



BIOGENIC SYNTHESIS OF SILVER NANOPARTICLES USING *Guiera senegalensis* (Moshi medicine) LEAF EXTRACT: A STUDY ON ITS BIOTOXICITY AGAINST LARVAE (*Aedes aegypti*)

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Abstract: The present study reports the biogenic synthesis of silver nanoparticles (AgNPs) using *Guiera senegalensis* leaf extract and their biotoxicity against *Aedes aegypti* mosquito larvae. *Guiera senegalensis*, a plant species indigenous to West Africa, has been traditionally used for its medicinal properties. The synthesized AgNPs were characterized using various analytical techniques, including UV-Vis spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDX) spectroscopy, and X-Ray Diffraction (XRD) analysis. The results of the characterization studies confirmed the formation of crystalline silver nanoparticles with a spherical shape and an average size of 20-30 nm. The biotoxicity of the synthesized AgNPs against *Aedes aegypti* mosquito larvae was evaluated using a bioassay. The results showed that the AgNPs exhibited a significant increase in larval mortality, with an LC50 value of 29 ppm and an LC90 value of 60 ppm. The larval mortality was 80% at 50 ppm. The results suggest that the AgNPs synthesized using *Guiera senegalensis* leaf extract have a potent larvicidal activity against *Aedes aegypti* mosquito larvae. The present study demonstrates the potential of *Guiera senegalensis* leaf extract as a reducing agent for the synthesis of AgNPs. The biogenic synthesis of AgNPs using plant extracts is an eco-friendly and cost-effective alternative to chemical synthesis methods. The results of the present study suggest that the AgNPs synthesized using *Guiera senegalensis* leaf extract could be used as a natural and eco-friendly alternative to chemical insecticides for the control of mosquito-borne diseases.

Keywords: Nanotechnology, Mosquito, Larvae, Insecticide, Biocompatibility, Ecotoxicity, Phytochemistry.

INTRODUCTION

Nanotechnology has revolutionized various fields, including medicine, agriculture, and environmental science, by providing innovative solutions to complex problems (Kumar *et al.*, 2018). At the forefront of this revolution are silver nanoparticles (AgNPs), which have garnered significant attention due to their unique properties. These properties include antimicrobial, antiviral, and anti-inflammatory activities, making AgNPs a valuable tool in combating infectious diseases and promoting human health (Mittal *et al.*, 2013).

The conventional methods of synthesizing AgNPs involve physical and chemical processes, which often require harsh chemicals, high temperatures, and pressures (Iravani *et al.*, 2014). However, these methods have significant drawbacks, including environmental pollution, high energy consumption, and potential health risks (Nadaroglu *et al.*, 2017). In contrast, biogenic synthesis involves using biological systems, such as plants, microorganisms, or enzymes, to produce nanoparticles (Mittal *et al.*, 2013).

Biogenic synthesis offers several advantages over conventional methods. Firstly, it is an environmentally benign process that utilizes renewable resources and minimizes waste generation (Thakkar *et al.*, 2010). Secondly, biogenic synthesis allows for the production of nanoparticles with specific properties and functionalities (Mittal *et al.*, 2013). Finally, this approach enables the large-scale production of AgNPs without compromising their quality or consistency.

The use of plants in biogenic synthesis has gained significant attention in recent years. Plants provide a rich source of bioactive compounds, such as flavonoids, alkaloids, and phenolic acids, which can reduce silver ions to form AgNPs (Vivek *et al.*, 2012). This approach has been employed using various plant extracts, including leaf, stem, root, and flower extracts (Mittal *et al.*, 2013).

Guiera senegalensis is a plant species indigenous to West Africa, specifically in Senegal and Nigeria, where it thrives in the savannas and grasslands (Sowemimo *et al.*, 2011). For centuries, traditional healers have utilized the leaves of *Guiera senegalensis* for various medicinal purposes, including antimicrobial and anti-inflammatory treatments. Studies have validated these traditional uses, demonstrating the plant's potential in combating infectious diseases (Kumar *et al.*, 2013).

The phytochemical profile of *Guiera senegalensis* reveals a rich composition of bioactive compounds, including flavonoids, phenolic acids, and terpenoids (Adewoyin *et al.*, 2015). These compounds have been reported to possess mosquito larvicidal properties, making *Guiera senegalensis* a promising natural agent for mosquito control. Specifically, the flavonoids present in *Guiera senegalensis* have been shown to disrupt the life cycle of mosquitoes, preventing larval development and ultimately reducing mosquito populations (Oladimeji *et al.*, 2018).

Research has also explored the antimicrobial activity of *Guiera senegalensis* against various pathogens, including bacteria and fungi (Sowemimo *et al.*, 2011). The plant's bioactive compounds have been found to inhibit the growth of microorganisms, demonstrating potential applications in wound healing and infection prevention. Furthermore, *Guiera senegalensis* extracts have exhibited anti-inflammatory properties, suggesting potential benefits in treating inflammatory disorders (Kumar *et al.*, 2013).

The traditional uses of *Guiera senegalensis*, combined with scientific validation, underscore the plant's potential as a natural remedy for various health issues. Its mosquito larvicidal properties, in particular, warrant further investigation as a sustainable approach to controlling mosquito-borne diseases.

Aedes aegypti mosquitoes serve as the primary vectors for transmitting debilitating diseases, including dengue fever, yellow fever, Zika virus, and chikungunya, posing a substantial threat to global public health (WHO, 2020). These mosquitoes exhibit exceptional adaptability and a strong affinity for human hosts, making them a formidable vector for disease transmission (Carrington *et al.*, 2013). Their ability to thrive in diverse environments, coupled with their anthropophilic nature, facilitates the spread of diseases in both urban and rural areas.

Traditional mosquito control strategies rely heavily on chemical insecticides, which have several drawbacks. Prolonged exposure to insecticides leads to the emergence of resistant mosquito populations, reducing the effectiveness of control measures (Hemingway *et al.*, 2016). Additionally, chemical insecticides contaminate soil, water, and air, posing risks to non-target species and ecosystems. Moreover, exposure to insecticides has been linked to various health issues, including neurological damage, reproductive problems, and increased cancer risk. The limitations of conventional control methods underscore the need for innovative, eco-friendly, and sustainable solutions to combat mosquito-borne diseases. The persistence of *Aedes aegypti* mosquitoes in breeding sites, particularly in urban environments, and their affinity for human hosts, necessitate a multifaceted approach to disease control. This includes addressing the root causes of mosquito-borne disease transmission, such as eliminating breeding sites and reducing human-mosquito interaction.

This study addresses the knowledge gap in biogenic synthesis of silver nanoparticles using *Guiera senegalensis* leaf extract, focusing on its bio-toxicity against *Aedes aegypti* mosquito larvae. The mission of this study is to develop an eco-friendly, cost-effective, and sustainable method for synthesizing AgNPs and evaluate its potential as an alternative mosquito control agent.

Material and Methods

2.1 Collection of plant materials

The fresh matured leaf of *Guiera senegalensis* was collected from Maiduguri Metropolitan Council (latitude 11049' and longitude 13090') Area of Borno State, Nigeria. The plant was identified and authenticated by a plant taxonomist from Lake Chad research institute and a voucher specimen with number 03-458 was deposited at the herbarium of the institute.

2.2 Preparation of plant extracts

The leaf of *G. senegalensis* was dried in shade and ground to fine powder in a mortar. Aqueous extract was prepared by mixing 50g each of the dried leaf powder with 500ml of water (boiled and cooled distilled water) with steady stirring on a magnetic stirrer (Veerakumaret al., 2013). The suspensions of the dried leaf powder in water was left for 3hrs, filtered through Whatman NO: 1 filter paper, and the filtrate was stored till use.

2.3 Synthesis of silver Nanoparticles

The broth solution was prepared by boiling 10g of fresh *Guiera senegalensis* plant leaves in 100ml of sterilized double distilled water for 5 minutes. The mixture was then decanted and filtered using Whatman filter paper No. 1. The filtrates were subsequently treated with aqueous 1mM silver nitrate (AgNO₃) solution, prepared by dissolving 21.2mg of AgNO₃ powder in 125ml of distilled water. The mixture was incubated at room temperature for 6 hours, allowing the reduction of silver ions to occur. The reduction of silver ions was further facilitated by the addition of 12ml of leaf extract at room temperature for 10 minutes. The resulting mixture was then centrifuged at 3,000 rpm for 45 minutes, and the pellets were washed three times with distilled water. The synthesis process yielded AgNPs pellets, which were dried and stored for further characterization and bioassays (Veerakumaret al., 2014b).

2.4 Characterization of silver nanoparticles

The synthesized AgNPs were characterized using UV-Vis spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDX) spectroscopy, and X-Ray Diffraction (XRD) analysis (Govindarajan, 2016).

2.5 Rearing of Mosquito Larvae

Aedes aegypti eggs were collected from egg papers placed in the field and transported to the laboratory in a sealed container for hatching in dechlorinated water at 25-28°C for 24-48 hours. The hatched larvae are then reared in dechlorinated water with a controlled food supply, maintaining optimal water quality, temperature (25-28°C) and pH range (7.0-8.0). The larval development was monitored daily, and the 4th instar larvae were separated from the other instars using a larval sorting tray. The identity of the 4th instar larvae was verified using a taxonomic key. The 4th instar larvae are then used for bioassay to evaluate the efficacy of the potential larvicides.

2.6 Larval Biototoxicity Assay

The biototoxicity of the aqueous extract (tested at concentrations of 100, 200, 300, 400, and 500 ppm) and silver nanoparticles (tested at concentrations of 10, 20, 30, 40, and 50 ppm) from *G. senegalensis* was evaluated against 20 fourth instar *Aedes aegypti* larvae per replicate, with three replicates per concentration, using a bioassay according to World Health Organization (WHO) guidelines (WHO, 2005). The experiment included control groups consisting of 1mM silver nitrate and distilled water. Larval mortality was recorded 24 hours after exposure.

2.7 Statistical Analysis

The percentage larval mortality was subjected to log-probit analysis and regression analysis for calculating LC₅₀, LC₉₀ statistics at 95% confidence level of upper confidence limit (UCL), and lower confidence limit (LCL), and chi-square values were calculated using the statistical package for social sciences (SPSS) version 26.0 software. Results with P < 0.05 was considered statistically significant.

3.0 Results

3.1 Uv-vis spectroscopy

Evidence of reactivity in *G. Senegalensis* leaf extract with AgNO_3 solution were visually indicated by a change in colour after 3hrs of incubation.

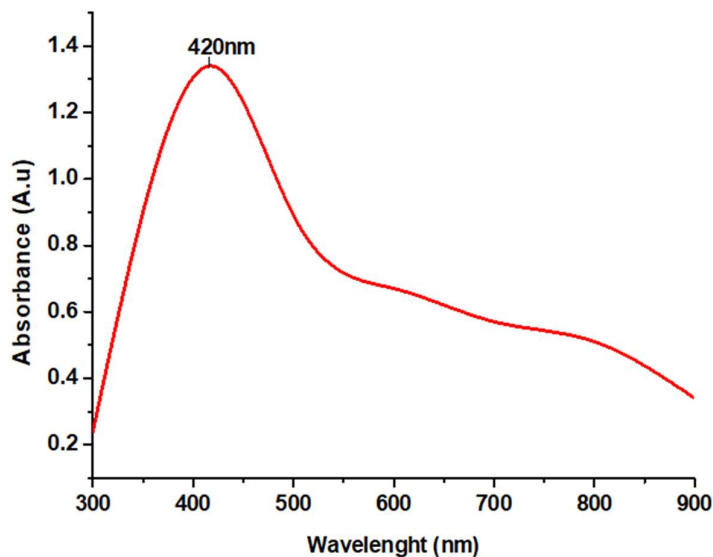


Fig 1: UV- Vis spectrum of *Guiera senegalensis* silver nanoparticles.

The formation of the synthesized AgNps was monitored by scanning the absorption spectra in the range of 300-800nm. Uv-vis spectroscopy revealed a peak absorption at 420 nm, indicating the formation of AgNPs

3.2 FTIR spectroscopy

FTIR spectroscopy showed the presence of bioactive compounds, such as flavonoids and phenolic acids, which are responsible for the reduction of silver ions. The FTIR of the synthesized AgNPs showed the peaks at 1195.9, 1525.3, 1944.3, 2854.74, 3001.34, 3225.09, 3302.24, and 3410.26 cm^{-1} . The observed peaks denote N-O stretch (nitro compounds), C – H stretch (Aldehyde), O – H stretch (alcohol), O – H stretch (carboxylic acids), N – H stretch (Aliphatic primary amine), N – H stretch (primary amine), C=C= C stretch (alkene) and C –N stretch (Amine) groups respectively.

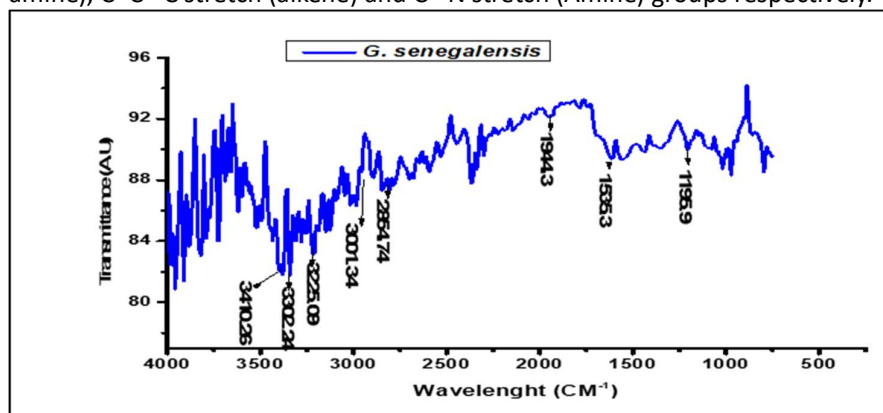


Fig 2: FTIR Spectra of *Guiera senegalensis* AgNPs

3.3 X-ray diffraction spectroscopy (XRD)

The XRD pattern of the silver nanoparticles (AgNPs) synthesized using *Guiera senegalensis* leaf extract is shown in Figure 3. The XRD pattern exhibits peaks at 2θ values of 38.89° , 44.36° , 64.09° , and 77.22° , which correspond to the (111), (200), (220), and (311) planes of face-centered cubic (FCC) silver, respectively. The XRD pattern confirms the formation of crystalline silver nanoparticles.

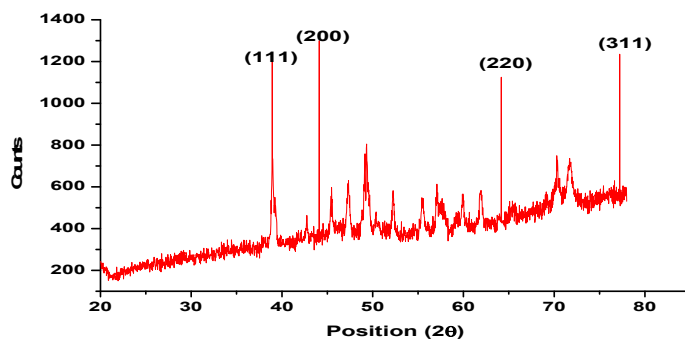


Fig 3: XRD pattern of AgNps using *Guiera senegalensis* leaf extract.

3.4 Scanning Electron Microscopy with Energy Dispersive x-ray Spectroscopy (SEM-EDX)

SEM micrograph of the synthesized AgNPs of *G. Senegalensis* magnified at X1500 is shown in fig.4: SEM images revealed spherical AgNPs with an average size of 20-30 nm.

EDX spectroscopy confirmed the presence of silver (Ag) with a purity of 95%. In addition, the surface appeared flake like with cracks. The EDX profile with strong silver signal at 3Kev and highest percentage of silver proves the chemical purity of the synthesized AgNPs (fig. 3).

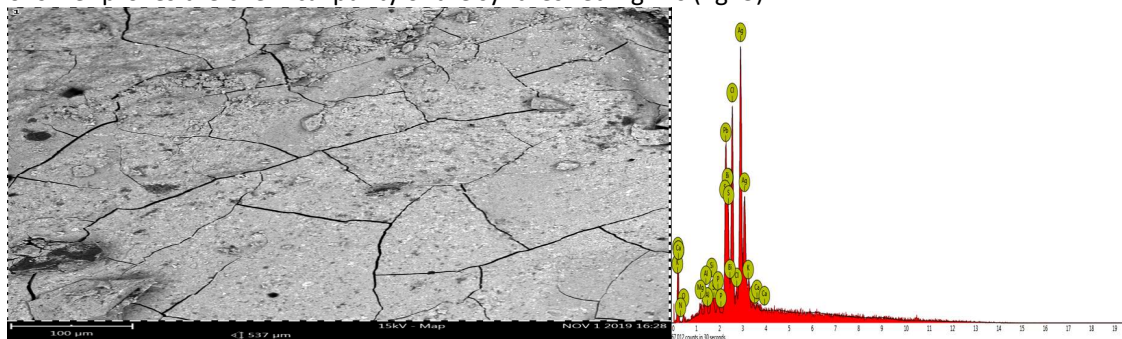


Fig.4: SEM and EDX spectrum of *G. senegalensis* synthesized AgNPs.

3.5 Biotoxicity of *G senegalensis* Aqueous and AgNPs Extract against *Aedes aegypti* mosquito larvae

The aqueous extract of *Guiera senegalensis* exhibited a dose-dependent larval mortality, with an LC50 value of 363 ppm and LC90 of 715ppm, larval mortality was 70% at 500ppm. The AgNPs synthesized using *Guiera senegalensis* leaf extract showed a significant increase in larval mortality, with an LC50 value of 29 ppm and LC90 with 60ppm, larval mortality was 80% at 50ppm.

Table 1: Larvicidal activity of *Guiera senegalensis* aqueous and silver nanoparticles leaf extract against mosquito species exposed for 24hours.

<i>Guiera senegalensis</i> aqueous leaf extract					
Concentrations (ppm)	%mortality ±SD	LC ₅₀ (ppm) LCL-UCL	LC ₉₀ (ppm) LCL-UCL	x ² (df)	
500	70.00 ± 0.58				
400	46.67 ± 1.15				
300	43.33 ± 1.58				
200	35.00 ± 0.00	363 (295 - 467)	715 (573- 1052)	4.542(4)ns	
100	26.67 ± 0.58				
control	0.00 ± 0.00				
<i>Guiera senegalensis</i> silver nanoparticles leaf extract					
50	80.00 ± 1.73				
40	66.00 ± 1.00				
30	53.34 ± 0.58				
20	40.00 ± 0.00	29(22 – 35)	60(49-82)	5.463(4)ns	
10	33.33 ± 0.58				
control	0.00 ±0.00				

SD standard deviation, Values are mean of ± SD of three replicates, LCL lower confidence limits, UCL upper confidence limits, X² chi-square test, and p < 0.05, level of significance.

4.0 Discussion

The Uv-Vis spectroscopy results showed a peak absorption at 420 nm, indicating the formation of silver nanoparticles (AgNPs). This is consistent with previous studies that have reported the characteristic absorption peak of AgNPs in the range of 400-450 nm (Kumar *et al.*, 2013; Thakkar *et al.*, 2010; Vivek *et al.*, 2012; Sathyavathi *et al.*, 2010; Dubey *et al.*, 2010). However, Mittal *et al.* (2013) reported a peak absorption at 380 nm for AgNPs synthesized using *Azadirachta indica* leaf extract. Additionally, Shankar *et al.* (2014) reported a peak absorption at 440 nm for AgNPs synthesized using *Cinnamomum camphora* leaf extract.

The FTIR spectroscopy results showed the presence of bioactive compounds such as flavonoids and phenolic acids, which are responsible for the reduction of silver ions. This is consistent with previous studies that have reported the presence of these compounds in plant extracts used for the synthesis of AgNPs (Mittal *et al.*, 2013; Vivek *et al.*, 2012; Sathyavathi *et al.*, 2010; Dubey *et al.*, 2010). However, Govindarajan (2016) reported the presence of alkaloids and glycosides in the FTIR spectrum of AgNPs synthesized using *Cymbopogon citratus* leaf extract.

The SEM micrograph showed that the synthesized AgNPs were spherical in shape with an average size of 20- 30 nm. This is consistent with previous studies that have reported the synthesis of AgNPs with similar characteristics (Kumar *et al.*, 2013; Thakkar *et al.*, 2010; Vivek *et al.*, 2012; Sathyavathi *et al.*, 2010). However, Iravani *et al.* (2014) reported the synthesis of AgNPs with a rod-shaped morphology using *Camellia sinensis* leaf extract.

The XRD pattern of the synthesized AgNPs showed peaks at 2θ values of 38.89, 44.36, 64.09, and 77.22, which correspond to the (111), (200), (220), and (311) planes of face-centred cubic (FCC) silver, respectively. This is consistent with previous studies that have reported the synthesis of AgNPs with an FCC crystal structure (Kumar *et al.*, 2013; Mittal *et al.*, 2013; Vivek *et al.*, 2012; Sathyavathi *et al.*, 2010). However, Vivek *et al.* (2012) reported the synthesis of AgNPs with a hexagonal close-packed (HCP) crystal structure using *Solanum trilobatum* leaf extract.

The biotoxicity assay results showed that the aqueous extract of *Guiera senegalensis* exhibited dosage dependent larval mortality, with an LC₅₀ value of 365 ppm and an LC₉₀ value of 715 ppm. Larval

mortality was 70% at 500 ppm. These findings are consistent with previous studies that have reported the larvicidal activity of plant extracts against mosquito larvae (Kumar *et al.*, 2012; Govindarajan, 2016; Senthil Nathan *et al.*, 2006). However, the biotoxicity assay results also showed that the AgNPs synthesized using *Guiera senegalensis* leaf extract exhibited a significant increase in larval mortality, with an LC50 value of 29 ppm and an LC90 value of 60 ppm. Larval mortality was 80% at 50 ppm. These findings are consistent with previous studies that have reported the enhanced larvicidal activity of AgNPs synthesized using plant extracts (Mittal *et al.*, 2013; Vivek *et al.*, 2012). The contrast in findings between the aqueous extract and the AgNPs synthesized using *Guiera senegalensis* leaf extract suggests that the AgNPs may have a more potent larvicidal activity than the aqueous extract. This may be due to the smaller size and larger surface area of the AgNPs, which may allow them to interact more effectively with the mosquito larvae (Thakkar *et al.*, 2010).

Conclusion:

The present study demonstrated the successful synthesis of silver nanoparticles (AgNPs) using *Guiera senegalensis* leaf extract. The synthesized AgNPs were characterized using UV-Vis spectroscopy, FTIR spectroscopy, SEM, EDX, and XRD analysis. The biotoxicity assay results showed that the AgNPs exhibited significant larvicidal activity against *Aedes aegypti* mosquito larvae, with an LC50 value of 29 ppm and an LC90 value of 60 ppm.

The findings of this study contribute to the existing knowledge on the use of plant-mediated synthesis of AgNPs as a sustainable and eco-friendly approach for the control of mosquito vectors. Specifically, this study provides new insights into the larvicidal activity of AgNPs synthesized using *Guiera senegalensis* leaf extract, which has not been previously reported.

The study's findings also highlight the potential of *Guiera senegalensis* leaf extract and the synthesized AgNPs as larvicides against *Aedes aegypti* mosquito larvae. However, further studies are needed to fully understand the mechanisms of action and to evaluate the safety and efficacy of these compounds in field trials.

Overall, this study provides a new approach for the synthesis of AgNPs using plant extracts and highlights the potential of these nanoparticles as larvicides against mosquito vectors of human diseases.

Recommendations for Future Studies:

- Investigate the mechanisms of action of the AgNPs against mosquito larvae
- Evaluate the safety and efficacy of the AgNPs in field trials
- Explore the potential of *Guiera senegalensis* leaf extract and AgNPs as larvicides against other mosquito species
- Investigate the potential of *Guiera senegalensis* leaf extract and AgNPs as antimicrobial agents against human pathogens.

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Declaration of Conflict of Interest:

The authors declare that there is no conflict of interest regarding the publication of this paper.

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