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Research Article

Larvicidal Efficacy of Green-Synthesized Silver Nanoparticles from *Alstonia boonei* and *Terminalia catappa* against *Anopheles gambiae*

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About Article

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ABSTRACT

This study investigates the eco-friendly synthesis of silver nanoparticles (AgNPs) using aqueous extracts of *Alstonia boonei* (AA) and *Terminalia catappa* (TCA) and evaluates their larvicidal efficacy against the fourth-instar larvae of *Anopheles gambiae*. The bio-reduction of silver ions (Ag^+) to elemental silver (Ag^0) was confirmed by a color change and the Surface Plasmon Resonance (SPR) peak at 435 nm, indicating successful AgNP formation. FTIR analysis identified functional groups, such as O-H, C-H, and N groups, which acted as both reducing and capping agents. Scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDXs) confirmed the presence of silver (77.20 %) in the biosynthesized nanoparticles. The larvicidal activity was evaluated following WHO (2005) protocol, different concentrations of the nanoparticles: 50, 100, 150, 200 and 250 mgL^{-1} was used, 25 fourth-instar larvae of was exposed to each concentration, with three replicate for each test. Both AA and TCA-synthesized AgNPs exhibited concentration-dependent larvicidal activity. AA proved to be the most potent, with a lethal concentration (LC_{50}) of 65.2 % CI after just one hour of exposure. Upon the initial treatment, the TCA was not very effective as compared to AA. After 12 hours, it became much more potent, with an LC_{50} of 34.5 %. The tiny size of the nanoparticles helped them get through the larval membrane easily. Once inside, they invade the cells, which caused the larvae to die. This shows that the silver nanoparticles, made from these plant extracts, could be a great, eco-friendly way to control mosquitoes.

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1. INTRODUCTION

The increasing incidence of malaria-related mortality, primarily caused by *Anopheles* mosquitoes, has made it of great necessity to find new alternative ways that are both safe for the environment and sustainable to control these mosquito vectors. The common chemical sprays which have been used for a long time are facing a bunch of problems which include insect resistance, environmental hazards, and toxicity to both humans and other helpful creatures. Hence, the quest to find a better, ecofriendly, cost-friendly, and more targeted way to manage these pests (Kumar & Yadav, 2020; Suman *et al.*, 2021).

One of the best way to get rid of mosquitoes is to target them at their larvae stage in stagnant water. At this stage, they are easy to find and can't move around much, unlike adult mosquitoes (Benelli, 2015; Murugan *et al.*, 2017). This study focused on creating nanoparticles in an eco-friendly way to use as a powerful weapon against mosquito larvae through green synthesis of nanoparticles as a novel larvicidal agent. It specifically explores the use of aqueous leaf extracts from *Alstonia boonei* and *Terminalia catappa*, two plants with well-documented medicinal properties, to synthesize silver nanoparticles. The objective is to characterize these nanoparticles and evaluate their potential as a groundbreaking, plant-based, and environmentally sustainable solution for controlling *An. gambiae* mosquitoes, thereby offering a viable alternative to synthetic chemical insecticides.

Despite the growing body of research on green-synthesized nanoparticles for pest control, a significant research gap remains in the exploration of specific, regionally abundant medicinal plants. While various plant extracts have been used to create larvicidal nanoparticles, there is a distinct lack of studies focusing on the potential of *Alstonia boonei* and *Terminalia catappa* for this purpose. These plants are known to contain a rich variety of phytochemicals with proven biological activities, yet their specific application in synthesizing silver nanoparticles for targeted *An. gambiae* larval control remains largely unexplored. This study, therefore, addresses this gap by investigating the larvicidal potential of nanoparticles synthesized using these two specific plant extracts, thereby contributing a novel, plant-based solution to the field of sustainable vector control.

2. LITERATURE REVIEW

The use of medicinal plants in nanotechnology is on the increase as they both are eco-friendly and have cost-effective potential for nanoparticle synthesis (Makarov *et al.*, 2014). Among these plants, *A. boonei* and *T. catappa* are known for their rich phytochemical composition, which mediate the green synthesis of metallic nanoparticles with potent bioactive properties.

A. boonei, which is commonly known as fetish tree, is a medicinal plant of Africa origin. The leaf extracts are known to have bioactive compounds such as alkaloids, polyphenols, and terpenoids, which play very important role in the green synthesis of nanoparticles, particularly silver nanoparticles (AgNPs) (Adebayo *et al.*, 2020). The polyphenols present in the leaf and their hydroxyl (-OH) groups, aid in reducing, converting metal ions into nanoparticles, while alkaloids and other phytochemicals serve as capping agents, ensuring stability and stopping aggregation, qualifying *A. boonei* as an

efficient natural medium for producing larvicidal nanoparticles. *T. catappa* (Indian almond tree) leaves are rich in polyphenols, flavonoids, and tannins, these bioactive compounds are essential for nanoparticle synthesis (Jeeva *et al.*, 2014). These compounds perform dual functions as reducing and capping agents, resulting to the formation of constant and stable nanoparticles. The dual-action mechanism of *T. catappa* leaf extracts ensures the production of highly effective nanoparticles with efficient larvicidal properties, thereby emphasizing its potential in nanotechnology.

While other green synthesis methods have been explored, including those using microorganisms like bacteria and fungi, or other non-plant biological materials such as yeast and algae, plant-based synthesis offers distinct advantages (Patil *et al.*, 2020). For example, microbial methods can be time-consuming and require specific culture conditions, which can increase production costs and complexity (Duran *et al.*, 2011). In contrast, plant extracts provide a more straightforward and rapid single-step process. Within plant-based synthesis, many studies have focused on well-known sources like neem (*Azadirachta indica*) and basil (*Ocimum sanctum*) due to their known insecticidal properties (Murugan *et al.*, 2017). However, our study distinguishes itself by leveraging the unique phytochemical profiles of *A. boonei* and *T. catappa*. The specific combination of alkaloids, polyphenols, and flavonoids in these two plants offers a novel and highly effective pathway for creating potent larvicidal silver nanoparticles. This approach expands the library of viable plant sources for sustainable nanoparticle synthesis, providing a crucial alternative to more commonly studied plants and non-plant methods, thus filling a significant gap in the current literature.

3. METHODOLOGY

3.1. Collections of plant material

Fresh *T. catappa* and *A. boonei* leaves were collected in their full thriving stage around Omun, Coker Osogbo (Longitude: E 4.60388, Latitude: N 7.76117). They were validated in the taxonomy section of the Department of Plant Biology of Osun State University, Osogbo. The plants were washed to remove dust, dried under room temperature for 7-14 days, the leaves were then collected and blended separately into fine particles and preserved in different airtight container till use. Other parts (Leaves, fruits, flowers and bark) of the plants were collected and taken to the Department of Plant Biology Laboratory, Osun state University, Osogbo for further identification, a voucher was deposited in the lab.

3.2. Preparation of plant extract

To prepare the aqueous extract, 10 grams of each of the already blended leaf extract was diluted with 1000 ml of distilled water, stirred allowed for 24 hours under room temperature, the solution was then be filtered using Whatman filter paper NO. 1 and refrigerated till use.

3.3. Green-synthesis of silver nanoparticles

Green synthesis of AgNPs using *A. boonei* and *T. catappa* leaf extract was done following the procedures reported by Lateef *et al.*, (2016). The reduction of silver ions was achieved by adding



7 mL of the extract with 293 mL of AgNO_3 solution (1mM), while the reduction of ferrous ions was achieved by adding 10 mL of the extract with 1 mL of FeSO_4 solution and both were incubated at room temperature. The transparent brown color seen indicates the formation of silver nanoparticles while the amber color in FeNPs gives the formation of iron nanoparticles. The solution was stored in a clean air-tight container and refrigerated until use.

3.4. Characterization of Nanoparticles

3.4.1. UV-visible spectroscopy

The synthesized nanoparticles were analyzed for Surface Plasmon Resonance with wavelength ranging from 200 - 1000 nm at room temperature using a UV-Visible spectrophotometer (Biobase BK-UV1900 P spectrometer, China).

3.5. Fourier transform-infrared spectrometer (FTIR) analysis

The FTIR spectrum of synthesized nanoparticles was mediated to identify the characteristic functional groups of bioactive components using an infrared spectrum analyzer (SHIMADZU FTIR-8400S). The spectra were measured between 400 and 4000 cm^{-1} in frequency.

3.6. Scanning electron microscope (SEM) analysis

SEM (Phenom PRO X SEM-MVE01570775) was used to determine synthesized nanoparticles size and shape. The constituent of the nanoparticles was analyzed with Energy Dispersive X-ray Fluorescence (EDXRF) Spectrum (ARL QUANT'X EDXRF Analyser, Serial No.9952120).

3.7. Larvae collection and maintenance

The third and fourth larvae instars of *Anopheles gambiae s.l* were collected from in and around Freedom park area of Osogbo, Osun state, with the help of "O" type brush. These larvae were brought to the laboratory and maintained in 500 mL of water. Further identification was carried out at the Department of Animal and Environmental Biology, Osun State University, Nigeria.

3.8. Preparation of plant extract for larvicides

To prepare the aqueous extract, 50 g of powdered leaf and 500 mL of distilled water was heated for 15 min using a magnetic stirrer/hot plate at 70°C (78HW-1, JINOTECH). The extract was filtered and refrigerated in an airtight bottle for preservation until use.

3.9. Larvicidal bioassay

The larvicidal activity was evaluated following the guidelines

for laboratory and field testing of mosquito larvicides (WHO,2005). From the stock solution, different concentrations of aqueous extracts of nanoparticles: 50, 100, 150, 200 and 250 mg/L were prepared and 25 fourth-instar larvae were exposed to each concentration. Larvae in the control were exposed to 250 mL of distilled water. Three replicates were performed for each test. After 24 hours, immovable larvae will be considered dead and their number were recorded respectively.

3.10. Statistical analysis

Data from all replicates will be pooled for analysis. LC_{50} and LC_{90} values are calculated from a log dosage-probit mortality regression line using SPSS version 25.0. Standard deviation or confidence intervals of the means of LC_{50} values were calculated and recorded on a form. A test series is valid if the relative standard deviation (or coefficient of variation) is less than 25% or if confidence limits of LC_{50} overlap (significant level at $P < 0.05$). The potency of the chemical against the larvae of a particular vector and strain can then be compared with the LC_{50} or LC_{90} values of other insecticides.

4. RESULTS AND DISCUSSION

4.1. Green-synthesis and characterization of AgNPs synthesized from both *A. boonei* and *T. catappa*

After an incubation period, the yellow solution gradually turned transparent brown, indicating the bio-reduction of silver ions (Ag^+) to elemental silver (Ag^0). This gradual change in color over time evidently reflects the nucleation and growth stages of the synthesized silver nanoparticles (AgNPs).

UV-visible spectroscopy analysis was between 190 and 700 nm revealed a distinct absorption peak at 435 nm. This peak is characteristic of Surface Plasmon Resonance (SPR) and confirms the formation of AgNPs.

The presence of specific functional groups from the bioactive constituents was identified through analysis. Prominent bands were observed at 3256, 3924, 2353, 1559, 1385, and 1072 cm^{-1} . These correspond to the O-H stretching of alcohols, C-H stretching of alkanes, C=C stretching, C=O stretching of alkenyls, C-H bending of the methylene group, and a nitrogen group, respectively. These functional groups are responsible for both the synthesis and capping of the nanoparticles.

Scanning Electron Microscopy with energy-dispersive X-ray spectroscopy (SEM-EDXs) images showed a fine, whitish-ash cloudy pattern, with the EDXs analysis confirming a substantial amount of silver and also the presence of other elemental components were identified as Ag (77.20 %), O (10.20 %), N (8.30 %), Si (2.20 %), and S (2.10 %). The significant presence of silver in the EDXs pattern provides definitive confirmation of the successful formation of AgNPs.



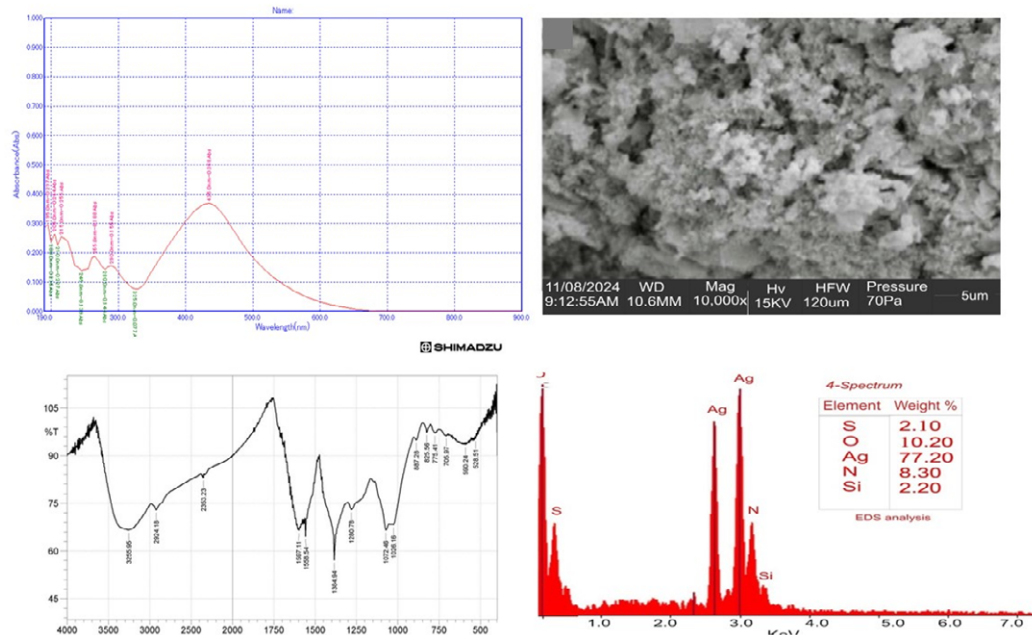


Figure 1. 1a-d UV-vis spectrum; b. FTIR Spectroscopy; c. SEM image; d. EDS spectrum of AgNPs of *A. boonei*

Table 1. Larvicidal activity of *terminalia catappa* and *alstonia boonei* silver nanoparticle against *anopheles gambiae* s.L larvae upon exposure at 1 and 6 hours respectively

Time taken	Extract	Mortality (%)		LC ₅₀ (50 %CI)	LC ₉₀ (90%CI)	LC ₉₅ (95%CI)	X ²	P value
<i>Anopheles</i>								
1hr	AA	Control	0.0 ± 0.0	65.2 (36.9 to 86.7)	215 (154.3 to 464.2)	322.6 (209.3 to 984.7)	16.56	<.0001
		50	11.7 ± 2.9c				.	.
		100	60.0 ± 10.0b				.	.
		150	63.3 ± 12.6b				.	.
		200	88.3 ± 10.4a				.	.
		250	95.0 ± 5.0a				.	.
6hrs	TCA	Control	0.0 ± 0.0	67.7 (19 to 100.5)	455.9 (246.3 to >>1000)	871.9 (372.9 to >>1000)	8.74	0.003
		50	30.0 ± 5.0c				.	.
		100	51.7 ± 7.6b				.	.
		150	51.7 ± 7.6b				.	.
		200	73.3 ± 7.6a				.	.
		250	85.0 ± 5.0a				.	.
12hrs	TCA	Control	0.0 ± 0.0	34.5 (2.3 to 60.1)	190.1 (122.4 to >>1000)	339.6 (185.7 to >>1000)	7.62	0.006
		50	61.7 ± 7.6b				.	.
		100	66.7 ± 5.8b				.	.
		150	68.3 ± 7.6b				.	.
		200	88.3 ± 2.9a				.	.
		250	98.3 ± 2.9a				.	.

LC₅₀: Lethal concentration for 50% mortality of larvae, LC₉₀: Lethal concentration for 90% mortality of larvae, LC₉₅: Lethal concentration for 95% mortality of larvae, 95% CI; 95% confidence interval, SD; Standard deviation. Means with different superscript are statistically different ($P < 0.05$) based on Duncan's multiple range tests.



4.2. Discussion

The findings from this study has proficiently showed the bio-reduction of silver ions (Ag^+) to silver nanoparticles (AgNPs) using an aqueous extract of both *A. boonei* and *T. catappa* respectively. The observed gradual change in color from yellow to a see through brown after an incubation period is a basic indicator of this process. This can be attributed to presence of the special bioactive compounds in the solution (aqueous leaf extract) which acted as reducing agents, common in green synthesis (Ibrahim, 2015; Kanwal *et al.*, 2019). The change in color with time is attributed to the different stages of nucleation and growth of the nanoparticles, agrees with the findings of Kanwal *et al.* (2019), where it was also note that the final color is a function of the size, shape, and concentration of the synthesized AgNPs.

The UV-visible spectroscopy analysis was used to confirm the formation of AgNPs revealing different absorbance peak. A distinct absorption peak appeared at 435 nm which is indicative of the Surface Plasmon Resonance (SPR) phenomenon, which is a collective oscillation of conduction electrons in response to incident light. This peak falls within the typical range for AgNPs formation at 400 to 450 nm, confirming the successful synthesis. This finding largely agrees with Mittal *et al.* (2014) and Sankar *et al.* (2013) stating that SPR is a primary method for confirming the presence of noble metal nanoparticles.

Fourier-transform infrared (FTIR) spectroscopy helps in identifying the functional groups responsible for both the reduction and stabilization of the AgNPs. The prominent bands observed at 3256, 3924, 2353, 1559, 1385, and 1072 cm^{-1} reveal a bunch of different chemical groups like O-H (from alcohols), C-H (from alkanes), C=C stretching, C=C stretching of alkenyls, C-H bending of the methylene group, and a nitrogen group, respectively. These functional groups, which is from the plant extract are notable in mediating the nanoparticles and also act as capping agents to keep them from clumping together also act as both reducing agents for the Ag^+ ions and as capping agents for the newly formed AgNPs (Mittal *et al.*, 2014). The presence of these capping agents is of great importance as they prevent agglomeration and provide stability to the nanoparticles. The O-H groups from polyphenols, in particular, are widely cited in the literature for their role in the bio-reduction process (Sankar *et al.*, 2013).

Finally, the Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM-EDXs) images showed a cloudy, whitish-ash pattern, and the analysis confirmed a whopping 77.20 % silver, with smaller amounts of oxygen, nitrogen, and sulfur, which further supports the idea that the capping agents from the plant extract were stuck to the surface (Sankar *et al.*, 2013). Although, tiny bit of silicon was seen, this can be attributed presences of an impurity. The clear evidence from the SEM-EDs analysis was the final confirmation that we successfully made the AgNPs. The detection of other elements like oxygen, nitrogen, and sulfur further supports the FTIR data, suggesting that compounds containing these elements from the plant extract are adsorbed onto the surface of the nanoparticles, acting as capping agents.

Alstonia boonei-synthesized silver nanoparticles (AA) were the most effective against fourth-instar *Anopheles gambiae* larvae. After a one-hour exposure to concentrations ranging from 50

–250 mg/gL, mortality rates were observed at 11.7 % to 95.0%, with specific values of 11.7 ± 2.9 %, 60.0 ± 10.0 %, 63.3 ± 12.6 %, 88.3 ± 10.4 %, and 95.0 ± 5.0 %. The lethal concentration required to kill 50% of the larvae (LC_{50}) was 65.2 % CI, while the LC_{90} and LC_{95} were 215 % CI and 332.6 % CI, respectively.

Terminalia catappa-synthesized silver nanoparticles (TCA) showed varying efficacy depending on the exposure time. At 6 hours, the mortality rates were 30.0 ± 5.0 %, 51.7 ± 7.6 %, 51.7 ± 7.6 %, 73.3 ± 7.6 %, and 85.0 ± 5.0 %. For this exposure time, the LC_{50} was 67.7 % CI, the LC_{90} was 455.9 % CI, and the LC_{95} was 871.9 % CI. After 12 hours of exposure, mortality increased significantly to 61.7 ± 7.6 %, 66.7 ± 5.8 %, 68.3 ± 7.6 %, 88.3 ± 2.9 %, and 98.3 ± 2.9 %. The corresponding lethal concentrations were much lower, with an LC_{50} of 34.5 % CI, an LC_{90} of 190.1 % CI, and an LC_{95} of 339.6 % CI.

The both nanoparticles exhibited more larvicidal potency at higher concentrations. This finding resonates with other studies in similar relationship between nanoparticle concentration and lethality in different types of mosquito larvae. The key to their effectiveness seems to be their tiny size. The nanoparticles are small enough to easily get through the larvae's protective cellular membranes, which is the first step in their fatal attack. Once inside the cell, the nanoparticles go on the offensive. They actively seek out and bind to crucial cellular components, particularly proteins and DNA. When they bind to these molecules, they cause them to lose their shape and become completely useless. This cellular chaos ultimately leads to the breakdown of the cell membrane, which disrupts its power supply. With no way to maintain its energy, the cell can no longer function, leading to its death and, eventually, the death of the mosquito larva.

It is important to acknowledge that while these silver nanoparticles show great promise as a targeted larvicidal agent, their potential impact on non-target species in aquatic ecosystems needs to be thoroughly investigated. The same properties that make them effective against mosquito larvae—their small size and high reactivity—could pose a risk to other beneficial aquatic organisms such as plankton, fish, and beneficial insects. The release of silver ions from the nanoparticles and their accumulation in the environment are critical areas for future research to ensure the long-term ecological safety and sustainability of this approach. Therefore, a comprehensive eco-toxicological assessment is a crucial next step to fully validate these nanoparticles as a safe and viable alternative to conventional chemical insecticides.

5. CONCLUSION

In conclusion, this study successfully demonstrated the larvicidal efficacy of green-synthesized silver nanoparticles (AgNPs) derived from *A. boonei* and *T. catappa* against *An. gambiae* mosquito larvae. The findings indicate a clear dose-dependent relationship, with increased larval mortality observed at higher concentrations of both types of nanoparticles. This potent effect is attributed to the small size of the nanoparticles, which facilitates their penetration of the larval membrane and subsequent disruption of cellular functions through the denaturation of essential proteins and DNA. This ultimately leads to a collapse of the cell's energy



system and, consequently, the death of the larva. The results highlight the potential of these eco-friendly nanoparticles as a promising, sustainable, and highly effective alternative for controlling this critical malaria vector.

RECOMMENDATIONS

Based on these findings, I recommend more research to deeply understand the long-term environmental impact and non-target effects on other animals of these green-synthesized nanoparticles is crucial. Also, future studies should seek to conduct a controlled field trials to validate the efficacy of these agents under natural conditions. The results of this study provide a strong base for the development of a sustainable, eco-friendly larvicide that could serve as a promising alternative to conventional chemical insecticides for effective and responsible mosquito control.

- *Data Availability Statement:* The data that support the findings of this study are available from the author upon reasonable request.

- *Conflict of interest statement:* The author states that there is no conflict of interest.

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