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Green synthesis of silver nanoparticles from *Cassia roxburghii*—a most potent power for mosquito control

Udaiyan Muthukumaran[1] · Marimuthu Govindarajan[1] · Mohan Rajeswary[1]

Abstract Mosquitoes transmit serious human diseases, causing millions of deaths every year. The use of synthetic insecticides to control vector mosquitoes has caused physiological resistance and adverse environmental effects in addition to high operational cost. Insecticides of synthesized natural products for vector control have been a priority in this area. In the present study, silver nanoparticles (AgNPs) synthesized using *Cassia roxburghii* plant leaf extract against *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* were determined. Larvae were exposed to varying concentrations of synthesized AgNPs (12, 24, 36, 48, and 60 μg/mL) and aqueous leaf extracts (60, 120, 180, 240, and 300 μg/mL) for 24 h. The synthesized AgNPs were characterized by UV–Vis spectrum, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), with energy-dispersive X-ray spectroscopy analysis (EDX), transmission electron microscopy, and X-ray diffraction analysis (XRD). Compare to aqueous extracted synthesized AgNPs showed extensive mortality rate against *An. stephensi*, *Ae. aegypti*, and *C. quinquefasciatus* with the LC_{50} and LC_{90} values that were 26.35, 28.67, 31.27 and 48.81, 53.24, and 58.11 μg/mL, respectively. No mortality was observed in the control. This is the first report on mosquito larvicidal activity of plant-synthesized nanoparticles. Thus, the use of *C. roxburghii* to synthesize silver nanoparticles is a rapid, eco-friendly, and a single-step approach, and the AgNPs formed can be potential mosquito larvicidal agents. Therefore, this study proves that *C. roxburghii* is a potential bioresource for stable, reproducible nanoparticle synthesis (AgNPs) and also can be used as an efficient mosquito control agent. This is the first report on the larvicidal activity of the plant extract and AgNPs.

Keywords *Cassia roxburghii* · Leaf extract · Synthesis · AgNPs · Larvicidal activity · Mosquitoes

Introduction

Mosquito vectors of human disease are responsible for the transmission of pathogens of malaria, yellow fever, chikungunya, dengue, lymphatic filariasis, and encephalitis. Interruption of disease transmission by killing or preventing infected mosquitoes from biting human beings is one of the approaches for the control of mosquito-borne diseases. Mosquitoes also cause allergic responses in humans that include local skin and systemic reactions such as angioedema (Peng et al. 1999). *Aedes aegypti*, a vector of dengue, is widely distributed in tropical and subtropical zones. Dengue fever incidence has increased fourfold since 1970, and nearly half the world's population is now at risk. In 1990, almost 30 % of the world population, 1.5 billion people lived in regions where the estimated risk of dengue transmission was greater than 50 % (Hales et al. 2002). An outbreak of chikungunya virus infection emerged in the southwest Indian Ocean islands in 2005, spread out to India, and resulted in an ongoing outbreak that has involved >1.5 million patients, including travelers who have visited these areas (Taubitz et al. 2007). *Anopheles stephensi* are major malaria vectors in India. With an annual incidence of 300–500 million clinically manifested cases and a death toll of 1.1–2.7 million, malaria is still one of the most important communicable diseases. Currently, about

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40 % of the world's population lives in areas where malaria is endemic (Wernsdorfer and Wernsdorfer 2003). *Culex quinquefasciatus*, a vector of lymphatic filariasis, is widely distributed in tropical zones with around 120 million people infected worldwide and 44 million people having common chronic manifestation (Bernhard et al. 2003).

The synthesized nanoparticles, which are less likely to cause ecological damage, have been identified as potential replacement of synthetic chemical insecticides, hence the need to use green synthesized silver nanoparticles for the control of disease vectors. Plants and microbes are currently used for nanoparticle synthesis. The use of plants for synthesize of nanoparticles is rapid, low cost, eco-friendly, and a single-step method for biosynthesis process (Huang et al. 2007). It has been reported that medicinally valuable angiosperms have the greatest potential for synthesis of metallic nanoparticles with respect to quality and quantity (Song and Kim 2009). Among the various known synthesis methods, plant-mediated nanoparticle synthesis is preferred as it is cost-effective, environmentally friendly, and safe for human therapeutic use (Kumar and Yadav 2009). Biological control or control through natural derivatives deserves favor over control by synthetic chemicals as they are more target-specific, relatively nontoxic, easily biodegradable, and less expensive, and even can provide effective and environmentally friendly solution of controlling the population of these harmful vectors. But, those factors cannot nullify the usefulness of chemical insecticides, especially at the time of outbreak when rapid control of vectors is indispensable. At this point, application of different nanoparticles (NPs) synthesized by green chemical route opens up new scope of research to find its utility in mosquitocidal activity expecting that these nanoparticles should be more susceptible to mosquito body system due to its biogenic nature as well as less harmful to the environment due to minimal dosage. AgNPs may be released into the environment from discharges at the point of production, from erosion of engineered materials in household products (antibacterial coatings and silver-impregnated water filters), and from washing or disposal of silver-containing products (Benn and Westerhoff 2008).

The larvicidal potential of silver nanoparticles synthesized using fungus *Cochliobolus lunatus* against *Ae. aegypti* and *An. stephensi* have been observed (Salunkhe et al. 2011). *Nelumbo nucifera* synthesized silver nanoparticles using aqueous leaf extract against the larvae of *Anopheles subpictus* and *C. quinquefasciatus* have been observed (Santhoshkumar et al. 2011). The larvicidal activity of synthesized silver nanoparticles (AgNPs) utilizing aqueous extract from *Eclipta prostrata*, a member of the Asteraceae, has been investigated against fourth instar larvae of filariasis vector, *C. quinquefasciatus*, and malaria vector, *An. subpictus* (Rajkumar and Rahuman 2011). The larvicidal efficacy of the crude leaf extracts of *Ficus benghalensis*, with three

different solvents like methanol, benzene, and acetone, was tested against the early second, third, and fourth instar larvae of *C. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi* (Govindarajan 2010a). The leaf extract of *Acalypha indica* with different solvents—benzene, chloroform, ethyl acetate, and methanol—has been tested for larvicidal, ovicidal activity, and oviposition attractancy against *An. stephensi* (Govindarajan et al. 2008a). The larvicidal and repellent properties of essential oils are from various parts of four plant species *Cymbopogon citratus*, *Cinnamomum zeylanicum*, *Rosmarinus officinalis*, and *Zingiber officinale* against *Culex tritaeniorhynchus* and *An. subpictus* (Govindarajan 2011a). Focus on use of nanomaterials for benefit of human health, energy, defense, catalysis, agriculture, and environment is increasing at a rapid pace. Recent advances in this field, particularly the ability to prepare highly ordered nanoparticles of different sizes and shapes, have led to the development of new biocidal agents (Jones et al. 2008). Larvicidal activity of silver nanoparticle synthesized using various plant extracts and fungi against mosquito vector is well documented (Veerekumar et al. 2013, 2014; Muthukumaran et al. 2015). *Cassia roxburghii* was found in most parts of central and southern India, often along the roads for shade. *Cassia roxburghii* leaves are used for ring worm infections (Swarbrick and Boylan 2002). The presence of Anthraquinone glycosides and Chrysophanol, Physcion, Rhein, and Roxburghinol has been reported earlier (Duggal and Misra 1982). *Cassia* species are well known in folk medicine for their laxative and purgative uses (Rastogi and Mehrotra 2002). They are also used for treating skin diseases such as ring worm, scabies, eczema, and wounds (Mohanty and Das 2006). However, there was no scientific evidence justifying the mosquito larvicidal activity of *Cassia roxburghii*. In view of this, a laboratory study was carried out to assess the effectiveness of AgNPs synthesized with the help of *C. roxburghii* against the larvae of *An. stephensi*, *Ae. aegypti*, and *C. quinquefasciatus* mosquitoes.

Materials and methods

Collection of materials

Fresh leaves of *C. roxburghii* (Family: Fabaceae) (Fig. 1) were collected from Kodiyakarai, Tamil Nadu, India, and the taxonomic identification was made by Dr. V. Vengatesalu, Professor, 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.

Fig. 1 *Cassia roxburghii* plant

Preparation of plant extracts

The leaves (*C. roxburghii*) 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 (Veerekumar et al. 2013). 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.

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. *Ae. aegypti* feeding was done from 12 noon to 4.00 p.m. and *An. 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, 70–

85 % relative humidity, with a photo period of 12-h light and 12-h dark.

Synthesis of silver nanoparticles

The fresh leaves of *C. roxburghii* broth solution 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 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 leaf extract at room temperature for 10 min, resulting in a brown-yellow solution indicating the formation of AgNPs (Veerekumar et al. 2014).

Characterization of the synthesized AgNPs

Synthesis of AgNP solution with leaf extract may be easily observed by UV–Vis spectroscopy. The bio-reduction 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 $60,000 \times g$ 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.

Larvicidal activity

Larvicidal activity of the aqueous crude extract and AgNPs from *C. roxburghii* was evaluated according to WHO protocol (2005). Based on the wide range and narrow range tests, aqueous crude extract was tested at 60, 120,180, 240, and 300 μg/mL concentrations, and AgNPs was tested at 12, 24, 36, 48,

and 60 μg/mL concentrations. Twenty numbers of late third instar larvae were introduced into a 500-mL glass beaker containing 249 mL of dechlorinated water, and 1 mL of desired concentrations of leaf extract and silver nanoparticles was added. For each concentration, five replicates were performed, for a total of 100 larvae. Larval mortality was recorded at 24 h after exposure, during which no food was given to the larvae. Each test included a set control groups (silver nitrate and distilled water) with five replicates for each individual concentration. The lethal concentrations (LC_{50} and LC_{90}) were calculated by probit analysis (Finney 1971).

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90}, and other statistics at 95 % confidence limits of upper confidence limit and lower confidence limit, and chi-squared 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

Larvicidal activity of aqueous extract and synthesized AgNPs

The results of larvicidal activity of *C. roxburghii* aqueous leaf extract and synthesized AgNPs against late third instar *An. stephensi*, *Ae. aegypti*, and *C. quinquefasciatus* were noted and presented in Tables 1 and 2. Considerable mortality was evident after the treatment of *C. roxburghii* for all three important vector mosquitoes. The LC_{50} and LC_{90} values of *C. roxburghii* aqueous leaf extract appeared to be effective against *An. stephensi* (LC_{50} 133.69 μg/mL and LC_{90} 243.59 μg/mL), followed by *Ae. aegypti* (LC_{50} 149.68 μg/mL and LC_{90} 274.63 μg/mL), and *C. quinquefasciatus* (LC_{50} 163.48 μg/mL and LC_{90} 293.43 μg/mL). Most considerable mortality was evident after the treatment of silver nanoparticles. AgNPs against the vector mosquitoes of *An. stephensi*, *Ae. aegypti*, and *C. quinquefasciatus* had the following LC_{50} and LC_{90} values: *An. stephensi* had LC_{50} and LC_{90} values of 26.35 and 48.81 μg/mL, *Ae. aegypti* had LC_{50} and LC_{90} values of 28.67 and 53.24 μg/mL, and *C. quinquefasciatus* had LC_{50} and LC_{90} values of 31.27 and 58.11 μg/mL. The control showed nil mortality in the concurrent assay. χ^2 value was significant at $p < 0.05$ level.

Characterization of silver nanoparticles

Color change was noted by visual observation in the *C. roxburghii* leaf extracts when incubated with $AgNO_3$

solution. *C. roxburghii* 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 a 6-h incubation period after which no significant change occurred (Fig. 2a, b). The absorption spectrum of *C. roxburghii* leaf extracts at different wavelengths ranging from 300 to 800 nm revealed a peak at 460 nm (Fig. 2c). FTIR analysis of the purified nanoparticles showed the presence of bands due to O–H group Stretch hydrogen bonded (3222.44), C-H Stretch Alkenes (2922.23), C-H Stretch Alkanes (2853.35), C (triple bond) N Stretch (2209.12), C–C Stretch Aromatics (1593.43), C–H stretch Aromatics (1514.24), N–O Stretch Nitro (1384.18), C–O Stretch Acid (1240.74), C–H alkyl halides (1167.81), C–O Stretch Alcohol (1102.98), and C– C1 Stretch alkyl halides (833.39) (Fig. 3). SEM micrographs of the synthesized AgNPs of *C. roxburghii* magnified at ×60,000 and measured at 500 nm are shown in Fig. 4a. The triangular, pentagonal, and hexagonal structures are clear. EDX proves the chemical purity of the synthesized AgNPs (Fig. 4b). 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 57 to 95 nm with an average size of 32 nm (Fig. 5). XRD analysis (Fig. 6) showed intense peaks at 2θ values of 38.13°, 44.32°, 49.12°, 64.53°, and 77.38° corresponding to (461), (103), (10), (99), and (91) Bragg's reflection based on the face-centered cubic structure of silver nanoparticles.

Discussion

Mosquito-borne diseases are one of the most public health problems in the developing countries. Many approaches have been developed to control mosquito menace. One such approach to prevent mosquito-borne disease is by killing mosquito at larval stage. Management of this disease vector using synthetic chemicals has failed because of insecticide resistance, vector resurgence, and environmental pollution (Wondji et al. 2009). In the present study to assess the larvicidal properties of *C. roxburghii* leaf extract and synthesized AgNPs against *An. stephensi*, *Ae. aegypti*, and *C. quinquefasciatus*. This result is also comparable to earlier reports of Priyadarshini et al. (2012) who observed that the highest larval mortality was found in the synthesized AgNPs against the first to fourth instar larvae and pupae with LC_{50} values of 10.14, 16.82, 21.51, and 27.89 ppm, respectively, LC_{90} values of 31.98, 50.38, 60.09, and 69.94 ppm, respectively, and LC_{50} and LC_{90} values of pupae of 34.52 and 79.76 ppm, respectively. Govindarajan (2010b) reported that the larvicidal activity of the crude extract of *Sida acuta* against three important mosquitoes with LC_{50} values ranges between 38 and 48 mg/L. The crude extract had strong

Table 2 *Larvicidal activity of silver nanoparticles against* Anopheles stephensi, Aedes aegypti *and* Culex quinquefasciatus

Mosquitoes	Concentration	24 h mortality (%)$\pm$SD[a]	LC$_{50}$(μg/mL) (LCL-UCL)	LC$_{90}$(μg/mL) (LCL-UCL)	χ^2
An. stephensi	Control	0.0$\pm$0.0	26.35 (18.01–34.05)	48.81 (39.85–68.09)	21.567*
	12	30.2$\pm$0.5			
	24	49.6$\pm$1.2			
	36	68.3$\pm$1.6			
	48	82.4$\pm$1.4			
	60	100.0$\pm$0.0			
Ae. aegypti	Control	0.0$\pm$0.0	28.67 (21.37–35.66)	53.24 (44.47–70.43)	16.188*
	12	26.2$\pm$0.5			
	24	47.6$\pm$1.6			
	36	64.2$\pm$1.8			
	48	77.8$\pm$1.6			
	60	96.3$\pm$1.8			
C.quinquefasciatus	Control	0.0$\pm$0.0	31.27 (24.36–38.24)	58.11 (48.89–75.84)	13.767*
	12	24.6$\pm$0.4			
	24	42.5$\pm$1.5			
	36	59.6$\pm$1.8			
	48	73.3$\pm$1.6			
	60	91.8$\pm$1.8			

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

*$p<0.05$, level of significance

[a] Values are mean$\pm$SD of five replicates

Table 1 *Larvicidal activity of* Cassia roxburghii aqueous leaf extract against *Anopheles stephensi, Aedes aegypti,* and *Culex quinquefasciatus*

Mosquitoes	Concentration	24-h mortality (%)$\pm$SD[a]	LC$_{50}$ (μg/mL) (LCL-UCL)	LC$_{90}$ (μg/mL) (LCL-UCL)	χ^2
An. stephensi	Control	0.0$\pm$0.0	133.69 (95.89–169.20)	243.59 (201.46–329.25)	19.158*
	60	28.8$\pm$0.7			
	120	47.6$\pm$1.3			
	180	67.5$\pm$1.7			
	240	83.4$\pm$1.5			
	300	100.0$\pm$0.0			
Ae. aegypti	Control	0.0$\pm$0.0	149.68 (119.47–179.40)	274.63 (235.15–344.21)	11.700*
	60	24.2$\pm$0.8			
	120	43.4$\pm$1.2			
	180	62.7$\pm$1.5			
	240	78.3$\pm$1.4			
	300	93.6$\pm$1.8			
C.quinquefasciatus	Control	0.0$\pm$0.0	163.48 (134.75–193.15)	293.43 (252.75–364.62)	10.554*
	60	20.8$\pm$0.9			
	120	38.2$\pm$1.3			
	180	57.5$\pm$1.4			
	240	72.3$\pm$0.8			
	300	91.6$\pm$1.6			

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

*$p<0.05$, level of significance

[a] Values are mean$\pm$SD of five replicates

Fig. 2 Photographs showing the change in color after adding AgNO₃. **a** Before the reaction and **b** 6 h after the reaction. **c** UV–Vis spectra of aqueous silver nitrate with *C. roxburghii* leaf extract
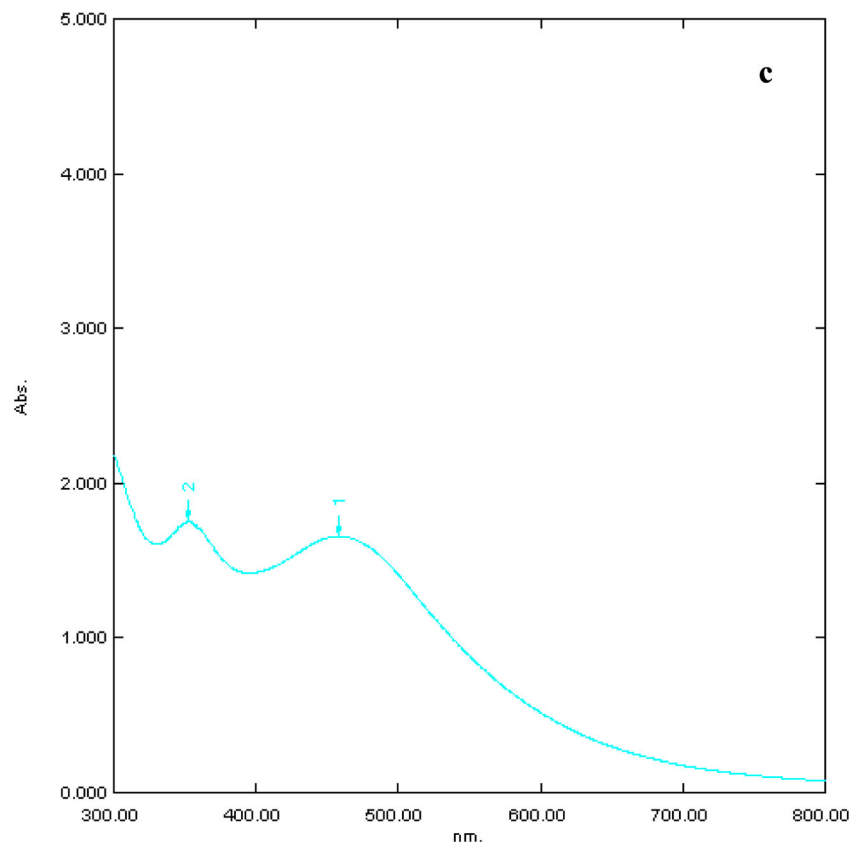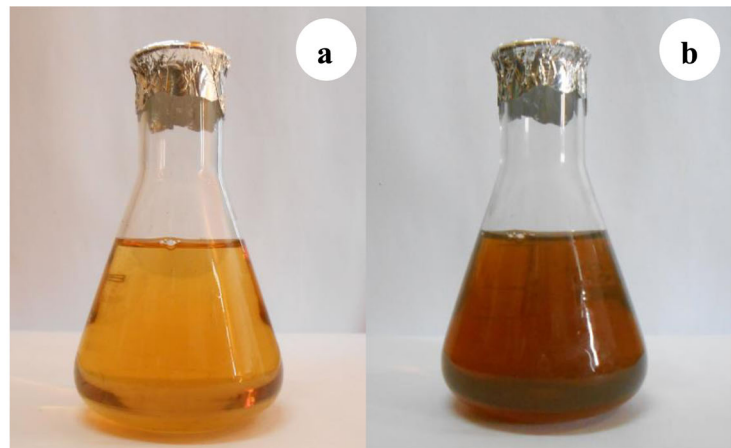
repellent action against three species of mosquitoes as it provided 100 % protection against *An. stephensi* for 180 min followed by *Ae. aegypti* (150 min) and *C. quinquefasciatus* (120 min), respectively. The methanol extract of *Cassia fistula* exhibited LC_{50} values of 17.97 and 20.57 mg/L, *An. stephensi* and *C. quinquefasciatus*, respectively (Govindarajan et al. 2008b). The maximum efficacy 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 *An. subpictus* (LC_{50} 27.85 and 5.14 mg/L; LC_{90} 71.45 and 25.68 mg/L), respectively. A biological method has

been used to synthesize stable silver nanoparticles that were tested as mosquito larvicides against *Ae. aegypti*, *An. stephensi*, and *C. quinquefasciatus* (Arjunan et al. 2012).

Biosynthesized silver nanoparticles using fungus *Cochliobolus lunatus* was used for the control of *Ae. aegypti* and *An. stephensi* and reported that the nanoparticles are significantly effective against second, third, and fourth instar larvae of *Ae. aegypti* (LC_{50} 1.29, 1.48, and 1.58; LC_{90} 3.08, 3.33, and 3.41 ppm) and against *An. stephensi* (LC_{50} 1.17, 1.30, and 1.41; LC_{90} 2.99, 3.13, and 3.29 ppm) (Salunkhe et al. 2011). Sathyavathi et al. (2010) was in good agreement
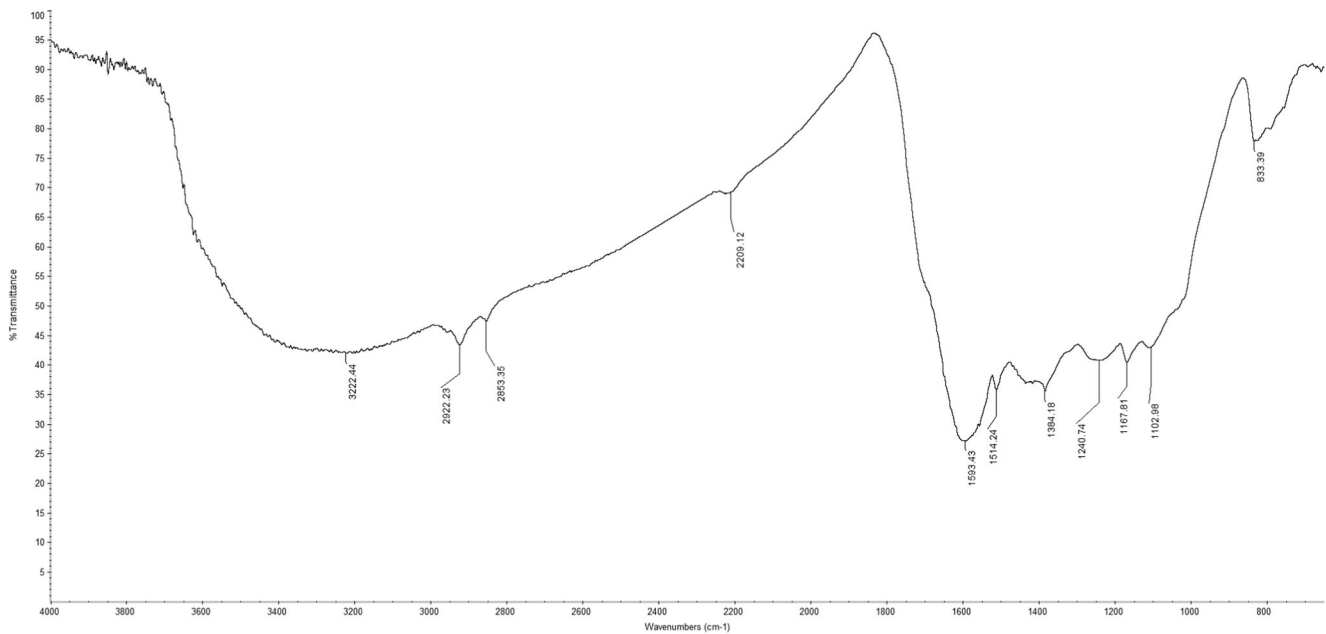
Fig. 3 FTIR spectrum of synthesized AgNPs using *C. roxburghii* leaf extract

with the results of present study with the diffraction peaks at 44.50, 52.20, and 76.7 corresponding to the (111), (200), and (220) facets of the face-centered cubic crystal structure. Senthil Nathan et al. (2008) reported that fourth instar larvae of *An. stephensi* are highly sensitive to the ethyl acetate extract of the leaves of *Dysoxylum malabaricum*. Miao et al. (2010) demonstrated, for the first time, that silver-engineered nanoparticles can be taken in and accumulated inside the *Ochromonas danica* cells, where they exerted their toxic effects. Ecotoxicity study was determined by Ag NPs in 48-h effective concentration 50 (EC_{50}) values for *Daphnia magna* of suspensions of 60 and 300 nm Ag NPs that were 1.0 (95 % confidence interval (CI)=0.1–1.3) and 1.4 mg Ag/L (95 % CI=0.3–2.1), respectively. Earthworms (*Eisenia fetida*) were exposed to $AgNO_3$ (94.21 mg/kg) and Ag NPs (727.6 mg/kg) with similar size ranges coated with either polyvinylpyrrolidone (hydrophilic) or oleic acid (amphiphilic) 773.3 mg/kg during a standard subchronic reproduction toxicity test (Shoults-Wilson et al. 2010). Marimuthu et al. (2011) reported the larvicidal effect of aqueous crude leaf extracts, Ag NPs, and synthesized Ag NPs using *Mimosa pudica* showed highest mortality in synthesized Ag NPs against the larvae of *An. subpictus* (LC_{50}=8.89, 11.82, and 0.69 ppm) and against the larvae of *C. quinquefasciatus* (LC_{50}=9.51, 13.65, and 1.10 ppm), respectively.

The larvicidal efficacy of benzene, hexane, ethyl acetate, methanol, and chloroform leaf extract of *Cardiospermum halicacabum* against *C. quinquefasciatus* and *Ae. aegypti*. The LC_{50} values were 174.24, 193.31, 183.36, 150.44, and 154.95 ppm, and 182.51, 200.02, 192.31, 156.80, and 164.54 ppm, respectively (Govindarajan 2011b). Tiwary et al. (2007) observed the larvicidal activity of linalool-rich

essential of *Zanthoxylum armatum* against different mosquito species *viz.*, *C. quinquefasciatus* (LC_{50} 49 ppm), *Ae. aegypti* (LC_{50} 54 ppm), and *An. stephensi* (LC_{50} 58 ppm). Cheng et al. (2003) examined plant EOs against *Ae. aegypti* larvae with LC_{50} values ranging from 36.0 to 86.8 μg/mL. Cavalcanti et al. (2004) reported that the larvicidal activity of EOs from Brazilian plants with LC_{50} values ranging from 60 to 69 μg/mL against *Ae. aegypti* larvae. Rahuman et al. (2000) also found that nhexadecanoic acid in *Feronia limonia* dried leaves was effective against fourth-instar larvae of *C. quinquefasciatus*, *An. stephensi*, and *Ae. aegypti* with LC_{50} values of 129.24, 79.58, and 57.23 μg/mL, respectively. The EO from the leaves of *C. anisata* exhibited significant larvicidal activity, with 24-h LC_{50} values of 140.96, 130.19, and 119.59 ppm, respectively (Govindarajan 2010c). The highest larvicidal activity was observed in the EO from *Z. officinale* against *C. tritaeniorhynchus* and *An. subpictus* with the LC_{50} and LC_{90} values as 98.83 and 57.98 ppm, and 186.55 and 104.23 ppm, respectively (Govindarajan 2011a). The larvicidal activity of crude benzene and ethyl acetate extracts of leaf of *Ervatamia coronaria* and *Caesalpinia pulcherrima* against *An. stephensi*, *Ae. aegypti*, and *C. quinquefasciatus*. The highest larval mortality was found in benzene extract of *E. coronaria* against the larvae of *An. stephensi*, *Ae. aegypti*, and *C. quinquefasciatus* with the LC_{50} and LC_{90} values were 79.08, 89.59, 96.15 ppm, and 150.47, 166.04, 174.10 ppm, respectively (Govindarajan et al. 2011). Amer and Mehlhorn (2006) examined 41 plant extracts and 11 oil mixtures against the *Ae. aegypti*, *An. stephensi*, and *C. quinquefasciatus* using the skin of human volunteers to find out the protection time and repellency. The five most effective oils were those of Litsea (*Litsea cubeba*), Cajeput

Fig. 4 Scanning electron micrographs of AgNPs synthesized with *C. roxburghii* leaf extract and 1.0-mM AgNO₃ solution and incubated at 60 °C for 6 h at pH 7.0. **a** Magnified ×60,000. *Inset bar* represents 500 nm. **b** EDX image showing chemical composition
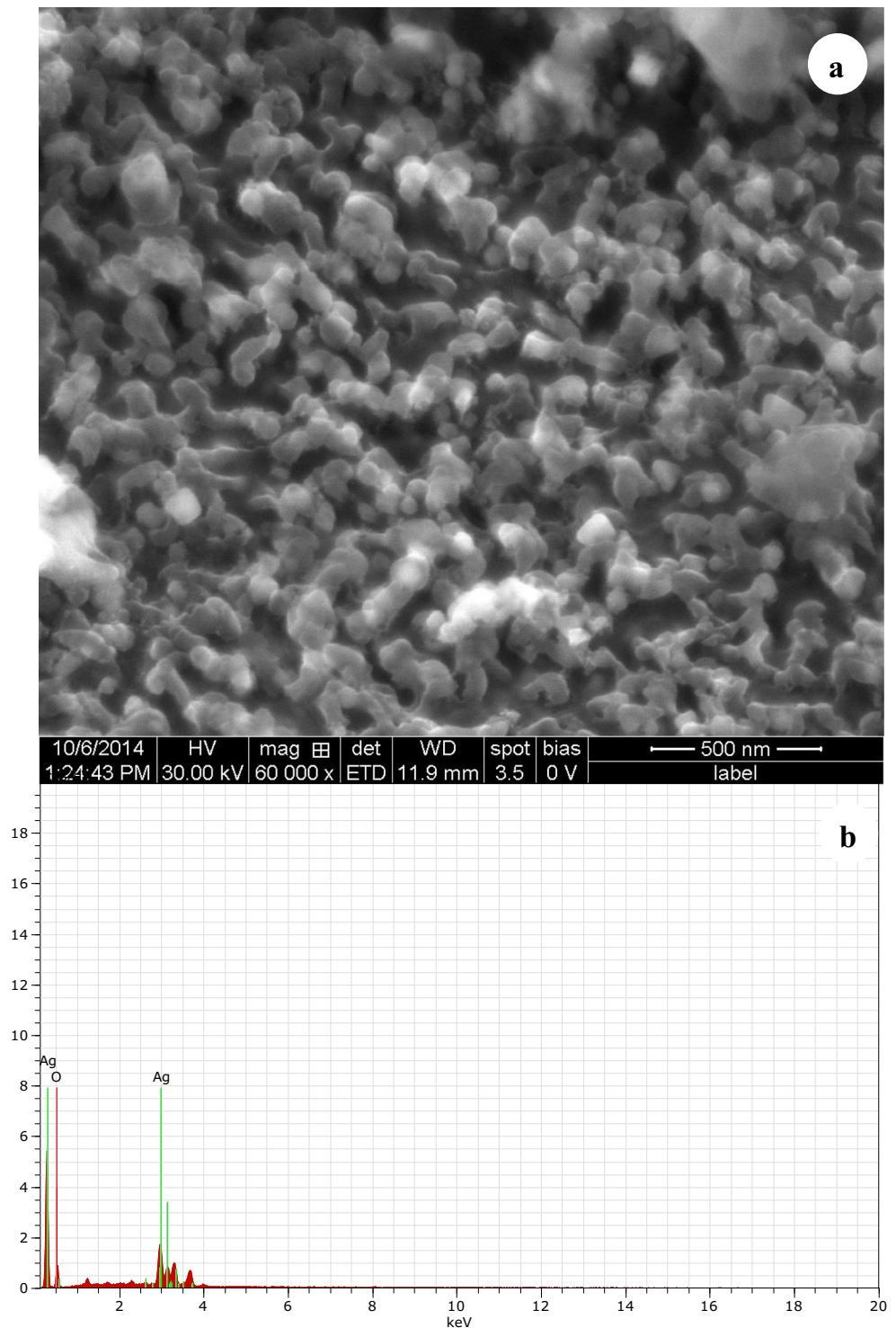
(*Melaleuca leucadendron*), Niaouli (*Melaleuca quinquenervia*), Violet (*Viola odorata*), and Catnip (*Nepeta cataria*), which induced a protection time of 8 h at the maximum and a 100 % repellency against all three species.

The maximum efficacy in the aqueous extract of *M. paradisiaca* against the larvae of hematophagous *Haemaphysalis bispinosa*, *Hippobosca maculata*, the larvae of *An. stephensi*, and *C. tritaeniorhynchus* with LC$_{50}$ values of 28.96, 31.02, 26.32, and 20.10 mg/mL, respectively (Jayaseelan et al. 2011), was observed. The higher mortality rates at lower doses are comparable with earlier reports of AgNPs produced by plant *N. nucifera* leaf extracts (LC$_{50}$= 0.69 ppm, LC$_{90}$=2.15 ppm) against *An. subpictus* and *C. quinquefasciatus* (LC$_{50}$=1.10 ppm, LC$_{90}$=3.59 ppm)
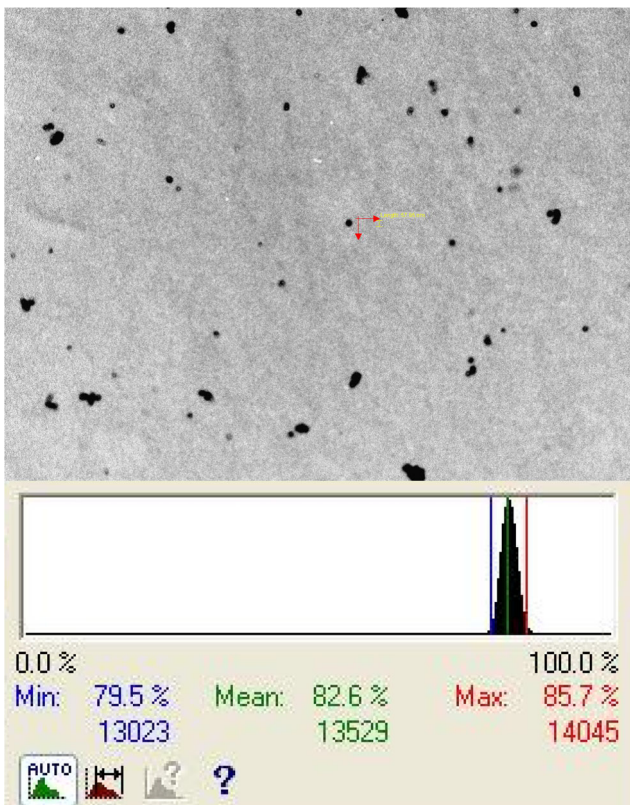
Fig. 5 Transmission electron microscopic image and histogram showing synthesized AgNPs from *C. roxburghii*

(Santhoshkumar et al. 2011). The LC_{50} and LC_{90} values of *Cassia tora* leaf extracts against adulticidal activity of hexane, chloroform benzene, acetone, and methanol (*C. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi*) were the following: For *C. quinquefasciatus*, LC_{50} values were 338.81, 315.73, 296.13, 279.23, and 261.03 ppm and LC_{90}
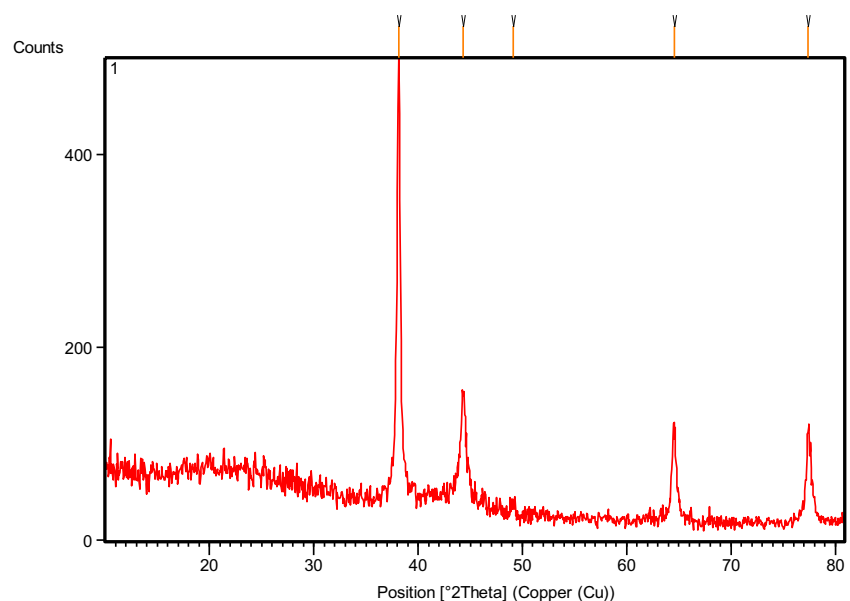
values were 575.77, 539.31, 513.99, 497.06, and 476.03 ppm; for *Ae. aegypti*, LC_{50} values were 329.82, 307.3, and 252.03 ppm and LC_{90} values were 563.24, 528.33, 496.92, 477.61, and 448.05 ppm; and for *An. stephensi*, LC_{50} values were 317.28, 300.30, 277.51, 263.35, and 251.43 ppm and LC_{90} values were 538.22, 512.90, 483.78, 461.08, and 430.70 ppm, respectively (Amerasan et al. 2012).

Essential oils obtained from aromatic plants like Eucalyptus (*E. sideroxylon*, *E.globulus ssp globulus*, and *E. globulus ssp maidenii*) were good and safe alternatives due to their low toxicity to mammals and easy biodegradability with knockdown time 50 % (KT_{50}) values of 24.75, 27.73, and 31.39 min (Toloza et al. 2010). Subarani et al. (2013) reported that the *Vinca rosea*-synthesized silver nanoparticles did not exhibit any noticeable toxicity on *Poecilia reticulata* after 24, 48, and 72 h of exposure. These results suggest that the synthesized AgNPs have the potential to be used as an ideal eco-friendly approach for the control of the *Ae. aegypti* larvae. Bansal et al. (2009) reported that the 24-h LC_{50} values as observed for aqueous extracts for green unripe and yellow ripe fruits were 112.7, 498.2, and 846.3 mg/L and 104.7, 267.7, and 832.2 mg/L for *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*, respectively. In conclusion, the synthesis of AgNPs with plant leaves shows a "green approach" that can be used as an effective reducing agent for the synthesis of silver nanoparticles. This biological reduction of metal would be a boon for the development of clean, nontoxic, and environmentally acceptable metal nanoparticles; the formed silver nanoparticles are hydrophilic in nature, disperse uniformly in water, are highly stable, and had significant mosquito larvicidal activity against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*. This is the first report on the mosquito larvicidal activity of synthesized nanoparticles from *C. roxburghii*.

Fig. 6 X-ray diffraction showing synthesized AgNPs from *C. roxburghii*

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