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Larvicidal potential of green-synthesized silver nanoparticles from *Alstonia scholaris* against mosquito larvae

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Abstract

Mosquito-borne illnesses persist as substantial global health threats, necessitating safer and eco-friendly control strategies. This study evaluates the larvicidal efficacy of green-synthesized silver nanoparticles (AgNPs) derived from the aqueous leaf extract of *Alstonia scholaris* against the larvae of *Aedes aegypti* under laboratory conditions.

AgNPs were synthesized using *A. scholaris* leaf extract as a reducing and stabilizing agent and characterized by UV–Vis spectroscopy, FTIR, XRD, and HR-SEM. Larvicidal bioassays were performed on third-instar larvae of *A. aegypti* at nanoparticle concentrations ranging from 10–200 ppm. Mortality was recorded at 24, 36, 48, and 60 hours post-exposure, and LC_{50} and LC_{90} values were calculated using probit analysis.

Characterization confirmed spherical, crystalline AgNPs of 10–15 nm size. Larvicidal assays showed dose- and time-dependent mortality, with LC_{50} decreasing from 227.58 ppm (24 h) to 49.45 ppm (60 h). Morphological deformities in treated larvae confirmed nanoparticle-induced damage. This study demonstrates that *A. scholaris*-mediated AgNPs exhibit potent larvicidal activity against mosquito vectors. Their eco-friendly synthesis and efficacy highlight potential applications in integrated vector management strategies.

Keywords: *Aedes aegypti*, *Alstonia scholaris*, Integrated Vector Management, Larvicidal activity, silver nanoparticles

Introduction

Mosquito-borne diseases remain one of the most serious global public health concerns, particularly in tropical and subtropical regions where nearly half of the world's population is at risk of infection (Vega-Rúa & Okech, 2019) [36]. Illnesses such as malaria, dengue, chikungunya, Zika virus, and filariasis collectively affect millions each year, resulting in high morbidity and mortality. According to the World Health Organization, malaria alone caused approximately 249 million cases and 608,000 deaths in 2022, while dengue infects more than 390 million individuals annually in endemic countries (Fatima *et al*., 2025) [10]. These alarming statistics highlight the urgent need for effective vector management strategies, particularly in regions where *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* are the dominant disease vectors (Derua *et al*., 2018) [7].

Conventional vector control relies heavily on adulticidal insecticides, but larval source management has emerged as a more sustainable and preventive approach. Since larvae remain confined to aquatic habitats, larviciding provides an opportunity to intervene early in the mosquito life cycle and reduce adult emergence, thereby interrupting transmission cycles (Chanda *et al*., 2017) [5]. Integrated Vector Management (IVM) strategies increasingly prioritize larval control because of its effectiveness in suppressing populations and preventing disease outbreaks.

Chemical larvicides such as temephos, malathion, and insect growth regulators have long been applied for mosquito control (Larvicides, 2005) [19]. However, continuous use of these chemicals raises major concerns including resistance, bioaccumulation, harmful effects on non-target organisms, and risks to human health (Senthil-Nathan, 2020) [33]. Resistance to temephos in *Aedes* species, for example, has been reported in many endemic regions, reducing the efficacy of these interventions (Diédhiou *et al*., 2017) [8]. These challenges underscore the urgent need for novel, eco-friendly, and biologically safe alternatives to conventional larvicides.

Plant-derived phytochemicals and nanotechnology-based solutions have emerged as promising candidates in this direction. Medicinal plants are abundant sources of secondary metabolites such as alkaloids, flavonoids, terpenoids, and phenolics, many of which display insecticidal and growth-inhibiting activities (Bensemmane *et al*., 2021) [4]. Parallelly, advances in green nanotechnology have opened avenues for environmentally friendly synthesis of metallic nanoparticles using plant extracts as reducing and stabilizing agents (Asif *et al*., 2022) [1]. Such biogenic nanoparticles combine the pharmacological potential of plants with the unique physicochemical properties of nanomaterials, enhancing their biological activity (Bamal *et al*., 2021) [3].

Among these, silver nanoparticles (AgNPs) have attracted special attention for their potent antimicrobial, antioxidant, and larvicidal properties. Green-synthesized AgNPs show advantages over chemically synthesized ones due to their eco-compatibility and functionalization by phytochemicals (Govindarajan *et al*., 2016) [16]. Extracts of plants such as *Azadirachta indica, Ocimum sanctum,* and *Phyllanthus* *niruri* have been successfully used for AgNP synthesis, demonstrating strong larvicidal potential against vectors of malaria and dengue (Kamaraj *et al*., 2012; Santhoshkumar *et al*., 2011; Prabakaran *et al*., 2024) [16, 26, 32]. The synergistic role of bioactive metabolites during nanoparticle formation enhances stability and larvicidal efficacy while ensuring lower toxicity to non-target organisms.

Alstonia scholaris (Devil's tree), a medicinally important evergreen distributed across Asia, has drawn increasing research attention for nanoparticle synthesis. Traditionally used in Ayurveda and folk medicine to treat malaria, respiratory ailments, and fever, the plant is rich in alkaloids, iridoids, and flavonoids (Pandey *et al*., 2020) [24]. Several reports indicate its phytochemicals possess antimicrobial and larvicidal properties (Nawaz *et al*., 2019; Rizwan *et al*., 2021) [22, 31]. The plant's phytoconstituents therefore provide an effective platform for the green synthesis of nanoparticles with enhanced biological activity (Prajapati *et al*., n.d.).

Nanoparticles derived from *Alstonia scholaris* extracts could play a dual role—leveraging the medicinal properties of the plant itself while enhancing larvicidal potential through nanoscale effects. Green-synthesized AgNPs exhibit high surface-to-volume ratios, which improve their interactions with larvae, causing oxidative stress, membrane disruption, and enzymatic inhibition

(Joudeh & Linke, 2022) [15]. Importantly, studies have reported that biogenic AgNPs demonstrate reduced toxicity to aquatic non-target organisms when compared with synthetic larvicides, aligning with sustainability goals (Mehmood *et al*., 2024) [21]. Given the rising incidence of mosquito-borne diseases and the limitations of synthetic larvicides, research into phytochemical-assisted nanotechnology offers a practical and sustainable alternative. The present study explores the synthesis of AgNPs using *Alstonia scholaris* leaf extracts, their physicochemical characterization, and their larvicidal efficacy against mosquito larvae. The objective is to establish whether *A. scholaris*-mediated AgNPs can serve as effective, eco-friendly larvicidal agents, thereby contributing to integrated mosquito management and reducing reliance on conventional insecticides.

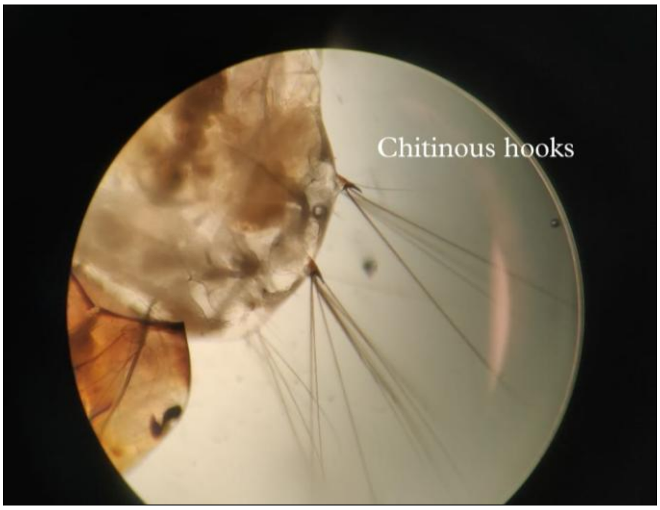


Fig 1: Aedis Aegypti Chitinous hooks presence

Fig: Aedis Aegypti Larval stage

Materials and Methods

Plant Selection and Authentication

Fresh, mature leaves of *Alstonia scholaris* (L.) R. Br. were collected from Jalna district, Maharashtra, India, during the summer season. The plant was taxonomically identified and authenticated by a qualified botanist, and a voucher specimen was deposited at the Parasitology Research Laboratory, JES College, Jalna for reference. The leaves were first rinsed thoroughly with tap water to remove dust and surface contaminants and subsequently washed with distilled water to ensure removal of adhering particles. The cleaned leaves were shade-dried under ambient laboratory conditions (25–30 °C) for approximately seven days and then oven-dried at 40 °C for 48 hours. Dried leaves were pulverized into fine powder using a sterile mortar and pestle and stored in airtight containers until further extraction.

Preparation of Aqueous Leaf Extract

Ten grams of powdered leaf material was suspended in 100 mL of double-distilled water and heated at 60 ± 1 °C for one hour using a magnetic stirrer to facilitate phytochemical extraction. After cooling to room temperature, the solution was filtered through Whatman No. 1 filter paper. The filtrate obtained was stored in amber-colored bottles at –15 °C to prevent phytochemical degradation. Extracts were freshly prepared every week to ensure maximum bioactivity and reproducibility of results.

Green Synthesis of Silver Nanoparticles

For nanoparticle synthesis, 90 mL of 1 mM silver nitrate ($AgNO_3$) solution was mixed with 10 mL of aqueous leaf extract in a sterile conical flask. The mixture was incubated at room temperature (25 ± 2 °C) under dark conditions to avoid photoreduction. A distinct color change from pale yellow to brown was observed within minutes to hours, indicating the reduction of Ag^+ ions and the formation of silver nanoparticles. All reactions were carried out in triplicate to validate reproducibility.

Characterization of Silver Nanoparticles

UV–Visible Spectroscopy: Absorbance spectra of the synthesized AgNPs were recorded using a UV–Vis spectrophotometer in the range of 300–700 nm. The appearance of a surface plasmon resonance (SPR) peak between 420–450 nm confirmed the reduction of silver ions and formation of nanoparticles.

Fourier Transform Infrared (FTIR) Spectroscopy: FTIR spectra of dried AgNP samples were recorded in the region 4000–400 cm^{-1} to identify phytochemical functional groups involved in reduction and stabilization. Shifts in O–H, C=O, and N–H stretching vibrations confirmed the role of polyphenols, flavonoids, and alkaloids in nanoparticle capping.

X-Ray Diffraction (XRD): XRD analysis was performed using Cu-Kα radiation (λ = 1.5406 Å). Distinct Bragg reflection peaks observed at 2θ values of 38.1°, 44.3°, 64.5°, and 77.6° corresponded to the (111), (200), (220), and (311) planes of a face-centered cubic crystalline structure of silver. The crystallite size was estimated using the Debye–Scherrer equation.

High-Resolution Scanning Electron Microscopy (HR-SEM): Morphological features and particle size distribution were examined using HITACHI S-530 HR-SEM. The images revealed predominantly spherical nanoparticles with smooth surfaces, with sizes ranging from 10–50 nm. Minimal agglomeration was observed, suggesting effective stabilization by phytoconstituents.

Larvicidal Bioassay

Larvae of *Aedes aegypti* were collected from natural breeding sites and reared under laboratory conditions. Third-instar larvae were identified morphologically using standard taxonomic keys and selected for experiments. Larvicidal bioassays were conducted following WHO guidelines. Groups of 25 larvae were exposed to different concentrations of AgNPs (10–200 ppm) in 250 mL glass beakers containing 100 mL of test solution. Distilled water served as negative control. Each treatment and control were replicated three times.

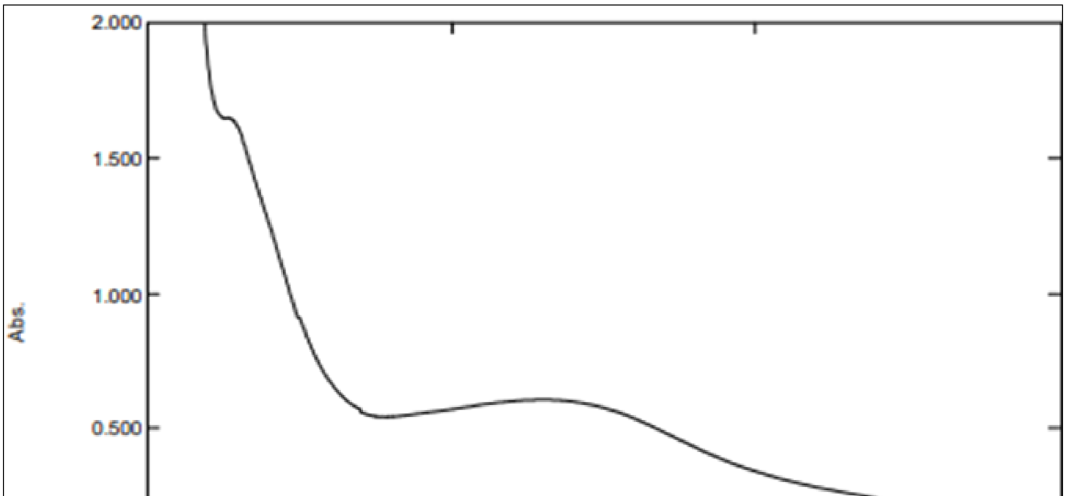
Larval mortality was assessed after 24, 36, 48, and 60 hours. Larvae that did not move after gentle probing were considered dead.

Statistical Analysis: Larval mortality data were subjected to probit analysis to calculate lethal concentrations (LC50 and LC90) along with 95% confidence intervals. Regression equations and correlation coefficients were determined for each exposure period. One-way analysis of variance (ANOVA) followed by post hoc tests were used to evaluate significant differences between treatments. A p-value of less than 0.05 was considered statistically significant. All analyses were performed using SPSS software version 26.

Results

Spectroscopic Characterization by UV–Visible Analysis

The UV–Vis spectral analysis of the aqueous extract of *Alstonia scholaris* revealed a prominent absorption peak at 200 nm, indicative of the presence of phytochemicals such as flavonoids and phenolic compounds. After reaction with silver nitrate, the solution exhibited a distinct surface plasmon resonance (SPR) peak between 420–450 nm, confirming the successful reduction of Ag^+ ions to Ag^0 and the synthesis of silver nanoparticles. The sharpness and intensity of the peak suggested nanoparticle stability and narrow size distribution.



UV–Vis spectra of *A. scholaris*-derived AgNPs.

Functional Group Analysis by FTIR Spectroscopy

FTIR spectra provided evidence of bioactive functional groups responsible for reduction and stabilization of nanoparticles. The aqueous extract showed strong absorption bands at 3282 cm⁻¹ corresponding to O–H stretching, 1637 cm⁻¹ for C=O stretching, and 1419 cm⁻¹ for C=C vibrations. In the AgNP spectrum, slight shifts in these bands along with the emergence of metal-oxygen vibrations confirmed that hydroxyl, carbonyl, and amine groups from phytochemicals were directly involved in nanoparticle capping and stabilization.

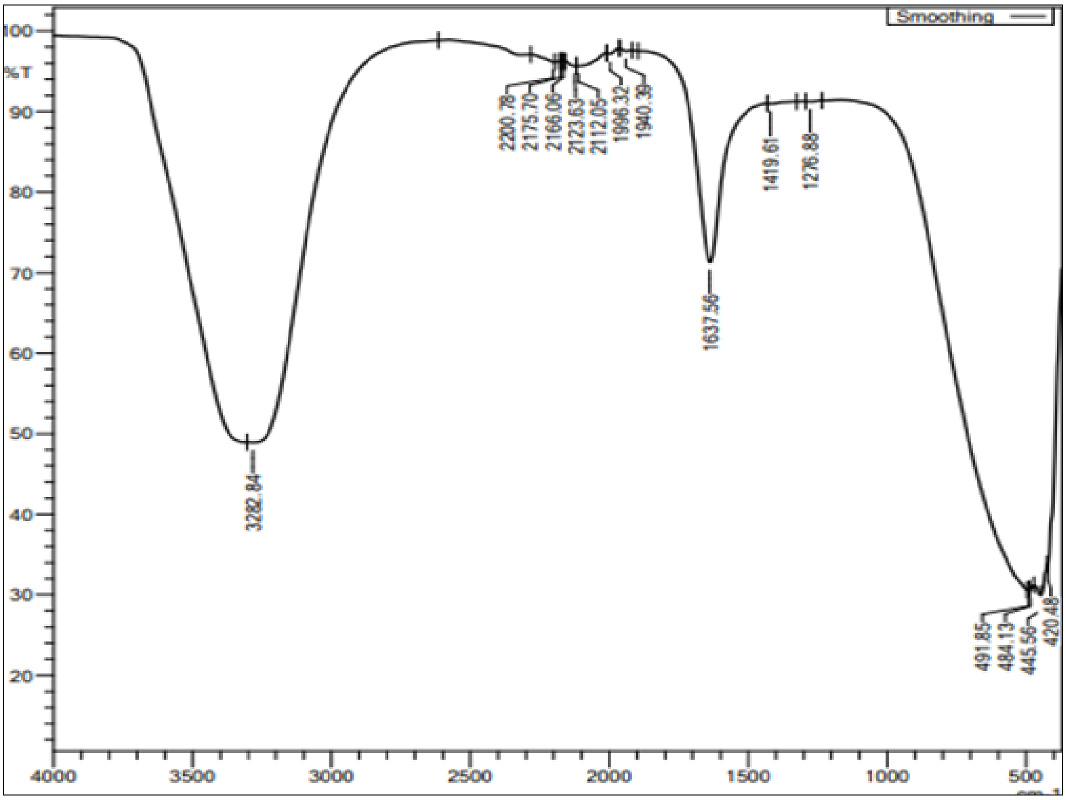


Fig 2: FTIR spectra showing functional groups involved in nanoparticle stabilization.

X-ray Diffraction (XRD)

The crystalline nature of synthesized AgNPs was confirmed by XRD analysis. Distinct diffraction peaks were observed at 2θ values of 38°, 44°, 64°, and 77°, corresponding to the (111), (200), (220), and (311) planes of a face-centered cubic lattice of elemental silver. The absence of extraneous peaks indicated high purity of the synthesized nanoparticles. The average crystallite size was calculated using the Debye–Scherrer equation and was found to be approximately 15 nm.

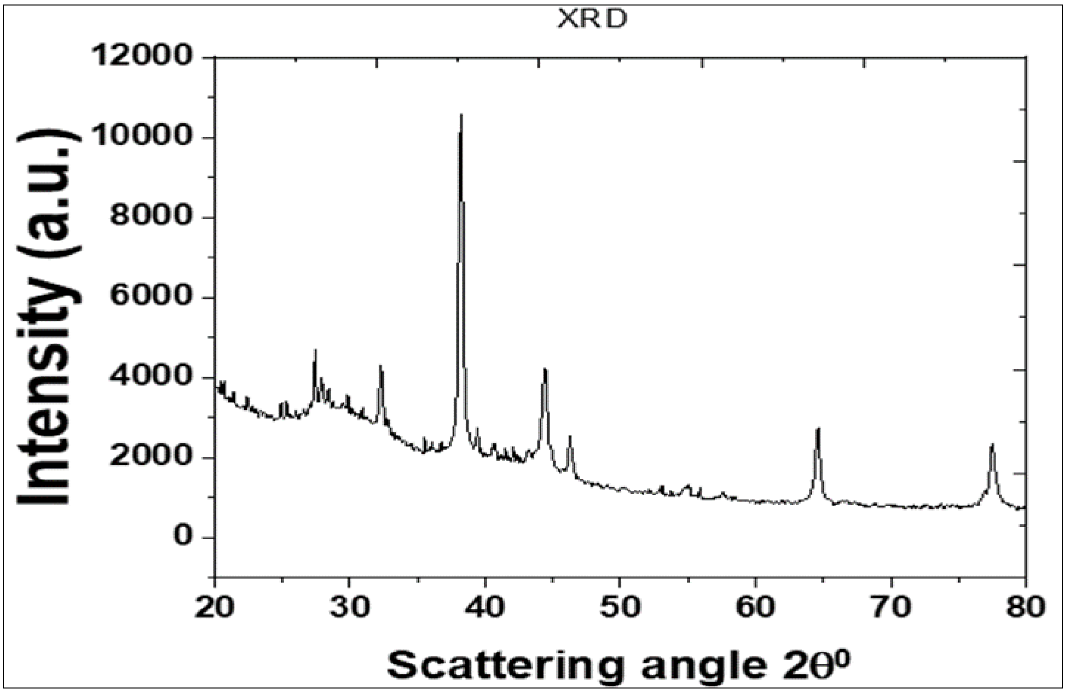


Fig 3: XRD diffraction pattern confirming crystalline structure of AgNPs.

High-Resolution Scanning Electron Microscopy (HR-SEM)

HR-SEM analysis revealed that the AgNPs were predominantly spherical with smooth surfaces and uniform morphology. Particle sizes ranged between 10–50 nm with minimal aggregation, suggesting good colloidal stability conferred by plant-derived biomolecules. Phytochemicals likely provided both steric and electrostatic stabilization.

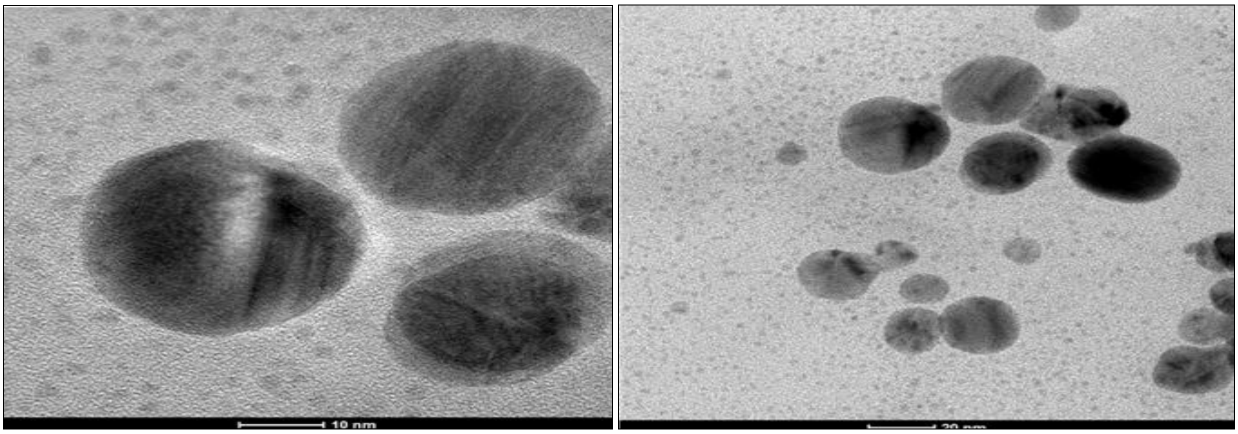


Fig 4: Morphological analysis of Larvicidal activity Larvicidal Bioassay

The larvicidal efficacy of *Alstonia scholaris*-mediated AgNPs was assessed over time using probit analysis to derive LC50 and LC90 values. Mortality increased with both concentration and exposure time. At 24 hours, LC50 and LC90 values were 227.58 ppm and 811.38 ppm, respectively, with a regression coefficient R = 0.9915 (p = 0.0084). At 36 hours, LC50 decreased to 127.75 ppm and LC90 to 541.48 ppm, with R = 0.9874 (p = 0.0126). At 48 hours, LC50 further reduced to 73.86 ppm and LC90 to 313.35 ppm, supported by R = 0.9648 (p = 0.0351). After 60 hours, the lowest LC50 and LC90 values were recorded at 49.45 ppm and 154.85 ppm, respectively, with R = 0.9679 (p = 0.0320). These results clearly demonstrate a strong time- and dose-dependent larvicidal effect of the synthesized nanoparticles.

Table 1 presents the detailed regression equations, LC50 and LC90 values, and statistical parameters for each exposure duration. Figure 1 illustrates the UV–Vis spectra confirming nanoparticle synthesis, Figure 2 shows the FTIR spectra of the plant extract and AgNPs, Figure 3 presents the XRD diffraction patterns, and Figure 4 depicts SEM micrographs of control and treated larvae.

Table 1: LC_{50} and LC_{90} values of *A. scholaris*-mediated AgNPs against *Aedes aegypti* larvae Discussion

Table 1: LC50 and LC90 values (in ppm) for *Alstonia* plants over various time intervals.									
Period	**Test Concentration (PPM)**	**Mean % Mortality**	**Natural Log**	**Probit of Kill**	**Regression Equation**	**LC50**	**LC90**	**R**	**ANOVA**
24hr	50	7	3.91202	-1.4751	Y=-5.472+1.008X	227.583	811.378	0.99155	0.00845
24hr	100	18	4.60517	-0.915					
24hr	150	32	5.01064	-0.4674					
24hr	200	48	5.29832	-0.0499					
36hr	50	22	3.91202	-0.7719	Y=-4.304+0.887X	127.752	541.481	0.9874	0.0126
36hr	100	37	4.60517	-0.3316					
36hr	150	55	5.01064	0.12591					
36hr	200	68	5.29832	0.46798					
48hr	50	40	3.91202	-0.2531	Y=-3.815+0.887X	73.8596	313.348	0.96483	0.03517
48hr	100	55	4.60517	0.12591					
48hr	150	70	5.01064	0.52469					
48hr	200	85	5.29832	1.03686					
60hr	50	55	3.91202	0.12591	Y=-4.379+1.123X	49.4479	154.849	0.96793	0.03207
60hr	100	73	4.60517	0.61312					
60hr	150	87	5.01064	1.12686					
60hr	200	96	5.29832	1.75185					

The present study demonstrates the successful green synthesis of silver nanoparticles (AgNPs) using the aqueous leaf extract of *Alstonia scholaris* and highlights their significant larvicidal potential against *Aedes aegypti*. The synthesis process, confirmed by UV–Vis, FTIR, XRD, and HR-SEM analyses, produced stable, crystalline, and spherical nanoparticles, thereby validating the efficiency of A. scholaris phytochemicals as natural reducing and stabilizing agents. The larvicidal bioassays revealed a strong time- and dose-dependent mortality pattern, with LC50 values decreasing markedly from 227.58 ppm at 24 hours to 49.45 ppm at 60 hours. These results collectively emphasize the ecological and biological relevance of phytomediated nanotechnology in vector management.

The UV–Vis and FTIR analyses suggested the involvement of phenolics, flavonoids, and alkaloids in nanoparticle formation, corroborating previous studies where plant-derived biomolecules facilitated nanoparticle stability and biological activity (Bensemmane *et al*. 2021; Nawaz *et al*. 2019) [4, 22]. The observed Bragg peaks in XRD confirmed the face-centered cubic crystalline structure, consistent with earlier reports on plant-mediated AgNPs (Prasannaraj and Venkatachalam 2017; Rizwan *et al*. 2021) [28, 31]. HR-SEM images revealed predominantly spherical nanoparticles with sizes between 10–50 nm, aligning with findings of Balkrishna *et al*. (2023) [2], who emphasized the role of natural capping agents in preventing aggregation and ensuring uniformity.

The larvicidal bioassays demonstrated that nanoparticle efficacy increased with both concentration and exposure time, indicating cumulative toxicity. Similar time-dependent mortality trends were reported by Santhoshkumar *et al*. (2011) [32] with Nelumbo nucifera-mediated AgNPs and Karthi *et al*. (2020) [18] using Rhizophora mucronata leaf extracts. The morphological disruptions observed in treated larvae, such as ruptured cuticles and tissue degeneration, mirror the findings of Ragavendran *et al*. (2017) [29] and Derua *et al*. (2018) [7], who noted similar structural damage induced by fungal and microbial larvicides. These disruptions likely result from oxidative stress, enzyme inhibition, and nanoparticle-mediated interference with membrane integrity.

Interspecific variation in susceptibility is a well-established phenomenon in mosquito control. While the present study focused on *Aedes aegypti*, earlier research has shown that Anopheles stephensi and Culex quinquefasciatus may exhibit differing levels of sensitivity depending on nanoparticle type and phytochemical composition (Prabakaran *et al*. 2024; Wang *et al*. 2024) [26, 37]. Such variations underscore the importance of expanding larvicidal testing across multiple species to determine broad-spectrum applicability.

The novelty of this work lies in demonstrating, for the first time, the larvicidal potential of AgNPs synthesized using A. scholaris, a plant traditionally recognized for its medicinal value. By harnessing its phytochemical richness, this study bridges traditional ethnobotanical knowledge with modern nanotechnology. The eco-friendly synthesis route eliminates the need for toxic chemicals and presents a cost-effective strategy compatible with sustainable vector management approaches.

Nevertheless, certain limitations remain. The experiments were confined to laboratory conditions, which may not fully simulate environmental complexities. Only third-instar larvae were tested, leaving early instars, pupae, and non-target aquatic organisms unexamined. Furthermore, the specific phytochemicals responsible for larvicidal activity were not isolated or quantified. Future studies should include field trials, stage-specific toxicity assays, and detailed phytochemical characterization to identify the active compounds mediating nanoparticle synthesis and larvicidal action.

Overall, the findings of this study strongly support the potential application of A. scholaris-mediated AgNPs as an eco-friendly, effective larvicide. Incorporating such green nanotechnological approaches into integrated vector management strategies could significantly reduce reliance on synthetic insecticides, mitigate resistance development, and promote environmental sustainability.

Conclusion

The present study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using the aqueous leaf extract of *Alstonia scholaris* and established their larvicidal potential against *Aedes aegypti*. The synthesized nanoparticles were spherical, crystalline, and stable, as confirmed through UV–Vis, FTIR, XRD, and HR-SEM analyses. Larvicidal bioassays revealed a clear dose- and time-dependent mortality pattern, with LC50 values decreasing significantly over extended exposure durations. Morphological disruptions observed in treated larvae further substantiated the toxic effects of the AgNPs.

This is the first report to validate A. scholaris-mediated silver nanoparticles as effective larvicides. The integration of phytochemicals from a traditionally valued medicinal plant with nanotechnology highlights a novel approach for mosquito control. Importantly, the synthesis method is eco-friendly, cost-effective, and avoids the use of hazardous chemicals, making it compatible with sustainable vector management strategies.

While the laboratory results are promising, further research is needed to evaluate the efficacy of these nanoparticles under field conditions, across multiple mosquito species, and against different developmental stages. Additionally, detailed phytochemical profiling would help identify the active compounds responsible for nanoparticle synthesis and larvicidal action. Assessing the impact on non-target organisms will also be essential to ensure environmental safety.

In conclusion, A. scholaris-mediated AgNPs represent a potent, environmentally friendly alternative to conventional larvicides, with strong potential for incorporation into integrated vector management programs aimed at reducing mosquito-borne disease transmission.

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