Biogenic Synthesis of Silver Nanoparticles Using Guava (*Psidium guajava*) Leaf Extract and Its Larvicidal Action against *Anopheles gambiae*

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**Abstract**

The progress in the field of nanotechnology has contributed to the development of tools for combating the most critical problems in developing countries. The requirements that such tools should meet are low-cost and resource settings, environmental protection, ease of use, and availability. The use of plant properties for the generation of nanoparticles (NPs), which serve as bioinsecticides to combat the plasticity and resistance of mosquitoes and parasites, is considered possible. Here, we report for the first time the larvicidal activity of silver (Ag) NPs (AgNPs) synthesized from *Psidium guajava* (*P. guajava*) leaf extract and its larvicidal action against *Anopheles gambiae*. Concentrations of AgNPs between 0 and 200 ppm were used and their LC₅₀ at 24 h and 48 h were determined as 19.55 ppm and 8.737 ppm, respectively. The AgNPs were stable and highly effective against the larvae of *A. gambiae* and thereby we anticipate that they can be used to combat vector-borne diseases in developing countries.

**Keywords**

Vector Control, *Anopheles gambiae*, Silver Nanoparticles, *Psidium guajava*
Mosquitoes are the principal vector of vector-borne diseases, which affect human beings and animals [1]. Diseases transmitted by mosquitoes lead to commercial and labor output losses, particularly in countries with tropical and subtropical climates [2]. Mosquitoes represent a huge threat for millions of people worldwide, since they spread various tropical diseases, especially malaria, which is transmitted by female *A. gambiae* mosquitoes [3].

Infected female *Anopheles* mosquitoes transmit malaria parasites to people and animals via their bites during their blood meal. Marked progress has been achieved in malaria control, including the discovery of artemisinin (for which the Nobel Prize was awarded to Y. Tu), development of the first vaccine against *Plasmodium falciparum* malaria, and decrease in the rate of malaria infections worldwide and particularly in sub-Saharan Africa, which contributes to the bulk of malaria burden [4] [5] [6]. However, resistance to existing antimalarial drugs, such as the gold-standard medication artemisinin, particularly in the Greater Mekong sub-region in Southeast Asia, is a growing problem [7], which has hampered the global progress in malaria control. In 2016, the malaria cases were estimated to be 216 million, with an increase of about 5 million cases compared to 2015; the death rates in both years reached approximately 445,000. The majority of the malaria cases (92%) and related deaths (93%) occurred in Africa [5], where the principal vectors of malaria are *A. gambiae sensu stricto* (s.s.) and *Anopheles arabiensis* [8].

Synthetic insecticides are widely used to control insect spread as indoor insecticides and residual spraying in treated nets [9]. Their abuse leads to both human and environmental toxicity, thereby potentially eliminating non-target organisms [10]. The adaptation of mosquitoes to new environmental conditions is a result of the development of physiological resistance, and alternative selective measures to prevent such resistance are urgently needed. Vector control is a crucial necessity in epidemic situations. The new methods for mosquito control must be both economical and efficient, while being safe for non-target organisms and the environment. They must be adapted to the conditions prevailing in endemic countries [11]. The use of impregnated mosquito nets or indoor sprays are measures to slow the transmission of the disease by killing or preventing infected mosquitoes from biting humans [12]. Secondary metabolites of various plants including *Azadirachta indica* (neem), *Clerodendron infortunatum* (glorybower), *Schoenocaulon officinale* (neotropical lily), and *Chrysanthemum pyrethrum* (African daisy) [13] [14] have been used for controlling the spread of mosquitoes [1] [15]. Since most malaria-affected countries are poor, the main challenges are to reduce the costs of the toxicological tests and to make the biopesticides available despite the low incomes and economic weakness of these markets, as well as to limit intellectual property. Other factors include the quality control and lack of stability of these metabolites depending on the environmental conditions. In addition, there is competition with other biopesticides and
biocontrol agents which reduce their efficiency [14]. Moreover, movements in
the global distribution and burden of infectious diseases with climate change are
observed [16]. By generating NPs obtained from plant metabolites with therapeu-
tic potential, the scientific community is aiming to overcome these chal-
 lenges and to develop biocontrol agents against mosquitoes and microbes. Plant
extracts are considered eco-friendly bioreactors due to the simple process of Ag+ reduction. Studies have shown that when present in the reaction mixture sur-
face-active molecules or stabilizers such as ionic liquids create electrostatic in-
teractions, thereby increasing the stability of the NPs [17]. Controlling the NP/seco-
dary metabolite interface would make it possible to modulate the nanostructure
and to adapt the properties of the materials for specific applications. The num-
ber of studies focusing on the cost-effective use of nanomaterials for human
health is increasing rapidly [12]. Nanotechnologies have the potential to revol-
utionize pest control and larval management. The production of plant-based NPs is
advantageous over chemical and physical methods, since it is cheap, single-step,
and does not require high pressure, energy, temperature, or the use of highly
toxic chemicals [18]. In the present study, we report for the first time the larvici-
dal action of green Ag NPs synthesized from P. guajava L. leaf extract against
4th instar larvae of A. gambiae (s.l.). The efficacy of NPs was compared to that of
their precursors, namely, plant extract and Ag+. In the bioassay, the P. guajava
leaf extract was the dispersion medium, capping, and reducing agent.

**P. guajava and their AgNPs**

*P. guajava* (Myrtaceae) is a native bush species from South America known as
“goiaba”, which is commonly used in traditional medicine. Among the condi-
tions treated with goiaba are gastrointestinal infections; malaria, respiratory in-
fec tions, oral and dental infections, skin infections, diabetes, cardiovascular dis-
 ease and hypertension, cancer, malnutrition, gynecological issues, pain, fever,
and liver and kidney conditions [19]. The following two varieties of *P. guajava*
are commonly cultivated: *P. guajava* var. pomifera and *P. guajava* var. pyrifera.
The fruit of *P. guajava* is highly appreciated in the tropical and subtropical cui-
sine and used widely in traditional medicine [20]. The *P. guajava* is a
small-branched tree with smooth, mottled bark that can peel off in flakes. Its
leaves (6 inches long and 3 inches wide) are aromatic and oppositely arranged
along the stems with prominent lateral veins on the dorsal side [21]. A number
of compounds in the plant leaves including gallic acid, quercetin, morin, cate-
chin, epicatechin, rutin, naringenin, kaempferol, which are flavonoids, have
shown promising activity [22] [23]. Toxicity studies in mice and other animal
models as well as controlled human studies have demonstrated the safety of the
plant [24]. However, high concentrations of the aqueous extract of this plant
have previously yielded positive larvicidal activity [25]. The traditional uses of
this plant have been validated by scientific research. Extensive studies revealed
that the compounds of the extract exert antioxidant, antipyretic, antifungal, an-
timicrobial, hypotensive, analgesic, and anti-inflammatory effects [26]. Genom-
ma Lab International Laboratories produces tablets, distributed under the QG5 trademark, containing 166.6 mg dry extract of *P. guajava* leaves, with 0.8 to 1.2 mg quercetine. These tablets have been shown to relieve all 5 symptoms of colitis, including inflammation, lower abdominal pain, spasms, gas, and bloating. Moreover, QG5 helps against acute non-infectious diarrhea and menstrual colic.

Previous studies have characterized the synthesis process of AgNPs from *P. guajava* leaf extracts (Table 1 and references therein [27]-[50]). Potent antimicrobial action [46] [47] [49] [50], cytotoxicity [34], and dye fabric degradation [44] of AgNPs have been described. This has resulted in the formulation of the following guidelines: 1) plant extracts can be obtained by aging, soxhlet extraction, microwave, or ultrasound methods; 2) 1 mM Ag nitrate (AgNO₃) is a favorable concentration for the reaction; 3) the reaction condition and state of agglomeration have plasmon resonance bands between 380 and 490 nm, as obtained using UV-Vis spectroscopy; 4) the stability in water of the AgNPs obtained from *P. guajava* extract is up to 30 weeks; 5) rapid synthesis as the use of microwave heating tends to produce pure AgNPs; 6) TEM shows nanometer range spherical NPs while SEM shows aggregates; and 7) IR spectroscopy is an appropriate method to validate biomolecule presence at metallic interface.

### 2. Materials and Methods

**Plant collection and preparation of the extract**

Leaves of *Psidium guajava* L. ([Figure 1](#)) were collected at Massoumbou (N4°5’17.058’; E9°50’45.906”), Littoral region, Cameroon, in December 2018. They were authenticated by Dr. Barthelemy Tchiengue at the National Herbarium, Yaounde and compared to a voucher specimen previously deposited (no. 2885/SRFK). The plant extract was obtained according to a previously published method [29]. The plant reactor was used for not more than 1 week to avoid the gradual loss of viability due to prolonged storage [51]. The extract concentration was determined as per previously reported procedures [52].

![Figure 1. *P. guajava*: left plant, right fruit and leaves.](#)
### Table 1. Ag nanoparticles from *Psidium guajava* leaf extracts.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Activity</th>
<th>Preparation extract</th>
<th>Preparation nanoparticles</th>
<th>UV-Vis</th>
<th>FTIR</th>
<th>DLS</th>
<th>DRX</th>
<th>SEM/EDX</th>
<th>TEM</th>
<th>AFM</th>
</tr>
</thead>
<tbody>
<tr>
<td>[27]</td>
<td>India</td>
<td>Antibacterial</td>
<td>10 g fresh/200 mL, microwave</td>
<td>10 mL AgNO₃ (1 mM) 50 mL, 90°C</td>
<td>490 nm</td>
<td>Molecules at surface</td>
<td>Ag pure</td>
<td>26 ± 5 nm</td>
<td>Ag, Al, C, O</td>
<td>26 ± 5 nm</td>
<td>Mostly spherical</td>
</tr>
<tr>
<td>[28]</td>
<td>India</td>
<td>Antibacterial</td>
<td>20 g fresh/100 mL, 100°C</td>
<td>1/100 dilution extract/complex Ag⁺ (10 mM)</td>
<td>380, 416 nm</td>
<td>Molecules at surface</td>
<td>Ag, Cu</td>
<td>0 - 50 nm, mean 24 nm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[29]</td>
<td>India</td>
<td>Antimicrobial</td>
<td>10 g fresh/100 mL, boiled</td>
<td>10 mL AgNO₃ (1 mM) 90 mL, 80°C</td>
<td>410 nm</td>
<td>Molecules at surface</td>
<td>Ag⁺ bioorganic crystallized</td>
<td>59 nm, spherical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[30]</td>
<td>India</td>
<td>Antibacterial</td>
<td>10 g fresh/100 mL, boiled</td>
<td>2.5 mL AgNO₃ (1 mM) 100 mL</td>
<td>438 - 430 nm</td>
<td>Molecules at surface</td>
<td>Ag⁺ bioorganic crystallized</td>
<td>15 - 35 nm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[31]</td>
<td>India</td>
<td>Antibacterial</td>
<td>100 g dry/ethanol soxhlet</td>
<td>10 mL AgNO₃ (0.1 M) 90 mL</td>
<td>460 nm</td>
<td>Molecules at surface</td>
<td>Not clear</td>
<td>0.1 μm - 0.5 μm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[32]</td>
<td>India</td>
<td>Antibacterial</td>
<td>5 g/100 mL, water boiled</td>
<td>1:1 extract, AgNO₃ (1 mM)</td>
<td>440 nm</td>
<td>Molecules at surface</td>
<td>Not clear</td>
<td>Ag (111)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>[33]</td>
<td>India</td>
<td>Antibacterial</td>
<td>5 g fresh/100 mL, boiled</td>
<td>3 mL AgNO₃ (1 mM) 40 mL</td>
<td>462 nm</td>
<td>Molecules at surface</td>
<td>Not clear</td>
<td>2 - 10 nm, spherical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[34]</td>
<td>India</td>
<td>Antimicrobial</td>
<td>Fresh, crushed, centrifuged</td>
<td>25 mL AgNO₃ (0.01 M) 50 mL</td>
<td>420 nm</td>
<td>Molecules at surface</td>
<td>Not clear</td>
<td>2 - 10 nm, spherical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[35]</td>
<td>India</td>
<td>Antimicrobial</td>
<td>dry dipped in ethanol and sodium hypochlorite and fungi</td>
<td>Medium free biomass incubated/1:1 AgNO₃ (1 mM) shaker 160 rpm</td>
<td>383 - 424 nm</td>
<td>Not clear</td>
<td>Not clear</td>
<td>2 - 10 nm, spherical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[36]</td>
<td>India</td>
<td>Antibacterial</td>
<td>20 g fresh/100 mL, 100°C</td>
<td>5 mL AgNO₃ (1 mM) 45 mL</td>
<td>420 - 470 nm</td>
<td>Molecules at surface</td>
<td>Not clear</td>
<td>0.2 - 5 nm, spherical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[37]</td>
<td>India</td>
<td>Antibacterial</td>
<td>100 g powder extracted methanol</td>
<td>5 mL AgNO₃ (1 M) 95 mL H₂O</td>
<td>480 nm</td>
<td>Molecules at surface</td>
<td>Not clear</td>
<td>0.2 - 5 nm, spherical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[38]</td>
<td>India</td>
<td>Antibacterial</td>
<td>5 g fresh/sea sand/60 mL H₂O</td>
<td>0.2 mL AgNO₃ (1 M), 20 mL H₂O, 30°C</td>
<td>435 nm</td>
<td>Not clear</td>
<td>Not clear</td>
<td>Mean 40, 10 - 90 nm, spherical few agglomerated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[39]</td>
<td>India</td>
<td>Antibacterial</td>
<td>10 g fresh/100 mL, boiled</td>
<td>5 mL AgNO₃ (1 M) 50 mL</td>
<td>420 - 490 nm</td>
<td>Molecules at surface</td>
<td>Ag pure, 30 - 35 nm</td>
<td>12 - 75 nm, spherical polydisperse</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[40]</td>
<td>India</td>
<td>Antibacterial</td>
<td>5 g fresh leaves/50 mL H₂O, 50°C, 5 min</td>
<td>5 mL AgNO₃ (0.01 M) 90 mL H₂O</td>
<td>439 nm</td>
<td>Not clear</td>
<td>Not clear</td>
<td>12 - 75 nm, spherical polydisperse</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[41]</td>
<td>India</td>
<td>Antibacterial</td>
<td>20 g powder/100 mL acetone</td>
<td>9 mL AgNO₃ (1 mM) 45 mL</td>
<td>435 nm</td>
<td>Not clear</td>
<td>Not clear</td>
<td>Mean 40, 10 - 90 nm, spherical few agglomerated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[42]</td>
<td>China</td>
<td>Antimicrobial</td>
<td>100 g dry/500 mL ethanol, hot water</td>
<td>10 mL AgNO₃ (1 mM) 100 mL</td>
<td>435 nm</td>
<td>Not clear</td>
<td>Not clear</td>
<td>Mean 40, 10 - 90 nm, spherical few agglomerated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[43]</td>
<td>China</td>
<td>Antimicrobial</td>
<td>2 g fresh/100 mL, 90°C</td>
<td>20 mL AgNO₃ (1 mM) 100 mL</td>
<td>438 nm</td>
<td>Not clear</td>
<td>Not clear</td>
<td>Mean 40, 10 - 90 nm, spherical few agglomerated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[44]</td>
<td>China</td>
<td>Dye degradation</td>
<td>100 g/1 L ethanol, ultrasound</td>
<td>5 mg/mL separated flavonoids solution/AgNO₃ (1 mM) 100 mL</td>
<td>420 nm</td>
<td>Not clear</td>
<td>Not clear</td>
<td>Mean 40, 10 - 90 nm, spherical few agglomerated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[45]</td>
<td>India</td>
<td>Antimicrobial</td>
<td>5 g fresh/100 mL, boiled</td>
<td>Microwave synthesis</td>
<td>54 nm</td>
<td>Not clear</td>
<td>Not clear</td>
<td>Mean 40, 10 - 90 nm, spherical few agglomerated</td>
<td></td>
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<tr>
<td>[46]</td>
<td>India</td>
<td>Antibacterial</td>
<td>20 g fresh/200 mL, 60°C</td>
<td>5 mL AgNO₃ (1 mM) 100 mL, stirred</td>
<td>430 - 456 nm</td>
<td>Molecules at surface</td>
<td>Ag⁺ bioorganic crystallized</td>
<td>55 nm, spherical</td>
<td></td>
<td></td>
<td></td>
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</table>

DOI: 10.4236/jbnb.2020.111004
<table>
<thead>
<tr>
<th>Country</th>
<th>Activity</th>
<th>Material</th>
<th>Treatment</th>
<th>Product Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>Antibacterial</td>
<td>10 g fresh/100 mL ethanol boiled</td>
<td>5 mL AgNO₃ (1 mM) 45 mL, stirred</td>
<td>419 nm Molecules at surface 62 nm Crystalline</td>
</tr>
<tr>
<td>India</td>
<td>Antibacterial</td>
<td>Fresh/100 mL hot</td>
<td>1 - 5 mL AgNO₃ (1 mM) 10 mL</td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>Antifungal</td>
<td>30 g powder/500 mL Hexane, Soxhlet</td>
<td>50 mL AgNO₃ (1 M), 50 mL H₂O, 50°C</td>
<td>10 - 35 nm, Spherical</td>
</tr>
<tr>
<td>Thailand</td>
<td>Antifungal, micelles</td>
<td>2 g dry/100 mL water</td>
<td>0.1 mg/mL, 70°C/1 mL AgNO₃ (10 mM) 455 nm hot stirred</td>
<td>96 ± 4 nm, Spherical, Ag, Cl, C</td>
</tr>
</tbody>
</table>


### Biosynthesis of AgNPs

The AgNPs were synthesized as previously described with slight modifications [27]. The bioreduction process was performed by adding 10 mL of freshly prepared aqueous extract to a 50 mL aqueous solution of AgNO₃ (1 mM). The mixture was incubated 5 h at 25°C - 28°C in dark to minimize the photo activation of AgNO₃. The incubation was performed under static conditions until the color changed to brown (Figure 2). The mixture was then centrifuged (D-7200; Hettich, Tuttlingen, Germany) at 7000 rpm for 1 h and washed twice with distilled water and once with 95% ethanol. Reaction was verified by treating the obtained filtrate with sodium chloride. Purified pellets were placed in a petri dish, dried in an oven at 60°C for 24 h, and used for NP characterization. The characterization of the AgNPs is in the supplement material: see Figure A1 (UV-Vis), Figure A2 (IR), Figure A3 (PXRD), Figure A4 (DLS) and Figure A5 (SEM and EDX).

### Evaluation of larvicidal activities

Eggs of the susceptible Anopheles gambiae (Kisumu strain) were obtained from the Organisation de Coordination pour la lutte contre les Endémies en Afrique central, Yaounde, Cameroon. They were maintained and reared in the Insectarium of the University of Douala, Faculty of Medicine and Pharmaceutical Sciences to obtain 4th instar larvae. The larvicidal activity of the AgNPs produced from Psidium guajava extract was determined following the standard test procedures of the WHO [53] with some modifications. For the bioassay, 20 4th instar larvae were placed in plastic bowls (6 cm diameter, 120 mL capacity) with distilled water in 4 replicates. The controls were set up with distilled water, Psidium guajava plant extract, and AgNO₃ at ambient temperature, or AgNO₃ in the dark. Different concentrations of AgNO₃ in the range of 0 - 200 ppm were prepared through serial dilutions of 100 mL each. The experiments were carried out at 27°C ± 2°C, relative humidity of 75% ± 5%, and a photoperiod of 14 h/10 h (light/dark). Larvae were considered dead if they did not respond to contact. The number of dead larvae was counted 24 h and 48 h after treatment and the percentage of mortality was computed as follows:
Figure 2. AgNO₃ aqueous solution (1), Psidium guajava leaf aqueous extract (2), Ag nanoparticle aqueous solution (3).

Percentage of mortality = (number of dead individuals/number of treated individuals) × 100.

Statistical analysis

Data were analyzed using the GraphPad Prism software version 5.01 for Windows (GraphPad Software, Inc., San Diego, CA, USA) and LC₅₀ was calculated at 95% fiducial limits of both upper and lower confidence limits.

3. Results and Discussion

Larvicidal activity of synthesized AgNPs

$P. \text{guajava}$ plant was selected for this study because of its accessibility and word wide distribution, thereby allowing easy translation of the results from lab scale to industrial scale. Different AgNP synthetic schemes have been previously developed in India, China, and Thailand (Table 1). The synthetic schema, which we selected, is oriented toward environment preservation; in the current study, water was used as solvent and the NP production method used was aging. We obtained 2.42 g/L concentration of $P. \text{guajava}$ plant extract, which was used for the synthesis of AgNPs and AgClNPs (supplement 1). Possible reaction schemes leading to the mixtures of Ag and AgCl were described by Awwad and coworkers [54] and by our group [55]. Early 4th instar larvae of Anopheles gambiae were treated with biosynthesized AgNPs in various concentrations ranging between 0 and 200 ppm and the mortality percentage was assessed. The LC₅₀ values of AgNPs were determined as 19.55 ppm and 8.737 ppm at 24 h and 48 h, respectively (Figure 3). The analysis of the larvicidal activity is shown in Table 2 and the mortality percentage is depicted in Figure 4.

$P. \text{guajava}$ plant extract did not cause larval mortality at all tested concentrations. When used at different concentrations, both photo-activated AgNO₃ and AgNO₃ in the dark killed all larvae of $A. \text{gambiae}$. Mondal and colleagues have previously described the mortality of Culex quequefasciatus in response to a 10 ppm AgNO₃ solution. At 24 h the mortality rate was 12.5%, at 48 h was 13.04%,
and at 72 h was 21.74% [56]. The Ag⁺ are accumulated in various organisms (plants, herbivorous organisms, or fishes) isn’t environment friendly [57]. Since the AgNPs aggregate and agglomerate quickly, their isolation and resuspension in water appeared unsuccessful. The plant extract, which we used here, served as a green dispersant and played a capping role, as proved by infrared or energy-dispersive X-ray spectroscopy experiments. Nowadays, environmental safety is crucial when developing novel strategies for combating vector-borne diseases. An insecticide should be ecofriendly in nature and acceptable by the community to cause the desired mortality against target organisms [2].

The advantages of using the developed here AgNPs as larvicidal substances are that small active quantities are required and that the resistance due to the excessive use of pesticides can be overcome [56]. Ponraj and colleagues have elucidated the mechanism of larval toxicity caused by NPs. They proposed that the binding of AgNPs to sulphur-containing proteins or to phosphorus-containing molecules similar to DNA leads to the denaturation of enzymes, decrease in membrane permeability, disturbance of proton transfer, and degradation of organelles, which eventually causes loss of cellular function and finally cell death [1].

Figure 3. Percentage of dead larvae as function of the logarithmic concentrations at 24 and 48 h. Hillslope with R squared of 0.9422. (a) 24 h; (b) 48 h.
Figure 4. Percentage mortality of *Anopheles gambiae* 4th instar larvae as a function of Ag nanoparticle concentration (in ppm) after 24 h (blue) and 48 h (brown).

Table 2. Larvicidal activity of Ag nanoparticles obtained from *Psidium guajava* L. and the precursors: plant extract and AgNO₃.

<table>
<thead>
<tr>
<th>Time duration</th>
<th>Samples</th>
<th>Concentration (ppm)</th>
<th>Mean number of death</th>
<th>% mortality</th>
<th>LC₅₀ ppm</th>
<th>95% confidence limits</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LCL</td>
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<tr>
<td>24 h</td>
<td>Nanosilver</td>
<td>0</td>
<td>13</td>
<td>5.4</td>
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<td></td>
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<td>7.5</td>
<td>57</td>
<td>23.8</td>
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<td>15.1</td>
<td>100</td>
<td>41.7</td>
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<td>158</td>
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NA: not applicable.
4. Conclusion

Vector control is one of the most serious concerns in developing countries and local synergetic interventions are favored. Main limitation before translation to environmental uses is the toxicity study on non-target organisms affected by the obtained nanoparticles. In the current study, we synthesized AgNPs using fresh leaves of *P. guajava*. The secondary metabolites of this plant act as effective capping and reducing agents. The method is cost effective and environment friendly. The synthesized NPs displayed larvicidal effects against the larval stage of the malaria vector *A. gambiae*. LC$_{50}$ after 24 h and 48 h of 19.55 ppm and 8.737 ppm where obtained with studied concentration range of AgNPs between 0 and 200 ppm. The synthesized NPs were found stable and highly effective against the 4$^{th}$ instar larvae of *A. gambiae*. We anticipate that *P. Guajava* mediated AgNPs can be used as a novel biopesticide for controlling the spread of mosquitoes and vector-borne diseases in tropical countries. Future work includes the study of other mosquito development stages, the macroscopic and microscopic impact of the NPs on the organisms.

Funding

CSC provided help for PXRD and DAAD provide support for SEM, EDX, and DLS.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article and its additional files.

Authors’ Contributions

AAN, FEM, CJ, and LGL conceived and designed the study. AAN, WEK, LPKF, ENH, PBEK, and JYSF screened the literature and performed data extraction. AAN analyzed and interpreted the results with the help of FEM, WEK, GEL, and LPKJ. CS provided microscopy data and BM performed dynamic light scattering. AAN, FEM, CJ, and LGL drafted the manuscript and all authors revised the manuscript. FEM and LGL supervised the work at all stages. All authors have read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References


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List of Abbreviations


Supplementary Material

Characterization of silver nanoparticles.

A1 Ultraviolet visible spectroscopic measurement (UV-Vis)

The bioreduction of Ag-nanoparticles was observed by measuring the UV–vis spectrum of 2.5 ml samples of the reaction suspension at different time intervals. The absorption maxima was scanned with an UV-visible Uviline 9100 spectrophotometer operated at 1 nm resolution and optical length of 10 mm. UV-visible analysis of the reaction mixture was observed for a period of 300 s. Distilled water was used as a blank.

A2 Fourier-transform infrared spectroscopy (FTIR)

FTIR spectrum was recorded at room temperature through potassium bromide pellet method. Samples were grinded with KBr pellets and kept in infrared path, and the spectrum was measured using a Nicolet IS5 model of Thermo Scientific operating by scanning in the range 400 - 4000 cm⁻¹ at a resolution of 0.4 cm⁻¹.

A3 Powder X-ray spectroscopy (PXRD)

The PXRD spectroscopy measurements of purified silver nanoparticles were carried out using a Panalytical Empyrean Serie 2 X-ray diffractometer (Cu K-Alpha1 [Å] 1.54060, KAlpha2 [Å] 1.54443, K-Beta [Å] 1.39225) by preparing a thin film on silicon substrate. Powder X-ray diffraction was used for the crystal structure characterization and composition of the nanoparticles. Their PXRD pattern, shown in Figure 5 was compared to Joint Committee on Powder Diffraction Standards files (JCPDS 65-2871 and 31-1238) and found composed of pure silver and silver chloride nanograins.

A4 Dynamic light scattering (DLS)

Particle sizes and size distributions were evaluated using a Zetasizer (Malvern Nano S Zetasizer) operating with a He–Ne laser at a wavelength of 633 nm. Each analysis was performed in triplicate and the mean value is reported. In each run, 10 - 15 measurements were made.

A5 Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDX)

Scanning electron microscopy images and energy dispersive X-ray spectrometric measurements where done on a Jeol scanning electron microscope JSM-6510 with a tungsten cathode and an EDX unit. The samples were coated with Au for 20 s at 30 mA by using a Jeol JFC-1200 sputter coater (JSM-6510). Microscopy provides detailed characterization of the distribution and morphology of the
nanoparticles and the presence of nano-silver elements was confirmed by EDX at 20 keV. EDX qualitative spectrum shows a strong silver peak (3 keV) along with chloride, oxygen, carbon as main elements.

Figure A1. Ultraviolet-visible spectra 1 hour analysis of synthesized nanoparticles.

Figure A2. Fourier transform infrared spectrum for synthesized silver nanoparticles using dry plant power (up) and silver nanoparticles (down).

Figure A3. X-ray diffraction pattern of the nanoparticles from *Psidium guajava*. ● represents silver nanocrystallites and □ represents silver chloride nanocrystallites.
A. A. Ntoumba et al.

Figure A4. DLS histogram of aqueous solutions of silver nanoparticles mediated *Psidium guajava* plant extract together with a Gaussian fitting (red curve).

Figure A5. SEM capture (left) and EDX element mapping (right) of the silver nanoparticles mediated *Psidium guajava* Plant extract.

Graphical abstract.