zeta, pH, appearance |
| Room temperature | 25 ± 2 °C | 0, 7, 15, 30, 60, 90 d | Same + functional assay |
| Elevated temperature | 40 ± 2 °C | 0, 7, 15, 30 d | Stress aggregation and corona stability |
| Photostress | Defined light exposure | Before/after exposure | SPR, size/PDI, chemical fingerprint |
| Freeze-thaw | 3 cycles | Each cycle | Size/PDI, redispersibility |

***Table 4. Proposed nanoparticle stability-screening matrix for pharmaceutical research.***

ICH Q1A(R2) provides general principles for stability testing of drug substances and products, including stress testing and the need for stability-indicating analytical procedures [13]. Green metallic nanoparticle dispersions are not automatically covered as conventional drug products, so ICH conditions should be used as a scientific framework and adapted to the dosage form, intended route and nanoparticle-specific failure modes rather than treated as a direct regulatory recipe.

## 8. Literature-Derived Analytical Synthesis of O. indicum AgNP Evidence

The strongest way to interpret the existing O. indicum evidence is to place each reported observation beside the analytical question it answers. This avoids overclaiming from any single technique and highlights which

measurements remain absent.

| Analytical domain | Reported observation | Interpretation |
|---|---|---|
| Botanical source | Aqueous bark extract; leaf extract also reported | Establishes O. indicum as a direct plant-mediated synthesis source [7,8] |
| Precursor | 1 mM AgNO3 in bark study | Defined silver-ion starting concentration [8] |
| Optimization | pH 11 gave pronounced symmetric SPR; 1:9 extract:precursor selected | Shows strong dependence on process conditions [8] |
| UV-Vis | SPR λmax ≈ 403 nm | Supports AgNP formation and relatively small spherical particles [8] |
| FTIR | O–H, C=O, C–O/C–N regions shifted after synthesis | Supports phytochemical participation in reduction/capping [8] |
| XRD | 38.12°, 44.28°, 64.43°, 77.47°; mean crystallite ≈10.43 nm | Confirms crystalline FCC Ag phase [8] |
| SEM | Predominantly spherical; slight agglomeration; ≈24.93 nm grain size | Direct morphology/size evidence [8] |
| Stability | Minimal UV-Vis variation over 15 days | Short-term colloidal stability evidence [8] |
| DPPH | Extract IC50 ≈29.91 µg/mL; AgNPs ≈119.0 µg/mL | Shows antioxidant behavior changes after nanoparticle formation [8] |

***Table 5. Consolidated literature-derived evidence for O. indicum-mediated AgNP synthesis and characterization.***

8.1 Cross-technique interpretation

The 403-nm SPR band, FCC XRD pattern and spherical SEM morphology are mutually consistent with formation of metallic silver nanoparticles. The difference between XRD crystallite size (~10 nm) and SEM grain size (~25 nm) is not contradictory: an imaged particle can contain more than one coherent crystallite, and sample preparation can promote limited aggregation. FTIR changes add evidence that extract-derived molecules remain associated with the nanoparticle surface. The 15-day UV-visible stability result suggests that this organic corona is functionally meaningful, but quantitative zeta-potential and DLS time-course data would be needed to establish the electrokinetic and hydrodynamic basis of stability.

8.2 Analytical gaps

- Quantitative marker analysis of the exact extract batch used for nanoparticle synthesis is generally missing.
- Residual ionic silver should be measured after purification to distinguish nanoparticle-associated effects from free Ag+.
- DLS/PDI and zeta-potential values are needed for pharmaceutical colloid specifications.
- Longer stability studies should connect physical stability with chemical marker stability and biological performance.
- Batch-to-batch reproducibility should be demonstrated using independently collected plant material.

## 9. Quality Control, Safety and Regulatory Considerations

9.1 Identity and purity

A nanoparticle intended for pharmaceutical research requires more than confirmation of a plasmon band. Identity should combine elemental composition, crystalline phase and particle morphology. Purity testing should consider residual Ag+, nitrate, extraction-derived impurities, processing solvents, microbial contamination and, where relevant, endotoxin. The purification method should be validated for its ability to remove soluble precursor without stripping the stabilizing corona excessively.

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9.2 Particle-size specification

Size should be reported by method. TEM or SEM gives a dry-state primary-particle or grain dimension; DLS gives an intensity-weighted hydrodynamic diameter that includes the solvation/capping layer and is disproportionately influenced by larger aggregates. A single 'particle size' number without method and distribution is therefore analytically inadequate. PDI should accompany DLS data, and number-based microscopy distributions should include the number of particles measured.

9.3 Zeta potential and colloidal stability

Zeta potential is a useful but medium-dependent indicator of electrostatic stability. Reviews commonly treat larger absolute zeta potentials as evidence of stronger electrostatic repulsion, while emphasizing that pH and ionic strength can alter the value [12,15]. For phytochemically capped nanoparticles, steric stabilization can also be important; consequently, a modest zeta potential does not necessarily imply immediate instability. The measurement medium, conductivity, temperature and pH must be reported.

9.4 Toxicological qualification

The safety profile of a medicinal plant extract cannot be transferred automatically to a metallic nanoparticle made from that extract. Nanoscale silver can release ions and can interact with membranes and intracellular components in ways that the parent botanical does not. A staged safety program should include hemocompatibility where relevant, mammalian cell viability, oxidative-stress markers, inflammatory responses, genotoxicity when justified, and route-specific in vivo evaluation. Dose should be expressed as mass of metal and, where feasible, particle number or surface area to improve cross-study comparability.

9.5 Proposed release specification for research batches

| Test | Research-stage acceptance concept |
|---|---|
| Appearance | Homogeneous dispersion; no irreversible visible sediment |
| UV-Vis | Defined SPR λmax window and spectral width |
| DLS/PDI | Target size range; PDI preferably controlled and justified |
| Zeta potential | Batch-specific stability window in defined medium |
| Element/phase | Ag by EDX/ICP; FCC phase by XRD |
| Residual Ag+ | Quantified after purification |
| Extract markers | Fingerprint/marker level in input extract and/or corona |
| Microbial quality | Appropriate to intended use |

***Table 6. Suggested research-stage control strategy; final limits must be established from validated process capability and intended use.***

## 10. Challenges, Limitations and Sources of Variability

10.1 Botanical variability

The largest source of hidden process variability is often the plant extract itself. Genotype, plant part, age, season, geography, drying and storage can change the abundance and redox state of flavonoids and other constituents. Two extracts prepared with the same mass-to-volume ratio may therefore behave as different reagents. Marker-based standardization and a reducing-capacity assay can transform the extract from an ill-defined ingredient into a measurable process input.

10.2 Ambiguous mechanistic assignments

Many green-synthesis study infer that 'flavonoids and phenolics' are responsible for nanoparticle formation from FTIR shifts. This is chemically plausible but analytically incomplete. FTIR bands are broad and shared by many plant constituents. Stronger evidence would combine depletion or oxidation of specific HPLC peaks, LC-MS identification of corona components, experiments with isolated baicalein/chrysin/oroxylin A, and mass-balance measurements of metal-ion reduction.

10.3 Inconsistent reporting of size and stability

Studies frequently compare TEM size, SEM grain size, XRD crystallite size and DLS hydrodynamic size as though they were interchangeable. They are not. Stability is also sometimes inferred from a single negative zeta-potential value or a visually unchanged suspension. Pharmaceutical reporting should define the measurement principle, dispersion medium, time after synthesis and statistical distribution for every size/stability metric.

10.4 Scale-up

At larger scale, mixing time, local pH gradients, heat transfer, light penetration and order of addition can change nucleation. A process that produces narrow particles in a 10-mL tube may yield broader distributions in a liter-scale vessel. Scale-up should therefore preserve dimensionless mixing or at least mixing-energy conditions, control addition rate and use in-process UV-visible monitoring to identify the reaction endpoint.

## 11. Proposed Standardized Experimental Protocol for Future Pharmaceutical Studies

11.1 Stage I: botanical qualification and extraction

Authenticate O. indicum, deposit a voucher specimen, select a renewable plant part where possible, and define drying/milling conditions. Prepare three independent extract batches. Measure pH, dry residue, TPC/TFC and a chromatographic fingerprint. Quantify at least one relevant flavonoid marker. Only batches within a pre-established chemical window should enter nanoparticle synthesis.

11.2 Stage II: screening synthesis

Use AgNO3 as the model precursor at 0.5, 1.0 and 2.0 mM. Screen extract:precursor ratios (1:3, 1:5, 1:7, 1:9), pH (5, 7, 9, 11) and temperature (25, 35, 45 °C) with a defined reaction time. Record UV-visible spectra at fixed intervals until the SPR response reaches a plateau. Include precursor-only and extract-only controls. The O. indicum literature supports 1 mM precursor, alkaline conditions and a 1:9 ratio as rational center/reference conditions [8].

11.3 Stage III: multivariate optimization

Select the three most influential variables and fit a response-surface design. Primary responses should include SPR peak width, DLS size, PDI, zeta potential, yield and residual Ag+. A composite desirability function should favor small, narrow, stable particles without excessive organic residue. Confirm the predicted optimum in at least three independent batches and report prediction intervals rather than only mean values.

11.4 Stage IV: full characterization

Characterize the optimized material by UV-visible spectroscopy, FTIR, XRD, TEM/SEM, EDX or ICP-based elemental analysis, DLS/PDI and zeta potential. If the material is intended for biological use, quantify residual Ag+ and evaluate the phytochemical corona by HPLC/LC-MS after desorption or extraction. Report particle concentration and process yield.

11.5 Stage V: stability and functionality

Store optimized colloids under at least refrigerated and room-temperature conditions, with an elevated-temperature stress arm. At each time point measure appearance, pH, UV-visible spectrum, DLS/PDI and zeta potential. Repeat one functionally relevant assay, such as antimicrobial potency or a validated analytical sensing response, at selected intervals. A stability failure should be defined prospectively, for example by irreversible sedimentation, substantial size/PDI increase, major SPR shift/broadening or loss of functional performance.

11.6 Stage VI: statistical analysis

Report results as mean ± SD for independent synthesis batches, not only technical replicate readings from one batch. Use analysis of variance for designed experiments, model lack-of-fit testing, residual diagnostics and adjusted $R^2$/predicted $R^2$ for response-surface models. Stability trends can be evaluated using regression or repeated-measures methods where assumptions are met. Predefine significance level and distinguish statistical significance from a practically meaningful change in nanoparticle quality.

| Control layer | Core measurement | Decision value |
|---|---|---|

| | | |
|---|---|---|
| Botanical CQA | Marker assay/fingerprint | Reduces extract-batch variability |
| Process CQA | pH, ratio, temperature, time | Controls nucleation/growth |
| Particle CQA | Size, PDI, zeta, phase | Defines physicochemical quality |
| Purity CQA | Residual Ag+, soluble organics | Separates nanoparticle effect from contaminants |
| Stability CQA | Size/SPR/zeta over time | Supports shelf-life development |
| Performance CQA | Route-specific bio/analytical assay | Links quality to intended function |

***Table 7. End-to-end control strategy for a reproducible O. indicum nanoparticle program.***

## 12. Discussion and Future Directions

The central analytical insight is that O. indicum-mediated nanoparticle synthesis should be treated as a coupled botanical-material process. The extract is both a reagent and a surface modifier. Consequently, reproducibility depends on controlling not only metal precursor chemistry but also phytochemical composition. This is particularly important in pharmaceutical science, where a visually successful color change is not an adequate definition of a reproducible material.

Existing O. indicum data are encouraging. The literature demonstrates AgNP formation from both leaf and bark extracts, characteristic FCC silver diffraction, nanoscale morphology and functional behavior [7,8]. The bark study provides an especially useful process anchor by linking alkaline pH and a 1:9 extract-to-precursor ratio with a strong SPR response and by documenting minimal UV-visible change over 15 days [8]. At the same time, the evidence base would be strengthened substantially by direct quantification of the extract markers used in the synthesis batch, DLS/PDI and zeta-potential time courses, residual Ag+ analysis and longer stability studies.

Future research should move from descriptive green synthesis to causal analytical chemistry. Purified baicalein, chrysin, baicalin and oroxylin A can be tested individually and in defined mixtures to determine reduction kinetics and capping efficiency. LC-MS-based corona analysis can identify which phytochemicals remain surface-associated after washing. Isothermal titration or spectroscopic binding studies may help separate reduction from capping. Such experiments would convert the general statement that 'phytochemicals mediate synthesis' into a quantitative structure-process relationship.

## 13. Conclusion

Oroxylum indicum is a phytochemically credible and experimentally demonstrated biological source for green synthesis of metallic nanoparticles, with the strongest direct evidence presently available for AgNPs. Its flavonoid-rich chemistry provides plausible reducing and capping functionality, while published O. indicum AgNP studies confirm characteristic optical, structural and morphological signatures. A pharmaceutical-quality development program should integrate botanical authentication, HPLC-based extract standardization, multivariate synthesis optimization, orthogonal nanoparticle characterization, residual-metal-ion control and time-resolved colloidal stability testing. Literature-reported conditions—particularly 1 mM $AgNO_3$, alkaline pH, a 1:9 extract-to-precursor ratio and an SPR maximum near 403 nm—provide rational starting points, not universal specifications. The field will advance most effectively by replacing single-batch descriptive synthesis with standardized, statistically designed and stability-indicating workflows that connect phytochemical composition to nanoparticle critical quality attributes and intended pharmaceutical performance.

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