ISSN: 1410-8917
Jurnal Kimia Sains & Aplikasi
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 29 (7) (2026): 505-514

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa

Biogenic Silver Nanoparticles from Plumeria Extract and Their Larvicidal Potential Against *Aedes aegypti*

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https://doi.org/10.14710/jksa.29.7.505-514

Article Info

Article history:

Received: 02nd June 2026
Revised: 24th July 2026
Accepted: 18th August 2026
Online: 31st August 2026

Keywords:
Plumeria leaves; Green synthesis; Larvicide; Silver nanoparticles; *Aedes aegypti*

Abstract

Larvicides are a class of insecticides that chemically control the growth of dengue vectors. Although synthetic larvicides remain effective for mosquito control, their prolonged and excessive use may contribute to insecticide resistance and potential environmental impacts. In alternative literature, silver nanoparticles can be used to reduce these negative impacts, as they exhibit larvicidal activity. Organic waste, such as plumeria leaves that have fallen, wilted, and lost economic value, can serve as a bioreductor in the synthesis of silver nanoparticles. Green synthesis of silver nanoparticles was carried out by mixing the water extract of plumeria leaves with a 1 mM $AgNO_3$ solution at a 1:9 (v/v) ratio. The concentration of the plumeria leaf water extract used was 1%, and the heating temperature was 60°C. Silver nanoparticles were characterized using a UV-Vis spectrophotometer, PSA, TEM, and SEM-EDS, and their larvicidal activity was tested. Silver nanoparticles provide a maximum wavelength of 430 nm. The TEM and SEM-EDS characterization results show irregular spherical shapes, as well as square and triangular shapes, and PSA confirmed an average particle size of 72.39 nm with a zeta potential of -21.51 mV. Biosynthesized silver nanoparticles exhibit larvicidal activity with an LC_{50} of 13 ppm.

1. Introduction

Nanoparticles are a subset of nanomaterials, which are nanometer-sized materials studied in nanotechnology. The concept of nanoparticles extends beyond particle size to encompass synthesis methods and the study of new properties of the resulting nanomaterials. Nanoparticle materials generally range in size from 1 to 100 nm. Modification of materials at the nanometer scale is carried out to produce smaller materials with more optimal and efficient structures and characteristics [1]. Silver nanoparticles are one of the most widely developed nanotechnology materials due to their unique properties and broad applications in various biomedical and industrial fields [2].

Broadly speaking, silver nanoparticle synthesis can be performed using top-down (physical) and bottom-up (chemical) methods. However, these methods often use harsh chemicals and are less environmentally friendly, prompting the development of green synthesis methods as a safer alternative [3]. Although green synthesis reduces the use of hazardous chemicals during nanoparticle production, the potential ecotoxicity of silver nanoparticles, particularly in aquatic environments, should also be considered. The use of plant extracts (green synthesis) for silver nanoparticle synthesis is advantageous because the materials are readily available and non-toxic, the chemicals used are relatively few, and hazardous chemicals are minimized [4]. Plant extracts that can be used as bioreductors are those that contain compounds that can reduce Ag^+ ions in solution to Ag^0 [5]. Compounds found in plants that can function as reducing agents are terpenoids, phenolics, flavonoids, tannins, steroids, saponins, and alkaloids [6].

Indonesia is a country rich in plants, among them the plumeria (*Plumeria acuminata* L.). Previous research has shown that the methanol extract of plumeria leaves contains various secondary metabolites, such as terpenoids, flavonoids, tannins, alkaloids, steroids, saponins, glycosides, and carbohydrates [7]. These metabolites act as bioreductors in the synthesis of silver nanoparticles [6]. Especially in Bali, plumeria is one of the plants favored for decorating yards, offices, and public parks. Balinese people commonly use fresh plumeria flowers for prayer purposes. The dried flowers that fall from the tree are used as an ingredient in making incense [8]. However, the utilization of fallen leaves is usually low, leaving plumeria leaves with no commercial value. The use of plumeria leaves as a bioreductor for silver nanoparticles has been reported by Rudrappa *et al.* [9]. The use of organic waste from fallen and wilted Plumeria leaves as a bioreductor for the synthesis of silver nanoparticles remains rare, indicating significant development potential.

The use of silver nanoparticles as nanoinsecticides has been explored for dengue vector control [10]. Dengue hemorrhagic fever (DHF) is an endemic disease in tropical and subtropical regions and remains a major public health problem in Indonesia. Dengue is transmitted primarily by Aedes mosquitoes, particularly *Aedes aegypti* and *Aedes albopictus*. In early 2022, Indonesia experienced a significant increase in dengue cases, including in Bali Province [11]. In May 2023, the number of dengue cases in Buleleng Regency reached 782 cases [12]. According to the Indonesian Ministry of Health [13], as of March 26, 2024, dengue cases in regencies and cities across Indonesia reached 53,131 cases, with 404 deaths. This number increased the following week to 60,296 cases with 455 deaths. Although dengue can be treated, delayed treatment may lead to severe complications, such as dengue shock syndrome (DSS), which can be fatal. Therefore, prevention remains important and includes regular monitoring of mosquito larvae and the use of insecticides to control adult mosquitoes and their larvae.

Larvicides are a class of synthetic insecticides that chemically control the growth of disease vectors. A popular larvicide currently used is abate powder (Temephos). Intensive use of temephos can have negative impacts on human health, such as poisoning [14]. An alternative to controlling dengue vectors is the use of silver nanoparticle larvicides [10]. In previous research, silver has potential as a nanoinsecticide, with an LC_{50} of 0.28 ppm and an LC_{90} of 0.43 ppm against *Aedes aegypti* larvae [15]. Based on dengue fever case data, researchers are interested in examining the larvicidal activity of silver nanoparticles synthesized from plumeria leaf extract. Although Rudrappa *et al.* [9] previously synthesized silver nanoparticles using plumeria leaf extract for antimicrobial and anticancer evaluations, this study focuses on utilizing organic waste from fallen and wilted *Plumeria acuminata* leaves as a bioreductant to produce silver nanoparticles specifically evaluated for their larvicidal potency against *Aedes aegypti* larvae.

2. Experimental

2.1. Tools and Materials

Glassware (Pyrex), blender (Philips), hot plate (IKA® C-MAG HS 7), analytical balance (OHAUS), filter paper (Whatman), UV-Vis spectrophotometer (Thermo Scientific), Particle Size Analyzer/PSA (Malvern), SEM-EDS (JSM-6510LA), TEM (H9500), oven, centrifugator, dropper pipette, cuvette, vial, and volumetric flask. Organic waste of yellow plumeria leaves (*Plumeria acuminata* L.), silver nitrate/$AgNO_3$ (PT Brataco), demineralized water (PT Brataco), *Aedes aegypti* instar III larvae, and abate.

2.2. Extract Preparation and Phytochemical Screening

Plumeria leaves were collected from the area around East Denpasar, Bali. The samples were wet-sorted to remove impurities, washed with clean water, and dried in indirect sunlight under a black cloth. Once dry, the leaves were ground using a blender to obtain a powder. Twenty g of the powdered plumeria was heated in 100 mL of demineralized water at 90°C for 15 minutes using the infusion method. The extract was then filtered while hot and diluted to a 1% concentration for use in silver nanoparticle synthesis. Qualitative phytochemical screening was performed to determine the secondary metabolite content of the plumeria leaf water extract [16].

For the flavonoid test, 0.5 g of magnesium (Mg) powder, 1 mL of concentrated HCl, and 1 mL of amyl alcohol were added to 10 mL of the filtrate, and the mixture was vigorously shaken. A positive flavonoid result was indicated by the formation of a red, yellow, or orange color in the amyl alcohol layer [17]. Terpenoid and steroid tests were performed using the Liebermann-Burchard reagent. The filtrate was placed in a test tube, then 2–3 drops of anhydrous acetic acid were added, and the mixture was gently stirred until nearly dry. Afterward, 1–2 drops of concentrated sulfuric acid were added. The formation of a red or reddish-purple color indicates the presence of terpenoids, while a green or blue color indicates the presence of steroids [18].

For the alkaloid test, 10 mL of chloroform and 4 drops of NH_4OH were added to the filtrate, and the mixture was filtered. The filtrate was placed in a closed test tube and shaken with 10 drops of 2 M H_2SO_4 until two layers formed. The acid layer was separated into another test tube, and Mayer, Wagner, and Dragendorff reagents were added. A positive alkaloid result was indicated by the formation of a white precipitate in Mayer's reagent, a yellowish-brown precipitate in Wagner's reagent, and an orange precipitate in Dragendorff's reagent [19]. The saponin test was performed by shaking the filtrate for approximately 30 seconds. A positive saponin result was indicated by the formation of a stable foam 1–10 cm high for no less than 10 seconds [20]. For the tannin test, 2–3 drops of a 5% $FeCl_3$ solution were added to the filtrate. A positive result was indicated by the formation of a dark blue, blue-black, or blackish-green color, indicating the presence of tannin compounds in the extract [19].

2.3. Green Synthesis of Silver Nanoparticles

Synthesis of silver nanoparticles began with preparing a 1 mM $AgNO_3$ solution by dissolving 170 mg of $AgNO_3$ powder in 1000 mL of demineralized water in a volumetric flask. Silver nanoparticle synthesis was carried out by mixing the 1 mM $AgNO_3$ solution with aqueous extract of *Plumeria acuminata* L. leaves. The treatment used a 1:9 (v/v) ratio of 1% leaf aqueous extract to 1 mM $AgNO_3$ solution. The mixture was heated at 60°C and replicated three times. A visual indicator of silver nanoparticle formation was a color change from clear yellow to reddish brown. The obtained silver nanoparticles were then characterized using a UV-Vis spectrophotometer, PSA, SEM-EDS, and TEM.

2.4. Silver Nanoparticles Characterization

Silver nanoparticles were characterized using a UV-Vis spectrophotometer, particle size analyzer (PSA), scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDS), and transmission electron microscopy (TEM). For UV-Vis characterization, the silver nanoparticle sample was placed in a cuvette, and its absorbance was measured. The UV-Vis spectrophotometer was used to determine the maximum absorption wavelength, which indicates the formation of silver nanoparticles and typically ranges from 400 to 500 nm [21].

TEM was used to determine the morphology and size of the nanoparticles. Silver nanoparticles may exhibit various morphologies, including spherical, triangular, crystalline, and anisotropic shapes, with particle sizes generally ranging from 1 to 100 nm [22]. The silver nanoparticle solution was dropped onto a copper grid and dried under vacuum. The grid was then placed in a specimen holder and analyzed using TEM [23].

SEM provides information on particle morphology, including shape and size. SEM-EDS characterization was performed to examine the morphology and elemental composition of the silver nanoparticles [24]. The sample solution was centrifuged at 10,000 rpm for 10 minutes. The resulting precipitate was washed with 10 mL of demineralized water and centrifuged again. This process was repeated until a sufficient precipitate was obtained. The precipitate was then dried in an oven at 50°C, and the resulting powder was analyzed using SEM-EDS [25].

A PSA was used to determine the particle size and zeta potential of the silver nanoparticles [26]. A total of 15 mL of the silver nanoparticle solution was diluted with 10 mL of distilled water. The diluted sample was transferred into a 2 cm-high measurement cell, and the particle size was measured using a PSA [27].

2.5. Larvicidal Testing

Larvicidal activity testing was conducted using third-instar *Aedes aegypti* larvae following a procedure adapted from Rundito *et al.* [28]. AgNPs were tested at several concentrations: 0.3085, 1.234, 2.468, 3.085, and 4.319 ppm. Abate served as the positive control, and distilled water as the negative control. Twenty-five larvae were placed in each vial containing the test solution, and each treatment was repeated three times. Larvae were incubated for 24 hours at room temperature without food. After the observation period, the number of dead larvae was counted in each treatment. Larvae were considered dead if they showed no movement after being touched with a pipette or stirring rod. The percentage of larval mortality was calculated to determine the effectiveness of the silver nanoparticles as a larvicide.

2.6. Data Analysis

This study used a quantitative measurement method with the parameter being the LC_{50} (Lethal Concentration 50) value. Data from the larvicidal activity test were obtained as the mortality percentage of *Aedes aegypti* larvae at various silver nanoparticle concentrations and in the positive control (Abate after 24 hours of observation. Mortality data were then analyzed using Microsoft Office Excel. The analysis was performed using linear regression, with the x-axis representing the concentration in logarithmic form (log ppm) and the y-axis representing the probit of the percentage of larval mortality, yielding the equation $y = a + bx$. This regression equation was used to determine the LC_{50} value by entering the probit value of 5 as the y-value, since probit 5 corresponds to 50% mortality. The x-value obtained from the regression equation is the log of the concentration, so the anti-log of this value was used to obtain the LC_{50}. Because 50% larval mortality was not reached within the tested concentration range, the reported LC_{50} represents an estimated value obtained by probit regression analysis rather than a directly observed experimental value.

The difference in larvicidal activity between the LC_{50} of silver nanoparticles and the LC_{50} of the positive control was analyzed using an Independent-Samples t-test in SPSS. A p-value > 0.05 indicates that H_0 is accepted and that there is no significant difference between the two treatments. If the data do not meet the homogeneity requirements, the analysis continues using the nonparametric Wilcoxon test.

3. Results and Discussion

3.1. Extract Preparation and Phytochemical Screening

Plumeria leaf extract was prepared by infusion in demineralized water. Demineralized water was chosen as a solvent because it is safe, readily available, and capable of extracting polar secondary metabolites. Furthermore, the infusion method was chosen for its simplicity and suitability for extracting active compounds that are soluble in demineralized water. The water extract of plumeria leaves was then used as a bioreductant in the synthesis of silver nanoparticles [29]. The results of the phytochemical screening test are presented in Table 1.

Phytochemical screening results showed that the plumeria leaf water extract contained flavonoids, saponins, and tannins, whereas alkaloids, terpenoids, and steroids were negative. Flavonoids, saponins, and tannins were well extracted because they are polar and readily soluble in demineralized water. Flavonoids were indicated by the formation of an orange color in the amyl alcohol layer due to the formation of flavylium salts from the reaction of Mg metal and concentrated HCl. Saponins produced a stable foam due to the presence of glycoside compounds that can form foam in demineralized water. In contrast, tannins produced a blackish-green color due to the formation of a complex between the tannin hydroxyl group and the Fe^{3+} ion [18].

Table 1. Phytochemical screening results of plumeria leaf water extract

Test	Reagents	Reaction results	Result
Flavonoids	Magnesium (Mg) powder, concentrated HCl, and amyl alcohol	Orange color solution in the amyl alcohol layer	+
Terpenoids	Lieberman-Burchard	Formation of a brownish-black color	-
Steroids			-
Alkaloids	Mayer	No white precipitate formed	-
	Dragendorff	No orange precipitate formed	-
	Wagner	No orange-brown precipitate formed	-
Saponins	Hot water	Stable foam (1–10 cm in height) persisting for ± 10 seconds	+
Tannins	5% $FeCl_3$	The formation of a blackish green or blue color	+

(+) sign: Compound present. (-) sign: No compound present

The negative results for alkaloids, steroids, and terpenoids may be influenced by the type of solvent used. Terpenoids and steroids are relatively nonpolar compounds and are therefore less soluble in polar solvents such as demineralized water. In addition, previous research has shown that methanol extracts of plumeria leaves contain terpenoids and alkaloids, as methanol is more effective at extracting both polar and nonpolar compounds [30].

The presence of flavonoids, saponins, and tannins in the aqueous plumeria leaf extract indicates its potential as a bioreductant for the green synthesis of silver nanoparticles by reducing Ag^+ ions to Ag^0. The reduction of Ag^+ to Ag^0 may be associated with polyphenolic compounds, particularly flavonoids and tannins. The phenolic hydroxyl groups (–OH) in these secondary metabolites can act as electron donors, facilitating the reduction of silver ions to Ag^0. Meanwhile, carbonyl groups (C=O) and other organic components may act as capping agents. This reduction process involves electron transfer that promotes nucleation, while oxidation of phenolic groups to quinone structures may contribute to the stabilization of the resulting nanoparticles and limit excessive agglomeration [30, 31].

3.2. Green Synthesis of Silver Nanoparticles

Based on research on the synthesis process, there is a visual color change. The color of the solution, which was originally clear and yellowish, changed to reddish-brown after heating, as shown in Figure 1. As an indicator of the formation of silver nanoparticles visually, there is a change in the color of the solution from clear yellow to yellow brown to reddish brown [32]. In theory, the formation of silver nanoparticles using plant extract bioreductants results from the reduction of Ag^+ ions [33].

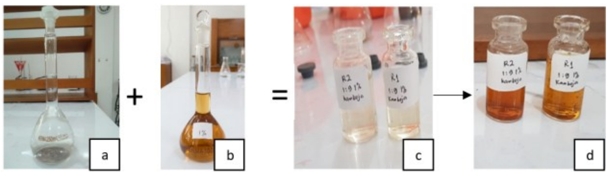

Figure 1. Formation of silver nanoparticles (AgNPs) colloids. (a) $AgNO_3$ solution, (b) 1% plumeria leaf water extract, (c) Solution before heating, (d) Color change after heating at 60°C

Plant extracts contain secondary metabolites, including phenolic compounds, flavonoids, tannins, alkaloids, saponins, and steroids, that may participate in silver nanoparticle biosynthesis. Among these metabolites, phenolic compounds, particularly flavonoids, are widely recognized as primary reducing agents because their hydroxyl groups readily donate electrons to reduce Ag^+ ions to Ag^0 atoms. Other phytochemicals, such as alkaloids and saponins, may also contribute to nanoparticle formation but are more commonly associated with nanoparticle stabilization and capping rather than reduction [6, 31]. The color change in the solution indicates the reduction of Ag^+ ions to Ag^0. The formation of silver nanoparticles begins with the interaction between silver ions and secondary metabolites contained in the plant extract. Functional groups such as hydroxyl, carbonyl, and amine play a role in forming complexes with Ag^+ ions.

Furthermore, flavonoids and other phenolic compounds reduce Ag^+ ions through electron transfer from their hydroxyl groups. Tannins, which are rich in phenolic hydroxyl groups, also contribute to this reduction process. In contrast, alkaloids and saponins are thought to play supportive roles in nanoparticle formation. They are more commonly associated with the stabilization and capping of synthesized nanoparticles, although some studies suggest they may also participate in the reduction process [31, 32].

3.3. Characterization of Silver Nanoparticles

3.3.1. UV-Vis Spectrophotometer

Characterization using a UV-Vis spectrophotometer was performed to determine the maximum absorption wavelength indicative of silver nanoparticle formation, which generally occurs in the range of 400–500 nm [34]. After mixing the $AgNO_3$ solution with the Plumeria leaf aqueous extract at a ratio of 1:9, a λ_{max} of 430 nm was observed (Figure 3). This absorption peak indicates the formation of AgNPs and can be attributed to surface plasmon resonance (SPR). The observed maximum wavelength is associated with the interaction of light with the AgNPs, which are stabilized by secondary metabolites containing chromophore groups. The observed SPR peak at 430 nm indicates the successful formation of AgNPs from the plumeria leaf extract.

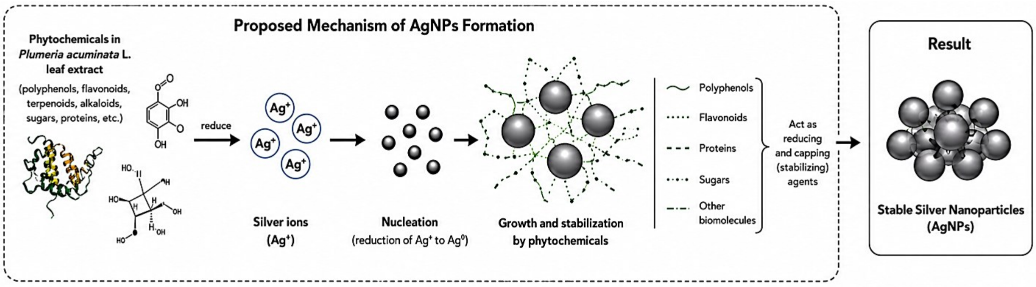

Figure 2. Estimated reduction reaction of Ag^+ to Ag^0

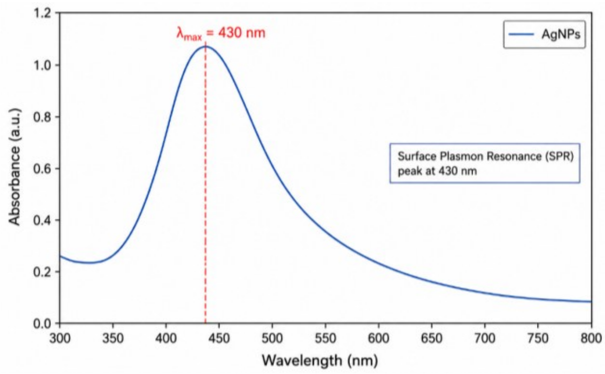

Figure 3. UV-Vis spectrum of AgNPs

3.3.2. Transmission Electron Microscopy

TEM analysis is an instrument used to determine the morphology of nanoparticles, including spherical, triangular, crystalline, and anisotropic shapes [22]. Figure 4 shows the results of the AgNPs successfully synthesized using a bioreductant from the aqueous extract of plumeria leaves.

Based on the research, the resulting AgNPs were irregularly spherical, with some also appearing square or triangular (Figure 4). This information indicates that plumeria leaf extract, which contains secondary metabolites, can act as a reducing and capping agent for Ag precursors, thereby stabilizing the size of the resulting nanoparticles. According to Zuhrotun *et al.* [32], plant extracts also act as capping agents in the synthesis of silver nanoparticles. According to research by Rudrappa *et al.* [9], particle sizes measured by TEM ranged from 20.12 to 45.40 nm.

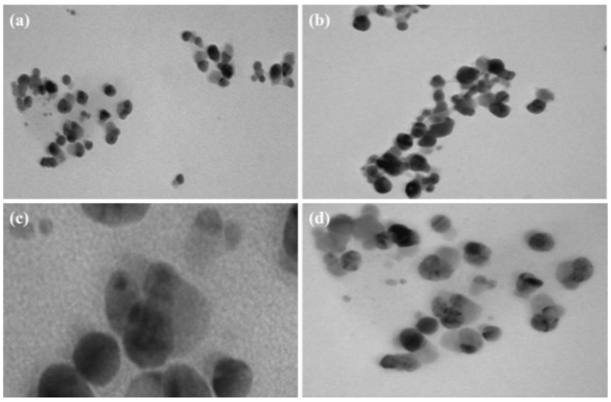

Figure 4. TEM images of AgNPs at (a, b) 60,000×, (c) 300,000×, and (d) 120,000× magnifications

3.3.3. Scanning Electron Microscopy - Energy Dispersive X-ray Spectroscopy

SEM provides information on particle morphology, including shape and size [24], while EDS identifies the constituent elements and their weight and atomic percentages. SEM-EDS analysis requires the sample to be dry, homogeneous, conductive, and stable. The sample prepared for SEM analysis is shown in Figure 5, while SEM images of the silver nanoparticles at magnifications of 250×, 400×, 2,000×, and 10,000× are presented in Figure 6.

Figure 6 confirms particle formation, with the 10,000× image showing closely packed and agglomerated particles (Figure 6d). The AgNPs exhibit irregular square and triangular morphologies. At lower magnifications (Figure 6a–c), the particle morphology is less clearly visible because of agglomeration, which makes the particles appear larger. This aggregation is attributed to van der Waals forces, which cause nanosized particles to combine into larger aggregates and result in a non-uniform particle size distribution [35].

The EDS spectrum analysis showed the elemental composition of the AgNPs, as presented in Figure 7. The analysis detected silver (Ag) at an energy peak of 2.983 keV. In EDS analysis, the characteristic X-ray emission of elemental silver typically appears at around 3 keV, confirming the presence of metallic silver in the synthesized nanoparticles [36]. The EDS spectrum displays signal intensities at different energy levels (keV), corresponding to the elemental composition of the sample. The strong signal further confirms the presence of silver in the synthesized nanoparticles, consistent with the findings reported by Ansar *et al.* [37].

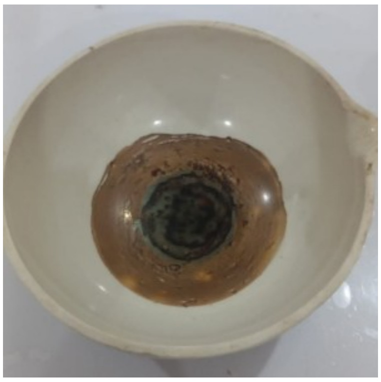

Figure 5. Dry sample of AgNPs

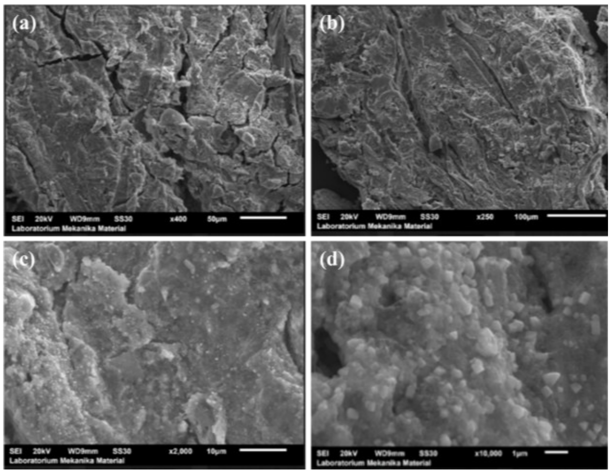

Figure 6. Morphological analysis of AgNPs using SEM (a) 250×, (b) 400×, (c) 2,000×, and (d) 10,000× magnifications

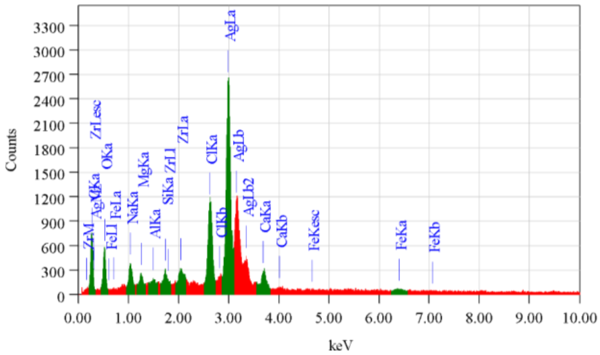

Figure 7. EDS spectrum of AgNPs

3.3.4. Particle Size Analyzer

The analysis using PSA aims to determine the nanoparticle colloid's particle size and the uniformity of the formed AgNPs [26]. The results of the PSA measurements are shown in Table 2. Table 2 shows that AgNPs synthesized using plumeria leaf water extract as a bioreductant have an average size of 72.39 nm. These results are consistent with the literature, which defines nanoparticles as particles with a size range of 1–100 nm [22]. The PDI value was 0.2744, indicating the particle size distribution within the sample. A lower PDI value indicates a more homogeneous particle size distribution, whereas a higher PDI may be associated with particle agglomeration and the formation of larger aggregates [35]. A PDI value close to 0 indicates a homogeneous particle size dispersion, while a PDI value greater than 0.5 indicates high particle heterogeneity, leading to non-uniform particle size [38]. Based on this, it can be concluded that the PDI results from AgNPs measurements using plumeria leaf water extract as a bioreductant indicate homogeneous particles, as evidenced by the resulting particle size distribution.

This study shows that the zeta potential of AgNPs synthesized using 1% plumeria leaf water extract as a bioreductant in a 1:9 ratio at 60°C was -21.51 mV (Table 2). Zeta potential is a parameter of the electrical charge between colloidal particles and is crucial in determining the stability and aggregation of nanoparticle colloids. A zeta potential with an absolute value of approximately ±20–30 mV generally indicates moderate colloidal stability, whereas values greater than ±30 mV are commonly associated with stronger electrostatic stabilization [39]. A negative zeta potential value indicates the surface charge acquired by the AgNPs during the synthesis process. The negative surface charge generates electrostatic repulsion between nanoparticles, thereby reducing particle aggregation and contributing to the colloidal stability of the suspension [39].

3.4. Larvicidal Testing

The results of the larvicidal activity test of AgNPs against *Aedes aegypti* larvae are shown in Figure 8. The results showed that increasing the concentration of AgNPs increased the percentage of larval mortality, with the highest concentration resulting in the highest number of larval deaths. These findings indicate that AgNPs exhibit larvicidal activity against *Aedes aegypti* larvae. The observed mortality may be attributed to the small size of the AgNPs, which allows them to enter the larval body through the body surface and digestive tract. Ag^+ ions released from the silver nanoparticles may disrupt cell membrane permeability, damage cellular proteins, and interfere with larval metabolism and respiration. In addition, secondary metabolites such as flavonoids and tannins present in the aqueous plumeria leaf extract may contribute to larvicidal activity due to their insecticidal properties [40].

Table 2. PSA data of AgNPs

Particle size (nm)	PDI	Zeta potential (mV)
72.39	0.2744	-21.51

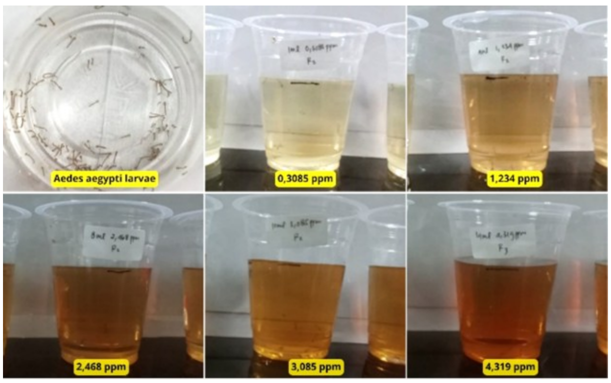

Figure 8. Observations of the larvicidal activity of AgNPs on *Aedes aegypti* larvae

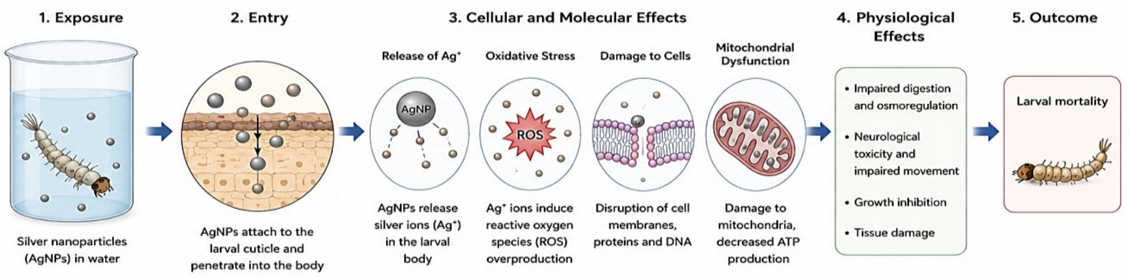

Figure 9. Illustration of the mechanism of AgNPs as a larvicide against *Aedes aegypti* larvae

During a 24-hour observation, larvae exposed to AgNPs exhibited behavioral changes, including slowed movement, curved bodies, floating at the surface, and ultimately immobility. These behavioral changes indicate disturbances in the nervous system and larval metabolism due to exposure to AgNPs. In the negative control, no larval mortality was found. In contrast, in the AgNP treatment and the positive control, abate, mortality increased with increasing treatment concentration. At the highest tested concentration (4.319 ppm), the observed larval mortality ranged from 36% to 44% across the three replicates. Since 50% larval mortality was not achieved within the tested concentration range, the LC_{50} values were estimated using probit regression analysis.

The calculated LC_{50} values for the three replicates were 13, 16, and 10 ppm, respectively, with a mean LC_{50} of 13 ppm. Therefore, the reported LC_{50} represents an extrapolated estimate obtained through probit regression analysis beyond the tested concentration range. The lower LC_{50} value for abate indicates that abate remains more effective than AgNPs, but AgNPs still show good potential as a natural biolarvicide. The results of this study align with previous research showing that plant extract-based silver nanoparticle biosynthesis is an effective, environmentally friendly larvicide against *Aedes aegypti* mosquito larvae. The larvicidal activity of AgNPs is also influenced by particle size and the stability of the resulting nanoparticles. Smaller nanoparticles have a larger surface area, thus optimizing interaction with the larval body [41]. The relatively larger particle size obtained in this study (72.39 nm) may have reduced the specific surface area available for interaction with mosquito larvae and decreased the release of Ag^+ ions. Consequently, particle size may have contributed to the relatively lower larvicidal activity observed in the present study.

AgNPs exhibit effective larvicidal activity against *Aedes aegypti* larvae via multiple biological and molecular mechanisms. AgNPs can adhere to the larval cuticle, enter the body through cuticle pores and the digestive tract, and then release toxic Ag^+ ions. Ag^+ ions interact with sulfhydryl (-SH) groups in proteins and enzymes, thereby inhibiting cellular metabolism and energy production. In addition, AgNPs trigger the formation of reactive oxygen species (ROS), which cause oxidative stress, membrane lipid damage, protein denaturation, and DNA damage. Silver nanoparticles can also damage cell membranes and mitochondria, resulting in impaired cellular respiration and decreased ATP production. This physiological damage disrupts the digestive system, osmoregulation, and nervous system of larvae, resulting in paralysis, growth retardation, and even death.

In green synthesis using plumeria extract, phytochemicals such as flavonoids, polyphenols, alkaloids, and terpenoids act as reducing and capping agents, thereby increasing the stability and bioactivity of AgNPs as larvicides. This mechanism has been widely reported in previous studies, showing that AgNPs act through a combination of Ag^+ release, oxidative stress induction, cell membrane damage, and mitochondrial dysfunction, ultimately leading to mosquito larval mortality [4, 42, 43, 44]. Therefore, AgNPs produced by green synthesis using an aqueous extract of plumeria leaves have the potential to be developed as an alternative biolarvicide, more environmentally friendly than those produced by chemical synthesis.

3.5. Data Analysis

The LC_{50} data for AgNPs and abate were then analyzed using an independent-samples t-test. Based on the results of the independent-samples t-test, AgNPs and the positive control were observed after 24 hours. The "Equal variances assumed" value was 0.002. This indicates a significant difference between the AgNPs and the positive control, as the p-value is <0.05. Therefore, the decision to reject H0 indicates a significant difference in the larvicidal LC_{50} values of AgNPs and the positive control. AgNPs from plumeria leaf water extract were less effective as a larvicide than abate. Several factors, including the concentration of silver nanoparticles, influence this.

4. Conclusion

Based on the research results, it can be concluded that the water extract of plumeria leaves can act as a bioreductant in the biosynthesis process of AgNPs. The resulting nanoparticles showed maximum absorbance at 430 nm. PSA characterization showed a hydrodynamic particle size distribution of 72.39 nm and a zeta potential of -21.51 mV, indicating moderate colloidal stability. The morphology of the nanoparticles, consisting of irregular spherical shapes and several other geometric shapes, was characterized using TEM and SEM. In addition, the biosynthesized AgNPs exhibited larvicidal activity against *Aedes aegypti* larvae, with an LC_{50} of 13 ppm. However, the larvicide effectiveness of AgNPs remained lower than that of abate, as evidenced by SPSS analysis, which showed a significant difference between the two treatments.

Acknowledgment

In this study, we would like to thank PT DKSH, especially Mrs. Wiji Rahayu, for assistance with the particle size analysis.

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