

GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM DALBERGIA SISSOO (ROXB.) ROOT EXTRACT AND ITS IN VITRO ANTI-INFLAMMATORY POTENTIAL EVALUATION.

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ABSTRACT

Medicinal plants provide chemically diverse matrices for the development of multifunctional anti-inflammatory candidates. Whereas plant-based nanoparticle synthesis offers a route to couple phytochemical activity with nanoscale physicochemical properties. *D. sissoo* roots collected in September 2025 were authenticated at Government College University Faisalabad, extracted with aqueous methanol, and used as the reducing/capping matrix for silver nanoparticle preparation. Comparative ATR-FTIR analysis showed a pronounced fingerprint-region envelope at 1450–1150 cm⁻¹ in DsR-AgNPs, with a minimum at 1318 cm⁻¹ and additional bands at 809 and 712 cm⁻¹. Dynamic light scattering gave 102.3 nm for the crude extract and 148.6 ± 17.7 nm for DsR-AgNPs (PDI 27.8 ± 0.9%, n = 2), while SEM of the dried material demonstrated irregular, angular, strongly agglomerated aggregates. In vitro bioassays showed concentration-dependent inhibition of protein denaturation and protection of human red-blood-cell membranes; at 100 µg/mL, DsR-AgNPs produced 82.4 ± 2.1% and 76.8 ± 1.9% inhibition, respectively, compared with 51.2 ± 1.7% and 47.5 ± 1.4% for the crude root extract. The research reports stronger COX-2 than COX-1 inhibition by DsR-AgNPs, reaching 88.7 ± 1.6% versus 36.5 ± 1.2% at 100 µg/mL and a source-defined selectivity index of 2.43. These findings support t that phytochemical capping and nanoscale formulation can reshape the biological profile of *D. sissoo* root constituents. TEM, EDX, XRD, zeta-potential and UV–Vis results were also measured; therefore, the article distinguishes measured evidence.

Keywords:

Dalbergia sissoo; silver nanoparticles; green synthesis; root extract; FTIR; dynamic light scattering; SEM; anti-inflammatory activity; protein denaturation; HRBC membrane stabilization; cyclooxygenase; phytochemicals.

1. INTRODUCTION

Inflammation is a coordinated physiological response that protects tissues from injury and

infection but becomes pathogenic when excessive, persistent, or inadequately resolved. The inflammatory response integrates vascular

changes, leukocyte recruitment, lipid mediators, cytokines, reactive oxygen species and transcriptional programs that collectively determine whether tissue returns to homeostasis or progresses toward chronic injury [1–4]. Prostaglandins generated through cyclooxygenase (COX) pathways are central mediators of pain, fever and vascular responses, while leukotrienes generated through lipoxygenase (LOX) pathways contribute to chemotaxis and vascular permeability [2]. Consequently, pharmacological interruption of inflammatory mediator production remains a major therapeutic strategy. Conventional anti-inflammatory medicines are effective but are constrained by dose and duration dependent safety concerns. Non-selective NSAIDs inhibit COX-dependent prostaglandin formation but can compromise gastrointestinal, renal and cardiovascular physiology. Whereas glucocorticoids can cause metabolic, skeletal and immune adverse effects during prolonged use [4,5]. The continuing burden of inflammatory disorders and the limitations of existing agents sustain interest in chemically diverse natural products that may modulate several biological nodes simultaneously [6].

Dalbergia sissoo (Roxb.), commonly known as shisham or Indian rosewood, is an important Fabaceae species distributed across the Indian subcontinent and widely represented in Pakistan. Its medicinal history encompasses inflammatory, painful, gastrointestinal and skin-related conditions. Modern phytochemical studies show that *D. sissoo* contains flavonoids, isoflavonoids, phenolic compounds and other secondary metabolites, providing a plausible chemical basis for anti-inflammatory and antioxidant activity [8–12]. Farag et al. identified isoflavonoid glycosides, including biochanin and tectorigenin related compounds, from *D. sissoo*, while experimental work has demonstrated anti-inflammatory activity of *D. sissoo* extracts in established models [8,9]. A 2023 pharmacology review and a 2025 monograph further emphasize the chemical diversity and broad pharmacological potential of the species [10,11].

Green nanotechnology provides a conceptual bridge between pharmacognosy and

nanomedicine. Plant extracts can supply electron-donating metabolites capable of reducing metal ions, while hydroxyl-, carbonyl-, amide- and related functionalities can remain adsorbed at the nanoparticle surface and contribute to colloidal stabilization and biological interactions [14–20]. For silver nanoparticles (AgNPs), surface chemistry, aggregate state, hydrodynamic size and colloidal stability influence biological activity and toxicity; therefore, synthesis and characterization cannot be considered independently [19–24]. Recent reviews also emphasize that plant-mediated AgNPs require careful standardization and toxicity assessment before translation [19–21].

Although *D. sissoo* leaves have been explored for AgNP synthesis, the root has received substantially less attention as a phytochemical feedstock for anti-inflammatory nanomaterial development [12,13]. The thesis underlying this article therefore addressed a focused question: can a root extract of *D. sissoo* support the preparation of silver nanoparticles whose physicochemical profile and in vitro anti-inflammatory activity differ meaningfully from the crude extract? The present article reorganizes that work into a concise, conceptually integrated manuscript and interprets the findings against current literature while retaining the source numerical data.

2. Materials and Methods

2.1 Plant material and authentication:

Mature, healthy roots of *D. sissoo* (Roxb.) were collected from fields in village Bhawani Pur, Tehsil Shakargarh, District Narowal, Punjab, Pakistan, in September 2025. A representative specimen was submitted to the Department of Pharmacognosy, Government College University Faisalabad for identification and authentication. A voucher specimen was deposited for laboratory record as DSR-2. Roots were washed, cut, shade-dried for 3 weeks at room temperature with periodic turning, powdered, sieved and stored in dark, airtight amber containers.

2.2 Extraction and preliminary phytochemical procedures:

Hot continuous extraction was performed using aqueous methanol (described in the thesis as

usually 80% methanol). The procedure used 100 g portions of root powder and was repeated until 1000 g powder had been processed. The combined material was shaken intermittently for 72 h, filtered through muslin cloth and Whatman No. 1 paper, and concentrated under reduced pressure. The thesis reports approximately 100 g of crude alcoholic extract. Qualitative screening targeted alkaloids, flavonoids, phenolics/tannins, saponins, steroids/terpenoids, glycosides, carbohydrates, proteins and amino acids using standard pharmacognostic tests. Total phenolics and total flavonoids were planned using Folin-Ciocalteu and aluminium-chloride colorimetric approaches, respectively; however, the thesis states that the quantitative TPC/TFC data were not

recorded as completed results and are therefore not invented in this article.

2.3 Green synthesis and reaction-variable framework:

A freshly prepared aqueous root-extract solution was mixed dropwise with aqueous AgNO_3 under magnetic stirring and protected from direct light. The source procedure describes a visible shift from pale yellow toward yellowish-brown/brown during nanoparticle formation. Reaction variables considered were AgNO_3 concentration, extract-to-precursor ratio, pH, temperature and time. The representative optimization matrix reported in the thesis is reproduced below without numerical modification.

Table 1. Optimization matrix for the green synthesis of DsR-AgNPs (transcribed from thesis Table 3.3).

Parameter	Range examined	Optimised value (representative)
AgNO_3 concentration	0.5–5.0 mM	1.0 mM
Extract : precursor ratio	1:5 to 1:20	1:10
pH of medium	4–10	8
Temperature	Ambient to 80 °C	60 °C
Reaction time	15 min to 24 h	120 min

2.4 Physicochemical characterization:

ATR-FTIR spectra were recorded for the crude root extract (sample C) and DsR-AgNPs (sample D) from 4000–650 cm^{-1} using an Agilent MicroLab system at 4 cm^{-1} resolution with 30 sample/background scans and Happ-Genzel apodization. Dynamic light scattering (Anton Paar Litesizer 500) was performed in aqueous dispersion at 21 °C; the extract was measured once and DsR-AgNPs in duplicate. SEM imaging was performed on the dried nanoparticle powder at 2.3 kV across $\times 50$, $\times 100$, $\times 150$, $\times 300$, $\times 600$ and $\times 1,000$. The thesis states that the displayed SEM images were photographed from the instrument display and that dimensional calibration therefore carries an approximate $\pm 20\%$ uncertainty.

2.5 In vitro anti-inflammatory screening:

The completed biological dataset comprised protein-denaturation inhibition, human red blood cell (HRBC) membrane stabilization, proteinase inhibition, lipoxygenase inhibition and COX-

1/COX-2 inhibition. Concentration response assays were run in triplicate, using concentrations generally spanning 20–100 $\mu\text{g/mL}$ for the nanoparticle and 200–1000 $\mu\text{g/mL}$ for the crude extract in the protein-denaturation/HRBC dataset, with reference standards including diclofenac, indomethacin and celecoxib as specified in the individual tables. Protein-denaturation inhibition was calculated as $100 \times (\text{A}_{\text{control}} - \text{A}_{\text{test}}) / \text{A}_{\text{control}}$. For HRBC assays, membrane protection was expressed relative to a fully haemolysed control. The source reports calculated IC_{50} values by regression/interpolation but provides no complete regression outputs or p-values in the supplied thesis.

2.6 Data-integrity and interpretation framework:

Because the thesis contains internal inconsistencies, this article follows a conservative hierarchy: (i) numerical values that are explicitly presented in source tables/figures are preserved verbatim; (ii) characterization claims are treated as

measured only when the corresponding result is actually presented; and (iii) where later narrative text conflicts with earlier or later statements of pending work, the conflict is disclosed rather than silently reconciled. This is particularly important for the reported TEM core size, zeta potential, UV-Vis/SPRs, XRD and EDX findings. The resulting manuscript does not claim clinical efficacy, in vivo efficacy, or therapeutic safety.

3. Results

3.1 FTIR profile and evidence of a changed surface chemical environment:

The comparative FTIR dataset showed a relatively feature-poor spectrum for the crude extract and a

substantially more structured spectrum for DsR-AgNPs in the fingerprint region. The extract had only shallow inflections near 1653, 1560, 1508 and 1457 cm^{-1} , each less than about 1% deep. In contrast, DsR-AgNPs displayed a broad absorption envelope from approximately 1450–1150 cm^{-1} with a minimum at 1318 cm^{-1} and a transmittance of 22.0%, a sharp band at 809 cm^{-1} with transmittance 25.7%, and a weaker feature at 712 cm^{-1} with transmittance 32.0%. The doublet around 2340–2080 cm^{-1} occurred in both samples and was therefore treated as a likely diamond-ATR instrumental artifact rather than a sample-specific band.

Table 2. Comparative FTIR observations for the root extract (C) and DsR-AgNPs (D); values preserved from thesis Table 4.1.

Band / feature	Extract (C)	DsR-AgNPs (D)	Change on synthesis / interpretation
2324 / 2080 cm^{-1} (C); 2344 / 2113 cm^{-1} (D)	Sharp doublet	Sharp doublet, near-identical position	Diamond-ATR instrumental artefact; not sample-derived
~1653, 1560, 1508, 1457 cm^{-1}	Very shallow inflections (<1% deep)	Not separately resolved	Weak in C; not separately resolved in D
1450–1150 cm^{-1}	Absent as pronounced envelope	Broad intense envelope; minimum at 1318 cm^{-1} ; T = 22.0%	Pronounced in D
809 cm^{-1}	Absent	Sharp band; T = 25.7%	Present in D
712 cm^{-1}	Absent	Weaker band; T = 32.0%	Present in D
Baseline transmittance	C: ~58–75%	D: ~36–49%	Overall offset; contact/optical-density and absorption effects possible

band positions and transmittance values obtained by peak-picking the files.

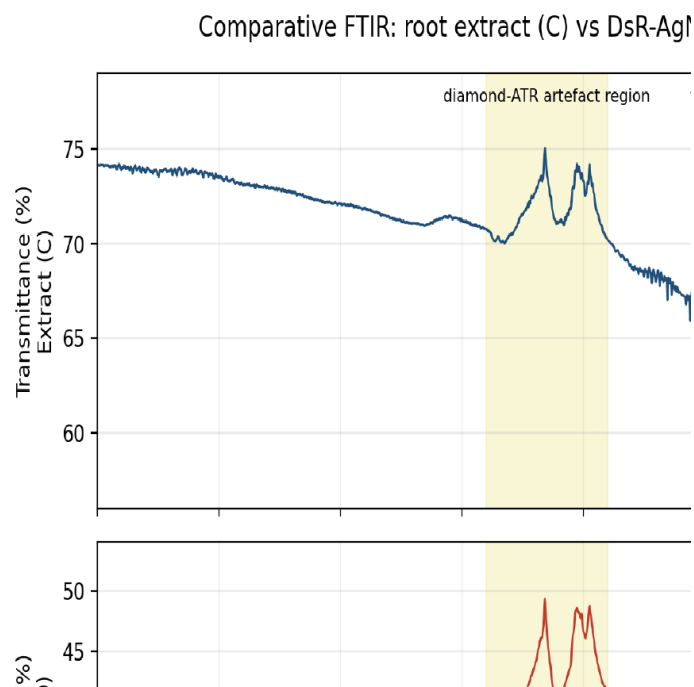


Figure 1. Comparative FTIR spectra of root extract (C) and DsR-AgNPs (D), reproduced from the thesis without changing the plotted data or annotations.

3.2 Hydrodynamic size, polydispersity and DLS data quality:

DLS measured a hydrodynamic diameter of 102.3 nm for the crude extract and 136.0 nm and 161.1 nm for the two DsR-AgNP runs, giving 148.6 ± 17.7 nm (PDI $27.8 \pm 0.9\%$, $n = 2$) as the reported nanoparticle mean. The intensity weighted distributions were bimodal. The major population accounted for approximately 94–97% of scattered intensity, while a minor faster-diffusing population accounted for approximately 3–6% and appeared at approximately 10–20 nm, with secondary peaks at 9.9 nm for C, 13.2 nm for D and 20.4 nm for D Rep. The source appropriately notes that DLS cannot distinguish a truly large

particle from an aggregate plus hydration/capping shell. This interpretation is reinforced by the DLS quality metrics: correlation-function intercepts were 0.667, 0.703 and 0.574 for C, D and D Rep, with fitted baselines of 0.9996, 1.0042 and 1.0349. Cumulant fit errors were 5.0×10^{-5} , 8.0×10^{-5} and 7.3×10^{-5} , respectively. The scattering-intensity traces had coefficients of variation of 6.3%, 11.0% and 22.0%, with peak-to-mean ratios of 1.20, 1.38 and 1.81. These metrics support the thesis conclusion that the D Rep run was more unstable and that larger- n replication is warranted before the reported hydrodynamic mean is treated as definitive.

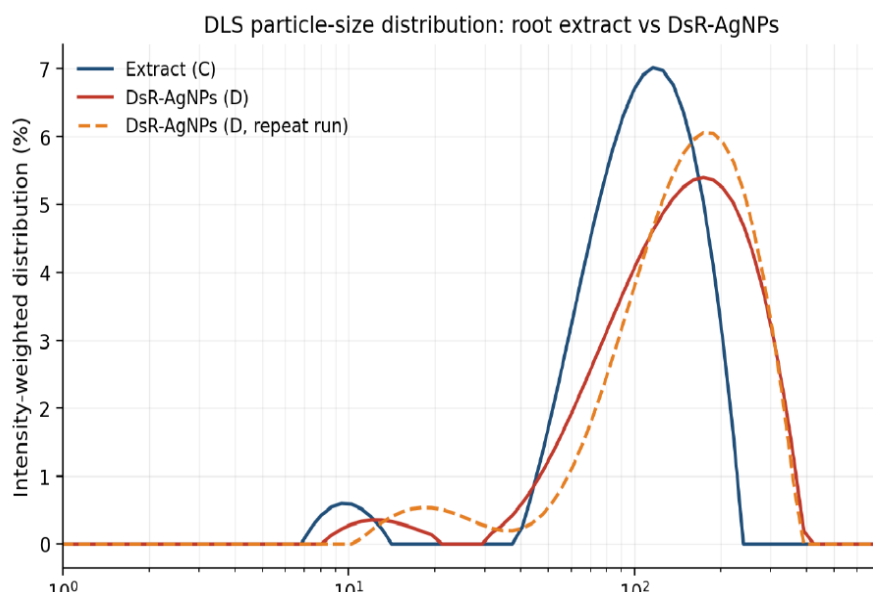


Figure 2. Intensity-weighted DLS particle-size distributions for the extract (C) and DsR-AgNPs (D, D Rep), reproduced unchanged from the thesis.

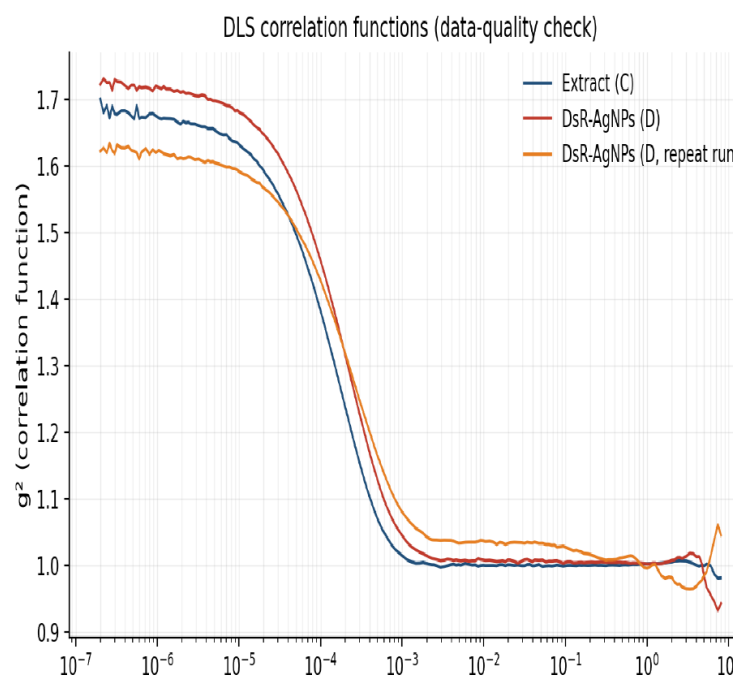


Figure 3. DLS correlation functions (g^2 vs delay time) for the extract (C) and DsR-AgNPs (D, D Rep), reproduced unchanged from the thesis.

Table 3. Particle-size, PDI and zeta-potential summary, transcribed from thesis Table 4.2. Conflicting narrative statements are disclosed in the text.

Parameter	Value	Inference
Aggregate morphology (SEM)	Irregular, angular aggregates, ~ tens to ~ 300 μm	Dried product is strongly agglomerated
TEM core size	$10 \pm 4 \text{ nm}$	Thesis-reported range 6–14 nm; narrative elsewhere states TEM was pending
Hydrodynamic diameter (DLS)	C: 102.3 nm; D: $148.6 \pm 17.7 \text{ nm}$ (n = 2)	Includes capping/hydration shell and possible aggregation
Polydispersity index	C: 24.0%; D: $27.8 \pm 0.9\%$ (n = 2)	Moderately broad distribution
Zeta potential	–15 mV and –30 mV	Reported in thesis Table 4.2; narrative elsewhere states zeta measurement pending

3.3 SEM morphology:

SEM imaging of the dried DsR-AgNP sample showed a dense, heterogeneous powder composed of bright, angular, plate-like or blocky masses against a finer matrix. At $\times 50$ and $\times 100$, the largest visible structures were of the order of 100–300 μm , with smaller masses in the tens-of-micrometres range. At $\times 150$, representative masses were clearly aggregates rather than monolithic particles and had rough granular surfaces. With increasing magnification, the granular substructure

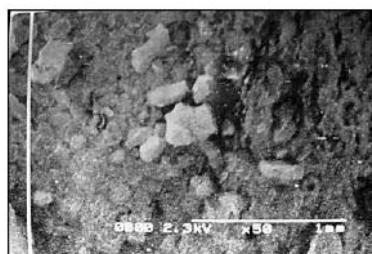
dominated the field, but the $\times 600$ and $\times 1,000$ images did not resolve individual primary nanoparticles. The source therefore treats the morphology as evidence of substantial agglomeration rather than as a direct measurement of primary nanoparticle size. This distinction is critical because dried plant-mediated AgNP powders can form secondary aggregates during solvent removal, whereas DLS measures the hydrodynamic state in dispersion [19–24].

Table 4. SEM acquisition parameters and morphological observations for dried DsR-AgNPs (sample D); accelerating voltage 2.3 kV throughout; field widths carry the stated $\pm 20\%$ uncertainty.

Panel	Mag.	Scale bar	Field width	Principal observation
(a)	$\times 50$	1 mm	$\sim 5 \text{ mm}$	Densely packed powder bed; bright angular masses up to $\sim 300 \mu\text{m}$ in loose clusters against a finer matrix
(b)	$\times 100$	500 μm	$\sim 2.5 \text{ mm}$	Individual aggregates resolved; irregular, plate-like and blocky outlines, several in mutual contact
(c)	$\times 150$	200 μm	$\sim 1.7 \text{ mm}$	Single aggregate fills much of field; dense granular surface sub-structure, lobate margins, adhering fragments
(d)	$\times 300$	100 μm	$\sim 800 \mu\text{m}$	Aggregate boundaries reduced to broad tonal variation; granular texture dominant
(e)	$\times 600$	50 μm	$\sim 400 \mu\text{m}$	Uniform fine-grained texture; scattered small bright points; no aggregate outlines resolved
(f)	$\times 1,000$	50 μm	$\sim 250 \mu\text{m}$	As (e); no further morphological detail resolved at the limiting resolution

from the instrument scale bars and carry the $\pm 20\%$ uncertainty discussed in

(a) $\times 50$ — scale bar 1 mm



(b) $\times 100$ — scale bar 500 μm



(c) $\times 150$ — scale bar 200 μm



(d) $\times 300$ — scale bar 100 μm



(e) $\times 600$ — scale bar 50 μm



(f) $\times 1.0\text{k}$ — scale bar 50 μm



Figure 4. SEM micrographs of dried DsR-AgNPs (sample D) at $\times 50$, $\times 100$, $\times 150$, $\times 300$, $\times 600$ and $\times 1,000$, reproduced unchanged from the thesis.

3.4 Protein-denaturation inhibition and HRBC membrane stabilization:

The completed in vitro screening showed a clear concentration-response pattern. At 20–100 $\mu\text{g/mL}$, DsR-AgNPs increased protein-denaturation inhibition from $34.5 \pm 1.2\%$ to $82.4 \pm 2.1\%$ and HRBC membrane stabilization from $28.9 \pm 1.1\%$ to $76.8 \pm 1.9\%$. By comparison, the crude root extract required 200–1000 $\mu\text{g/mL}$ to

rise from $15.2 \pm 0.6\%$ to $51.2 \pm 1.7\%$ for protein denaturation and from $11.4 \pm 0.5\%$ to $47.5 \pm 1.4\%$ for HRBC stabilization. Diclofenac at 100 $\mu\text{g/mL}$ produced $89.1 \pm 1.5\%$ and $84.3 \pm 1.1\%$, respectively. The reported assay-level IC_{50} values were 54.2 $\mu\text{g/mL}$ for the DsR-AgNP preparation, 112.4 $\mu\text{g/mL}$ for crude extract and 32.8 $\mu\text{g/mL}$ for diclofenac. These data indicate stronger activity per unit concentration for the nanoparticle

preparation than for the crude extract, while the standard drug remained more potent on the reported IC₅₀ metric.

Table 5. Protein-denaturation and HRBC membrane-stabilization results reproduced from the thesis bioassay dataset.

Treatment	Concentration (µg/mL)	Protein denaturation inhibition (%)	HRBC membrane stabilization (%)	Reported IC ₅₀ (µg/mL)
<i>Dalbergia sissoo</i> Root NPs	20	34.5 ± 1.2	28.9 ± 1.1	54.2
	40	48.2 ± 0.9	42.1 ± 1.6	
	60	59.4 ± 1.5	55.3 ± 0.8	
	80	71.1 ± 1.1	66.4 ± 1.4	
	100	82.4 ± 2.1	76.8 ± 1.9	
Crude Root Extract	200	15.2 ± 0.6	11.4 ± 0.5	112.4
	400	24.8 ± 1.1	20.3 ± 0.9	
	600	33.1 ± 1.0	29.7 ± 1.2	
	800	44.5 ± 1.3	38.2 ± 1.0	
	1000	51.2 ± 1.7	47.5 ± 1.4	
Standard Drug (Diclofenac)	100	89.1 ± 1.5	84.3 ± 1.1	32.8

3.5 Proteinase and lipoxigenase inhibition:

The proteinase/LOX dataset likewise showed concentration-dependent inhibition. At 100 µg/mL, DsR-AgNPs produced 74.1 ± 1.5% proteinase inhibition and 79.3 ± 1.8% LOX inhibition, versus 43.8 ± 1.1% and 48.6 ± 1.5%

for crude root extract. Indomethacin at 100 µg/mL produced 81.6 ± 1.4% and 85.4 ± 1.2%. The table reports IC₅₀ values of 62.4 µg/mL for DsR-AgNPs, 121.9 µg/mL for the crude extract and 35.2 µg/mL for indomethacin.

Table 6. Proteinase and lipoxigenase inhibition results reproduced from the thesis dataset.

Treatment	Concentration (µg/mL)	Proteinase inhibition (%)	Lipoxigenase inhibition (%)	Reported IC ₅₀ (µg/mL)
<i>Dalbergia sissoo</i> Root NPs	20	29.4 ± 1.0	31.8 ± 1.1	
	40	41.5 ± 1.3	44.2 ± 1.4	
	60	53.8 ± 0.9	57.6 ± 1.0	
	80	64.2 ± 1.2	68.9 ± 1.6	
	100	74.1 ± 1.5	79.3 ± 1.8	62.4
Crude Root Extract	20	9.1 ± 0.4	12.3 ± 0.5	
	40	18.4 ± 0.7	21.7 ± 0.9	
	60	26.9 ± 1.1	30.4 ± 1.2	
	80	35.3 ± 0.8	39.1 ± 1.0	
	100	43.8 ± 1.1	48.6 ± 1.5	121.9
Standard (Indomethacin)	100	81.6 ± 1.4	85.4 ± 1.2	35.2

3.6 COX-1/COX-2 inhibition and source-defined selectivity index:

The COX dataset showed the strongest separation between nanoparticle and crude-extract performance. For DsR-AgNPs, COX-2 inhibition increased from $38.2 \pm 1.3\%$ at $20 \mu\text{g/mL}$ to $88.7 \pm 1.6\%$ at $100 \mu\text{g/mL}$, whereas COX-1 inhibition increased from $12.4 \pm 0.8\%$ to $36.5 \pm 1.2\%$. The crude extract increased from $14.6 \pm 0.7\%$ to $53.1 \pm 1.7\%$ for COX-2 and from $8.3 \pm 0.4\%$ to $28.4 \pm 1.3\%$ for COX-1. Celecoxib at $100 \mu\text{g/mL}$ gave

$92.4 \pm 1.1\%$ COX-2 inhibition and $22.1 \pm 0.9\%$ COX-1 inhibition. The thesis calculates a selectivity index (SI) as the ratio of COX-2 inhibition to COX-1 inhibition and reports values of 2.43 for DsR-AgNPs, 1.87 for crude extract and 4.18 for celecoxib. Because this SI is based on endpoint inhibition percentages rather than enzyme-specific IC_{50} values, it should be interpreted as a study-defined comparative index rather than a validated clinical selectivity metric.

Table 7. COX-1/COX-2 inhibition and selectivity index, reproduced from the thesis dataset.

Treatment	Conc. ($\mu\text{g/mL}$)	COX-1 inhibition (%)	COX-2 inhibition (%)	Source-defined SI
<i>Dalbergia sissoo</i> Root NPs	20	12.4 ± 0.8	38.2 ± 1.3	
	40	20.1 ± 1.1	51.4 ± 1.0	
	60	26.5 ± 0.9	66.7 ± 1.5	
	80	31.8 ± 1.4	78.1 ± 1.2	
	100	36.5 ± 1.2	88.7 ± 1.6	2.43
Crude Root Extract	20	8.3 ± 0.4	14.6 ± 0.7	
	40	14.1 ± 0.6	23.9 ± 1.1	
	60	19.5 ± 0.8	34.2 ± 1.0	
	80	23.2 ± 1.1	44.8 ± 1.4	
	100	28.4 ± 1.3	53.1 ± 1.7	1.87
Standard Drug (Celecoxib)	100	22.1 ± 0.9	92.4 ± 1.1	4.18

The in vitro cytotoxicity results that uses COX-1/COX-2 inhibition includes viability-like values and positive controls. Because the assay label and columns are internally discordant, those numerical entries are not reinterpreted as a validated cytotoxicity endpoint in the present article.

4. Discussion:

The central conceptual finding is not simply that the nanoparticle preparation was “more active” than the crude extract; rather, the dataset suggests that converting a chemically complex botanical matrix into a silver-containing nanoform altered both its physicochemical behavior and its concentration-response profile. The FTIR comparison supports a changed interfacial chemical environment, while DLS and SEM show that the material existed as a colloidal system with

substantial aggregation in the dried state. The biological assays then reveal a repeated direction of effect: at the concentrations tested, DsR-AgNPs produced greater inhibition of protein denaturation, greater HRBC membrane stabilization, stronger proteinase/LOX inhibition and more pronounced COX-2 inhibition than the crude extract. This cross-assay coherence is the strongest part of the evidence base.

The phytochemical context of *D. sissoo* is mechanistically compatible with this observation. Flavonoids, isoflavonoids and phenolic constituents have both redox activity and protein/membrane interaction capacity. The isolation of biochanin and tectorigenin related isoflavonoid glycosides from *D. sissoo* demonstrates that the species contains structurally diverse compounds capable of interacting with biological systems [9]. At the same time, green AgNP

literature shows that polyphenols and other plant metabolites can participate in metal ion reduction and subsequently remain associated with the nanoparticle surface [14–20]. Thus, the most defensible interpretation is that the nanoparticle is a composite interfacial system rather than “silver alone” or “extract alone.”

The apparent increase in activity should, however, not be reduced to a generic statement that nanoparticles always improve bioavailability. In the present dataset, the measured hydrodynamic diameter of 148.6 ± 17.7 nm and PDI of $27.8 \pm 0.9\%$. The SEM images further show pronounced secondary agglomeration. Such behavior is consistent with the known distinction between primary particle size and hydrodynamic/aggregate size [19–24]. The result is scientifically useful because it highlights a product-development challenge: the observed biological performance was achieved by a material whose colloidal state was not fully standardized. Translation therefore requires control of aggregate state, batch to batch variability and surface chemistry, not simply replication of a nominal synthesis recipe.

The FTIR results should likewise be interpreted at the level of functional group participation rather than compound identification. The strong new spectral envelope from $1450\text{--}1150\text{ cm}^{-1}$, together with bands at 1318 , 809 and 712 cm^{-1} , is consistent with a changed surface associated biomolecular environment. Nevertheless, FTIR alone cannot establish that a specific metabolite such as dalbergin or biochanin was responsible for reduction, capping or pharmacological activity. Hence attributing the effect to individual metabolites and phytochemical literature supports their presence generally in the species [9–12].

The anti-inflammatory screening provides complementary rather than interchangeable biological signals. Protein-denaturation inhibition is a useful rapid screen, but it is not equivalent to therapeutic anti-inflammatory efficacy in living tissue [26]. HRBC membrane stabilization is a surrogate for membrane-protective activity and should not be interpreted as direct evidence of lysosomal stabilization in vivo. Proteinase and LOX inhibition probe different biochemical dimensions, while COX-1/COX-2 inhibition

introduces an enzyme-selectivity concept relevant to NSAID pharmacology. Agreement across these orthogonal readouts increases confidence that the nanoformulation has a reproducible in vitro anti-inflammatory phenotype, but it does not establish a single molecular mechanism.

The COX findings at $100\text{ }\mu\text{g/mL}$, DsR-AgNPs inhibited COX-2 by $88.7 \pm 1.6\%$ versus $36.5 \pm 1.2\%$ for COX-1, whereas the crude extract produced $53.1 \pm 1.7\%$ and $28.4 \pm 1.3\%$, respectively. Celecoxib remained more COX-2 selective on the source-defined SI metric (4.18) and reached $92.4 \pm 1.1\%$ COX-2 inhibition. The nanoparticle result therefore demonstrates strong relative preference for the COX-2 endpoint in this experimental system, not a proof of reduced gastrointestinal risk in humans. Recent systematic reviews of AgNP anti-inflammatory research likewise emphasize promising modulation of inflammatory pathways alongside the need for physicochemical standardization and toxicological characterization [20,21].

The literature on *D. sissoo* supports the biological plausibility of the present results. Leaf extracts have demonstrated anti-inflammatory activity in acute, subacute and chronic experimental models [8], while *D. sissoo* has also been used as a feedstock for green AgNP synthesis. Singh et al. reported biocompatible silver and gold nanoparticles using *D. sissoo* leaf extract, and more recent work described leaf-mediated silver nanoparticles with antioxidant, antibacterial and catalytic applications [12,13]. The current research therefore extends an existing species level nanotechnology literature toward root derived material and an anti-inflammatory screening framework. The novelty is best stated as a root extract focused comparative in vitro evaluation rather than as proof of a clinically superior nano drug.

From a translational perspective, the major opportunity is standardization. A reproducible botanical nanoformulation would require a defined botanical identity and harvest specification, extraction parameters, chemical fingerprinting, quantitative marker compounds, silver content, primary particle size, hydrodynamic size, PDI, zeta potential, residual precursor

characterization, aggregation state, and biological potency specifications. Current reviews emphasize that plant-mediated AgNP research is moving from proof of concept synthesis toward characterization, toxicity assessment and reproducibility [19–24]. The present data are therefore valuable as a prototype dataset but should be considered an intermediate stage in product development.

4.6 Integrated pharmacognosy–nanomedicine framework:

The combined dataset supports a useful pharmacognosy-to-nanomedicine framework in which plant identity, extraction, metal reduction, surface association and biological testing form one continuous causal chain. The root is not merely a convenient green reagent source: it is the source of a chemically complex reducing environment, and the resulting silver material is therefore best viewed as a phytochemical–metal composite whose biological behavior emerges from both components. This perspective is consistent with the expanding literature on plant-mediated AgNPs, where extract composition can alter nucleation, growth, surface chemistry, aggregation and biological effects [14–20].

At the upstream level, botanical identity and provenance are fundamental quality variables. The present roots were authenticated and assigned voucher code DSR-2, but the article cannot determine whether the subsequent biological effect is attributable to one marker metabolite, a broader phytochemical class, or the interaction between the silver core and a mixed surface corona. For future work, HPLC-DAD, LC-MS/MS or related metabolomic approaches should be used to establish a chemical fingerprint before and after nanoparticle formation. Such work could identify which metabolites are depleted from the extract, which remain associated with the nanoparticle fraction, and which correlate with potency. The objective would not be to replace the extract with a single isolated molecule but to define a reproducible chemical signature for the nanoformulation.

At the nanoparticle level, the current DLS/SEM combination illustrates why multiple orthogonal

techniques are essential. DLS measures a hydrodynamic entity in liquid, including solvation and any associated biomolecules and aggregates, whereas SEM of dried material measures a solid-state morphology that can be altered by drying. The difference between the 148.6 ± 17.7 nm hydrodynamic diameter and the tens-to-300 μ m SEM aggregates should therefore not be interpreted as contradictory particle sizes. Instead, it is a signal that dispersion state is a critical quality attribute. Transmission electron microscopy of a freshly dispersed sample, nanoparticle tracking analysis, repeated DLS across independent batches, and standardized zeta-potential measurements would provide a more coherent description of the primary and secondary particle populations.

The biological dataset also suggests that a single endpoint is insufficient to describe “anti-inflammatory activity.” The protein-denaturation and HRBC assays are relatively broad screening systems; proteinase and LOX inhibition add enzyme level information; and COX-1/COX-2 testing adds pathway selectivity. A future mechanistic study should extend this panel toward cytokine release, NF- κ B/MAPK signaling, reactive oxygen species, prostaglandin/leukotriene generation and macrophage polarization. Such experiments would help distinguish a general membrane/protein-protective effect from pathway specific suppression and would allow the proposed phytochemical silver synergy to be tested rather than assumed.

The source defined COX selectivity index provides an attractive hypothesis but is not yet a validated translational marker. Because the reported SI is calculated from endpoint inhibition percentages, it can be distorted by concentration choice and nonlinear enzyme-response behavior. A stronger pharmacological design would determine separate IC₅₀ or preferably IC₅₀/IC₉₀ parameters for COX-1 and COX-2 under identical assay conditions, use multiple independent batches, and calculate selectivity from matched potency estimates. This approach would make comparison with reference NSAIDs more meaningful and would avoid conflating endpoint percentage

inhibition with clinically established isoform selectivity.

Toxicological testing should proceed in parallel with efficacy optimization rather than after an efficacy claim is established. AgNPs can produce beneficial biological effects and adverse effects through overlapping physicochemical drivers, including particle size, surface chemistry, silver-ion release and dose [19–24]. Therefore, future studies should quantify total silver, soluble Ag⁺, residual AgNO₃, colloidal stability in biological media, hemolysis, cytotoxicity across relevant cell types, oxidative stress, genotoxicity and inflammatory cytokine responses. A green synthesis route should not automatically be equated with safety; the current article intentionally uses “potential” rather than “biocompatible” when discussing therapeutic development.

Finally, reproducibility and scalability should be treated as scientific questions. The reported optimization matrix identifies AgNO₃ concentration, extract-to-precursor ratio, pH, temperature and time as tunable parameters, but the thesis does not present a factorial or response-surface analysis linking those variables to particle size and activity. A statistically designed experiment could therefore identify an operating window rather than a single “optimal” point. The desired response variables should include primary particle diameter, PDI, zeta potential, silver yield, extract-marker retention and at least one biological potency measure. Such a design would connect process conditions directly to product performance and would make the green nanotechnology platform more reproducible and journal-ready.

In this sense, the major value of the present study is its integrated hypothesis: botanical chemistry can serve as both a synthetic tool and a source of bioactivity, while nanoscale organization can change how those molecules behave in a biological assay. The evidence already supports this as a working model. The next stage is to experimentally separate the contributions of the silver core, the phytochemical corona and the aggregate state. This shift from demonstrating that a green nanoparticle can be synthesized to determining why it performs as it does is the most important

conceptual step toward a mechanistically credible nano-phytopharmaceutical.

4.7 Comparative positioning of the present study:

The present findings occupy an intermediate position between traditional plant pharmacology and modern nanoformulation. Classical *D. sissoo* studies established anti-inflammatory activity of plant extracts, including leaf preparations in multiple inflammatory models [8], while phytochemical investigations demonstrated isoflavonoid diversity [9]. Separate nanotechnology studies established that *D. sissoo* leaf extracts can reduce Ag⁺ and stabilize metallic nanoparticles [12,13]. The present study connects these two literatures by using a root extract as the feedstock and by directly comparing the crude extract with the derived nanoparticle material across several in vitro anti-inflammatory assays. This comparative design is important because green synthesis should not be justified only by the existence of a nanoparticle signal; it should demonstrate whether the transformation produces a meaningful functional change.

5. Limitations and Data-Reconciliation Notes

No therapeutic safety, cytotoxicity mechanism, pharmacokinetics, biodistribution or clinical benefit can be inferred from the available in vitro dataset. Silver nanoparticle toxicity is strongly dependent on size, surface chemistry, dose and exposure context [20–24].

6. Conclusion:

This article presents a integrate interpretation of the *Dalbergia sissoo* root derived silver nanoparticle study. The strongest evidence is the convergence of altered FTIR surface chemistry, measurable hydrodynamic nanoparticles with moderate polydispersity, SEM-confirmed aggregate morphology, and concentration-dependent in vitro anti-inflammatory activity across protein denaturation, HRBC stabilization, proteinase/LOX inhibition and COX endpoints. At 100 µg/mL, DsR-AgNPs substantially exceeded the crude extract on the reported biological readouts, although reference drugs retained lower

reported IC₅₀ values or stronger endpoint activity in several comparisons. The findings support further investigation of *D. sissoo* root-derived AgNPs as a pharmacognostically grounded nanoformulation, but they should be regarded as proof of concept rather than evidence of a ready to use therapeutic product. The next experimental priorities are rigorous primary particle characterization, replicated DLS, chemically resolved surface analysis, standardized phytochemical markers, mechanistic inflammatory assays, validated cytotoxicity and genotoxicity testing, and appropriately designed in vivo studies.

7. Declarations:

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