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Article

Larvicidal Efficacy of Plant-Mediated Silver Nanoparticles from *Gmelina arborea* and *Hyptis suaveolens* Against Wild *Anopheles* Vectors

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Abstract

Malaria remains a leading cause of morbidity and mortality in sub-Saharan Africa, with escalating insecticide resistance among *Anopheles* vectors threatening the sustainability of current control interventions. The search for novel, environmentally benign larvicidal agents has increasingly focused on nanotechnology innovations derived from medicinal plants. This study assessed the larvicidal potential of silver nanoparticles (AgNPs) synthesized using aqueous leaf extracts of *Gmelina arborea* and *Hyptis suaveolens* against wild *Anopheles* larvae collected from Lagos State, Nigeria. Fresh plant samples were authenticated botanically, and green synthesis of AgNPs was confirmed by a characteristic honey-brown colour change and UV–visible spectrophotometric analysis. Larvicidal bioassays were performed according to WHO guidelines using six graded concentrations under controlled insectary conditions. Mortality was recorded at 24, 48, and 72 hours, and toxicological responses were analyzed using probit regression in IBM SPSS v20. After 72 hours, both AgNP formulations demonstrated a concentration-dependent increase in larval mortality, with *H. suaveolens* AgNPs showing greater bioefficacy than *G. arborea* AgNPs. Specifically, *H. suaveolens* AgNPs achieved LC_{50} and LC_{95} values of 0.292 ppm and 1.948 ppm respectively, whereas $LC_{50} = 0.469$ ppm and $LC_{95} = 3.919$ ppm were recorded for *G. arborea* AgNPs. Control mortality remained ≤10%, validating experimental reliability. The potent larvicidal activity displayed by both plant-derived AgNPs suggests that phytochemical-mediated nanoparticle synthesis may enhance toxicity against mosquito vectors while retaining environmental safety. These findings provide evidence supporting the integration of green nanotechnology into vector-control strategies to help mitigate resistance to conventional insecticides and strengthen malaria prevention efforts in endemic communities.

Keywords: silver nanoparticles; larvicidal activity; *Gmelina arborea*; *Hyptis suaveolens*; *Anopheles* larvae; green nanotechnology; malaria vector control

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Introduction

Malaria remains one of the most formidable public health challenges in sub-Saharan Africa, accounting for more than 95% of global cases and deaths, with Nigeria among the highest-burden nations [1–5]. The disease is primarily transmitted by *Anopheles* mosquitoes, whose widespread distribution, ecological adaptability, and increasing resistance to conventional insecticides undermine prevention and control efforts [6]. Despite substantial progress achieved through long-lasting insecticide-treated nets (LLINs), indoor residual spraying (IRS), and chemotherapeutic approaches, vector populations continue to persist, highlighting the need for innovative, sustainable, and environmentally safe larval control strategies. Larval source management (LSM), which includes

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larvicides, is a key component of integrated vector management (IVM) because it targets mosquitoes at aquatic immature stages before they emerge as biting adults. However, the persistent use of synthetic chemical larvicides presents several ecological and operational limitations—such as toxicity to non-target organisms, environmental persistence, cost constraints, and the rapid emergence of insecticide resistance among *Anopheles* populations [6–10]. As a consequence, there is growing scientific interest in exploring biogenic alternatives derived from plant resources that may offer improved efficacy, reduced toxicity, and better biodegradability [11–13].

Nanotechnology-based mosquito control has recently gained attention due to the distinctive physicochemical properties of nanoparticles, which enable improved stability, targeted action, and enhanced larvicidal potency at lower concentrations. Particularly, green-synthesized silver nanoparticles (AgNPs) have demonstrated broad-spectrum antimicrobial and vector control capabilities [14,15]. Biosynthesis of AgNPs using plant extracts represents a safer, cost-effective, and eco-friendly approach compared with conventional chemical synthesis methods, while also integrating the phytochemical properties of medicinal plants that may synergistically enhance larvicidal activity.

Gmelina arborea (Verbenaceae) and *Hyptis suaveolens* (Lamiaceae) are medicinal plants widely distributed in tropical regions and traditionally used for antimicrobial, anti-inflammatory and antiparasitic applications. Their phytochemical richness—particularly in flavonoids, terpenoids, phenolics and alkaloids—provides strong rationale for their potential in the biosynthesis of bioactive AgNPs. Although *G. arborea* extracts have shown larvicidal activity against *Aedes aegypti* larvae [16] and other pest insects [17], and *H. suaveolens* has been shown to support green synthesis of AgNPs [14,18–20], the larvicidal efficacy of silver nanoparticles derived specifically from these species against wild *Anopheles* larvae remains under-investigated.

Therefore, this study aims to evaluate the larvicidal potential of biosynthesized silver nanoparticles from *Gmelina arborea* and *Hyptis suaveolens* on wild *Anopheles* larvae. The findings may contribute to the development of novel, plant-based, nanotechnology-driven vector control interventions capable of strengthening malaria prevention and reducing dependence on synthetic insecticides in endemic settings.

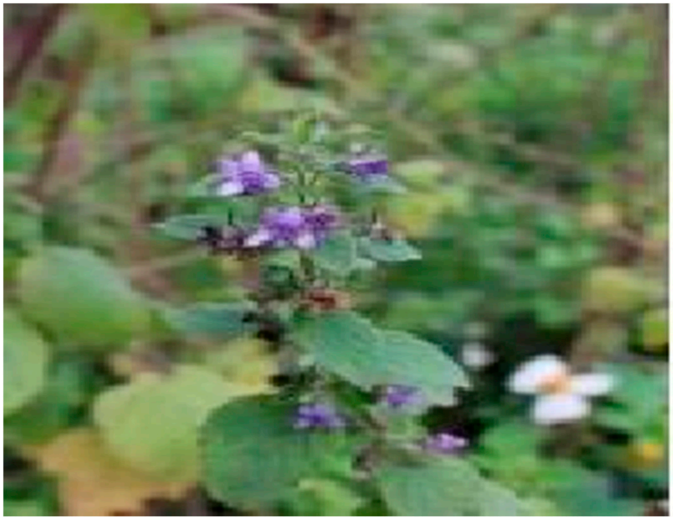

Figure 1. *Hyptis suaveolens* (L.).

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Figure 2. *Gmelina arboreal* (beech wood).

Methods

Source and Rearing of Test Organisms

Wild *Anopheles* larvae were collected from breeding sites, transported to the Laboratory and maintained under insectary conditions of 25–29 °C and 78–82% relative humidity.

A 24-hour acclimatization period was allowed before testing. Morphological identification was performed using the standard taxonomic keys of Gillies and Coetzee [21].

Preparation of Plant Extracts and Silver Nanoparticles

Fresh leaves of *G. arborea* and *H. suaveolens* were collected, washed with distilled water, air-dried, and pulverized into fine powder. This was stored in airtight containers until use.

The silver nanoparticles (AgNPs) were synthesized following the method of Umoren *et al*. [22] with modifications. Briefly, 1.019 g of $AgNO_3$ was dissolved in 160 mL deionized water and stirred magnetically for 5 minutes. A 40 mL aliquot of each plant extract was added dropwise under continuous stirring, and the mixture incubated in the dark for 48 hours to prevent photoreduction. Formation of AgNPs was confirmed by colour change from pale yellow to dark brown, indicative of Ag^+ reduction to Ag^0. The precipitates were centrifuged, washed, and oven-dried at 70 °C to obtain dry nanosilver powders.

Larvicidal Bioassay

Larvicidal tests were conducted following WHO standard protocols. For each plant-derived AgNP sample, six concentrations were prepared in 500 mL glass beakers containing 250 mL dechlorinated water. Thirty [30] healthy third-instar larvae were introduced into each beaker. Four replicates were used per concentration, with a parallel untreated control.

Experimental conditions were maintained at 27 ± 2 °C with a 12:12 h light–dark photoperiod over a 72-hour exposure. Larval mortality was recorded after 24, 48, and 72 hours. Larvae failing to respond to gentle probing were recorded as dead. Moribund larvae unable to reach the surface or exhibit diving reflex were also included in mortality counts. Tests were considered valid only if control mortality was ≤10%.

Toxicity Endpoints

Lethal concentration values (LC_{50} and LC_{95}) with 95% confidence limits were determined using probit regression analysis to describe concentration–mortality responses. Any test in which pupation occurred during exposure was excluded.

Statistical Analysis

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Mortality percentages were corrected using Abbott's formula where necessary, and data were subjected to probit analysis using IBM SPSS Statistics version 20.0. Toxicity parameters including LC_{50}, LC_{95}, slopes, and associated confidence limits were generated at a significance level of $p < 0.05$.

Results

Successful synthesis of silver nanoparticles was indicated by a visible colour change of the reaction mixture from colourless to honey brown, confirming the reduction of Ag^+ ions to Ag^0. This transformation was further validated using UV–visible spectrophotometry, which showed the characteristic surface plasmon resonance (SPR) peak of silver nanoparticles.

Larvicidal bioassays demonstrated that silver nanoparticles synthesized from both *Gmelina arborea* and *Hyptis suaveolens* exhibited concentration-dependent toxic effects against third-instar wild *Anopheles* larvae. Mortality consistently increased with rising nanoparticle concentrations and exposure time. Control groups recorded mortality rates ≤10%, validating experimental integrity.

Probit regression analysis (IBM SPSS v20.0) revealed the median lethal concentration (LC_{50}) and lethal concentration for 95% mortality (LC_{95}) after 72 hours of exposure, indicating potent larvicidal activity of both plant-derived AgNPs. Toxicity indices, including LC_{50}, LC_{95}, slope, and 95% confidence limits, are summarized in Tables 1 and 2.

Table 1. Results from bioassay of Silver nanoparticles of *Hyptis suaveolens* against *Anopheles* mosquito larva.

Silver Nanoparticles	**Concentration (ppm)**	**Mortality (%) ±SE**	**Lethal Concentration**
Control	0	0	72h LC_{50}(ppm)(LCL-UCL) 0.292(0.043-0.515)
Hyptis suaveolens	0.25	30±0.344	
	0.5	21±0.344	
	1.0	53.3±0.344	72h LC_{95}(ppm)(LCL-UCL) 1.948(1.069-17.152)
	1.5	56.6±0.344	
	2.0	96.6±0.344	
	2.5	100±0.344	

LC=Lethal Concentration, S.E=Standard Error.

Table 2. Results from bioassay of Silver nanoparticles of *Gmelina arboreal* against anopheles mosquito larva.

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Silver Nanoparticles	**Concentration(ppm)**	**Mortality (%)±SE**	**Lethal Concentration**
Control	0	0	LC50 (ppm) (LCL-UCL) 0.469(-)
Gmelina arboreal	0.25	30±0.311	
	0.5	70 ±0.311	
	1.0	53.3±0.311	LC95 (ppm) (LCL-UCL) 3.919(-)
	1.5	53.3±0.311	
	2.0	96.6±0.311	
	2.5	100±0.311	

LC=Lethal Concentration, S.E=Standard Error.

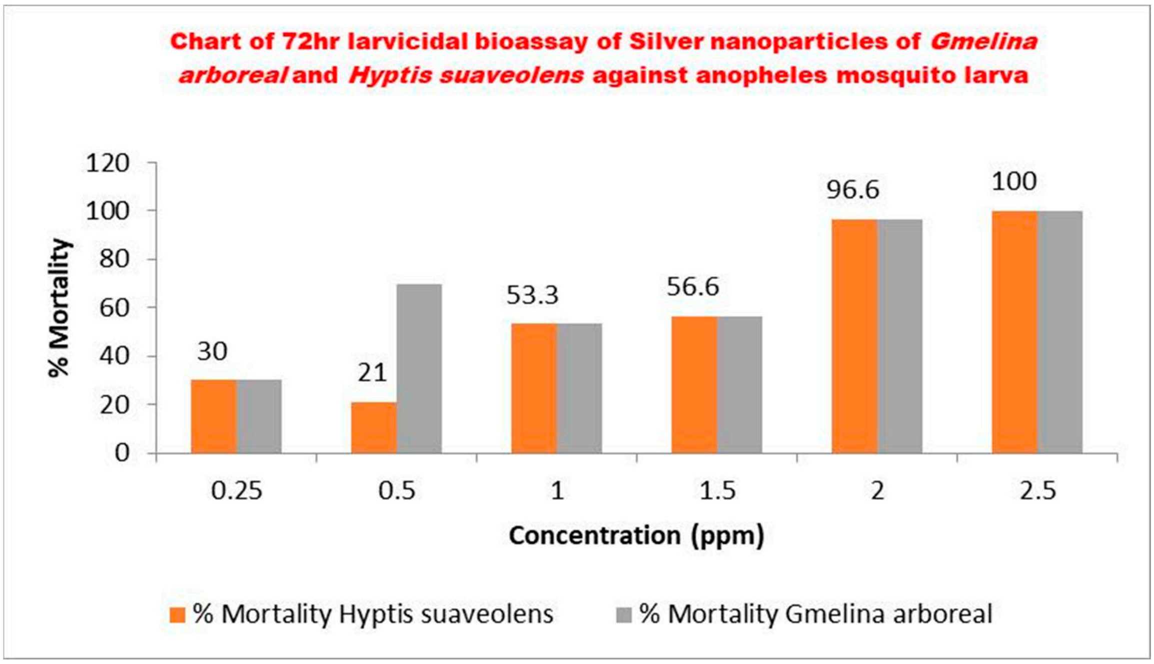

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Figure 3. Chart of 72hr larvicidal bioassay of Silver nanoparticles of *Gmelina arboreal* and *Hyptis suaveolens* against anopheles mosquito larva.

Discussion

The escalating global threat of antimicrobial resistance (AMR) is driving an urgent need for innovative approaches to vector control and infectious disease prevention [23–28]. Resistance to commonly used insecticides, including pyrethroids, organophosphates, and carbamates, has been widely reported in *Anopheles* populations across Nigeria and other malaria-endemic regions, undermining the effectiveness of current public health interventions

This study provides new evidence that plant-mediated silver nanoparticles (AgNPs) synthesized from *Gmelina arborea* and *Hyptis suaveolens* extracts possess strong larvicidal effects against wild *Anopheles* larvae, the primary malaria vector in Nigeria. The pronounced concentration-dependent and time-dependent mortality observed indicates that these biosynthesized nanomaterials act rapidly and effectively in disrupting larval physiology. The superior toxicity profile of *H. suaveolens* AgNPs, as reflected in its lower LC_{50} and LC_{95} values, suggests better bioactive synergy between the phytochemicals present in the plant and silver ions. These phytocompounds—including flavonoids, terpenoids, saponins, and phenolics—serve simultaneously as reducing and stabilizing agents during nanoparticle formation and may enhance larvicidal potency by improving nanoparticle stability, penetration, and interaction with larval tissues [14].

The findings of this study indicate that biosynthesized silver nanoparticles from *Gmelina arborea* and *Hyptis suaveolens* exhibited notable larvicidal potency, with LC_{50} values of 0.469 ppm and 0.292 ppm and LC_{95} values of 3.919 ppm and 1.948 ppm, respectively. These results are comparable to the larvicidal activity reported in previous nanoparticle-based studies. For example, Santhoshkumar *et al.*[29], synthesized AgNPs using *Nelumbo nucifera* leaf extract and demonstrated effective toxicity against *Culex quinquefasciatus*, with LC_{50} and LC_{90} values of 0.69 and 3.59 ppm. Likewise, AgNPs fabricated from *Turbinaria ornate* showed strong bioefficacy against *Aedes aegypti*, achieving an LC_{50} of 0.738 µg/mL. Azarudeen *et al.*[30], reported LC_{50} values of 9.20 µg/mL and 10.02 µg/mL for *Aedes aegypti* and *Culex quinquefasciatus* when using *Merremia emarginata*-derived AgNPs, while Govindarajan *et al.*[31], observed LC_{50} values of 15.56 µg/mL and 17.46 µg/mL from *Hugonia mystax*-mediated AgNPs against the same mosquito species. Additionally, AgNPs from *Curcuma zedoaria* demonstrated strong toxicity against *C. quinquefasciatus*, with a reported LC_{50} of 0.64 ppm. Collectively, these comparative studies reinforce the effectiveness of plant-based AgNPs as promising nanobiolarvicides with the potential to strengthen mosquito control in malaria-endemic regions.

Unlike conventional insecticides that act on a single target site (e.g., sodium ion channels for pyrethroids), AgNPs demonstrate multi-target mechanisms of toxicity—including disruption of cell membrane integrity, induction of oxidative stress through reactive oxygen species (ROS), interference with metabolic enzyme pathways, and impairment of respiration and osmoregulation. This multi-modal action makes the development of resistance more difficult and provides a critical advantage for future deployment in integrated vector management (IVM) programmes [32].

Moreover, green synthesis offers significant ecological advantages over synthetic insecticides and chemically synthesized nanoparticles. Using plant extracts eliminates hazardous by-products from production, reduces environmental persistence, and enhances biodegradability, making these nanoformulations highly suitable for larval habitats close to human populations. The reliance on widely available medicinal plants such as *G. arborea* and *H. suaveolens* also increases scalability and local acceptability, particularly in rural and peri-urban communities where malaria burden is highest. These factors align well with WHO recommendations calling for eco-friendly innovations that reinforce sustainability, reduce chemical dependency, and support public health policy goals [33–35].

While these findings are promising, future research should incorporate comprehensive nanoparticle characterization (e.g., TEM, FTIR, XRD, and zeta potential analysis) to fully elucidate structure–activity relationships. Field trials under natural breeding conditions, toxicological

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evaluation on non-target aquatic species, and formulation optimization are necessary to support regulatory approval and scale-up. Economic feasibility studies would also be valuable to ensure accessibility in resource-limited settings.

Conclusion

This study demonstrates that silver nanoparticles biosynthesized using *Gmelina arborea* and *Hyptis suaveolens* possess potent larvicidal activity against wild *Anopheles* larvae, with toxicity increasing across concentration and exposure duration. The lower LC_{50} and LC_{95} values recorded for *H. suaveolens* indicate greater bioefficacy, likely due to synergistic phytochemical interactions that enhance nanoparticle action. These findings provide compelling evidence that green-synthesized AgNPs represent a promising alternative to conventional insecticides currently facing widespread resistance in malaria vectors. Their multi-target mechanism of action, eco-friendly synthesis, and potential scalability support their integration into sustainable vector-control strategies within endemic regions of Nigeria and beyond. Further studies including nanoparticle characterization, evaluation of environmental safety on non-target organisms, formulation improvement, and field-based application trials are recommended to advance these nanobiolarvicides toward operational deployment in malaria prevention programs.

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