

Annona muricata leaf extract-mediated silver nanoparticles synthesis and its larvicidal potential against dengue, malaria and filariasis vector

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Abstract Mosquitoes transmit several diseases which cause millions of deaths every year. The use of synthetic insecticides to control mosquitoes caused diverse effects to the environment, mammals, and high manufacturing cost. The present study was aimed to test the larvicidal activity of green synthesized silver nanoparticles using *Annona muricata* plant leaf extract against third instar larvae of three medically important mosquitoes, i.e., *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. The different concentrations of green synthesized Ag Nanoparticles (AgNPs; 6, 12, 18, 24, 30 $\mu\text{g mL}^{-1}$) and aqueous crude leaf extract (30, 60, 90, 120, 150 $\mu\text{g mL}^{-1}$) were tested against the larvae for 24 h. Significant larval mortality was observed after the treatment of *A. muricata* for all mosquitoes with lowest LC₅₀ and LC₉₀ values, viz., *A. aegypti* (LC₅₀ and LC₉₀ values of 12.58 and 26.46 $\mu\text{g mL}^{-1}$), *A. stephensi* (LC₅₀ and LC₉₀ values of 15.28 and 31.91 $\mu\text{g mL}^{-1}$) and *C. quinquefasciatus* (LC₅₀ and LC₉₀ values of 18.77 and 35.72 $\mu\text{g mL}^{-1}$), respectively. The synthesized AgNPs from *A. muricata* were highly toxic than aqueous crude extract. The nanoparticle characterization was done using spectral and microscopic analysis, namely UV-visible spectroscopy which showed a sharp peak at 420 nm of aqueous medium containing AgNPs, X-ray diffraction (XRD) analysis revealed the average crystalline size of synthesized AgNPs (approximately 45 nm), and Fourier transform infrared spectroscopy (FTIR) study exhibited prominent peaks 3381.28, 2921.03, 1640.17, 1384.58, 1075.83, and 610.77 cm^{-1} . Particle size analysis

(PSA) showed the size and distribution of AgNPs (103 nm); field emission scanning electron microscopy (FE-SEM) and high-resolution transmission electron microscopy (HR-TEM) analysis showed a spherical shape, size range from 20 to 53 nm; and energy-dispersive X-ray spectroscopy (EDX) reflects the chemical composition of synthesized AgNPs. Heat stability of the AgNPs was confirmed between the temperatures 20 to 70 °C. The result suggests that green synthesized AgNPs from *A. muricata* has the potential to be used as a low-cost and eco-friendly approach for the control of selected mosquitoes.

Keywords Silver nanoparticles · *Annona muricata* · Larvicidal activity · *Anopheles stephensi* · *Culex quinquefasciatus* · *Aedes aegypti*

Introduction

Mosquito transmits several diseases such as dengue, malaria, lymphatic filariasis, chikungunya, and Japanese encephalitis. Mosquito-borne disease remains a major source of illness and death worldwide, and it has been increasing at an alarming rate and has become a serious problem. They are prevalent in more than 100 countries, both developed and developing countries across the world, infecting over 70 billion people every year universally and 40 million of the Indian population (Veerakumar et al. 2013). Mosquitoes can be found frequently due to lack of drainage system, fish pond, rice fields, and irrigation ditches (Komalamisra and Trongtokit 2005).

Aedes aegypti is recognized as a dengue and chikungunya vectors (WHO 2010). Dengue is widely distributed in tropical and subtropical zones. According to WHO (2009), there were 50 million people affected by dengue infection throughout the world. Recently, over 2.5 billion people are affected from dengue, and WHO (2012) estimated that there are 50 to 100

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million people affected by dengue every year. *Anopheles stephensi* is considered as an important vector of malaria in many countries, especially the Middle East and Indian subcontinent (Gayathri and Balakrishna Murthy 2006). According to WHO, 219 million people were affected by malaria in 2010. Malaria infects 500 million people every year and kills 1.2–2.7 million people per year globally. Moreover, 90 % of the malaria-related death occurs in Africa (Breman et al. 2004). *Culex quinquefasciatus* is an important vector of lymphatic filariasis, which is widely distributed in tropical and subtropical countries with nearly 1.4 billion people in 73 countries threatened worldwide by lymphatic filariasis, commonly known as elephantiasis. Approximately 120 million people are currently infected by the disease (WHO 2013).

Numerous chemical insecticides like permethrin and dieldrin are widely used to control the mosquitoes, but those insecticides have created a number of problems, viz., development of resistant insect strains, ecological imbalance, and harm to mammals. So that, the researchers started to give importance to the field of nanotechnology, which plays an important role in biology, medicine, clinical fields, conductive materials, and coating formulations (Templeton et al. 2000). Many researchers have reported the control of mosquito larvae using the biologically synthesized nanoparticles contains bio-active compounds which are responsible for controlling the mosquito larvae. The development of nanoparticles was increased using metals like silver, gold, zinc, copper, etc. (Evanoff and Chumanov 2005). Silver nanoparticles are reported to possess better anti-viral (Rogers et al. 2008), anti-fungal (Kim et al. 2009), anti-bacterial (Krishnaraj et al. 2010), anti-cancer (Venkatesan et al. 2014), and mosquito larvicidal (Chitra et al. 2015) properties.

Use of plant extracts for the synthesis of nanoparticles (NPs) could be more advantageous than other sources (Subarani et al. 2013). Recently, AgNPs have been synthesized using various medicinal plants like *Glycine max* (Vivekanandhan et al. 2009), *Pongamia pinnata* (Rajesh et al. 2010), *Annona squamosa* (Naresh Kumar et al. 2011), and *Murraya koenigii* (Suganya et al. 2013), respectively. In this study, we have chosen *Annona muricata* (L.) belonging to the family Annonaceae is a small, upright evergreen tree, 5–6 m in height with large, dark green leaves. *Annona muricata* extract was reported as anti-viral (Padma et al. 1998; Betancur-Galvis et al. 1999), anti-cancer (Gavamukulya et al. 2014), anti-diabetic (Florence et al. 2014), anti-fungal (Abubacker and Deepalakshmi 2013), anti-tubercular (Ragasa et al. 2013), and larvicidal properties (Abubacker et al. 2014). With this background, the present study was aimed to investigate the green synthesized silver nanoparticles from *A. muricata* and tested its

larvicidal properties against third instar larvae of three medically important mosquitoes *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus*.

Materials and methods

Collection of plant materials

Fresh leaves of *A. muricata* (Fig. 1) were collected from in and around Mettur Taluk (11.8000° N, 77.8000° E), Salem at, Tamilnadu, India. The voucher specimen was deposited in the laboratory for future use.

Preparation of plant extracts

The fresh leaves of *A. muricata* were washed with tap water followed by distilled water in order to remove the dust particles and air-dried for 15 days and grind to make a fine powder in an electric grinder. Aqueous extract was prepared by mixing 50 g of leaf powder with 500 mL of double distilled water with constant stirring on a magnetic stirrer. The dried leaf powder suspension was left for 3 h and filtered through a Whatman no. 1 filter paper, and the filtrate was stored at -4°C temperature by the use (Veerakumar et al. 2014).

Mosquito culture

Laboratory culture of mosquito larvae *A. stephensi* and *C. quinquefasciatus* was obtained from the National Centre for Disease Control (NCDC), Mettupalayam, Tamil Nadu, India. The wild *A. aegypti* larvae collected from Mettur, Tamil nadu, India were used in this study. All the larvae were kept in trays containing tap water and maintained in the laboratory. All the experiments were carried out at $27\pm 2^{\circ}\text{C}$ and 75–85 % relative humidity under 14:10 light/dark photoperiod



Fig. 1 *Annona muricata* plant

cycles. Larvae were fed with dog biscuits and yeast powder in the ratio of 3:1. The cultures were maintained and reared in the laboratory.

Synthesis of silver nanoparticles

The *A. muricata* broth solution was prepared by taking 10 g of distilled water washed and finely cut leaves in a 500-mL Erlenmeyer flask and along with 100 mL of double distilled water. Then, boiling the mixture for 10 min, and the extract was filtered through Whatman no. 1 filter paper and stored at -4°C , and it could be utilized within a week. After the extract preparation, 12 mL of filtrate was treated with 88 mL of 1 mM silver nitrate solution at room temperature for 15 min, resulting in a yellow to brown solution indicating the formation of silver nanoparticles.

Heat stability of AgNPs

The heat stability of the AgNPs was carried out by heating the AgNPs solution in different temperatures using a water bath (20, 30, 40, 50, 60, and 70°C). Each heated solution containing the silver nanoparticles was observed by UV-visible spectrometer, and the absorbance of the samples was measured at 300 to 700 nm.

Characterization of silver nanoparticles

The bioreduction of AgNO_3 in solution was monitored using UV-visible spectrometer, at the wavelength of 300–700 nm. The absorption spectra of synthesized NP concentrations were measured at different time intervals 0 min, 10 min, 30 min, 45 min, and 1 h (Shimadzu UV Spectrophotometer, model UV-1800). The colloidal solution containing the nanoparticles was centrifuged at 8000 rpm for 10 min. The supernatant was again centrifuged at 10,000 rpm for 20 min, and the pellet was obtained. This was followed by redispersal of silver nanoparticle pellets into 1 mL of deionized water. After, the purified suspension was freeze dried to obtain a dried powder. Finally, the dried silver nanoparticles were analyzed in the mid IR region of 400–4000 cm^{-1} by KBr pellet technique in Fourier transform infrared spectroscopy (FTIR) analysis (Vivek et al. 2011). Furthermore, the dried powder containing silver nanoparticles was subjected to X-ray diffraction (XRD) analysis to confirm the crystalline nature of AgNPs (Advance power X-ray diffractometer, Bruker, Germany model D8). The particle size distribution of silver nanoparticles was analyzed on particle size analyzer system (Zeta sizer, Malvern Instruments Ltd., USA). The images of nanoparticles were studied using Field emission Scanning Electron Microscope (FE-SEM; JEOL, Model JFC-1600), energy-dispersive spectroscopy (EDX), and high-resonance transmission electron microscopy (HR-TEM—Hitachi H-7100 using an accelerating voltage of

Table 1 Larvicidal activity of *A. muricata* aqueous leaf extract against third instar of *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* of 24-h observation

Mosquitoes	Concentration of ALE	24-h mortality (%)±SD	LC ₅₀ µg/mL (LCL–UCL)	LC ₉₀ µg/mL (LCL–UCL)	χ^2
<i>Aedes aegypti</i>	Control	0.0±0.0	51.13	82.08	3.323*
	30	4.0±0.0	(40.62–78.60)	(62.10–135.73)	
	60	6.6±2.3			
	90	9.3±2.3			
	120	13.3±2.4			
	150	17.3±2.3			
<i>Anopheles stephensi</i>	Control	0.0±0.0	61.38	156.55	79.34*
	30	40.0±4.0	(34.33–85.83)	(121.39–247.86)	
	60	52.0±10			
	90	66.6±6.1			
	120	77.3±4.6			
	150	85.3±8.3			
<i>Culex quinquefasciatus</i>	Control	0.0±0.0	88.72	199.67	72.12*
	30	24.0±4.0	(63.77–126.54)	(151.30–344.18)	
	60	36.0±0.0			
	90	49.3±2.3			
	120	61.3±2.3			
	150	78.6±2.3			

UCL upper confidence limit, LCL lower confidence limit, χ^2 chi-square test

*Significant at $p < 0.05$ level

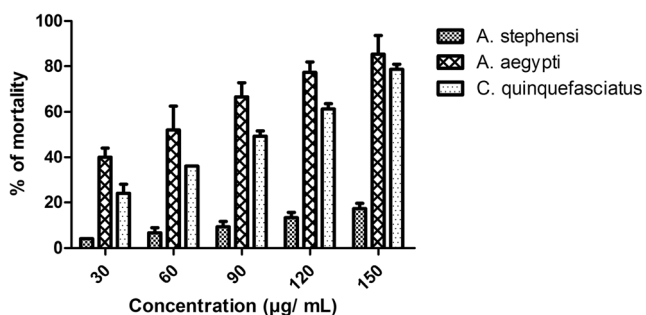


Fig. 2 The percentage mortality of *A. muricata* aqueous leaf extract against *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus*

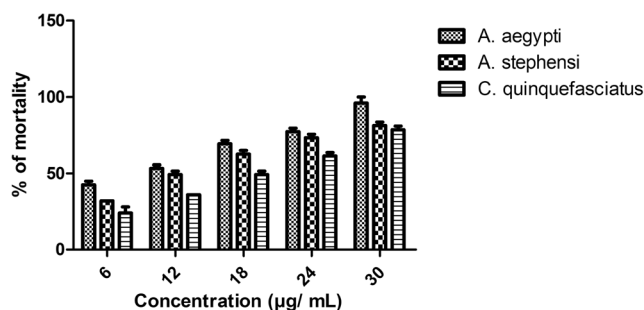


Fig. 3 The percentage mortality of *A. muricata* leaf-mediated AgNPs against *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus*

120 kV and water as solvent) to magnify the lattice arrangements of atom and shape of the AgNPs.

Larvicidal activity

Larvicidal bioassay of plant extracts and green synthesized nanoparticles was carried out according to (WHO 1996) protocol with minor modifications. Twenty-five numbers of third instars larvae were introduced into a 500-mL glass beaker containing 249 mL of dechlorinated water; the different concentrations of aqueous plant extract and AgNPs were added. The control was set up with dechlorinated tap water. After 24-

h exposure, the number of dead larvae was counted and the LC_{50} and LC_{90} values were calculated.

Synthesized AgNP leaf extract toxicity tests were carried out using a multi-concentration test, consisting of control and different concentrations of AgNP leaf extract. Each test was performed by placing 25 mosquito larvae into 250 mL of sterilized double distilled water with silver nanoparticles into a glass beaker. A nanoparticle solution was diluted using double distilled water as a solvent in order to make different concentrations (6, 12, 18, 24, 30 $\mu\text{g mL}^{-1}$). Each test included a set control group (silver nitrate and distilled water) with three replicates for each individual concentration. Mortality was assessed after 24 h to determine the acute toxicities on third instar larvae of three mosquitoes.

Table 2 Larvicidal activity of *A. muricata* synthesized AgNPs against third instar of *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* of 24-h observation

Mosquitoes	Concentration of AgNPs	24-h mortality (%)±SD	LC_{50} $\mu\text{g/mL}$ (LCL–UCL)	LC_{90} $\mu\text{g/mL}$ (LCL–UCL)	χ^2
<i>Aedes aegypti</i>	Control	0.0±0.0	12.58	26.46	26.84*
	6	42.6±2.3	(10.37–14.63)	(23.45–30.91)	
	12	53.3±2.3			
	18	69.3±2.3			
	24	77.3±2.3			
	30	96.0±4.0			
<i>Anopheles stephensi</i>	Control	0.0±0.0	15.28	31.91	19.31*
	6	32.0±0.0	(13.58–16.97)	(28.96–35.96)	
	12	49.3±2.3			
	18	62.6±2.3			
	24	73.3±2.3			
	30	81.3±2.3			
<i>Culex quinquefasciatus</i>	Control	0.0±0.0	18.77	35.72	12.05*
	6	24.0±4.0	(17.06–20.64)	(32.33–40.49)	
	12	36.0±0.0			
	18	49.3±2.3			
	24	61.3±2.3			
	30	78.6±2.3			

UCL upper confidence limit, LCL lower confidence limit, χ^2 chi-square test

*Significant at $p < 0.05$ level

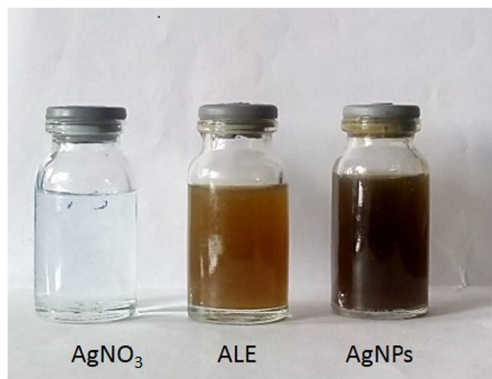


Fig. 4 Nanoparticle synthesis of *A. muricata* leaf extract and its color change after adding AgNO_3

Dose-response bioassay

Based on the preliminary screening results, aqueous plant extract and synthesized AgNPs were subjected to dose-response bioassay for larvicidal activity against third instar larvae of mosquitoes. Different concentrations of aqueous extract ($30\text{--}150\ \mu\text{g mL}^{-1}$) and AgNPs ($6\text{--}30\ \mu\text{g mL}^{-1}$) were prepared. Each concentration of aqueous extract and AgNPs were added to all the glass beakers which contains the sterile double distilled water and mosquito larvae. The number of dead larvae was counted after 24 h of exposure. The LC_{50} and LC_{90} values were calculated from the average of three replicates.

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50} and LC_{90} (lethal concentration) values, and their statistics at 95 % confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL) values were estimated by fitting a probit regression model. All the analysis were calculated using the SPSS (Statistical Package of Social Sciences) software package 16.0 version. Results with $p < 0.05$ were considered to be statistically significant.

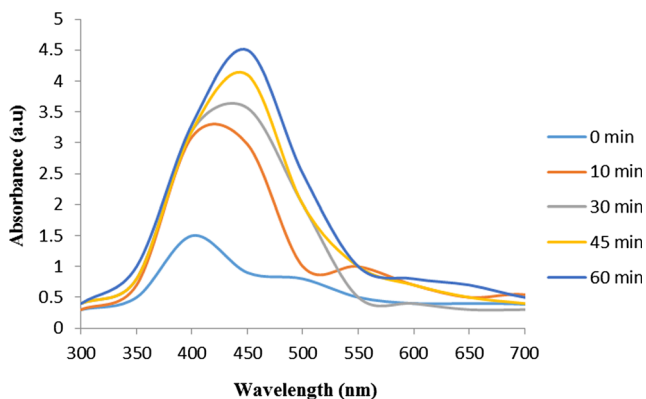


Fig. 5 UV-vis absorption spectra of silver nanoparticles synthesized using *A. muricata* leaf extract in different time intervals

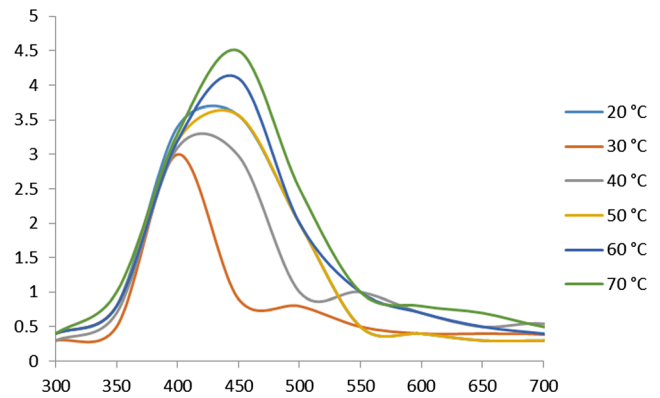


Fig. 6 UV-vis absorption spectra of silver nanoparticles synthesized using *A. muricata* leaf extract in different temperatures

Results

Mosquito larvicidal activity of aqueous leaf extract and synthesized AgNPs

The results of larvicidal activity of *A. muricata* aqueous leaf extract was tested against third instar larvae of *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus*. Mortality of *A. aegypti* showed the maximum numbers (LC_{50} $51.13\ \mu\text{g/mL}$; LC_{90} $82.08\ \mu\text{g/mL}$) followed by *A. stephensi* (LC_{50} $61.38\ \mu\text{g/mL}$; LC_{90} $156.55\ \mu\text{g/mL}$) and *C. quinquefasciatus* (LC_{50} $88.72\ \mu\text{g/mL}$; LC_{90} $151.30\ \mu\text{g/mL}$), respectively (Table 1). Percentage of mortality was also calculated (Fig. 2). The synthesized AgNPs were tested against third instar larvae of *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* that exhibited significant mortality against the selected mosquitoes with the following LC_{50} and LC_{90} values: *A. aegypti* (LC_{50} $12.58\ \mu\text{g/mL}$; LC_{90} $26.46\ \mu\text{g/mL}$), *A. stephensi* (LC_{50} $15.28\ \mu\text{g/mL}$; LC_{90} $31.91\ \mu\text{g/mL}$), and *C. quinquefasciatus* (LC_{50} $18.77\ \mu\text{g/mL}$; LC_{90} $35.72\ \mu\text{g/mL}$) (Table 2), and the percentage of mortality was calculated using standard deviation (Fig. 3). The nil mortality was observed in control and the χ^2 values was significant at $p \leq 0.05$ level.

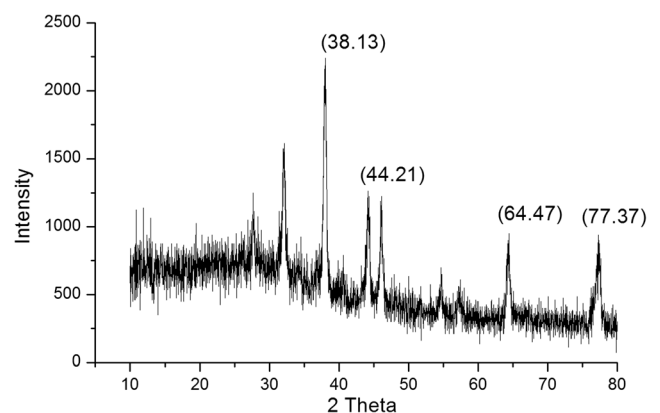


Fig. 7 XRD pattern of AgNPs synthesized using *A. muricata*

Table 3 Functional group analysis of synthesized AgNPs using FTIR

Frequency (cm ⁻¹)	Bond	Functional groups
3381.28	N–H stretch	Amides
2921.03	C–H stretch	Alkanes and alkyls
1640.17	–C=C stretch	Alkenes
1384.58	–CH ₃ –C–H bend	Alkanes and alkyls
1075.83	C–N stretch	Aliphatic amines
610.77	≡C–H bend	Alkynes

Characterization of AgNPs

Color changes were monitored by visual observation of the *A. muricata* aqueous leaf extracts when incubated with AgNO₃ solution. *Annona muricata* aqueous leaf extract without AgNO₃ did not reflect any change in color. The color of the extract that changed into yellow to brown within an hour revealed the reduction silver ions (Fig. 4). The absorption spectrum of synthesized AgNPs at different wavelengths ranging from 300 to 700 nm revealed a highest peak at 420 nm (Fig. 5). The heat stability of the synthesized AgNPs from 20 to 70 °C absorbed by spectral analysis at different wavelengths ranging from 300 to 700 nm (Fig. 6). XRD analysis showed sharp peaks with various 2θ intense degree values and the formation of the sharp peaks at 38.13, 44.21, 64.47, and 77.37° might be due to the stabilization of the synthesized AgNPs (Fig. 7). The FTIR analysis of the dried nanoparticles showed the presence of following functional groups, namely N–H stretch (3381.28 cm⁻¹), C–H stretch (2921.03 cm⁻¹), C=C stretch (1640.17 cm⁻¹), CH₃–C–H bend (1384.58 cm⁻¹), C–N stretch (1075.83 cm⁻¹), and ≡C–H bend (610.77 cm⁻¹) (Table 3 and Fig. 8). The particle size analyzer result shows the maximum size distribution of the nanoparticles (103 nm) present in the sample (Fig. 9). FE-SEM

micrographs of the synthesized AgNPs of *A. muricata* show a clear spherical shape and structure magnified at ×3000 and ×10,000, and measured at 20 to 70 nm (Fig. 10). EDX analysis result clearly shows the presence and purity of the synthesized AgNPs in the sample (Fig. 11). HR-TEM analysis reflected synthesized AgNPs were spherical in shape and (ranging from 20 to 53 nm) with an average size of 35 nm (Fig. 12).

Discussion

Plants contain enormous phytochemicals and compounds which have the incredible biological activity including mosquitocidal properties. Present study observed that the synthesized AgNPs had a higher mortality in all the three species when compared to the aqueous leaf extract with lowest LC₅₀ and LC₉₀ values. *Annona muricata* aqueous leaf extract that was tested against third instar larvae of *A. aegypti* showed significant LC₅₀ and LC₉₀ values (LC₅₀ 51.13 µg/mL; LC₉₀ 82.08 µg/mL) followed by *A. stephensi* (LC₅₀ 61.38 µg/mL; LC₉₀ 156.55 µg/mL) and *C. quinquefasciatus* (LC₅₀ 88.72 µg/mL; LC₉₀ 151.30 µg/mL). Many researchers who contribute the scientific evidences on Medicinal plants and their bioactive extracts are act as potent larvicidal agents against several mosquito species. For example, Patil et al. (2012) reported toxicity assay of *Pergularia daemia* latex showed a better effect against different stages of larvae *A. aegypti* and *A. stephensi* that had LC₅₀ values of 55.13, 58.81, 75.66, and 94.31 and 81.47, 92.09, 96.07, 101.31 mg/L. This report was comparable to *A. muricata* aqueous leaf extract having low LC₅₀ and LC₉₀ values. On the other hand, the LC₅₀ values of hexane, chloroform, ethyl acetate, acetone, and methanol extract of *Orthosiphon thymiflorus* tested with third instar larvae of *A. stephensi* which showed the LC₅₀

Fig. 8 FTIR spectrum of silver nanoparticles synthesized by *A. muricata* leaf extract

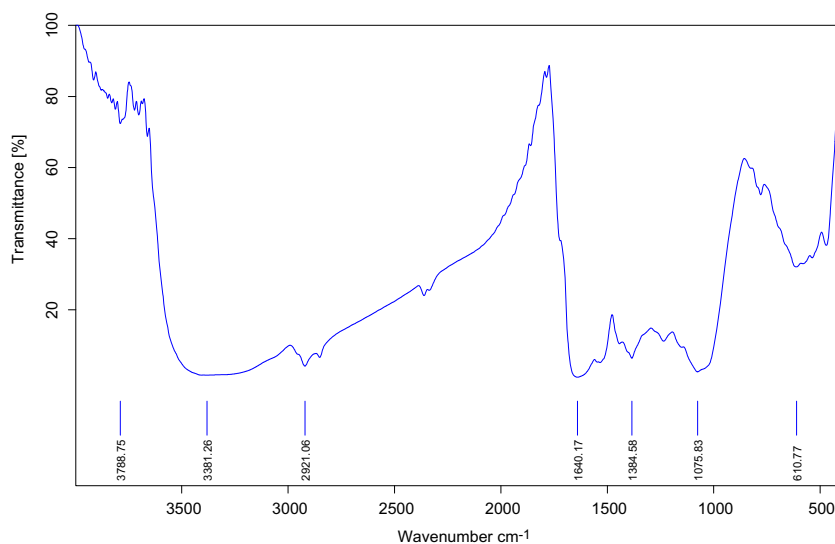
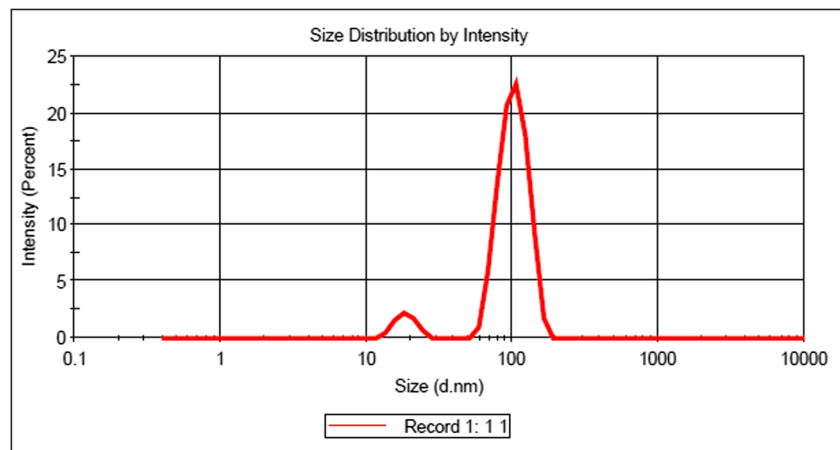
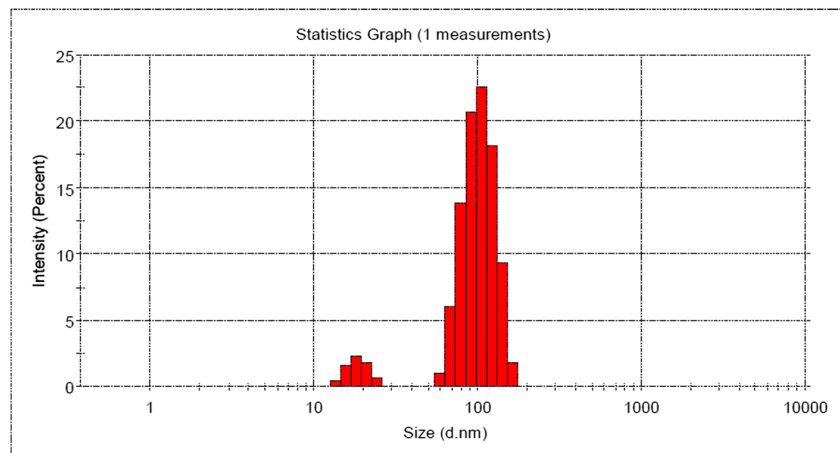


Fig. 9 The particle size distribution histogram of AgNPs shows size distribution of AgNPs



	Size (d.nm...)	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 260.6	Peak 1: 103.6	93.2	22.67
Pdl: 0.306	Peak 2: 18.64	6.8	2.968
Intercept: 0.885	Peak 3: 0.000	0.0	0.000



values were 201.39, 178.76, 158.06, 139.22, and 118.74 ppm; *C. quinquefasciatus* were 228.13, 209.72, 183.35, 163.55, and 149.96 ppm; and *A. aegypti* were 215.65, 197.91, 175.05, 154.80, and 137.26 ppm, respectively (Kovendan et al. 2012). The significant larval mortality was observed in leaf ethyl acetate extracts of *Aegle marmelos* and *Eclipta prostrata*, hexane, and methanol extract of *Andrographis paniculata* and *Cocculus hirsutus* showing LC₅₀ values of 167.00, 78.28, 67.24, and 142.83 ppm and LC₉₀ values of 588.31, 360.75, 371.91, and 830.01 ppm, respectively (Elango et al. 2009). The highest mortality was observed in the leaf ethyl acetate extract of *Achyranthes aspera*, leaf chloroform extract of *Anisomeles malabarica*, flower methanol of *Gloriosa superba*, and leaf methanol extract of *Ricinus communis* exhibited LC₅₀ values of 48.83, 135.36, 106.77, and 102.71 ppm and LC₉₀ of 225.36, 527.24, 471.90, and 483.04 ppm, respectively (Zahir et al. 2009). Murugan et al. (2012) reported that larval and pupal toxicity effects of the orange peel ethanol extract of *Citrus sinensis* against

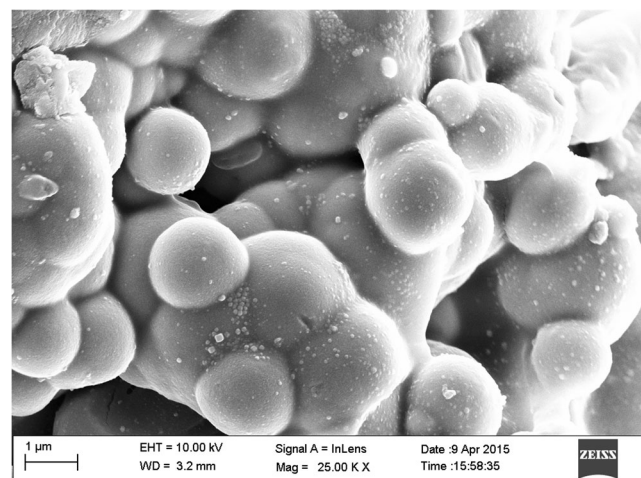
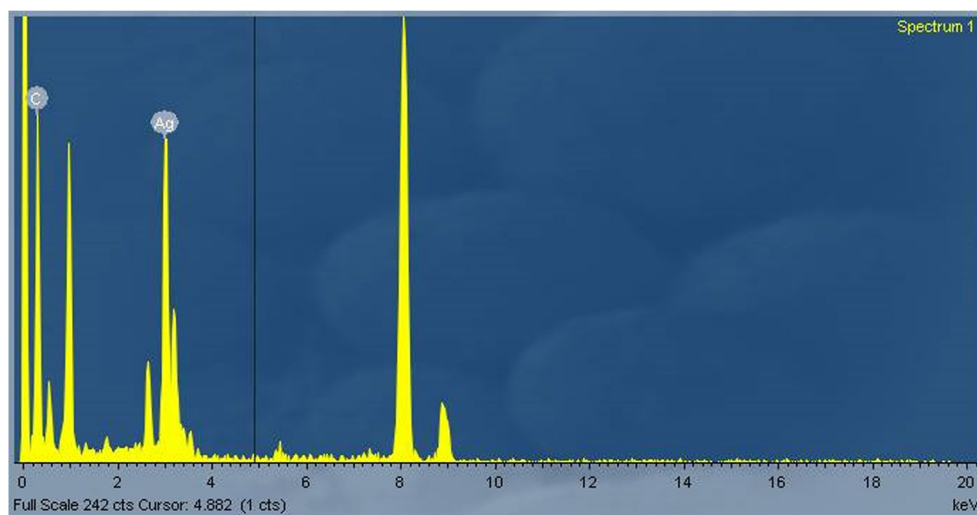


Fig. 10 Scanning electron microscopy image of synthesized silver nanoparticles (AgNPs) from *A. muricata* leaves

Fig. 11 EDX showing the chemical constituents of the synthesized nanoparticles



A. stephensi had LC_{50} and LC_{90} values of 182.24, 227.93, 291.69, 398.00, and 490.84 and 452.44, 544.72, 659.31, 858.92, and 987.28. Kamaraj et al. (2009) reported that the leaf petroleum ether, flower methanol extracts of *Cassia auriculata*, flower methanol extracts of *Leucas aspera* and *Rhinacanthus nasutus*, leaf and seed methanol extracts of *Solanum torvum*, and leaf hexane extract of *Vitex negundo* were evaluated for larvicidal activity with LC_{50} values of 44.21, 44.69, 53.16, 41.07, 35.32, 28.90, and 44.40 ppm, respectively.

The results of green synthesized AgNPs through *A. muricata* leaf was tested against third instar larvae of *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* with the following LC_{50} and LC_{90} values: *A. aegypti* (LC_{50} 12.58 $\mu\text{g}/\text{mL}$; LC_{90} 26.46 $\mu\text{g}/\text{mL}$) followed by *A. stephensi* (LC_{50} 15.28 $\mu\text{g}/\text{mL}$; LC_{90} 31.91 $\mu\text{g}/\text{mL}$) and *C. quinquefasciatus* (LC_{50} 18.77 $\mu\text{g}/\text{mL}$; LC_{90} 35.72 $\mu\text{g}/\text{mL}$). Rajkumar and Rahuman (2011) reported that the larvicidal activity of synthesized AgNPs utilizing an aqueous extract from *E. prostrata*

was observed in crude aqueous and synthesized AgNPs against *C. quinquefasciatus* (LC_{50} 27.49 and 4.56 mg/L; LC_{90} 70.38 and 13.14 mg/L) and against *Anopheles subpictus* (LC_{50} 27.85 and 5.14 mg/L; LC_{90} 71.45 and 25.68 mg/L), respectively. The aqueous crude latex of *Plumeria rubra* and synthesized silver nanoparticles tested against second and fourth instar larvae of *A. aegypti* and *A. stephensi* had LC_{50} and LC_{90} values. The LC_{50} values of aqueous crude latex are 143.60, 170.58, 181.67, and 287.49, and the LC_{90} values are 352.64, 369.28, 445.31, and 641.52 ppm. The synthesized AgNP LC_{50} values were 1.10, 1.74, 1.49, and 1.82, and LC_{90} values were 2.42, 4.23, 3.75, and 4.32 ppm (Patil et al. 2012). Santhoshkumar et al. (2010) reported that the leaf methanol, aqueous extracts of *Nelumbo nucifera*, and its green synthesis of AgNPs have the potential to be used as an ideal eco-friendly approach for the control of *A. subpictus* and *C. quinquefasciatus* (LC_{50} =8.89, 11.82, and 0.69 ppm; LC_{90} =28.65, 36.06, and 2.15 ppm) (LC_{50} 9.51, 13.65, and

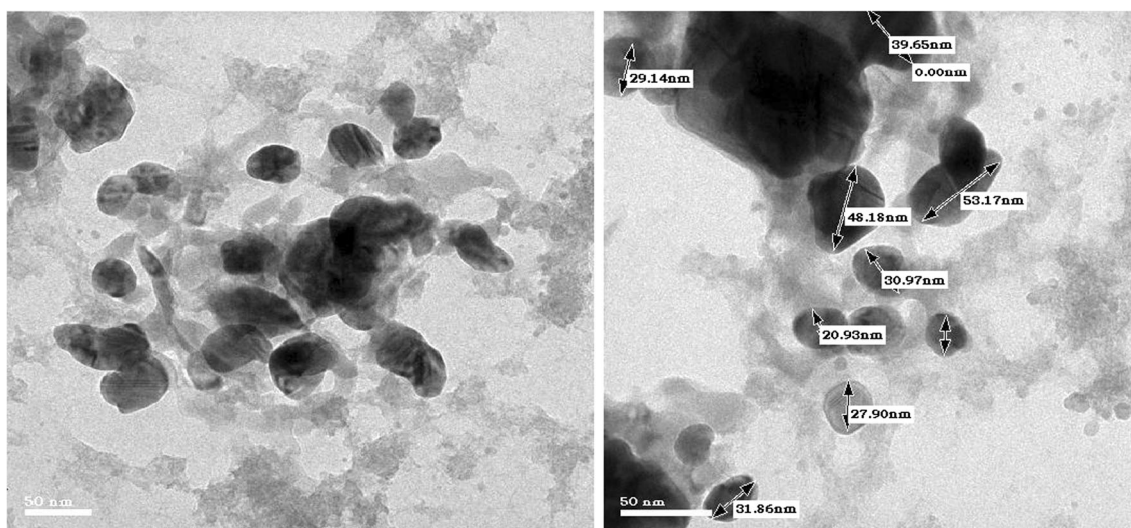


Fig. 12 Transmission electron microscopy image of synthesized silver nanoparticles (AgNPs) from *A. muricata* (scale bar corresponds to 50 nm) leaves

1.10 ppm; LC₉₀ 28.13, 35.83, and 3.59 ppm), respectively. The larvicidal aqueous crude leaf extract and synthesized AgNPs of *Mimosa pudica* showed the highest mortality in synthesized AgNPs against the larvae of *A. subpictus* and *C. quinquefasciatus* (LC₅₀ 013.90 and 11.73 mg/L; r 2 0.411 and 0.286), respectively (Marimuthu et al. 2011).

In our observation, the *A. aegypti* species had the highest mortality in both *A. muricata* leaf-mediated aqueous extract and AgNPs, which contains functional groups of alkenes, alkanes, and alkyls. Previous reports by Patil et al. (2012) in *P. rubra* and *P. daemia*; Kovendan et al. (2012) in *O. thymiflorus*; Kamaraj et al. (2009) in *R. nasutus* and *L. aspera*; Santhoshkumar et al. (2010) in *N. nucifera* which were produced the highest mortality against *A. aegypti*. It is agreement with the present investigation due to the presences of alkene, alkane and alkyl groups in *A. muricata* which might have been a strong insecticidal activity against *A. aegypti*. The presence of alkene, alkane, and alkyl groups in the leaf extract and synthesized AgNPs might be the reason of the mortality towards *A. aegypti* species.

Reduction of silver ions in the aqueous solution of silver during the reaction with the functional groups present in the plant leaf extract was observed in UV–vis spectroscopy. The UV–vis analysis showed absorbance for AgNPs (at 420 nm) that indicate formation of spherical AgNPs. Transmission of the X-diffraction on the sample showed bent reflection which depends upon layers of atoms/ions in the crystal and result of dark dots in the plate. The Bragg reflections showed four strong intense peaks at 2θ values at 38.13, 44.21, 64.47, and 77.37° which are corresponding to 111, 200, 220, and 311 planes, and this clearly indicates the crystalline nature of AgNPs and is comparable to the report of Chitra et al. (2015). FTIR spectra of AgNPs exhibited prominent peaks between 1384.58 and 1640.17 cm⁻¹ that shows the presence of alkane, alkene, and alkyl groups in the sample which is similar to the earlier report of Veerakumar et al. (2014). In this study, the constituents present in the plants might be responsible for the reduction and synthesis of metal nanoparticles. The particle size analysis provides evidence for size and size distribution of AgNPs which revealed that 90 % of the distribution of particles have a small size range from 18 to 260 nm with an average size of 103 nm. FE-SEM and HR-TEM were utilized to characterize the particle size, shape, and distribution by taking micrograph from drop coated films of the AgNPs that shows most of them are spherical in shape with the average size of 20 to 53 nm similar to Haldar et al. (2013).

Conclusion

The outcome of the present study is concluded that the extract of *A. muricata* are capable of producing AgNPs and are heat

stable from 20 to 70 °C. The green synthesized AgNPs show a better larvicidal efficacy as compared to other plant parts, since AgNPs are heat stable, nontoxic, simple, and eco-friendly that can be used as an effective approach to control mosquito larvae in any aquatic system. The *A. muricata* plant-mediated AgNPs has effectively controlled the third instar larvae of dengue vector *A. aegypti*, and this study extends the frontiers for nanoparticle-based technologies to control parasites which is highly recommendable for bio-insecticidal agents.

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