Green Synthesis of Silver Nanoparticles Using Jute Mallow (Corchorus olitorius) Foliar Extract: Physicochemical Characterization and Ecotoxicity Assessment on Artemia salina

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Abstract:

Phyto-mediated green nanotechnology provides an ecologically benign, cost-effective alternative to conventional physical and chemical nanomaterial synthesis. The present study successfully engineered silver nanoparticles (AgNPs) utilizing aqueous leaf extracts of jute mallow (Corchorus olitorius) and conducted an extensive toxicological evaluation against the aquatic bioindicator Artemia salina. Aqueous foliar extracts rapidly reduced silver ions (Ag+ to Ag0), evidenced by a visible bathochromic shift from pale yellow to dark brown and confirmed by a sharp Surface Plasmon Resonance (SPR) peak at 430 nm on UV-Visible spectrophotometry. FTIR analysis identified key functional bands between 3856.21 and 3631.77 cm-1 (hydroxyl groups) and 1624.77 cm-1 (carbonyl/amide vibrations), establishing the role of foliar flavonoids and polyphenols as capping and stabilizing agents. Morphological imaging via FE-SEM revealed predominantly spherical nanostructures ranging from 50 to 100 nm, while Dynamic Light Scattering (DLS) confirmed a primary hydrodynamic intensity peak at 95.17 nm with a zeta potential of -8.08 mV. Computational ProTox-3.0 modeling categorized the synthesized metallic silver as Toxicity Class 3 with an estimated oral LD50 of 100 mg/kg. In vivo bioassays against Artemia salina nauplii demonstrated time- and concentration-dependent toxicity, yielding a 48-h LC50 of 40 μg/mL. Exposed nauplii exhibited progressive somatic degeneration, gut lumen aggregation of nanoparticles, loss of swimming setae, and complete motor paralysis at higher concentrations (50–200 μg/mL). These multi-analytical findings confirm that while Corchorus olitorius is an efficient biological reducer for AgNP production, the resulting nanoparticles retain potent biological reactivity, underscoring the necessity of aquatic ecotoxicity screening prior to environmental release.

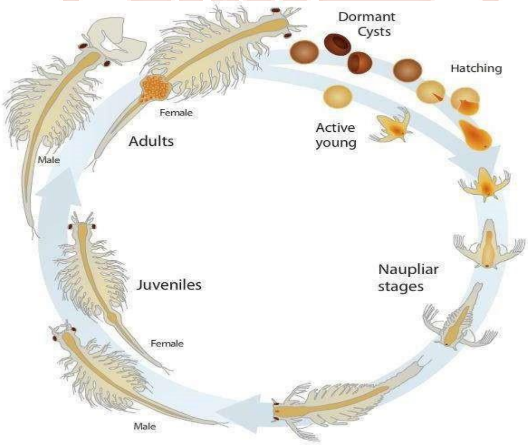
1. Introduction:

Nanotechnology represents an expanding frontier across biomedicine, environmental remediation, industrial catalysis, and antimicrobial coatings. Among engineered metal nanomaterials, silver nanoparticles (AgNPs) have attracted intense research interest due to their unique localized surface plasmon resonance, high surface-to-volume ratio, and broad-spectrum biocidal potency. However, conventional physical and chemical synthesis routes frequently involve hazardous reducing agents, toxic organic solvents, high energy inputs, and generate harmful surface by-products that hinder biological applications.

To mitigate these environmental and toxicological drawbacks, green synthesis utilizing botanical extracts has gained significant momentum. Plant extracts are rich in diverse bioactive secondary metabolites—such as polyphenols, flavonoids, terpenoids, and alkaloids—that act simultaneously as natural electron donors for metal ion reduction and steric capping agents to prevent particle coalescence. Corchorus olitorius L. (commonly known as jute mallow, Figure 1), a widely consumed medicinal plant in tropical and subtropical regions, contains high concentrations of phenolic acids, glycosides, and water-soluble antioxidants, rendering its foliar extract an exceptional candidate for green nanomanufacturing.

[Figures 1 & 2: Foliar specimen of Corchorus olitorius and Artemia salina nauplius]

Despite their eco-friendly synthesis, green-synthesized AgNPs inevitably enter aquatic ecosystems, where their nanospecific reactivity may exert unintended ecotoxicity on non-target microcrustaceans. The brine shrimp Artemia salina (Figures 2 & 3) serves as a premier ecotoxicological model organism to evaluate nanoparticle safety owing to its rapid development, non-selective filter-feeding behavior, and high physiological sensitivity.



[Figure 3: Life cycle diagram of Artemia salina]

The present investigation addresses two core objectives: (1) synthesizing and comprehensively characterizing AgNPs produced via aqueous leaf extracts of Corchorus olitorius using UV-Vis, FTIR, DLS, Zeta Potential, and FE-SEM; and (2) evaluating their aquatic safety, LC50 thresholds, and induced histomorphological deformities using Artemia salina nauplii, corroborated by in silico ProTox-3.0 toxicity predictions.

2. Materials and Methods

2.1 Botanical Material and Aqueous Extraction

Healthy foliar specimens of Corchorus olitorius were harvested from the botanical grounds of Veer Narmad South Gujarat University (VNSGU), Surat, Gujarat, India. The leaves were washed thoroughly with running tap water followed by double-distilled water to eliminate surface particulates. Cleaned leaves were shade-dried at room temperature (25 ± 2°C) for 10 days and pulverized into fine powder using a sterile mechanical grinder. For aqueous extraction, 10.0 g of powder was mixed with 100 mL double-distilled water (1:10 w/v) and heated at 60°C for 10 minutes under continuous magnetic stirring. The cooled suspension was filtered through Whatman No. 1 filter paper, and the clear filtrate was immediately utilized for nanoparticle fabrication.

2.2 Biogenic Synthesis and Centrifugal Purification of AgNPs

An analytical stock of 1 mM silver nitrate (AgNO3) was prepared in double-distilled water. The aqueous C. olitorius extract and AgNO3 solution were combined in a 1:9 volumetric ratio (10 mL extract added dropwise to 90 mL AgNO3) under continuous stirring at ambient room temperature for 60 minutes. Nanoparticle synthesis was monitored by visual color transition from light yellow to dark brown. The colloidal AgNPs were separated by centrifugation at 12,000 rpm for 15 minutes, washed twice with distilled water to remove unbound phytochemical residues, redispersed in deionized water, and stored in amber glass vials at 4°C.

2.3 Spectroscopic and Morphological Characterization

- **UV-Visible Spectroscopy:** Spectral absorption was recorded from 300 to 700 nm using a UV-Vis spectrophotometer to detect the characteristic Surface Plasmon Resonance (SPR) peak.
- **FTIR Spectroscopy:** Chemical functional groups on the AgNP surface were identified using a Bruker FTIR spectrometer over the range of 4000 to 500 cm^{-1}.
- **Dynamic Light Scattering (DLS) and Zeta Potential:** Hydrodynamic size distribution, polydispersity index (PDI), and net surface electrostatic charge were determined via a Malvern Zetasizer.

- **Field Emission Scanning Electron Microscopy (FE-SEM):** Nanoparticle surface morphology and dimensional distributions were visualized on a carbon-coated copper grid using FE-SEM.

2.4 In Silico Toxicity Profiling

Computational toxicology was modeled using the ProTox-3.0 platform to predict median lethal dose (LD50), acute toxicity class, ecotoxicity indicators, and target organ endpoints.

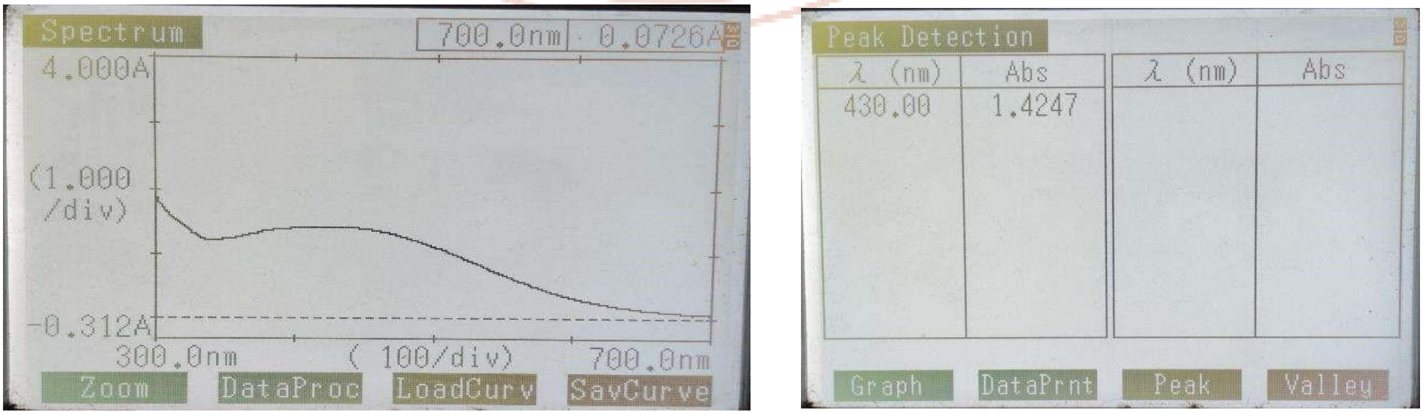
2.5 In Vivo Artemia salina Ecotoxicity Assay

Artemia salina cysts were hatched in artificial seawater (25 g/L sea salt + 1.5 g/L NaHCO3, pH 8.2) under continuous illumination and aeration for 24 hours. Actively swimming instar-I nauplii were harvested for toxicity testing. Bioassays were conducted across two concentration regimes: Low Range (10, 20, 30, and 40 µg/mL) and High Range (50, 100, 150, and 200 µg/mL). Ten active nauplii were transferred into 2 mL test solutions in 24-well microplates in triplicate, accompanied by seawater controls. Mortality percentages were recorded at 24 h and 48 h, from which the 48-h LC50 was calculated. Behavioral changes (motility loss, sinking) and morphological alterations (gut blockage, appendage loss) were documented under compound microscopy.

3. Results and Discussion

3.1 Visual Confirmation and UV-Vis Spectroscopy

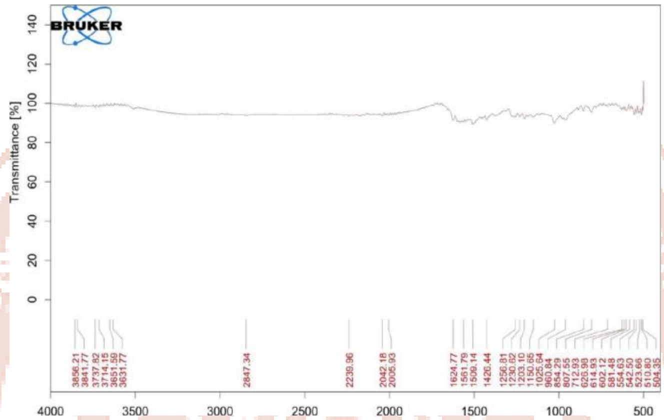
The reaction mixture displayed a distinct visual transformation from pale yellow to deep colloidal brown within 24 hours of adding C. olitorius foliar extract to the silver nitrate solution. This bathochromic shift indicates the reduction of Ag+ cations to metallic Ag0 nanoparticles, driven by the collective excitation of surface conduction electrons. UV-Vis spectral scanning showed a strong, symmetric SPR absorption band centered at 430.0 nm with an absorbance value of 1.4247 (Figures 4 & 5). The sharpness and single peak symmetry confirm the formation of predominantly spherical, isotropic silver nanoparticles.



[Figures 4 & 5: UV-Vis spectrum and instrument peak readout]

3.2 FTIR Surface Chemistry and Phytochemical Capping

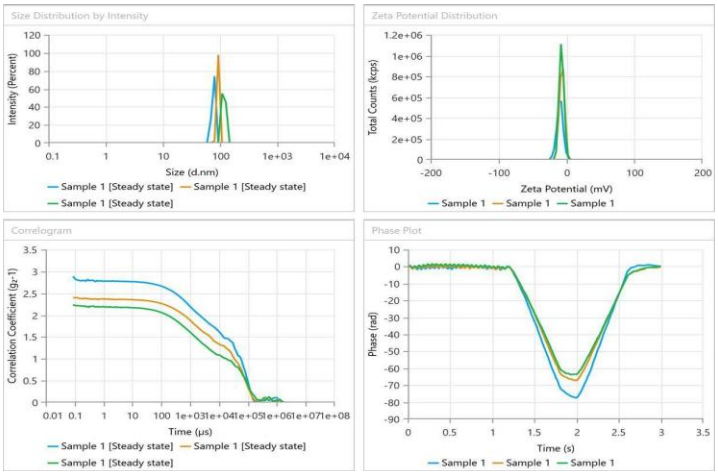
FTIR spectral analysis revealed characteristic absorption bands at 3856.21, 3737.82, 3631.77, 2847.34, 1624.77, 1561.79, 1426.44, 1256.81, 1203.10, and 1025.64 cm-1 (Figure 6). The high-frequency bands between 3856 and 3631 cm-1 correspond to O-H and N-H stretching vibrations of polyphenols and flavonoids. The intense peak at 1624.77 cm-1 represents carbonyl (C=O) and amide-I stretching, while the peaks around 1256–1025 cm-1 denote C-O phenolic stretching. These functional groups confirm that foliar polyphenols and proteins of C. olitorius actively mediate Ag+ reduction and form a protective organic capping shell around the nanoparticles.

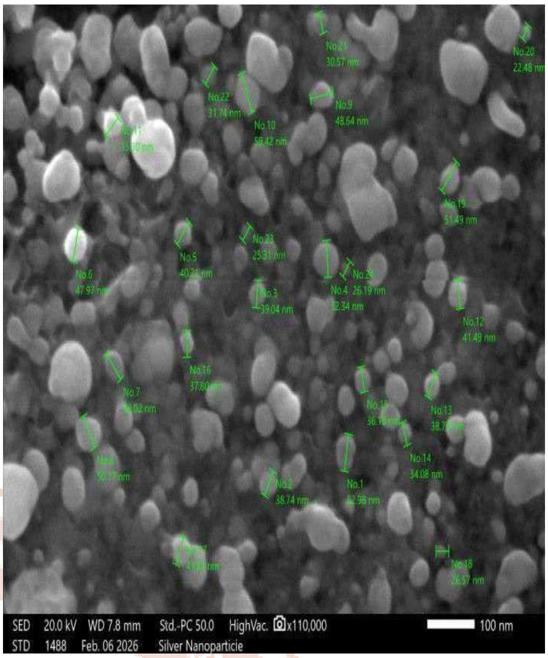


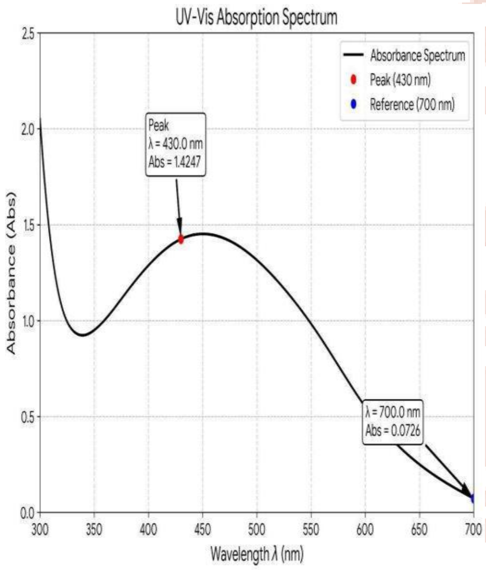
[Figure 6: Bruker FTIR spectrum]

3.3 DLS Particle Size, Zeta Potential, and FE-SEM Morphology

Dynamic Light Scattering (DLS) analysis documented a major intensity peak at 95.17 nm, corresponding to the primary hydrodynamic diameter of the nanoparticles (Figure 7). The overall Z-average was measured at 1725 nm with a PDI of 1.0, reflecting secondary colloidal aggregation in aqueous media due to macromolecular phytochemical bridging. The zeta potential was recorded as -8.08 mV. This negative surface charge originates from ionized phenolate and carboxylate functional groups on the capping layer, conferring moderate steric stabilization. FE-SEM micrographs demonstrated predominantly spherical AgNPs with core physical dimensions between 50 and 100 nm (Figure 8), showing close dimensional alignment with the primary DLS peak.



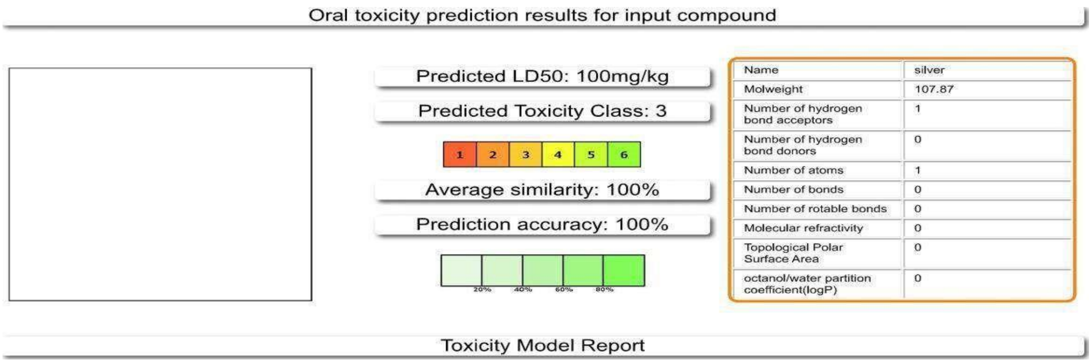




[Figure 7: DLS and Zeta Potential quadrant plot] | [Insert Figure 8: FE-SEM micrograph]

3.4 In Silico ProTox-3.0 Toxicity Prediction

Computational toxicology screening via ProTox-3.0 predicted an oral LD50 of 100 mg/kg, classifying the biogenic silver as Toxicity Class 3 (moderate toxicity, Figure 9). The model flagged positive activity for general ecotoxicity, blood-brain barrier permeation, and neurotoxic pathways, while showing negative profiles for mutagenicity and carcinogenicity, reinforcing the necessity of empirical aquatic testing.



[Figure 9: ProTox-3.0 in silico model report]

3.5 Artemia salina Ecotoxicity and LC50 Determination

In vivo toxicity testing using Artemia salina nauplii revealed marked concentration- and exposure-time-dependent lethality:

- **Low-Range Exposure (10–40 µg/mL):** Mortality ranged from 22% to 38% after 24 hours, rising to 38–50% after 48 hours. At 40 µg/mL, exactly 50% mortality was recorded at 48 hours, establishing the 48-h LC_{50} as 40 µg/mL.
- **High-Range Exposure (50–200 µg/mL):** Severe lethality occurred, ranging from 33% to 47% at 24 hours and escalating to 53–72% after 48 hours (maximum mortality of 72% at 150 µg/mL).

3.6 Sublethal Morphological and Behavioral Deformities

Microscopic examination revealed progressive structural degeneration in exposed nauplii compared to transparent, actively swimming controls. Surviving nauplii exhibited heavy accumulation of dark nanoparticle aggregates in the digestive tract, severe shrinkage of abdominal segments, loss of swimming antennae/setae, and cellular lysis. Behaviorally, exposed organisms displayed erratic spinning, loss of equilibrium, bottom sinking, and eventual paralysis, demonstrating that green-synthesized AgNPs induce significant physical obstruction and oxidative stress in microcrustaceans.

4. Conclusion

Aqueous leaf extracts of Corchorus olitorius provide an effective, eco-friendly biological medium for the rapid synthesis of stable, spherical silver nanoparticles (50–100 nm, SPR peak at 430 nm). However, toxicological bioassays revealed that these green AgNPs possess significant biological reactivity, exhibiting a 48-h LC50 of 40 µg/mL and causing severe gut blockage and appendage deformities in Artemia salina. These findings demonstrate that green nanomanufacturing does not inherently guarantee aquatic environmental safety, highlighting the vital importance of integrating ecotoxicity screening into nanomaterial development.

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