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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*) extract, which targets the 4th instar larvae of *Anopheles gambiae*. Concentrations of AgNPs between 0 and 200 ppm were used and their LC_{50} 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*

1. Introduction

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 spays 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 infortunatumis* (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 therapeutic potential, the scientific community is aiming to overcome these challenges 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 surface-active molecules or stabilizers such as ionic liquids create electrostatic interactions, thereby increasing the stability of the NPs [17]. Controlling the NP/secondary metabolite interface would make it possible to modulate the nanostructure and to adapt the properties of the materials for specific applications. The number of studies focusing on the cost-effective use of nanomaterials for human health is increasing rapidly [12]. Nanotechnologies have the potential to revolutionize 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 larvicidal action of green Ag NPs synthesized from *P. guajava* L. leaf extract against 4^{th} 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 conditions treated with goiaba are gastrointestinal infections; malaria, respiratory infections, oral and dental infections, skin infections, diabetes, cardiovascular disease 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 cuisine 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, catechin, 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, antimicrobial, 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, sohxlet extraction, microwave, or ultrasound methods; 2) 1 mM Ag nitrate ($AgNO_3$) 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.

Reference	Country	Activity	Preparation extract	Preparation nanoparticles	UV-Vis	FTIR	DLS	DRX	SEM/EDX	TEM	AFM
[27]	India		10 g fresh/200 mL microwave	10 mL/$AgNO_3$ (1 mM) 50 mL microwave	490 nm	Molecules at surface		Ag pure	26 ± 5 nm Ag, Al, C, O	26 ± 5 nm Mostly spherical	
[28]	India	Antibacterial	20 g fresh/100 mL, 100˚C	1/100 dilution extract/complex Ag^+ (10 mM)	380, 416 nm	Molecules at surface			Ag, Cu	0 - 50 nm, mean 24 nm	
[29]	India	Antimicrobial	10 g fresh/100 mL boiled	10 mL/$AgNO_3$ (1 mM) 90 mL, 80˚C	410 nm					59 nm, spherical	
[30]	India		10 g fresh/100 mL boiled	2.5 mL/$AgNO_3$ (1 mM) 100 mL	438 - 430 nm	Molecules at surface	15 - 200 nm, mean 21 nm, 80% 50 nm	Ag^+ bioorganic crystallized			15 - 35 nm
[31]	India	Antibacterial	100 g dry/ethanol sohxlet	10 mL/$AgNO_3$ (0.1 M) 90 mL	460 nm	Molecules at surface			0.1 µm - 0.5 µm		
[32]	India	Antibacterial films	5 g/100 mL water boiled	1:1 extract, $AgNO_3$ (1 mM)	440 nm			Ag (111)			
[33]	India	Antibacterial	5 g fresh/100 mL boiled	3 mL/$AgNO_3$ (1 mM) 40 mL	462 nm	Molecules at surface			Not clear		
[34]	India	Antimicrobial Cytotoxicity	Fresh, crushed, centrifuged	25 mL/$AgNO_3$ (0.01 M) 50 mL	420 nm	Molecules at surface				2 - 10 nm, spherical	
[35]	India	Antimicrobial	dry dipped in ethanol and sodium hypochlorite and fungi	Medium free biomass incubated/1:1 $AgNO_3$ (1 mM) shaker 160 rpm	383 - 424 nm						
[36]	India	Antibacterial	20 g fresh/100 mL, 100˚C	5 mL/$AgNO_3$ (1 mM) 45 mL	420 - 470 nm		2.01 - 6.5 nm			0.2 - 5 nm, spherical	
[37]	India	Antibacterial	100 g powder extracted methanol	5 mL/$AgNO_3$ (1 M) 95 mL H_2O	480 nm	Molecules at surface					
[38]	India	Antibacterial	5 g fresh/sea sand/60 mL H_2O	0.2 mL $AgNO_3$ (1 M), 20 mL H_2O, 30˚C	435 nm					Mean 40, 10 - 90 nm, spherical few agglomerated	
[39]	India		10 g fresh/100 mL boiled	5 mL/$AgNO_3$ (1 mM) 50 mL	420 - 490 nm			Ag pure, 30 - 35 nm		12 - 75 nm, spherical polydispersed	
[40]	India	Antibacterial	5 g fresh leaves/50 mL H_2O, 50˚C, 5 min	10 mL/$AgNO_3$ (C:variable), 90 mL H_2O	439 nm						
[41]	India	Antibacterial	20 g powder/100mL acetone	9 mL/$AgNO_3$ (1 mM) 45 mL					Si, K, Ag, C, O		
[42]	China	Antimicrobial	100 g dry/500 mL ethanol, hot water	10 mL/$AgNO_3$ (1 mM) 100 mL	435 nm	Molecules at surface	10 - 100 nm	Ag 25 nm	Ag, C, O 98% spherical	20 - 35 nm, presence of large molecules	
[43]	China	Antimicrobial	2 g fresh/100 mL 90˚C	20 mL/$AgNO_3$ (1 mM) 100 mL	438 nm	Molecules at surface		Ag 25 nm	Ag, O, C 99% 20 - 35 nm	20 - 25 nm	
[44]	China	Dye degradation	100 g/1 L ethanol, ultrasound	5 mg/mL separated flavonoids solution/$AgNO_3$ (1 mM) 100 mL	420 nm	Molecules at surface	10 - 100 nm	Ag 20 nm	Ag, O, C Spherical 15 - 20 nm	15 - 20 nm	
[45]	India	Antimicrobial	5 g fresh/100 mL boiled	Microwave synthesis			54 nm				
[46]	India	Antibacterial	20 g fresh/200 mL 60˚C	5 mL/$AgNO_3$ (1 mM) 100 mL, stirred	430 - 456 nm	Molecules at surface		Ag 55 nm + bioorganic crystallized	Spherical, mean 80 nm, Ag, O	55 nm, spherical	

Continued

[47]	India	Antibacterial	10 g fresh/100 mL ethanol boiled	5 mL/ $AgNO_3$ (1 mM) 45 mL, stirred					
[48]	India		Fresh/100 mL hot	1 - 5 mL/$AgNO_3$ (1 mM) 10 mL	419 nm	Molecules at surface	62 nm	Crystalline	
[49]	India	Antibacterial, Antifungal	30 g powder/500 mL Hexane, Soxhlet	50 mL/$AgNO_3$ (1 M), 50 mL H_2O, 50˚C					10 - 35 nm, Spherical
[50]	Thailand	Antifungal, micelles	2 g dry/100 mL water	0.1 mg/mL, 70˚C/1 mL $AgNO_3$ (10 mM) hot stirred	455 nm			96 ± 4 nm, Spherical, Ag, Cl, C	
This work	Cameroon	Larvicidal	10 g fresh/100 mL water 90˚C (2.42 g/L)	10 mL/$AgNO_3$ (1 mM) 50 mL	419 - 432 nm	Molecules at surface	16 - 79 nm. Center 28.6 nm	Ag 35.2 nm, AgCl 17.3 nm	Ag, C, O, Cl, Spherical and cuboids

UV-Vis: Ultraviolet Visible spectroscopy, FTIR: Fourier Transform Infrared Spectroscopy, DLS: Dynamic Light Scattering, DRX: Powder X-ray Diffraction, SEM: Scanning Electron Microscopy, EDX: Energy Dispersive X-Ray Spectrometry, TEM: Transmission Electron Microscopy, AFM: Atomic Force Microscopy.

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 $AgNO_3$ (1 mM). The mixture was incubated 5 h at 25˚C - 28˚C in dark to minimize the photo activation of $AgNO_3$. 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 $AgNO_3$ at ambient temperature, or $AgNO_3$ in the dark. Different concentrations of $AgNO_3$ 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/10h (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:

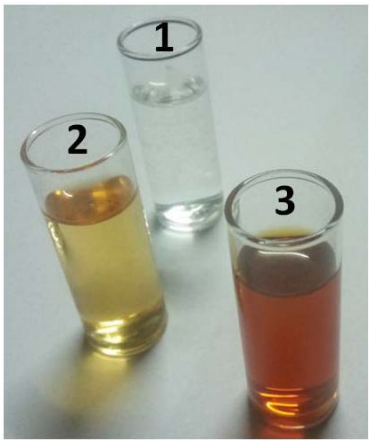


Figure 2. $AgNO_3$ aqueous solution (1), *Psidium guajava* leaf aqueous extract (2), Ag nanoparticle aqueous solution (3).

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 LC_{50} was calculated at 95% fiducial limits of both upper and lower confidence limits.

3. Results and Discussion

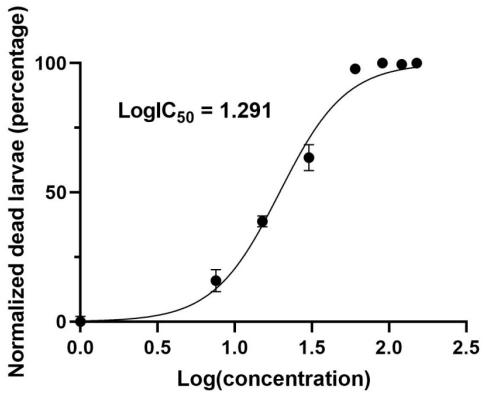
Larvicidal activity of synthesized AgNPs

P. 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. 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 4^{th} 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 LC_{50} 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**.

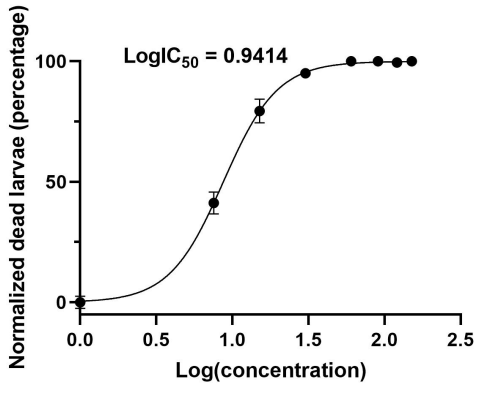
P. guajava plant extract did not cause larval mortality at all tested concentrations. When used at different concentrations, both photo-activated $AgNO_3$ and $AgNO_3$ in the dark killed all larvae of *A. gambiae*. Mondal and colleagues have previously described the mortality of *Culex quiquefasciatus* in response to a 10 ppm $AgNO_3$ 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 fiendly [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].



(a)



(b)

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.

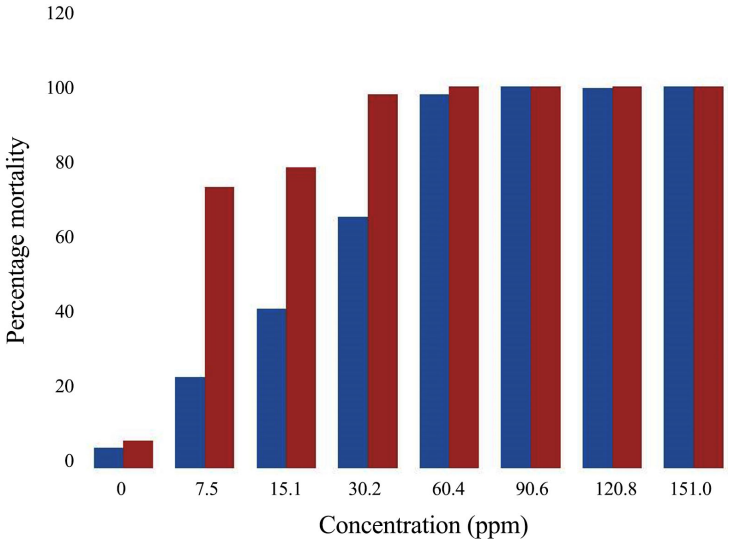


Figure 4. Percentage mortality of *Anopheles gambiae* 4th instar larvae as a function of Ag nanoparticle concentration (in ppm) after 24 h (blue) and 48 h (brown).

Table 2. Larvicidal activity of Ag nanoparticles obtained from *Psidium guajava* L. and the precursors: plant extract and $AgNO_3$.

Time duration	Samples	Concentration (ppm)	Mean number of death	% mortality	LC_{50} ppm	95% confidence limits	
						LCL	UCL
24 h	Nanosilver	0	13	5.4	19.55	18.00	21.19
		7.5	57	23.8			
		15.1	100	41.7			
		30.2	158	65.8			
		60.4	235	97.9			
		90.6	240	100			
		120.8	239	99.6			
		151.0	240	100			
	Aqueous extract	7.5 - 151	0	0	NA		
	Silver ions	7.5 - 151	240	100	NA		
48 h	Nanosilver	0	17	7.1	8.737	8.110	9.361
		7.5	177	73.8			
		15.1	189	78.8			
		30.2	235	97.9			
		60.4	240	100			
		90.6	240	100			
		120.8	240	100			
		151.0	240	100			
	Aqueous extract	7.5 - 151	0	0	NA		
	Silver ions	7.5 - 151	240	100	NA		

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.

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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

[1] Ponraj, G.K., Kumar, R. and Saravanan, V. (2017) Biosynthesis of Silver Nanoparticles from *Allium cepa* Leaf Extract and Its Larvicidal Activity against *Culex quinquefasciatus* and *Aedes aegypti*. *International Journal of Biosciences and Nanosciences*, **4**, 75-82.

[2] Prusty, A.K., Kajol, S.J., Datta, R.S. and Mishra, P.P. (2016) Mosquito Larvicidal Activity of Biogenically Synthesized Silver Nanoparticles Using *Aponogeton natans* Leaf Extract. *Indo American Journal of Pharmaceutical Research*, **6**, 5401-5406.

[3] Benelli, G., Caselli, A. and Canale, A. (2017) Nanoparticles for Mosquito Control: Challenges and Constraints. *Journal of King Saud University—Science*, **29**, 424-435. https://doi.org/10.1016/j.jksus.2016.08.006

[4] White, N.J. (2015) Declining Malaria Transmission and Pregnancy Outcomes in Southern Mozambique. *New England Journal of Medicine*, **373**, 1670-1671. https://doi.org/10.1056/NEJMe1511278

[5] WHO (2018) World Malaria Report. The World Health Organization, Geneva. http://www.who.int

[6] Benelli, G. and Mehlhorn, H. (2016) Declining Malaria, Rising Dengue and Zika Virus: Insights for Mosquito Vector Control. *Parasitology Research*, **115**, 1747-1754. https://doi.org/10.1007/s00436-016-4971-z

[7] Pino, P., Caldelari, R., Mukherjee, B., Vahokoski, J., Klages, N., Maco, B., Collins, C.R., Blackman, M.J., Kursula, I., Heussler, V., Brochet, M. and Soldati-Favre, D. (2017) A Multistage Antimalarial Targets the Plasmepsins IX and X Essential for Invasion and Egress. *Science*, **358**, 522-528. https://doi.org/10.1126/science.aaf8675

[8] Bass, C., Williamson, M.S., Wilding, C.S., Donnelly, M.J. and Field, M.L. (2007) Identification of the Main Malaria Vectors in the Anopheles Gambiae Species Complex Using a TaqMan Real-Time PCR Assay. *Malaria Journal*, **6**, Article No. 155. https://doi.org/10.1186/1475-2875-6-155

[9] Chrustek, A., Hołynska-Iwan, I., Dziembowska, I., Bogusiewicz, J., Wróblewski, M., Cwynar, A. and Olszewska-Słonina, D. (2018) Current Research on the Safety of Pyrethroids Used as Insecticides. *Medicine*, **54**, 61. https://doi.org/10.3390/medicina54040061

[10] Mendonca, P.M., Lima, M.G., Albuquerque, L.R.M., Carvalho, M.G. and Queiroz, M.M.C. (2011) Effects of Latex from "Amapazeiro" (Apocynaceae) on Blowfly *Chrysomya megacephala* (Diptera: Calliphoridae) Post-Embryonic Development. *Veterinary Parasitology*, **178**, 379-382. https://doi.org/10.1016/j.vetpar.2011.01.002

[11] Arjunan, N.K., Murugan, K., Rejeeth, C., Madhiyazhagan, P. and Barnard, D.R. (2012) Green Synthesis of Silver Nanoparticles for the Control of Mosquito Vectors of Malaria, Filariasis, and Dengue. *Vector Borne and Zoonotic Diseases*, **12**, 262-268. https://doi.org/10.1089/vbz.2011.0661

[12] Barik, T.K., Kamaraju, R. and Gowswami, A. (2012) Silica Nanoparticle: A Potential New Insecticide for Mosquito Vector Control. *Parasitology Research*, **111**, 1075-1083. https://doi.org/10.1007/s00436-012-2934-6

[13] Rahman, S., Biswas, S.K., Barman, N.C. and Ferdous, T. (2016) Plant Extract as Selective Pesticide for Integrated Pest Management. *Biotechnology Research Journal*, **2**, 6-10.

[14] Arnason, J.T., Sims, S.R. and Scott, I.M. (2012) Natural Products from Plants as Insecticides. Phytochemistry and Pharmacognosy. http://www.eolss.net/Sample-Chapters/C06/E6-151-13.pdf

[15] Isman, M.B. (2000) Plant Essential Oils for Pest and Disease Management. *Crop Protection*, **19**, 603-608. https://doi.org/10.1016/S0261-2194(00)00079-X

[16] Escobar, L.E., Romero-Alvarez, D., Leon, R., Lepe-Lopez, M.A., Craft, M.E., Borbor-Cordova, M.J. and Svenning, J.-C. (2016) Declining Prevalence of Disease Vectors under Climate Change. *Scientific Reports*, **6**, Article No. 39150. https://doi.org/10.1038/srep39150

[17] Eya'ane Meva, F., Ntoumba, A.A., Belle Ebanda Kedi, P., Tchoumbi, E.E., Schmitz, A., Schmolke, L., Klopotowski, M., Moll, B., Kökçam-Demir, Ü., Mpondo Mpondo, E.A., Lehman, L.G. and Janiak, C. (2019) Silver and Palladium Nanoparticles Produced Using a Plant Extract as Reducing Agent, Stabilized with an Ionic Liquid: Sizing by X-Ray Powder Diffraction and Dynamic Light Scattering. *Journal of Material Research and Technology*, **8**, 1991-2000. https://doi.org/10.1016/j.jmrt.2018.12.017

[18] Kumar, S., Viney, L. and Deepti, P. (2015) Green Synthesis of Therapeutic Nanoparticles: An Expanding Horizon. *Nanomedicine*, **10**, 2451-2471. https://doi.org/10.2217/nnm.15.112

[19] Daswani, P.G., Gholkar, M.S. and Birdi, T.J. (2017) *Psidium guajava*: A Single Plant for Multiple Health Problems of Rural Indian Population. *Pharmacognosie Review*, **11**, 167-174. https://doi.org/10.4103/phrev.phrev_17_17

[20] Almeida, C.E., Karnikowski, M.G.O., Foleto, R. and Baldisserotto, B. (1995) Analysis of Antidiarrhoeic Effect of Plants Used in Popular Medicine. *Revista de Saude Publica*, **29**, 428-433. https://doi.org/10.1590/S0034-89101995000600002

[21] Joseph, B. and Priya, R.M. (2011) Phytochemical and Biopharmaceutical Aspects of *Psidium guajava* (L.) Essential Oil: A Review. *Research Journal of Medicinal Plants*, **5**, 432-442. https://doi.org/10.3923/rjmp.2011.432.442

[22] Nantitanon, W. and Okonogi, S. (2012) Comparison of Antioxidant Activity of Compounds Isolated from Guava Leaves and a Stability Study of the Most Active Compound. *Drug Discovery and Therapeutics*, **6**, 38-43. https://doi.org/10.5582/ddt.2012.v6.1.38

[23] Díaz-de-Cerio, E., Verardo, V., Gómez-Caravaca, A.M., Fernández-Gutiérrez, A. and Segura-Carretero, A. (2015) Determination of Polar Compounds in Guava Leaves Infusions and Ultrasound Aqueous Extract by HPLC-ESI-MS. *Journal of Chemistry*, **2015**, Article ID: 250919. https://doi.org/10.1155/2015/250919

[24] Shruthi, S.D., Roshan, A., Timilsina, S.S. and Sunita, S. (2013) A Review on the Medical Plant *Psidium guajava* Linn. (Myetaceae). *Journal of Drug Delivery and Therapeutics*, **3**, 162-168. https://doi.org/10.22270/jddt.v3i2.404

[25] Rwang, P.G., Effoim, O.E., Merc, K.P. and Etokakpan, A.M. (2016) Effects of *Psidium guajava* (Guava) Extracts on Immature Stage of Mosquito. *International Journal of Complementary and Alternative Medicine*, **4**, Article ID: 00132. https://doi.org/10.15406/ijcam.2016.04.00132

[26] Gutierrez, R.M.P., Mitchell, S. and Solis, R.V. (2008) *Psidium guajava*: A Review of Its Traditional Uses, Phytochemistry and Pharmacology. *Journal of Ethnopharmacology*, **117**, 1-27. https://doi.org/10.1016/j.jep.2008.01.025

[27] Raghunandan, D., Mahesh, B.D., Basavaraja, S., Balaji, S.D., Manjunath, S.Y. and Venkataraman, A. (2011) Microwave-Assisted Rapid Extracellular Synthesis of Sta-

ble Bio-Functionalized Silver Nanoparticles from Guava (*Psidium guajava*) Leaf Extract. *Journal of Nanoparticles Research*, **13**, 2021-2028. https://doi.org/10.1007/s11051-010-9956-8

[28] Parashar, U.K., Kumar, V., Bera, T., Saxena, P.S., Nath, G., Srivastava, S.K., Giri, R. and Srivastava, A. (2011) Study of Mechanism of Enhanced Antibacterial Activity by Green Synthesis of Silver Nanoparticles. *Nanotechnology*, **22**, Article ID: 415104. https://doi.org/10.1088/0957-4484/22/41/415104

[29] Prasad, T.N.V.K.V., Elumalai, E.K. and Khateeja, S. (2011) Evaluation of the Antimicrobial Efficacy of Phytogenic Silver Nanoparticles. *Asian Pacific Journal of Tropical Biomedicine*, **1**, S82-S85. https://doi.org/10.1016/S2221-1691(11)60130-5

[30] Priya, R.S., Geetha, D. and Ramesh, P.S. (2014) Green Synthesis of Silver Nanoparticles Using *Psidium guajava* Leaf Extract and Their Characterization. *Indian Streams Research Journal*, **4**, 1-7.

[31] Sriram, T. and Pandidurai, V. (2014) Synthesis of Silver Nanoparticles from Leaf Extract of *Psidium guajava* and Its Antibacterial Activity against Pathogens. *International Journal of Current Microbiology and Applied Sciences*, **3**, 146-152.

[32] Shinde, N.M., Lokhande, A.C. and Lokhande, C.D. (2014) A Green Synthesis Method for Large Area Silver Thin Film Containing Nanoparticles. *Journal of Photochemistry and Photobiology B*: *Biology*, **136**, 19-25. https://doi.org/10.1016/j.jphotobiol.2014.04.011

[33] Moteriya, P., Padalia, H. and Chanda, S. (2014) Green Biosynthesis of Silver Nanoparticles Using *Psidium guajava* L. Leaf Extract and Antibacterial Activity against Some Pathogenic Microorganisms. *Journal of Pharmaceutical Research*, **8**, 1579-1585.

[34] Lokina, S., Stephen, A., Kaviyarasan, V., Arulvasu, C. and Narayanan, V. (2015) Cytotoxicity and Antimicrobial Studies of Silver Nanoparticles Synthesized Using *Psidium guajava* L. Extract. *Synthesis and Reactivity in Inorganic*, *Metal-Organic and Nano-Metal Chemistry*, **45**, 426-432. https://doi.org/10.1080/15533174.2013.831881

[35] Manogaran, S., Krishnan, N., Senthilkumar, V.S., Manivannan, S., Veerababhu, V. and Kannan, K.P. (2015) Green Synthesis of Silver Nanoparticles and Their Antimicrobial Property of Endophytic Fungi Isolated from *Mentha arvensis* L. and *Psidium guajava* L. *Journal of Chemical and Pharmaceutical Research*, **7**, 340-346.

[36] Rochlani, K., Vadakkekara, R., Chakraborty, M. and Dasgupta, S. (2016) Antibacterial Activity of Biostabilized Silver Nanoparticles. *Indian Journal of Chemical Technology*, **23**, 520-526.

[37] Dama, L.B., Mane, P.P., Pathan, A.V., Chandarki, M.S., Sonawane, S.R., Dama, S.B., Chavan, S.R., Chondekar, R.P. and Vinchurkar, A.S. (2016) Green Synthesis of Silver Nanoparticles Using Leaf Extract of *Lawsonia inermis* and *Psidium guajava* and Evaluation of Their Antibacterial Activity. *Scientific Research Reporter*, **6**, 89-95.

[38] Bose, D. and Chatterjee, S. (2016) Biogenic Synthesis of Silver Nanoparticles Using Guava (*Psidium guajava*) Leaf Extract and Its Antibacterial Activity against *Pseudomonas aeruginosa*. *Applied Nanosciences*, **6**, 895-901. https://doi.org/10.1007/s13204-015-0496-5

[39] Ravindra, B.K. and Patil, N.G. (2017) Synthesis and Characterization of Silver Nanoparticles Using *Psidium guajava* Leaves. *International Journal of Innovative Research and Reviews*, **5**, 72-77.

[40] Banik, I., Som, M., Bhattacharjee, S., Bhattacharyya, S. and Rai, C. (2017) Bacteriostatic Activities of Antioxidant Silver Nanoparticles Prepared from Leaves of *Azadirachta indica* and *Psidium guajava*. *European Journal of Pharmaceutical and Medi-*

cal Research, **4**, 223-228.

[41] Geetha, V. (2017) Green Synthesis of Silver Nanoparticles from *Psidium guajava* Leaves and Its Antibacterial Activity. *International Journal of Bioassays*, **6**, 5441-5443. https://doi.org/10.21746/ijbio.2017.07.003

[42] Wang, L., Xie, J., Huang, T., Ma, Y. and Wu, Z. (2017) Characterization of Silver Nanoparticles Biosynthesized Using Crude Polysaccharides of *Psidium guajava* L. Leaf and Their Bioactivities. *Materials Letters*, **208**, 126-129. https://doi.org/10.1016/j.matlet.2017.05.014

[43] Wang, L., Wu, Y., Xie, J., Wu, S. and Wu, Z. (2018) Characterization, Antioxidant and Antimicrobial Activities of Green Synthesized Silver Nanoparticles from *Psidium guajava* L. Leaf Aqueous Extracts. *Materials Science and Engineering*: *C*, **86**, 1-8. https://doi.org/10.1016/j.msec.2018.01.003

[44] Wang, L., Lu, F., Liu, Y., Wu, Y. and Wu, Z. (2018) Photocatalytic Degradation of Organic Dyes and Antimicrobial Activity of Silver Nanoparticles Fast Synthesized by Flavonoids Fraction of *Psidium guajava* L. Leaves. *Journal of Molecular Liquids*, **263**, 187-192. https://doi.org/10.1016/j.molliq.2018.04.151

[45] Parveen, A., Yalagatti, M.S., Abbaraju, V. and Deshpande, R. (2018) Emphasized Mechanistic Antimicrobial Study of Biofunctionalized Silver Nanoparticles on Model *Proteus mirabilis*. *Journal of Drug Delivery*, **2018**, Article ID: 3850139. https://doi.org/10.1155/2018/3850139

[46] Sharmila, C., Ranjith Kumar, R. and Chandar Shekar, B. (2018) *Psidium guajava*: A Novel Plant in the Synthesis of Silver Nanoparticles for Biomedical Applications. *Asian Journal of Pharmaceutical and Clinical Research*, **11**, 341-345. https://doi.org/10.22159/ajpcr.2017.v11i1.21999

[47] Radhakrishnan, S., Komathi, S. and Rajalakshmi, G. (2018) Synthesis of Silver Nanoparticles from *Psidium guajava* Leaf Extract and Their Antibacterial Activity. *European Journal of Biomedical and Pharmaceutical Sciences*, **5**, 783-786.

[48] Pagadala, R.S., Mekala, R., Sapara, S.H., Tenkayala, S.R. and Gangadhara, R. (2018) Green Synthesis and Characterization of Silver Nanoparticles by Using *Psidium guajava* Leaf Extract. *Journal of Drug Delivery and Therapeutics*, **8**, 301-305. https://doi.org/10.22270/jddt.v8i5-s.2025

[49] Bhatt, K.D., Desai, Y.R., Sharma, N.K., Sukhadiya, P.C. and Zalaiya, M.R. (2019) Aqueous Stable Silver Nanoparticles Derived from Nimble Leaves of *Psidium guajava*. *International Journal Conference Proceedings*, **1**, ICP.000519.2019.

[50] Suwan, T., Khongkhunthian, S. and Okonogi, S. (2019) Antifungal Activity of Polymeric Micelles of Silver Nanoparticles Prepared from *Psidium guajava* Aqueous Extract. *Drug Discovery and Therapeutics*, **13**, 62-69. https://doi.org/10.5582/ddt.2019.01024

[51] Eya'ane Meva, F., Okalla Ebongue, C., Véronique Fannang, S., Segnou, M.L., Ntoumba, A.A., Belle Ebanda Kedi, P., Njike Loudang, R.-E., Yonga Wanlao, A., Mang, E.R. and Mpondo Mpondo, E.A. (2017) Spectroscopic Synthetic Optimizations Monitoring of Silver Nanoparticles Formation from *Megaphrynium macrostachyum* Leaf Extract. *Journal of Nanomaterials*, **2017**, Article ID: 6834726. https://doi.org/10.1155/2017/6834726

[52] Belle Ebanda Kedi, P., Eya'ane Meva, F., Kotsedi, L., Nguemfo, E.L., Bogning Zangueu, C., Ntoumba, A.A., Mohamed, H.E.A., Dongmo, A.B. and Maaza, M. (2018) Eco-Friendly Synthesis, Characterization, *in Vitro* and *in Vivo* Anti-Inflammatory Activity of Silver Nanoparticle-Mediated *Selaginella myosurus* Aqueous Extract. *International Journal of Nanomedicine*, **13**, 8537-8548.

https://doi.org/10.2147/IJN.S174530

[53] WHO—World Health Organization (2005) Guidelines for Laboratory and Field Testing of Mosquito Larvicides. WHO/CDS/WHOPES/GCDPP/2005.13.

[54] Awwad, A.M., Salem, N.M., Ibrahim, Q.M. and Abdeen, A.O. (2015) Phytochemical Fabrication and Characterization of Silver/Silver Chloride Nanoparticles Using *Albizia julibrissin* Flowers Extract. *Advanced Materials Letters*, **6**, 726-730. https://doi.org/10.5185/amlett.2015.5816

[55] Eya'ane Meva, F., Avom Mbeng, J.O., Okalla Ebongue, C., Schlüsener, C., Kökçam-Demir, Ü., Ntoumba, A.A., Belle Ebanda Kedi, P., Elanga, E., Njike Loudang, E.-R., Nko'o, M.H.J., Tchoumbi, E.E., Deli, V., Chick Nanga, C., Mpondo Mpondo, E.A. and Janiak, C. (2019) *Stachytarpheta cayennensis* Aqueous Extract, a New Bioreactor towards Silver Nanoparticles for Biomedical Applications. *Journal of Biomaterials and Nanobiotechnology*, **10**, 102-119. https://doi.org/10.4236/jbnb.2019.102006

[56] Mondal, N.K., Chowdhury, A., Dey, U., Mukhopadhya, P., Chatterjee, S., Das, K. and Datta, J.K. (2014) Green Synthesis of Silver Nanoparticles and Its Application for Mosquito Control. *Asian Pacific Journal of Tropical Diseases*, **4**, S204-S210. https://doi.org/10.1016/S2222-1808(14)60440-0

[57] Rate, H.T. (1999) Bioaccumulation and Toxicity of Silver Compounds: A Review. *Environmental Toxicology and Chemistry*, **18**, 89-108. https://doi.org/10.1002/etc.5620180112

List of Abbreviations

Nps: nanoparticles, Ag: silver, AgNPs: silver nanoparticles; P: Psidium, UV-Vis: ultraviolet visible, IR: infrared spectroscopy, PXRD: powder X-ray diffraction, DLS: dynamic light scattering, SEM: scanning electron microscopy, EDX: energy dispersive X-ray spectroscopy, LC_{50}: 50% lethal concentration; WHO: World Health Organization.

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–Nelaser 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.

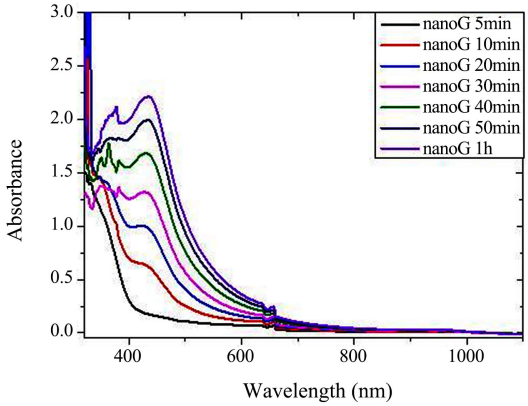


Figure A1. Ultraviolet-visible spectra 1 hour analysis of synthesized nanoparticles.

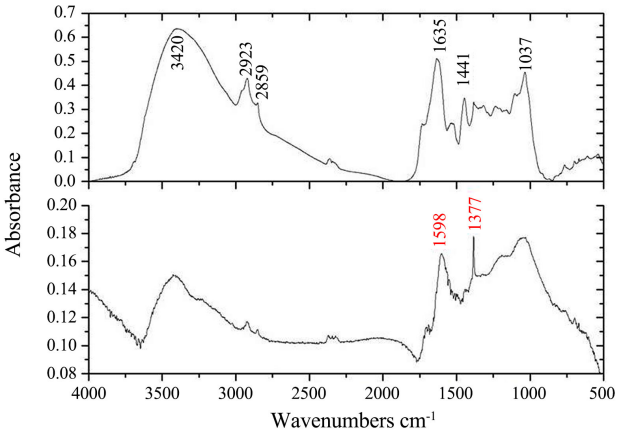


Figure A2. Fourier transform infrared spectrum for synthesized silver nanoparticles using dry plant power (up) and silver nanoparticles (down).

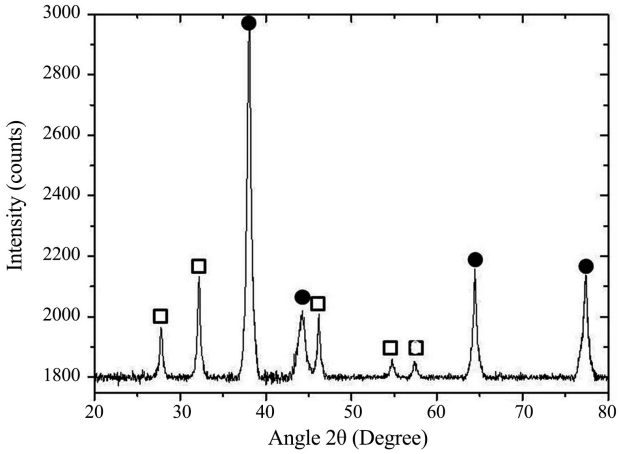


Figure A3. X-ray diffraction pattern of the nanoparticles from *Psidium guajava*; ● represents silver nanocrystallites and □ represents silver chloride nanocrystallites.

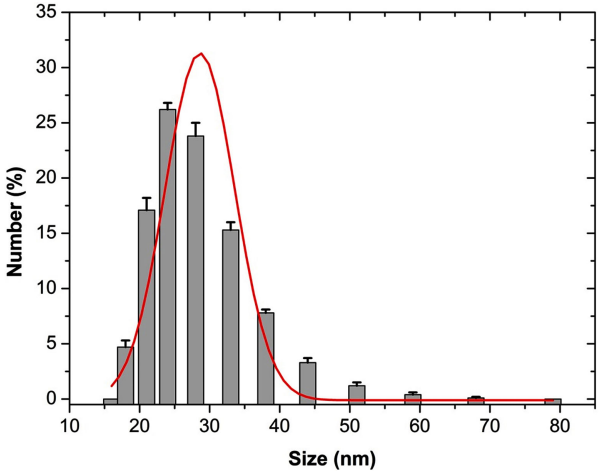


Figure A4. DLS histogram of aqueous solutions of silver nanoparticles mediated *Psidium guajava* plant axtract together with a Gaussian fitting (red curve).

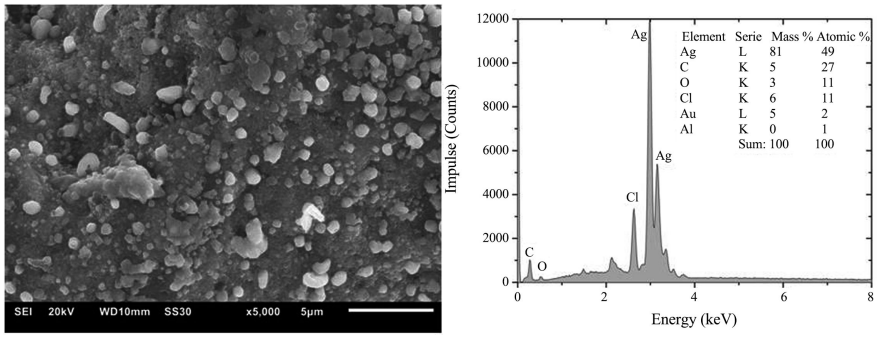
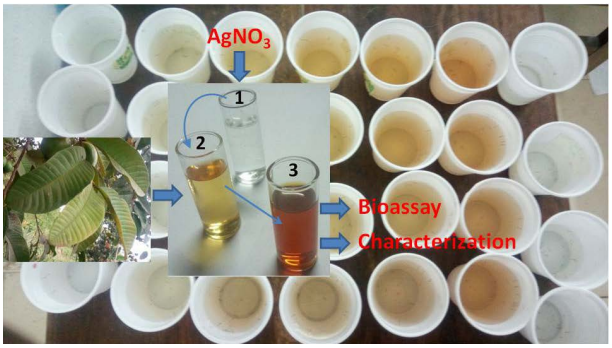


Figure A5. SEM capture (left) and EDX element mapping (right) of the silver nanoparticles mediated *Psidium guajava* Plan extract.



Graphical abstract.