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Utilization of *Boerhavia erecta* (L.) in the Green Synthesis of Silver Nanoparticles and its Potential Application in Control of Vector Mosquitoes

Kalyanasuntharam Elakkiya[1] and Marimuthu Govindarajan[1, 2*]

[1]Unit of Natural Product and Nanotechnology, Department of Zoology, Government Arts College (Autonomous) (Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu), Kumbakonam 612 002, Tamil Nadu, India

[2]Unit of Vector Control, Phytochemistry and Nanotechnology, Department of Zoology, Annamalai University, Annamalainagar 608002, Tamil Nadu, India

**Corresponding Author*

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Abstract: In public health, mosquitoes are considered the most dangerous vectors for spreading deadly pathogens and parasites to humans and animals. Many initiatives have been developed to combat mosquitoes. Research on plant-based products that prevent mosquito vectors has been suggested as an alternative to the commonly used synthetic chemicals pesticides which are dangerous for the environment, human health, and non-target species. The present study aimed to determine the toxicity of *Boerhavia erecta* mediated silver nanoparticles against the vector mosquitoes *Ae. albopictus*, *An. subpictus* and *Cx. tritaeniorhynchus* and its non-target impact on aquatic fish *Gambusia affinis*. Ag NPs have been studied by the Surface Plasmon Resonance. Various functional groups present in plant extract, crystalline nature, size and morphology of nanoparticles were analysed by UV-vis, FTIR, XRD, SEM and HR-TEM analysis. Larvicidal bioassay was conducted on III instar larvae of mosquitoes following the WHO guidelines. Lethal concentrations (LC_{50} and LC_{90}) were observed at 40, 80, 120, 160 and 200 µg/ ml (aqueous leaf extract) and 8, 16, 24, 32 and 40 µg/ ml (Ag NPs). *Ae. albopictus* larvae revealed 100% mortality and Ag NPs effectively inhibited the *Ae. albopictus*, *An. subpictus* and *Cx. tritaeniorhynchus* mosquitoes with LC_{50} values of 16.76, 17.75 and 18.88 µg/ml, respectively. As a consequence of these findings, *B. erecta*-fabricated Ag NPs offer a non-toxic, cost-effective and alternative solution to chemical insecticides for vector management.

Keywords: *Boerhavia erecta*, Silver nanoparticles, Vector management, Larvicidal, Bioassay, Non-toxic, Mortality

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Introduction

Arboviruses are diseases caused by pathogens transmitted by bites of hematophagous arthropods, and their transmission to humans occurs primarily through dipteran insects. Among these, mosquitoes (Diptera: Culicidae) play a crucial role in the spread of many fatal diseases, including important arbovirus diseases such as dengue virus, Zika virus, chikungunya virus, West Nile virus, Japanese encephalitis virus and parasitic diseases malaria and lymphatic filariasis (Aghayan *et al.*, 2024). They are a major threat to global public health, cause economic damage and impede rural and urban areas' development (Dey *et al.*, 2023). By the end of 2050, about half of the world's population could be in danger of contracting an arbovirus (Yagoo *et al.*, 2023). Recently, 89 countries and territories have documented cases of Zika virus infection spread by mosquitoes, but there is still no worldwide surveillance (WHO, 2022). According to WHO, JEV transmission is endemic in 24 countries, representing over 3 billion people at risk of infection (WHO, 2024). An estimated 80 million people suffer from lymphatic filariasis, and 30 million people have the clinical condition annually. Malaria remains an enormous harm to public health, including 228 million cases globally with 213 million (93%) occurring in Africa alone (Amato *et al.*, 2018).

Mosquito vectors breed in water, which makes it easy to control them there, making larval mosquitoes ideal targets for insecticides in this environment. A number of synthetic chemical products are commercially accessible in aquatic environments which possess a high safety profile, but toxicity to human skin and the nervous system can lead to various major problems, including rashes, swelling and irritation (Shukla *et al.*, 2018). Moreover, studies have indicated that chemical insecticides create serious threat to biodiversity due to their harmful effects on non-target organisms (Pathak *et al.*, 2022). New strategies and vector control tools are immediately needed for targeting aquatic stages. In this regard, natural insecticides, especially those developed from plants, appear to have greater promise.

Green nanotechnology is a burgeoning branch of nanotechnology and the most appealing and meritorious topic of research with the greatest number of applications. At present, due to their non-taxic nature, green synthesized metal nanoparticles have drawn a lot of attention in the fields of biomedical research, catalysis and environmental remediation (Shafey, 2020; Chihal *et al.*, 2022). Many industries, including tissue engineering, pharmaceuticals, aerospace, and microelectronics sectors, as well as the manufacturing, processing, preservation, and packaging of food, can benefit from the usage of nanoparticles. When compared to other metals, silver is a great option for creating nanomaterials because of its conductivity, chemical stability, biocompatibility, antimicrobial and insecticidal potential (Gomes *et al.*, 2021). Moreover, Ag NPs have demonstrated remarkable applications in a variety of fields, including electronics, paints, textiles, water purification, food preservation, cosmetics, drug delivery, wound healing, and bio-labeling (Zhang *et al.*, 2016).

A commonly used method for nanoparticle production in an environmentally sustainable manner involves biological materials, particularly plant extracts, which contain a wide range of biologically active chemicals that help both the reduction and stabilisation of nanoparticle formation (Jadoun *et al.*, 2021). Plant extract generated nanoparticles are cost effective, eco-friendly, health benefit and efficient. Recent studies have highlighted the silver nanoparticles synthesized from plants such as *Plumbago auriculata* (Govindan *et al.*, 2020), *Echinochloa stagnina* (Shehabeldine *et al.*, 2021), *Aristolochia bracteolate* Lam (Narayanan *et al.*, 2022) which effectively control vector mosquitoes.

Boerhavia erecta L. (Erect spiderling) is a perennial medicinal herb, belonging to family Nyctaginaceae. Genus *Boerhavia* contains more than 100 species in tropical regions of Asia and Africa (Chen and Wu, 2007). Traditionally, the entire plant has been used to treat a wide range of ailments, including gastrointestinal, reproductive, fungal infections as well as paediatric convulsions (Wajid *et al.*, 2017). Significantly, *B. erecta* plant contain alkaloids, flavonoids, saponins, steroids, and glycosides, in addition to more polar substances including proteins, minerals, sugar and vitamins (Suriyavathana *et al.*, 2012). Various natural products and synthesis compound derived from *B. erecta* have demonstrated potent antimicrobial activity (Nguyen *et al.*, 2022), cytotoxicity (Palle, 2020), antidiabetic and antihyperlipidemic activity (Nisha, 2018), anticancer and antioxidant properties (Shareef *et al.*, 2017). In the present investigation, we evaluated the larvicidal and biotoxicity effect of the *B. erecta* mediated Ag NPs against *Ae. albopictus*, *An. subpictus* and *Cx. tritaeniorhynchus* mosquito vector and non-target fish species *Gambusia affinis*.

Materials and Methods

Materials

The matured leaves of *Boerhavia erecta* (Tamil--Seemai mookarattai) were collected from in and around the Kumbakonam region, Thanjavur District, Tamil Nadu, India. The plant herbarium was numbered and stored in our research laboratory for future reference. Silver nitrate ($AgNO_3$) was purchased from Merck company in India. Using Millipore Milli-Q water, the glasswares were entirely cleaned and disinfected.

Mosquito Larvae

Ae. albopictus, *An. subpictus* and *Cx. tritaeniorhynchus* mosquito eggs were obtained from Vector Control Research Centre (VCRC), Pondicherry, India. The eggs were reared in our research laboratory following the standard method by Govindarajan and Benelli (2016). After the eggs hatched, larvae were fed a 3:1 ratio of powdered dog biscuits and yeast tablets mixtures. The female adult mosquitoes were fed a blood meal via a membrane feeder, while the male adult mosquitoes were fed honey and a 5.0% glucose solution.

Preparation of B. erecta leaf extract

Boerhavia erecta leaves were properly cleaned with tap water to remove any soil and debris, and they were allowed to dry for 10 to 15 days in the shade under laboratory condition (28±2°C). A mechanical grinder was used for grounding the dried leaves into a fine powder. To make aqueous extract, 50 g of dried leaves powder was put in 500 ml of distilled water, this solution was boiled by agitating constantly using a magnetic stirrer, then permitting it to cool to room temperature. After 3 h, the resulting solution was filtered using a Whatman No.1 filter paper. Finally, yellowish green colour solution was produced which was kept in a sealed container at 10 °C for further evaluation.

Biofabrication and characterization of Ag NPs

The Govindarajan *et al.* (2016) method was slightly modified to synthesize silver nanoparticles from *B. erecta* plant leaf extract. For the preparation of the extract, 10 g of thoroughly cleaned and chopped leaves were heated with 100 ml of distilled water for 30 min at 60°C. The mixture was then filtered through Whatman No. 1 filter paper. The colloidal extract was kept at -15°C and tested within a week. At room temperature (30 ± 2°C), the biosynthesis was then completed by reacting around 12 ml of aqueous extract with 88 ml of a 1 mM silver nitrate solution to fabricate Ag NPs. The biosynthesis was monitored by observing colour changes (greenish yellow to dark brown) in every 30 min.

Furthermore, characterization of the biosynthesized Ag NPs was done by UV-vis spectrophotometer (200-800 nanometer), (Shimadzu, Tokyo, Japan). FTIR spectroscopy (wavenumber between 500-4000 cm^{-1}) analysis determined which biomolecules were responsible for the synthesis. Through the use of X-ray diffraction analytical studies, the crystalline nature of Ag NPs was confirmed. The elemental components of the synthesised Ag NPs were examined using EDX. In addition, aggregation pattern, size, and morphology of the *B. erecta* Ag NPs were employed using the SEM and HR-TEM analytical techniques.

Larvicidal bioassay

Mosquito larvicidal efficacy of *B. erecta* leaf extract and synthesised Ag NPs was determined following the procedure described by World Health Organization, (2005). A batch of 25 larvae of the late third instars of *Ae. albopictus*, *An. subpictus* and *Cx. tritaeniorhynchus* were exposed to various concentrations of *B. erecta* aqueous extract (40, 80, 120, 160 and 200 μg/ ml) and synthesized Ag NPs (8, 16, 24, 32 and 40 μg/ ml) in a 250 ml water cup containing 200 ml tap water. For each concentration, five replicates were assessed, and equal numbers of the control group were maintained for comparison. During the treatment period food was not provided. Finally, the number of dead larvae and percentage of mortality was calculated after 24 h post-treatment.

Non-target toxicity assay

The biotoxicity effect of *B. erecta* aqueous extract and synthesized Ag NPs against non-target species *G. affinis* were evaluated at a level fifty times greater than the LC_{50} doses for mosquito larvae. 10 replicates of every concentration were examined, along with four replicates of the untreated control samples. After 48 h treatment period, the non-target species have been examined decreased activity, swimming ability and mortality (Sivagnaname and Kalyanasundaram, 2004).

Statistical analysis

Larval mortality data was obtained using probit statistical analysis to calculate LC_{50} and LC_{90} values (Finney, 1971). The SPSS program (version 26), was used to analyze all of the data. To evaluate the biotoxicity on non-target organisms, the following formula (Deo *et al.*, 1998) was applied to determine each non-target organism Suitability Index (SI). At $p < 0.05$, the significance level was determined.

Results and Discussion

Visual observation and UV- vis spectrum analysis

The leaf extract of *B. erecta* biosynthesized Ag NPs primarily confirmed by visual observation showed light yellowish green to dark brown colour after 240 min (Fig. 1a). The dark brown coloured silver nanoparticles have been extensively reported (Manimagalai *et al.*, 2020; Sivasakthi *et al.*, 2022). Moreover, the optical study is the best way to confirm the nanoparticles formation. Ag NPs synthesized solution was optically analysed by UV–vis spectrum range between 200–800 nm at time interval 30 to 240 min and maximum absorbance occurred at the wavelength of 434 nm after 240 min (Fig. 1b). It was found that the absorbance increased as the reaction time increased (Jan *et al.*, 2021). This peak, typical of Ag NPs, signifies the reduction of Ag^{+} ions to Ag^{0} (Azeez *et al.*, 2022).

FTIR spectrum analysis

The purpose of the FTIR analysis was to identify potential biomolecules that could be in the role of stabilising and capping the Ag NPs created from *B. erecta* leaf extract. FTIR spectrum of *B. erecta* leaf extract and synthesized Ag NPs is shown in Figure 2. In leaf extract, the broad peak at 3450 cm^{-1} is due to the N-H group of amines. The band at 2122 cm^{-1} is C =C stretching vibrations related to the alkynes group. The strong and medium absorption peaks of 1737 and 1633 cm^{-1} suggest the stretching of C=O (Carboxylic) and N-H bend (Primary amine). C-N aliphatic amine and C-H bending alkynes groups appeared at 1216 and 663 cm^{-1}. Moreover, FTIR spectrum of *B. erecta* mediated Ag NPs peaks observed at 3315, 2121, 1639 and 706 cm^{-1}. These peaks correspond to the stretching of O-H (Hydroxyl group), C=C (alkynes group), C=O (carbonyl groups) and C–Cl group, respectively. This finding aligns with the previous study of Govindan *et al.* (2020), who discovered the *Plumbago auriculata.* Lam mediated Ag NPs exhibited a strong band at 3381 cm^{-1} related to primary amines, a 2916 cm^{-1} peak responsible for the C-H stretching of alkanes, a band at 2848 cm^{-1} suggesting the presence of C-H-O stretching of aldehyde, and 1607 and 1207 cm^{-1} peaks related to the N-H bend and C-N stretching of primary amine and aliphatic amine. Ag NPs produced by *Terminalia chebula* showed unique absorption bands at different wavelengths, which were

(a)

(b)

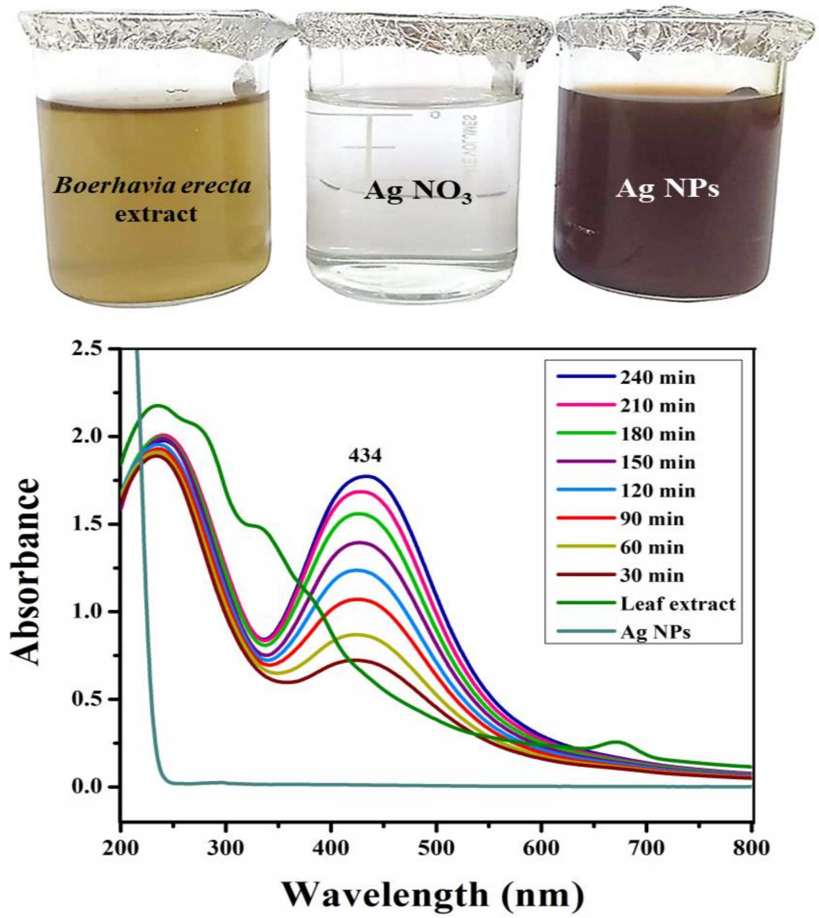

Fig. 1: (a) Colour variation of *B. erecta* leaf extract and Ag NPs (b) UV -vis spectrum of *B. erecta* mediated Ag NPs after 30 to 240 minutes from the reaction.

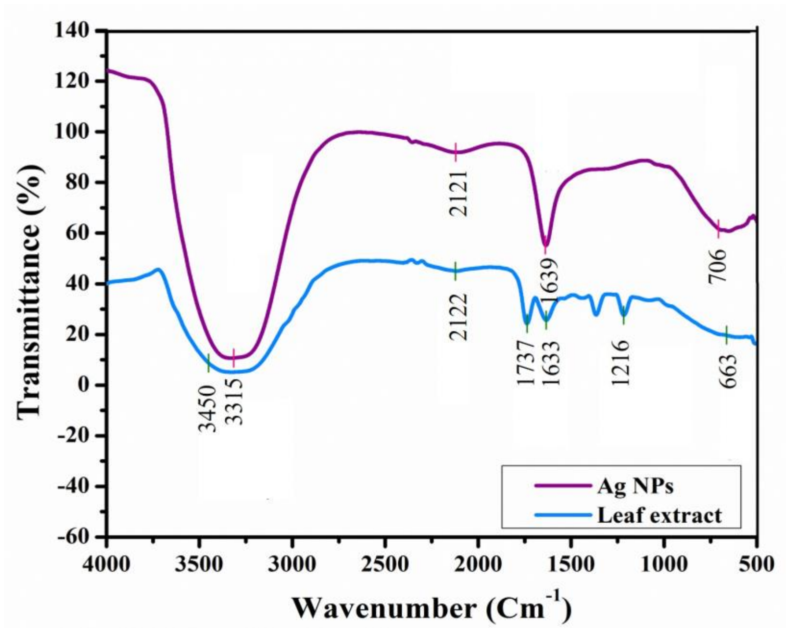

Fig. 2: FTIR spectrum of *B. erecta* leaf extract and Ag NPs.

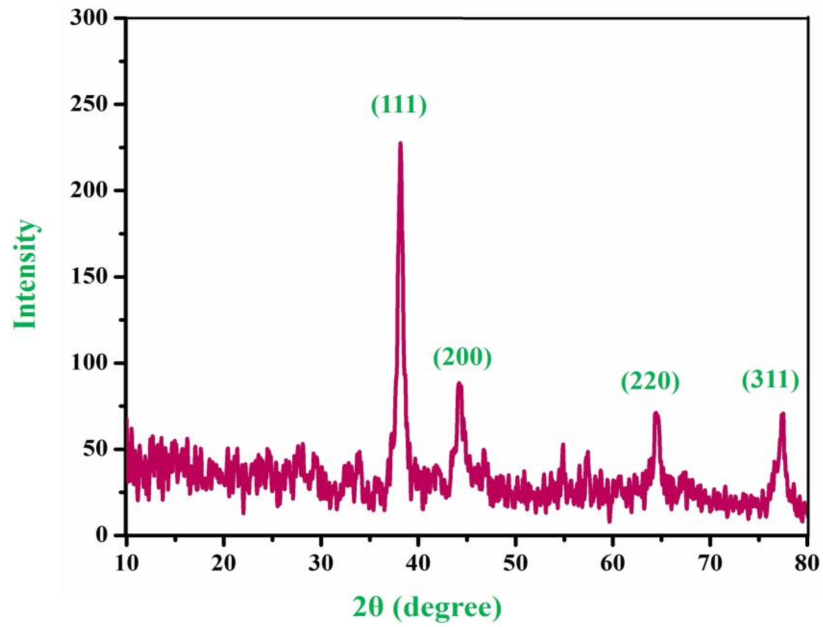

Fig. 3: XRD illustration of *B. erecta* fabricated Ag NPs.

suggested of certain functional groups used in the synthesis process. (Tharani *et al.*, 2023).

XRD spectrum analysis

The XRD data validated the Ag NPs size and crystalline phase (Fig. 3). Bragg's reflections of the 111, 200, 220, and 311 planes are represented by the XRD peaks that appear at 38.29°, 44.26°, 64.50°, and 77.55° 2θ positions, respectively. This could be described by the cubic crystal structure of Ag NPs. A report with previous studies, Ag NPs produced from *Azadirachta indica* and *Citrulla colocynthis* extract evidenced by peak of 38.5°, 46.5°, 64.7°, 77.1° and 37.9°, 46.2°, 67.5°, 77.1° which correspond to 111, 200, 220, and 311 planes, respectively and the Bragg reflection proved the Ag NPs formation with fcc crystal structure (Rasool *et al.*, 2020). In addition, XRD pattern of *Echinochloa stagnina* mediated Ag NPs confirmed nanocrystalline nature of metallic silver along with crystalline planes at 111, 200, 220, and 311 (Shehabeldine *et al.*, 2021).

SEM with EDX analysis

The surface morphology of the *B. erecta* mediated Ag NPs was observed by the Scanning Electron Microscopy (SEM) analysis. The outcome of the SEM image clearly indicated that Ag NPs were mostly agglomerated (Fig. 4a). The elemental composition of the Ag NPs synthesis from *B. erecta* leaf extract was further examined using Energy Dispersive X-ray (EDX) analysis. The obtained EDX spectrum (Figs. 4 b–h) demonstrated that the bio-fabrication of silver nanoparticles was confirmed by the strong signal in the silver region (21%) along with oxygen (53%), carbon (17%) and chlorine (10%). This observation is similar to previous reports (Sharma *et al.*, 2019; Narayanan *et al.*, 2022).

HR-TEM analysis

High-resolution Transmission Electron Microscopy analysis at various concentrations (Figs. 5 a-e) clearly demonstrated that the particles were mostly spherical in shape. Additionally, Figure 5 (f) presents the Selected Area Electron Diffraction (SAED) analysis of *B. erecta* Ag NPs, demonstrated the distinctive face-centred cubic crystalline structure of silver nanoparticles including their remarkable

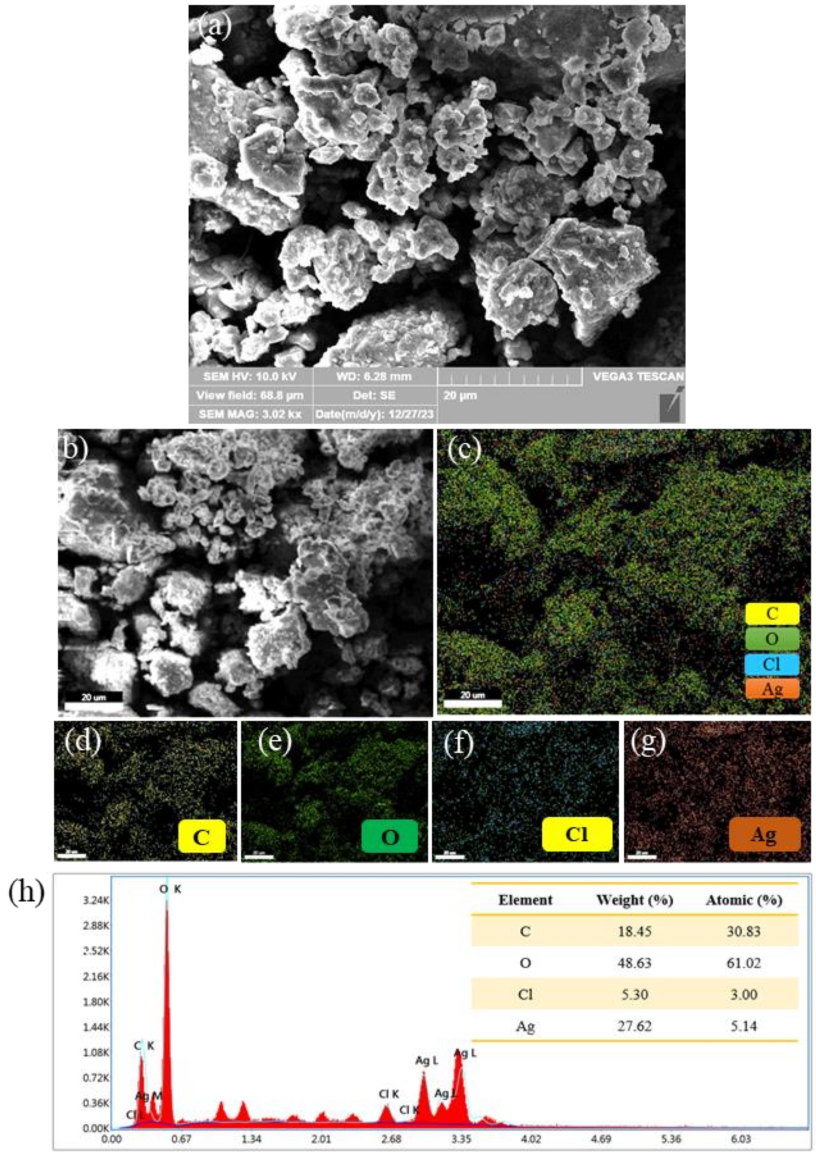

Fig. 4: (a) SEM image of *B. erecta* mediated Ag NPs, (b) Elements scan area, (c) Elements scan merged image, (d-g) element distribution of C, O, Cl and Ag, (h) EDX profile of synthesized Ag NPs.

crystallinity of the particles, diffraction pattern and the presence of ring outlines. These results concur with Elumalai *et al.* (2022), who indicated the spherical Ag NPs synthesized by *Atalantia monophylla* with sizes ranging between 18-25 nm, with an SAED pattern that indicated particles were highly crystalline in nature.

Larvicidal potential analysis

The late III instar larvae of *Ae. albopictus, An. subpictus,* and *Cx. tritaeniorhynchus* mosquitoes were used to evaluate the larvicidal potential of *B. erecta* aqueous extract and Ag NPs. Five different concentrations of *B. erecta* aqueous extract and Ag NPs were assessed, and the percentage of mortality was noted after 24 h exposure period. The results indicated that Ag NPs demonstrated excellent larvicidal toxicity on *Ae. albopictus*, *An. subpictus*, and *Cx. tritaeniorhynchus* larvae, with LC_{50} values of 16.76, 17.75, and 18.88 µg/ml; and LC_{90} values of 32.52, 34.05, and 35.82 µg/ml, respectively (Table 1). *B. erecta* leaf extract

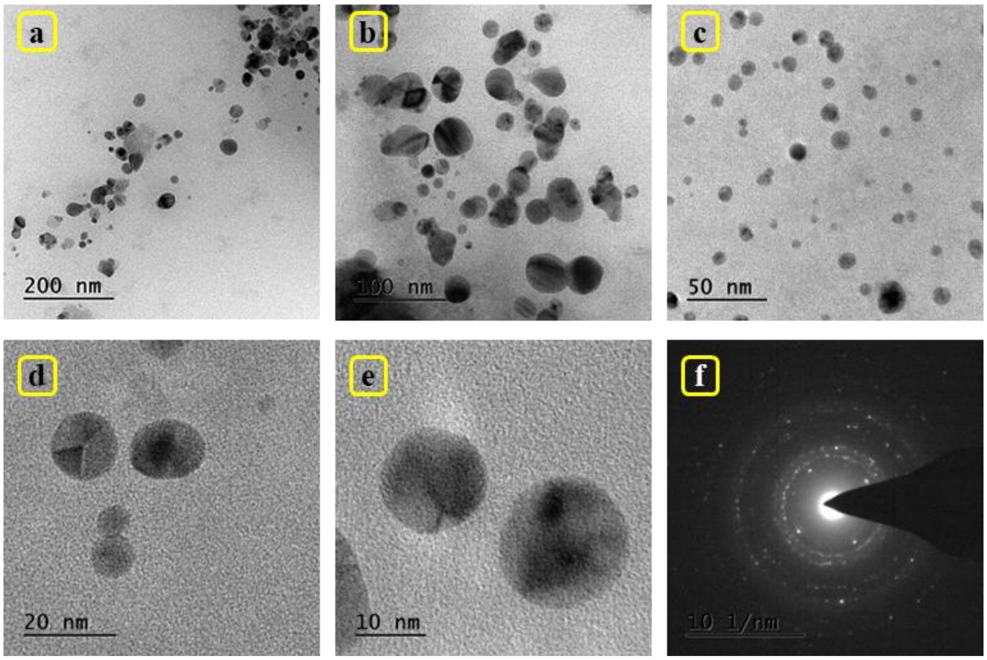

Fig. 5: (a-e) Different magnifications of HR-TEM images of *B. erecta* mediated Ag NPs, (f) SAED analysis of synthesized Ag NPs.

Table 1: Potential of *B. erecta* mediated Ag NPs against larvae of vector mosquitoes

Vector species	Concentration (µg/ml)	Mortality (%)±SD	LC_{50} (µg/ml) (LCL-UCL)	LC_{90} (µg/ml) (LCL-UCL)	Regression equation	χ^2
Ae. albopictus	Control	0±0	16.76 (14.93 – 18.40)	32.52 (30.18 – 35.57)	y= 9.84+ 2.330x	5.024*
	8	27.2±1.09				
	16	46.4±0.89				
	24	68.0±1.41				
	32	87.2±0.44				
	40	100.0±0.0				
An. subpictus	Control	0±0	17.75 (15.92 – 19.40)	34.05 (31.62 – 37.24)	y= 6.88+ 2.360x	2.636*
	8	24.8±0.44				
	16	43.2±1.09				
	24	66.4±0.89				
	32	84.8±1.09				
	40	98.4±0.44				
Cx. tritaeniorhynchus	Control	0±0	18.88 (17.04 – 20.56)	35.82 (33.25 – 39.21)	y= 4.08+ 2.370x	1.816*
	8	22.4±0.89				
	16	40.8±0.44				
	24	63.2±0.44				
	32	81.6±0.89				
	40	96.8±1.09				

*$p \leq 0.05$, Level of Significance

Table 2: Potential of *B. erecta* leaf aqueous extract against larvae of vector mosquitoes

Vector species	Concentration (μg/ml)	Mortality (%)±SD	LC_{50} (μg/ml) (LCL-UCL)	LC_{90} (μg/ml) (LCL-UCL)	Regression equation	χ^2
Ae. albopictus	Control	0±0	87.40 (78.09 – 95.75)	169.63 (157.35 – 185.83)	y= 8.32+ 0.464x	6.916*
	40	25.6±0.89				
	80	46.4±0.89				
	120	64.8±0.44				
	160	83.2±1.09				
	200	100.0±0.0				
An. subpictus	Control	0±0	92.00 (82.66 – 100.46)	177.11 (164.24 – 194.14)	y= 6.00+ 0.466x	4.789*
	40	23.2±1.09				
	80	44.8±0.44				
	120	62.4±0.89				
	160	80.8±0.44				
	200	98.4±0.89				
Cx. tritaeniorhynchus	Control	0.0±0.0	97.75 (88.39 – 106.37)	186.23 (172.57 – 204.44)	y= 3.44+ 0.466x	2.832*
	40	20.8±0.44				
	80	42.4±0.89				
	120	59.2±1.09				
	160	78.4±0.89				
	200	96.0±1.41				

*p≤0.05, Level of Significance

revealed effective larvicidal potential on the three vector mosquito larvae, with LC_{50} values of 87.40, 92.00, and 97.75 μg/ml; and LC_{90} values of 169.63, 177.11, and 186.23 μg/ml, respectively (Table 2). Notably, in both tested materials 100% mortality was observed in *Ae. albopictus* mosquitoes. There was no mortality observed in the mosquito larvae exposed to either $AgNO_3$ (1 mM) or deionized water, which served as the control. These results demonstrated the potential of biosynthesised Ag NPs and *B. erecta* aqueous extract as environmentally friendly mosquito control options by highlighting their promising larvicidal activity against mosquito vectors.

These findings are in agreement with findings of earlier investigators – (i) Ag NPs synthesized from leaf extract of *Leonotis nepetifolia* revealed maximum larvicidal activity on *Cx. quinquefasciatus* and *Ae. aegypti* with LC_{50} values 35.48, 47.44 ppm and LC_{90} values 142.29, 152.47 ppm, respectively (Manimegalai *et al.*, 2020); (ii) Sivasakthi *et al.* (2022) reported that *Ixora brachiate*-mediated Ag NPs exhibited high larvicidal ability against *Cx. quinquefasciatus* and *Ae. aegypti* along with LC_{50} values as 9.11 and 10.11 mg/ml, respectively. Collectively, these investigations demonstrated the effectiveness of plant-mediated Ag NPs as strong larvicidal agents against mosquito vectors, highlighting their potential for use in mosquito control approaches.

Biocompatibility impact on non-target species

Aquatic mosquito predator *G. affinis* was used to test the biotoxicity of *B. erecta* mediated Ag NPs and related aqueous extract. After 48 h experimental period, both aqueous leaf extract and synthesized Ag NPs revealed extremely low toxicity effects on *G. affinis*, along with LC_{50} values of 26515.73 and 2936.46 μg/ml, respectively (Table 3). The Suitability Index reported that, when comparing the toxicity of the target vector mosquitoes, the aquatic non-target organisms showed no toxicity (Table 4). Similar to this observation, *Cymodocea serrulata* seagrass extract fabricated Ag NPs exhibited no toxicity effect on non-target species *Othiona dissimilis* (Copepod) and *Artemia salina* (Amutha *et al.*, 2019). Leaf extract and Ag NPs from *Pimenta dioica* revealed no toxicity effect on non-intent species

Table 3: Biotoxicity of *B. erecta* aqueous leaf extract and synthesized Ag NPs against *G. affinis*

Test materials	Concentration (µg/ml)	Mortality (%)±SD	LC_{50} (µg/ml) (LCL-UCL)	LC_{90} (µg/ml) (LCL-UCL)	Regression equation	χ^2
Aqueous extract	Control	0±0	26515.73 (24025.41 - 28811.55)	49043.86 (45673.61 - 53406.30)	y= 6.87+ 0.001x	4.741*
	12000	24.6±0.48				
	24000	45.3±1.03				
	36000	66.0±0.87				
	48000	86.6±0.66				
	60000	100.0±0.0				
Ag NPs	Control	0±0	2936.46 (2601.81 - 3232.60)	5817.75 (5391.06 - 6380.98)	y= 10.81+ 0.013x	6.158*
	1400	28.6±0.48				
	2800	46.0±0.87				
	4200	67.3±0.87				
	5600	85.3±0.63				
	7000	100.0±0.0				

*p≤0.05, Level of Significance

Table 4: Suitability index of *G. affinis* over larvae of mosquitoes exposed to *B. erecta* leaf aqueous extracts and synthesized Ag NPs

Treatment	*Ae. albopictus*	*An. subpictus*	*Cx. tritaeniorhynchus*
Aqueous extract	303.38	288.21	271.26
Silver nanoparticles	175.20	165.43	155.53

Mesocyclops thermocyclopoides (Kumar *et al.*, 2022). These results underscore the importance of evaluating the potential ecological impact of novel mosquito control materials on non-target species to ensure environmental safety.

Conclusion

Green synthesis is more economically feasible since it requires fewer costly materials and equipment. This study assured that the *B. erecta* can be used as capping greener silver NPs. After synthesis, Ag NPs are confirmed through analysis using UV-vis, FTIR, XRD, SEM, and HR-TEM. *B. erecta* mediated Ag NPs and leaf extracts effectively controlled the larvae of *Ae. albopictus, An. subpictus,* and *Cx. tritaeniorhynchus* mosquitoes and no toxicity effect on non-target species *G. affinis*. The results essentially suggest an economically feasible and ecologically friendly process for producing Ag NPs, which may further the development of larvicidal application and other approaches related to the development of novel mosquitocidal properties.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Unit of Vector Control, Phytochemistry and Nanotechnology, Department of Zoology, Annamalai University, Annamalainagar, Tamil Nadu, India.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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