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ORIGINAL PAPER

Evaluation of plant-mediated synthesized silver nanoparticles against vector mosquitoes

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Abstract Diseases transmitted by blood-feeding mosquitoes, such as dengue fever, dengue hemorrhagic fever, Japanese encephalitis, malaria, and filariasis, are increasing in prevalence, particularly in tropical and subtropical zones. To control mosquitoes and mosquito-borne diseases, which have worldwide health and economic impacts, synthetic insecticide-based interventions are still necessary, particularly in situations of epidemic outbreak and sudden increases of adult mosquitoes. Green nanoparticle synthesis has been achieved using environmentally acceptable plant extract and eco-friendly reducing and capping agents. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, in the present study, the adulticidal activity of silver nanoparticles (AgNPs) synthesized using *Heliotropium indicum* plant leaf extract against adults of *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* was determined. Adult mosquitoes were exposed to varying concentrations of aqueous extract of *H. indicum* and synthesized AgNPs for 24 h. AgNPs were rapidly synthesized using the leaf extract of *H. indicum*, and the formation of nanoparticles was observed within 6 h. The results recorded from UV–vis spectrum, Fourier transform infrared, X-ray diffraction, scanning electron microscopy, and transmission electron microscopy support the biosynthesis and characterization of AgNPs. The maximum efficacy was observed in synthesized AgNPs against the adult of *A. stephensi* (lethal dose $(LD)_{50}$=26.712 μg/mL; LD_{90}=49.061 μg/mL), *A. aegypti* (LD_{50}=29.626 μg/mL; LD_{90}=54.269 μg/mL), and *C. quinquefasciatus* (LD_{50}=32.077 μg/mL; LD_{90}=58.426 μg/mL), respectively. No mortality was observed in the control. These results suggest that the leaf aqueous extracts of *H.indicum* and green synthesis of AgNPs have the potential to be used as an ideal eco-friendly approach for the control of the *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus*. This is the first report on the adulticidal activity of the plant extracts and AgNPs.

Keywords AgNPs · Adulticidal activity · *Heliotropium indicum* · Mosquitoes

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Introduction

Mosquito-borne diseases, such as malaria, filariasis, dengue, yellow fever, and Japanese encephalitis, contribute significantly to disease burden, death, poverty, and social debility in tropical countries. Mosquito-borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases. *Aedes aegypti* is generally known as a vector for an arbovirus responsible for dengue fever, which is endemic to Southeast Asia, the Pacific island area, Africa, and the Americas. This mosquito is also the vector of yellow fever in Central and South America and West Africa. Dengue fever has become an important public health problem as the number of reported cases continues to increase, especially with more severe forms of the disease, dengue hemorrhagic fever, and dengue shock syndrome or with unusual manifestations such as central nervous system involvement. Dengue is prevalent in more than 100 countries and threatens the health of approximately 2.5 billion people. Around 80 million people are infected annually at an attack rate of 4 % worldwide

(Pancharoen et al. 2002). Currently, resistant variety of the malaria parasite is commonly observed in almost all parts of the world where malaria is endemic (Cooper et al. 2005). *Anopheles stephensi* is an important vector of urban malaria in several countries of the Middle East and Indian subcontinent (Gayathri and Balakrishna Murthy 2006). Malaria continues to be a major public health problem in the tropical world. Of the total world population of about 5.4 billion people, 2,200 million are exposed to malarial infections in 90 countries or some areas. The recent estimates indicate that there may be 300–500 million clinical cases each year, with countries in tropical Africa accounting more than 90 % of these. Malaria is also the cause of an estimated 1–4 to 2–6 million deaths worldwide every year, with more than 90 % in Africa alone (World Health Organization 1995). Nowadays, the control of vector-borne diseases is more difficult due to the increased resistance of mosquito populations to synthetic insecticides and even to microbial control agents and because of the resistance of malaria parasites to chemotherapeutic drugs and some economic issues (Shelton et al. 2007). *Culex quinquefasciatus* acts as a vector for filariasis in India. Human filariasis is a major public health hazard and remains a challenging socioeconomic problem in many of the tropical countries. Lymphatic filariasis caused by *Wuchereria bancrofti* and transmitted by mosquito *C. quinquefasciatus* is found to be more endemic in the Indian subcontinent. It is reported that *C. quinquefasciatus* infects more than 100 million individuals worldwide annually (Rajasekariah et al. 1991). According to WHO, about 90 million people worldwide are infected with *W. bancrofti*, the lymphatic-dwelling parasite, and ten times more people are at the risk of being infected. In India alone, 25 million people harbor microfilaria and 19 million people suffer from filarial disease manifestations (Maheswaran et al. 2008).

Plants are rich source of alternative agents for control of mosquitoes, because they possess bioactive chemicals, which act against limited number of species including specific target insects and are eco-friendly (Sukumar et al. 1991). Traditionally, plant-based products have been used in human communities for many centuries for managing insects. Several secondary metabolites present in plants serve as a defense mechanism against insect attacks. These bioactive chemicals may act as insecticides, antifeedants, molting hormones, oviposition deterrents, repellents, juvenile hormone mimics, growth inhibitors, antimolting hormones, as well as attractants. Plant-based pesticides are less toxic, delay the development of resistance because of its new structure, and easily biodegradable (Ignacimuthu 2000). Several plant extracts and isolated compounds from different plant families have been evaluated for their promising larvicidal activities (Markouk et al. 2000). About 2,000 species of terrestrial plants have been reported for their insecticidal properties (Feinstein. 1952). Search for eco-safe, low-cost, and a highly potential insecticide for the control of mosquitoes needs the preliminary screening of plants to evaluate their insecticidal activities. Plant-based products do not have any hazardous effect on ecosystem. Recent research has proved that effectiveness of plant-derived compounds, such as saponine (Wiseman and Chapagain 2005), steroids (Chowdhury et al. 2008; Ghosh et al. 2008), isoflavonoids (Joseph et al. 2004), essential oils (Cavalcanti et al. 2004), alkaloids, and tannins (Khanna and Kannabiran 2007), has potential mosquito larvicides. Plant secondary metabolites and their synthetic derivatives provide alternative source in the control of mosquitoes (Yang et al. 2004).

Phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal, pupicidal, and adulticidal properties. Many synthetic insecticides and naturally occurring chemical cues have been shown to influence mosquito oviposition (Olagbemiro et al. 1999; Veerakumar and Govindarajan 2013). The chemicals derived from plants have been projected as weapons in future mosquito control program as they are shown to function as general toxicant, growth and reproductive inhibitors, repellents, and oviposition deterrent (Sukumar et al. 1991). However, the silica nanoparticles have been tested against the larvae and pupae of *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti* (Barik et al. 2012). The extracts of *Murraya koenigii*, *Coriandrum sativum*, *Ferula asafetida*, and *Trigonella foenum graceum* were found to be effective and showed encouraging results against *A. aegypti* (Harve and Kamath 2004). The pediculocidal and larvicidal activities of synthesized silver nanoparticles using the aqueous leaf extract of *Tinospora cordifolia* have been reported against the human capitis and fourth instar larvae of *Anopheles subpictus* and *C. quinquefasciatus* (Jayaseelan et al. 2011). The larvicidal and repellent properties of essential oils from various parts of four plant species *Cymbopogan citrates*, *Cinnamomum zeylanicum*, *Rosmarinus officinalis*, and *Zingiber officinale* against *Culex tritaeniorhynchus* and *A. subpictus* (Govindarajan 2011a). The larvicidal efficacy of the aqueous and methanol extracts from green unripe to yellow ripe fruits of *Solanum xanthocarpum* was effective in controlling *A. culicifacies*, *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* (Bansal et al. 2009). The larvicidal and ovicidal efficacy of different extracts of *Cardiospermum halicacabum* against *C. quinquefasciatus* and *A. aegypti* was determined (Govindarajan 2011b). Ovicidal and repellent activities of methanol leaf extract of *Ervatamia coronaria* and *Caesalpinia pulcherrima* were evaluated against *C. quinquefasciatus*, *A. aegypti*, and *A. stephensi* (Govindarajan et al. 2011). Biolarvicidal and pupicidal potential of silver nanoparticles synthesized with *Euphorbia hirta* has been screened against *A. stephensi* (Priyadarshini et al. 2012). The acetone, chloroform, ethyl acetate, hexane, and methanol leaf extracts of *Acalypha indica*, *Achyranthes*

aspera, *Leucas aspera*, *Morinda tinctoria*, and *Ocimum sanctum* were studied against the early fourth instar larvae of *A. aegypti* and *C. quinquefasciatus* (Bagavan et al. 2008).

Essential oils from plants like *Myrtus communis*, *Origanum syriacum*, and *Lavandula stoechos* and the pure compounds like thymol, carvacrol, and α-pienene have been documented for larvicidal activities toward *Culex pipiens molestus* (Traboulsi et al. 2002). There are larvicidal properties of *Lippia citriodora* against *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus*. Essential oils (EOs) derived from five plant species, celery (*Apium graveolens*), caraway (*Carum carvi*), zedoary (*Curcuma zedoaria*), long pepper (*Piper longum*), and Chinese star anise (*Illicium verum*), were subjected to investigation of adulticidal activity against mosquito vectors (Chaiyasit et al. 2006). Laboratory bioassays on insecticidal activity of EOs extracted from six Mediterranean plants (*Achillea millefolium*, *Lavandula angustifolia*, *Helichrysum italicum*, *Foeniculum vulgare*, *Myrtus communis*, and *R. officinalis*) were carried out against the larvae of the Culicidae mosquito *A. albopictus* (Conti et al. 2010). Mosquito larvicidal activity in crude hexane extracts from flower heads of *Spilanthes acmella*, *Spilanthes calva*, and *Spilanthes paniculata* was assessed (Pandey et al. 2007). Larvicidal activity of partially purified extracts of the leaves of *Vitex negundo* and *Nerium oleander* and seeds of *Syzygium jambolanum* on different instars of *C. quinquefasciatus* and *A. stephensi* was estimated, and *T. foenum* and *N. oleander* leaves which are used against mosquito larvae are found in Vellore City, India (Lokesh et al. 2010). The benzene and methanol extracts of *Artemisia vulgaris* have a repellent activity against *A. aegypti* (Yit et al. 1985). The protective action of *Andrographis paniculata* is proposed to be due to reactivation of the key antioxidant enzyme superoxide dismutase (Chander et al. 1995). *Heliotropium indicum* L. commonly known as Thel kodukku (Tamil), Hatisund (Oriya), Haathisoundha, Hattajuri (Hindi), Bhurundi, Duralabha, Srihatini (Sanskrit), India Trunsole, and Heliotrope (English) belongs to the family Boraginaceae, a small fragrant evergreen annual herb found throughout the hotter part of India along the roadside on the wasteland. It contains pyrrolizidine alkaloids (heliotrine, indicine-*N*-oxide), tannins, indicine, AC indicine, indicinine, indicinine-*N*-oxide, lupeol, rapnone, and estoadiol. The whole plant is used for high fever, throat infection, ulcer fever, gonorrhea, localized inflammation, rheumatism, ring worm, ulcers, wounds, aphrodisiac, astringent, bitter, and expectorant (Nadkarni 2007). So far, there are no reports on *H. indicum*-synthesized AgNPs on mosquito adulticidal activity. In this study, plant-mediated synthesized AgNPs were evaluated for the first time against adults of dengue (*A. aegypti*), malaria (*A. stephensi*), and filariasis (*C. quinquefasciatus*) vector mosquitoes.

Materials and methods

Collection of materials

Fresh leaves of *H. indicum* (Boraginaceae) (Fig. 1) were collected from in and around Dharmanallur, Virudhachalam, Tamil Nadu, India, and the taxonomic identification was made by the Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India. The voucher specimen was numbered and kept in our research laboratory for further reference. Silver nitrate was obtained from Qualigens Fine Chemicals, Mumbai, India.

Mosquitoes

The laboratory-bred pathogen-free strains of mosquitoes were reared in the vector control laboratory, Department of Zoology, Annamalai University. At the time of adult feeding, these mosquitoes were 3–4 days old after emergences (maintained on raisins and water) and were starved for 12 h before feeding. Each time, 500 mosquitoes per cage were fed on blood using a feeding unit fitted with parafilm as membrane for 4 h. *A. aegypti* feeding was done from 12 noon to 4.00 p.m., and *A. stephensi* and *C. quinquefasciatus* were fed during 6.00 p.m. to 10.00 p.m. A membrane feeder with the bottom end fitted with parafilm was placed with 2.0 mL of the blood sample (obtained from a slaughter house by collecting in a heparinized vial and stored at 4 °C) and kept over a netted cage of mosquitoes. The blood was stirred continuously using an automated stirring device, and a constant temperature of 37 °C was maintained using a water jacket circulating system. After feeding, the fully engorged females were separated and maintained on raisins. Mosquitoes were held at 28±2 °C and 70–85 % relative humidity, with a photoperiod of 12-h light and 12-h dark.

Fig. 1 *H. indicum* plant

Preparation of plant extracts

The leaves (*H. indicum*) were dried in shade and ground to fine powder in an electric grinder. Aqueous extract was prepared by mixing 50 g of dried leaf powder with 500 mL of water (boiled and cooled distilled water) with constant stirring on a magnetic stirrer (Minjas and Sarda 1986). The suspension of dried leaf powder in water was left for 3 h, filtered through Whatman no. 1 filter paper, and the filtrate was stored in amber-colored air-tight bottle at 10 °C temperature until use.

Synthesis of silver nanoparticles

The broth solution of fresh *H. indicum* leaves was prepared by taking 10 g of thoroughly washed and finely cut leaves in a 300-mL Erlenmeyer flask along with 100 mL of sterilized double-distilled water and then boiling the mixture for 5 min before finally decanting it. The extract was filtered with Whatman filter paper no. 1, stored at −15 °C, and could be used within 1 week. The filtrate was treated with aqueous 1 mM $AgNO_3$ (21.2 mg of $AgNO_3$ powder in 125 mL Milli-Q water) solution in an Erlenmeyer flask and incubated at room temperature. Eighty-eight-milliliter aqueous solution of 1 mM of silver nitrate was reduced using 12 mL of leaves extract at room temperature for 10 min resulting in a brown-yellow solution indicating the formation of AgNPs (Veerakumar and Govindarajan 2013).

Characterization of the synthesized nanoparticles

Synthesis of AgNP solution with leaf extract may be easily observed by UV–Vis spectroscopy. The bioreduction of the Ag ions in solutions was monitored by periodic sampling of aliquots (1 mL) of the aqueous component after 20 times dilution and measuring the UV–Vis spectra of the solution. UV–Vis spectra of these aliquots were monitored as a function of time of reaction on a Shimadzu 1601 spectrophotometer in the 300–800-nm range operated at a resolution of 1 nm. Further, the reaction mixture was subjected to centrifugation at 60,000×*g* for 40 min; the resulting pellet was dissolved in deionized water and filtered through Millipore filter (0.45 μm). An aliquot of this filtrate containing silver nanoparticles was used for Fourier transform infrared (FTIR) and transmission electron microscopy (TEM) analysis. For electron microscopic studies, 25 μL of sample was sputter-coated on a copper stub, and the images of the nanoparticles were studied using scanning electron microscopy (SEM; JEOL, model JFC-1600), and TEM (JEOL, model 1200EX) measurements were operated at an accelerating voltage of 120 kV and later with an XDL 3000 powder. FTIR spectra of the samples were measured using PerkinElmer Spectrum One instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets. An aliquot of this filtrate containing silver nanoparticles was used for X-ray diffraction (XRD) analysis.

Adulticidal activity

Adulticidal bioassay was performed by slightly modified method of World Health Organization (1981)) and Veerakumar and Govindarajan (2014). Based on the wide range and narrow range tests, aqueous crude extract was tested at 50, 100, 150, 200, and 250 μg mL^{-1} concentrations, and AgNPs were tested at 12, 24, 36, 48, and 60 μg mL^{-1} concentrations. Aqueous crude extract and AgNPs were applied on Whatman no. 1 filter papers (size 12×15 cm). Control papers were treated with silver nitrate and distilled water. Twenty female mosquitoes were collected and gently transferred into a plastic holding tube. The mosquitoes were allowed to acclimatize in the holding tube for 1 h and then exposed to test paper for 1 h. At the end of exposure period, the mosquitoes were transferred back to the holding tube and kept for 24-h recovery period. A pad of cotton soaked with 10 % glucose solution was placed on the mesh screen. Each test included a set control groups (silver nitrate and distilled water) with five replicates for each individual concentration. The lethal concentrations (lethal dose (LD_{50}, LD_{90}) were calculated by probit analysis (Finney 1971).

Statistical analysis

The average adult mortality data were subjected to probit analysis for calculating LD_{50}, LD_{90}, and other statistics at 95 % confidence limits of upper confidence limit and lower confidence limit, and chi-square values were calculated using the Statistical Package of Social Sciences 12.0 software. Results with $p<0.05$ were considered to be statistically significant.

Results

Adulticidal activity of aqueous crude extract and synthesized AgNPs

The results of the adulticidal activity of aqueous crude extract and synthesized AgNPs against the adult of *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* are presented in Tables 1 and 2 (Fig. 2). Considerable mortality was evident after the treatment of *H. indicum* for all three important vector mosquitoes. At higher concentrations, the adult showed restless movement for some times with abnormal wagging and then died. The rates of mortality were directly proportional to

Table 1 Adulticidal activity of *Heliotropium indicum* aqueous leaf extract against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*

Mosquitoes	Concentration	24 h mortality (%)±SD	LD_{50} (μg/mL) (LCL–UCL)	LD_{90} (μg/mL) (LCL–UCL)	χ^2
A. stephensi	Control	0.0±0.0	111.680	200.790	
	50	28.2±0.6	(81.368–140.328)	(166.855–268.371)	18.637*
	100	43.4±0.2			
	150	71.0±1.3			
	200	83.3±1.7			
	250	100.0±0.0			
A. aegypti	Control	0.0±0.0	123.452	219.180	16.805*
	50	26.4±0.8	(94.399–152.270)	(183.841-288.287)	
	100	36.2±0.4			
	150	63.1±1.6			
	200	79.3±1.9			
	250	98.2±0.2			
C. quinquefasciatus	Control	0.0±0.0	134.808	241.234	
	50	25.2±1.2	(105.065–165.859)	(201.943–336.278)	15.938*
	100	32.6±0.6			
	150	57.4±1.1			
	200	72.5±0.4			
	250	94.3±0.9			

Values are mean±SD of five replicates

SD standard deviation, *LCL* lower confidence limits, *UCL* upper confidence limits, χ^2 chi-square test

*$p<0.05$, level of significance

concentration. The maximum efficacy was observed in synthesized AgNPs against the adult of *A. stephensi* (LD_{50}=26.712 μg/mL; LD_{90}=49.061 μg/mL), *A. aegypti* (LD_{50}=29.626 μg/mL; LD_{90}=54.269 μg/mL), and *C. quinquefasciatus* (LD_{50}=32.077 μg/mL; LD_{90}=58.426 μg/mL), respectively. No mortality was observed in the control. χ^2 value was significant at the $p \leq 0.05$ level.

Table 2 Adulticidal activity of Silver nanoparticles against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*

Mosquitoes	Concentration	24 h (%)±SD mortality	LD50 (μg/mL) (LCL–UCL)	LD90 (μg/mL) (LCL–UCL	χ^2
A. stephensi	Control	0.0±0.0	26.712	49.061	
	12	29.5±0.2	(18.644–34.243)	(40.232–67.777)	20.797*
	24	49.2±0.6			
	36	68.4±1.2			
	48	87.3±1.6			
	60	100.0±0.0			
A. aegypti	Control	0.0±0.0	29.626	54.269	
	12	27.3±0.4	(22.498–36.648)	(45.422–71.610)	15.913*
	24	42.7±0.8			
	36	58.4±1.4			
	48	79.1±1.8			
	60	96.2±0.2			
C. quinquefasciatus	Control	0.0±0.0	32.077	58.426	
	12	25.2±1.8	(25.015–39.396)	(48.962–77.194)	14.944*
	24	25.2±1.8			
	36	38.6±0.2			
	48	54.1±1.6			
	60	73.3±0.3			

Values are mean±SD of five replicates

*$p<0.05$, level of significance

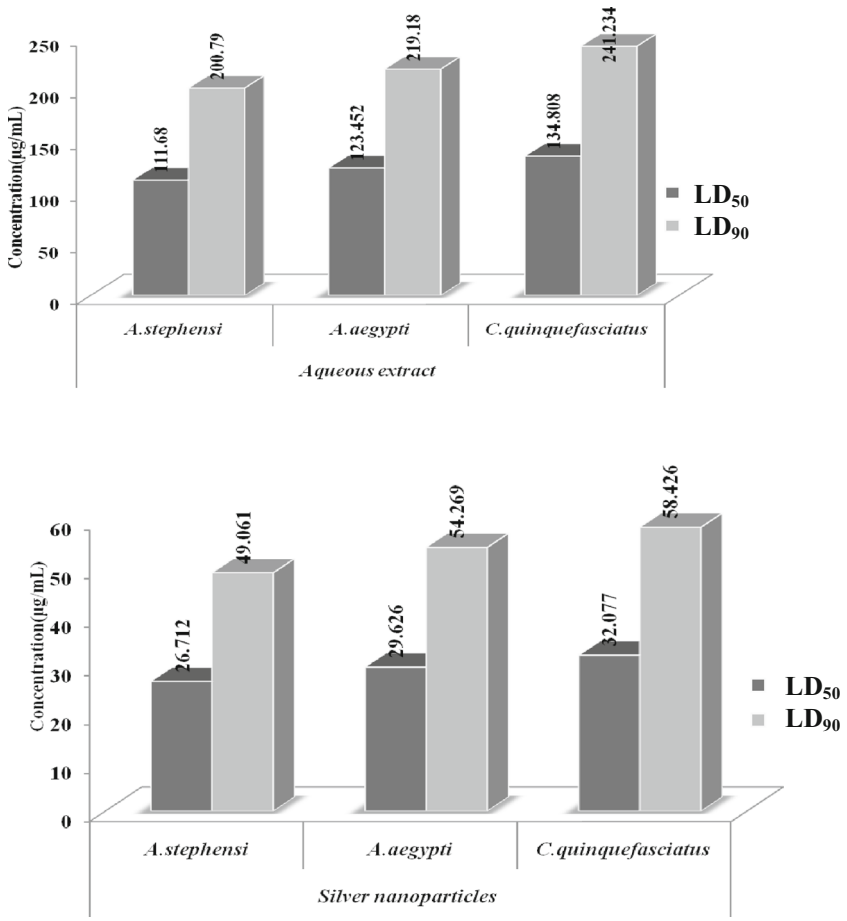

Fig. 2 Graph showing the LD_{50} and LD_{90} values of adulticidal activity of *H. indicum* aqueous leaf extract and silver nanoparticles against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*

Characterization of silver nanoparticles

Color change was noted by visual observation of the *H. indicum* leaf extracts when incubated with $AgNO_3$ solution. *H. indicum* leaf extract without $AgNO_3$ did not show any change in color. The color of the extract changed to light brown within an hour and, later, it changed to dark brown during the 6-h incubation period after which no significant change occurred (Fig. 3a, b). The absorption spectrum of *H. indicum* leaf extracts at different wavelengths ranging from 300 to 800 nm revealed a peak at 420 nm (Fig. 3c). FTIR analysis of the purified nanoparticles showed the presence of bands due to O–H group C=H bending (824.98), C=O stretching (1,094.71), N=H bending (1,603.81), –C=O stretching (1,765.45), C–H stretching (2,851.32), C–H stretching (2,932.36), and O–H stretching (3,396.59) (Fig. 4). SEM micrographs of the synthesized AgNPs of *H. indicum*, magnified at ×3,000, and measured at 20 to 60 nm are shown in Fig. 5a. The triangular, pentagonal, and hexagonal structures are clear. Energy-dispersive X-ray (EDX) proves the chemical purity of the synthesized AgNPs (Fig. 5b). The electron microscopic study of the nanoparticles using TEM revealed that the nano-Ag predominates with spherical, triangle, truncated triangles, and decahedral morphologies ranging from 18 to 45 nm with an average size of 32 nm (Fig. 6). The X-ray diffraction pattern of silver nanoparticles produced by leaf extract is shown in (Fig. 7). The control thin films of the leaf extract as well as the $AgNO_3$ did not show the characteristic peaks. The XRD pattern shows four intense peaks in the whole spectrum of 2θ values ranging from 25 to 60. The XRD spectrum compared with the standard confirmed spectrum of silver particles formed in the present experiments were in the form of nanocrystals, as evidenced by the peaks at 2θ values of 37.667°, 43.844°, 64.027°, and 76.957° corresponding to 439, 152, 90, and 125 planes for silver, respectively. The XRD pattern clearly shows that the silver nanoparticles formed by the reduction of $AgNO_3$ ions by *H. indicum* are crystalline in nature.

Discussion

Several approaches that have been employed to obtain a better biosynthesis of nanoparticles are advantageous over chemical and physical methods as they are a cost-effective and environment-friendly, where it is not necessary to use high pressure, energy, temperature, and toxic chemicals (Sinha et al.

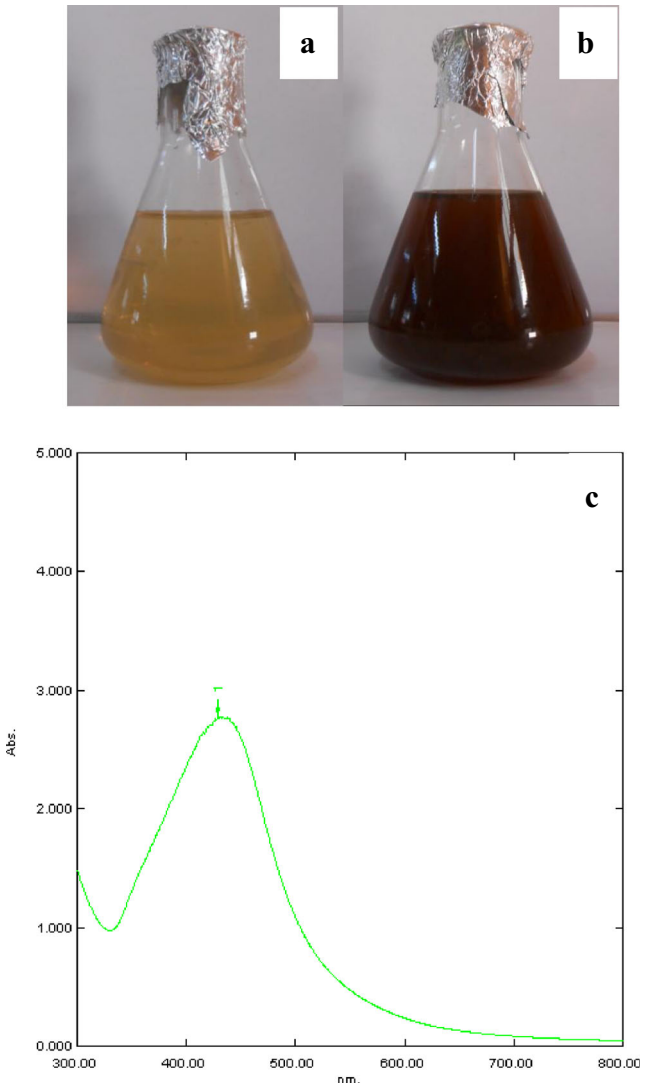

Fig. 3 **a** Photographs showing change in color after adding $AgNO_3$ before reaction and **b** after reaction time of 6 h. **c** UV–Vis spectra of aqueous silver nitrate with *H. indicum* leaf extract

2009). Plants are rich sources of bioactive compounds that can be used to develop environmentally safe vector and pest managing agents. Phyto-extracts are emerging as potential mosquito control agents, with low-cost, easy-to-administer, and risk-free properties. Simple crude extracts from plants have been used as insecticides in many countries for centuries (Crobsy 1971). The adulticidal and repellent activities of crude hexane, ethyl acetate, benzene, chloroform, and methanol extracts of leaf of *Eucalyptus alba* and *A. paniculata* were assayed for their toxicity against two important vector mosquitoes, viz., *C. quinquefasciatus* and *A. aegypti*. The highest adult mortality was found in methanol extract of *A. paniculata* against the adults of *C. quinquefasciatus* and *A. aegypti* with the LC_{50} and LC_{90} values of 149.81 and 172.37 ppm and 288.12 and 321.01 ppm, respectively (Govindarajan and Sivakumar 2012). The petroleum ether extract of *S. xanthocarpum* was observed to be the most toxic with LC_{50} of 1.41 and 0.93 ppm and LC_{90} of 16.94 and 8.48 ppm at 24 and 48 h after application, respectively, against *A. stephensi* (Mohan et al. 2007). Seed extract showed larvicidal activity (LC_{50}=116.8 and 195 ppm) after 24 h and (LC_{50}=065.2 and 154.5 ppm) after 48 h treatment against *A. stephensi* and *A. aegypti*, respectively. Larvicidal activity of flower methanol extract showed LC_{50} values of 233 and 302.5 ppm against *A. stephensi* and *A. aegypti*, respectively, after 48 h treatment. Methanol extract showed the lowest LD values against several instars of larvae and 50 adults (121.59, 142.73, 146.84, 202.98, 290.65, 358.42, and 300.03 μg/cm2, respectively) which indicates the highest toxicity or insecticidal activity (Ashraful Alam et al. 2009)

The maximum efficacy was observed in crude aqueous and synthesized AgNPs against *C. quinquefasciatus* (LC_{50} 27.49 and 4.56 mg L^{-1}; LC_{90} 70.38 and 13.14 mg L^{-1}1) and against *A. subpictus* (LC_{50} 27.85 and 5.14 mg L^{-1}; LC_{90} 71.45 and 25.68 mg L^{-1}), respectively. A biological method has been used to synthesize stable silver nanoparticles that were tested as mosquito larvicides against *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* (Arjunan et al. 2012). The larvicidal activity of AgNPs synthesized using *Sida acuta* plant leaf extract against late third instar larvae of *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti* was determined. The efficacies of synthesized AgNPs (10, 20, 30, 40, and 50 μg mL^{-1}) and aqueous leaf extract (50, 100, 150, 200, and 250 μg mL^{-1}) were tested against the larvae of *C. quinquefasciatus* (LC_{50} 26.13 and 130.30 μg mL^{-1}), *A. stephensi* (LC_{50} 21.92 and 109.94 μg mL^{-1}), and *A. aegypti* LC_{50} (23.96 and 119.32 μg mL^{-1}), respectively (Veerakumar and Govindarajan 2013). The ethyl acetate extract of *Eclipta prostrata* showed an LC_{50} value of 78.28 and LC_{90} value of 360.75 ppm against *A. subpictus* and LC_{50} 119.89 and LC_{90} 564.85 ppm against *C. tritaeniorhynchus*. *E. paniculata* were the most active with a LC_{90} of 17.2 mg L^{-1} and LC_{50} of 3.3 mg L^{-1} against the larvae of *Aedes fluviatilis* (Macedo et al. 1997).

Earlier authors reported that the larvicidal effect of aqueous crude leaf extracts, silver nitrate, and synthesized silver nanoparticles of *Mimosa pudica* showed that the highest mortality was found in synthesized AgNPs against the larvae of *A. subpictus* (LC_{50} 08.89, 11.82, and 0.69 ppm) and against the larvae of *C. quinquefasciatus* (LC_{50}=09.51, 13.65, and 1.10 ppm) (Marimuthu et al. 2010). The LC_{50} and LC_{90} values of the *Feronia elephantum* aqueous leaf extract appeared to be effective against *A. stephensi* (LC_{50} 54. 88 μg mL^{-1} and LC_{90} 97.38 μg mL^{-1}) followed by *A. aegypti* (LC_{50} 62.02 μg mL^{-1} and LC_{90} 110.71 μg mL^{-1}) and *C. quinquefasciatus* (LC_{50} 67.08 μg mL^{-1} and LC_{90} 117.85 μg mL^{-1}). Considerable mortality was evident after the treatment of silver nanoparticles. Synthesized AgNPs against the vector mosquitoes *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* had the

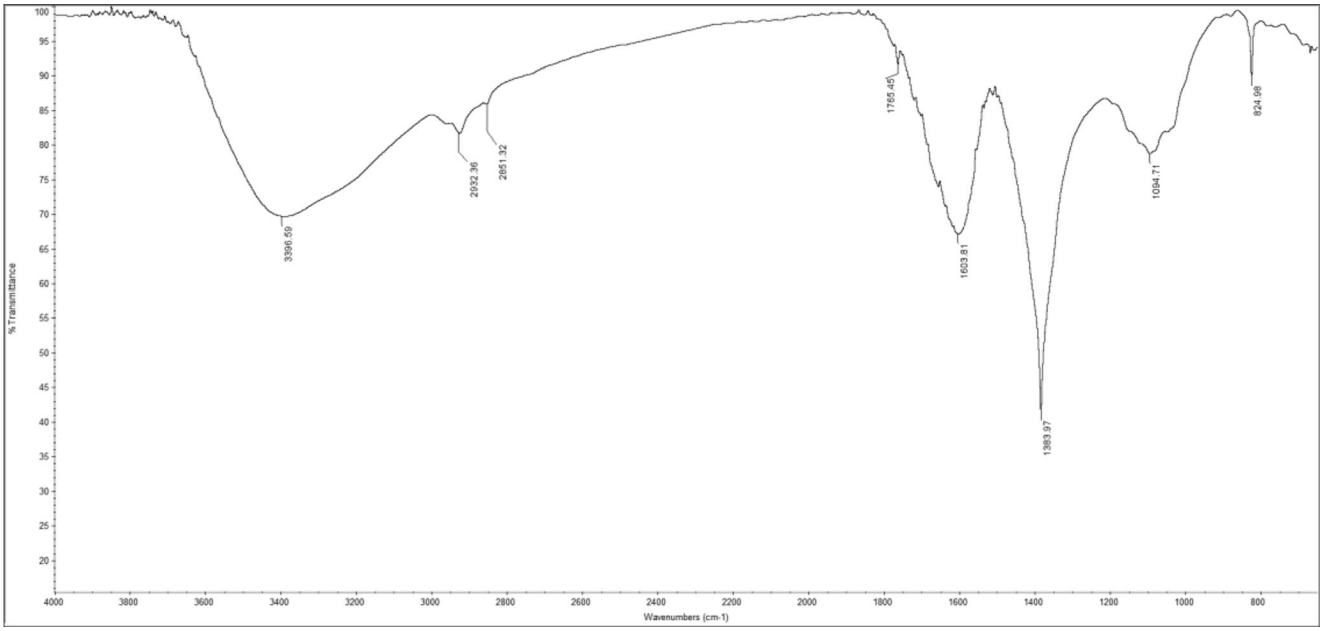

Fig. 4 FTIR spectrum of synthesized AgNPs using *H. indicum* leaf extract

following LC_{50} and LC_{90} values: *A. stephensi* had LC_{50} and LC_{90} values of 11.56 and 20.56 μg mL^{-1}; *A. aegypti* had LC_{50} and LC_{90} values of 13.13 and 23.12 μg mL^{-1}; and *C. quinquefasciatus* had LC_{50} and LC_{90} values of 14.19 and 24.30 μg mL^{-1} (Veerakumar et al. 2014). The synthesized zinc oxide nanoparticles against *Rhipicephalus microplus* and *Pediculus humanus capitis* and the larvae of *A. subpictus* and *C. quinquefasciatus* showed LC_{50} values of 29.14, 11.80, 11.14, and 12.39 mg/L, respectively (Kirthi et al. 2011).

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}

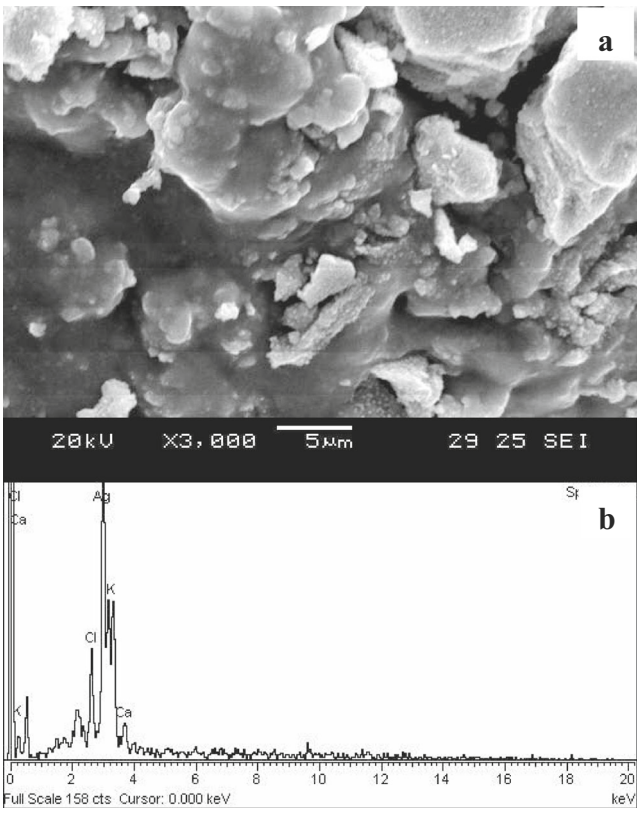

Fig. 5 Scanning electron micrographs of AgNPs synthesized with *H. indicum* leaf extract and 1.0 mM $AgNO_3$ solution and incubated at 60 °C for 6 h at pH 7.0. **a** Magnified ×3,000; *inset bar* represents 5 μm. **b** EDX image showing chemical composition

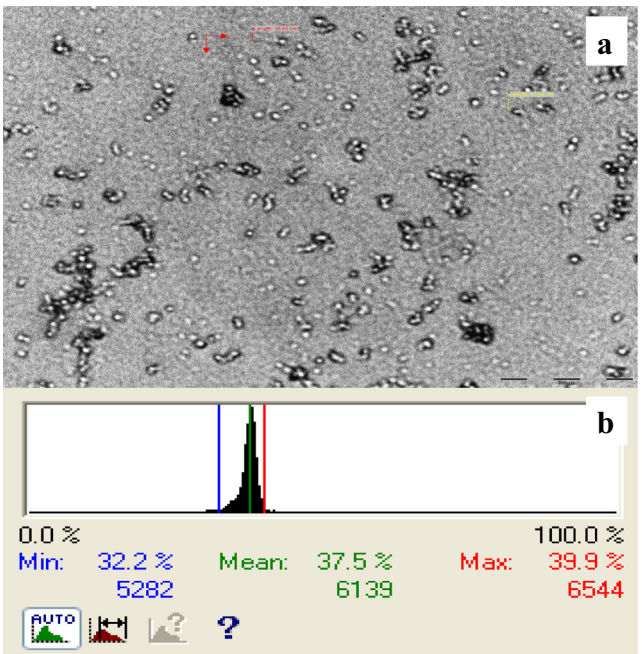

Fig. 6 Transmission electron microscopic image and histogram showing synthesized AgNPs from *H. indicum*

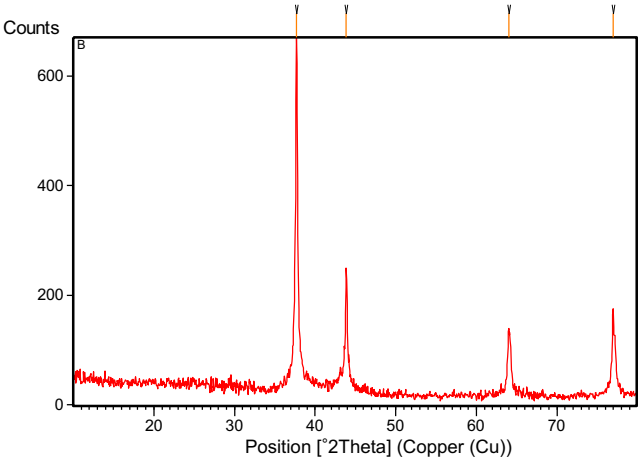

Fig. 7 X-ray diffraction showing synthesized AgNPs from *H. indicum*

27.49 and 4.56 mg/L; LC_{90} 70.38 and 13.14 mg/L) and against *A. subpictus* (LC_{50} 27.85 and 5.14 mg/L; LC_{90} 71.45 and 25.68 mg/L), respectively (Rajakumar and Rahuman 2011). The larvicidal aqueous crude leaf extracts and synthesized silver nanoparticles of *M. pudica* showed the highest mortality in synthesized AgNPs against the larvae of *A. subpictus* and *C. quinquefasciatus* (LC_{50} 13.90 and 11.73 mg/L; r=200.411 and 0.286), respectively (Marimuthu et al. 2011). The larvicidal activity of ethyl acetate extract of *M. pudica* showed the LD_{50}=134.66, 156.55, and 112.78 ppm; LD_{90}=921.14, 1,214.47, and 627.80 against *Culex gelidus* and LD_{50}=134.15, 152.64, and 115.66 ppm; and LD_{90}=633.38, 781.63, and 485.12 against *C. quinquefasciatus*, respectively (Kamaraj et al. 2010). In *Nelumbo nucifera* leaf, synthesized AgNPs' maximum efficacy was observed in crude methanol, aqueous, and synthesized AgNPs against the larvae of *A. subpictus* (LC_{50} 08.89, 11.82, and 0.69 ppm, respectively; LC_{90} 28.65, 36.06, and 2.15 ppm, respectively) and against the larvae of *C. quinquefasciatus* (LC_{50} 09.51, 13.65, and 1.10 ppm, respectively; LC_{90} 28.13, 35.83, and 3.59 ppm, respectively) (Santhoshkumar et al. 2011). The ethanolic extract of whole plant *L. aspera* against the first to fourth instar larvae and pupae values of LC_{50} of I instar was 9.695 %, that of II instar was 10.272 %, that of III instar was 10.823 %, that of IV instar was 11.303 %, and that of pupae was 12.732 %, respectively, against *A. stephensi* (Kovendan et al. 2012). The highest larval mortality was found in leaf ethyl acetate of *Aegle marmelos* and *E. prostrata*, hexane, and methanol of *A. paniculata* and *Cytisus hirsutus* showing LC_{50} values of 167.00, 78.28, 67.24, and 142.83 ppm and LC_{90} of 588.31, 360.75, 371.91, and 830.01 ppm, respectively (Elango et al. 2009). 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 LC_{50} values of 48.83, 135.36, 106.77, and 102.71 ppm and LC_{90} values of 225.36, 527.24, 471.90, and 483.04 ppm, respectively (Zahir et al. 2009). The efficacy of dried fruits of peppercorns against different instars of *A. aegypti* was found effective at LC_{50} values of 0.248, 0.356, and 0.405, respectively (Kumar et al. 2010).

The ethanol extract of *Apium graveolens* exhibited adulticidal activity against *A. aegypti* with LD_{50} and LD_{90} values of 6.6 and 66.4 mg/cm^2 (Choochote et al. 2004). Bioassay-guided fractionation and subtraction bioassays of the dichloromethane extract of the root barks of *Lantana viburnoides* sp. *viburnoides* contained active fractions of furanonaphthaquinones regio-isomers (LC_{50}=5.48–5.70 ppm in 72 h) and the lantadene triterpenoid camaric acid (LC_{50}=6.19 ppm in 72 h) as active principles, while the lupine triterpenoid betulinic acid (LC_{50}<10 ppm in 72 h) was obtained from the least active fraction against early fourth instar larvae of *Anopheles gambiae* (Innocent et al. 2008). Thymol induces toxicity in *Musca domestica* and *Spodoptera litura* (LD_{50}=25.4–29.0 μg/insect) (Lee et al. 1997). The adult mortality was found in ethanol extract of *Citrus sinensis* with the LC_{50} and LC_{90} values of 272.19 and 457.14 ppm, *A. stephensi* of 289.62 and 494.88 ppm, and *A. aegypti* of 320.38 and 524.57 ppm, respectively (Murugan et al. 2012). Green synthesis shows that the environmentally benign and renewable source of *H. indicum* is used as an effective reducing agent for the synthesis of AgNPs. This biological reduction of silver nanoparticle would be boon for the development of clean, nontoxic, and environmentally acceptable green approach to produce AgNPs, involving organisms even ranging to higher plants. The formed AgNPs are highly stable and have significant mosquito adulticidal activity. In conclusion, the present study clearly proved that the leaf extract of *H. indicum* has remarkable adulticidal efficacy against *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* vector mosquitoes. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plants. These leaf extracts of *H. indicum* have the potential to be used as an ideal eco-friendly approach for the vector control programs.

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