Research Article

K. Prabakaran, M. Baranitharan*, M. Mathiyazhagan, N. C. Sumedha, P. Surya, H. Irrusappan, Shobana Sampath, Mohammad Z. Ahmed, Perumal Asaithambi*

Eco-friendly synthesis of silver nanoparticles using *Phyllanthus niruri* leaf extract: Assessment of antimicrobial activity, effectiveness on tropical neglected mosquito vector control, and biocompatibility using a fibroblast cell line model

https://doi.org/10.1515/chem-2024-0089
received March 9, 2024; accepted August 12, 2024

Abstract: Mosquitoes are rapidly advancing as vectors of several severe diseases. The increasing resistance of mosquitoes and the environmental harm caused by insecticides pose significant challenges for eradicating mosquito vectors. In this study, 18 plant extracts were tested for larvicidal properties against *Aedes aegypti, Culex quinquefasciatus*, and *Anopheles stephensi* larvae. *Phyllanthus niruri* (Pn) showed enhanced larvicidal activity in both laboratory and field trials. The biosynthesis of silver nanoparticles (AgNPs) using Pn leaf methanol extract (Pn-LME) was confirmed by UV-visible spectroscopy, X-ray diffraction, transmission electron microscopy, scanning electron microscopy, and Fourier-transform infrared spectroscopy. Among various concentrations, 3 mM AgNPs exhibited significant LC90 values of 0.83, 1.46, and 9.11 ppm compared to 9.25, 93.48, and 14.60 ppm of Pn-LME against *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti*, respectively. This indicates the high mortality of mosquito vectors at low AgNP concentrations. Additionally, Pn-AgNPs showed enhanced antibacterial activity and no cytotoxicity in normal fibroblast cells (L929). Field trials demonstrated a 98.70% decrease in mosquito larval density at *A. stephensi* breeding sites, a 96.55% reduction at *C. quinquefasciatus* sites, and a 97.85% reduction at *Ae. aegypti* sites. This study presents an eco-friendly and cost-effective AgNP biopesticide synthesized from Pn leaves for controlling and preventing the transmission of filarial, dengue, and malaria vectors.

Keywords: silver nanoparticle, green synthesis, plant extract, *Phyllanthus niruri*, larvicidal activity, antibacterial

*** Corresponding author: M. Baranitharan,** Department of Zoology, St. Joseph University, Dimapur, 797 115, Nagaland, India,
e-mail: professorbarani@gmail.com
*** Corresponding author: Perumal Asaithambi,** Department of Water Supply and Environmental Engineering, Faculty of Civil and Environmental Engineering, Jimma Institute of Technology, Jimma University, Po Box - 378, Jimma, Ethiopia,
e-mail: asaithambi.perumal@ju.edu.et
K. Prabakaran: Department of Zoology, Kanchi Mamunivar Centre for Post Graduate Studies (Affiliated to Pondicherry University), Puducherry, 605 008, India
M. Mathiyazhagan: Department of Botany, Silapathar Science College (Affiliated to Assam Science & Technology University), Silapathar, 787 059, Assam, India
N. C. Sumedha: Department of Zoology, St. Joseph University, Dimapur, 797 115, Nagaland, India
P. Surya: Department of Research Analytics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, 600 077, Tamil Nadu, India
H. Irrusappan: Technical Support Unit, NCVBDC, New Delhi, 110 054, India
Shobana Sampath: Department of Biotechnology, Vel Tech Rangarajan Dr Sagunthala R&D Institute of Science and Technology, Chennai, 600 062, India
Mohammad Z. Ahmed: Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

1 Introduction

Among the anthropods, mosquitoes transmit more diseases, affecting millions of people worldwide. Very recently, the World Health Organization (WHO) announced mosquitoes as the “number one public enemy.” A mosquito acts as a vector for several life-threatening vector-borne diseases, such as encephalitis, chikungunya fever, yellow fever, malaria, dengue fever, filariasis, and West Nile virus infection. In addition, they pose a significant health hazard in the southeastern countries of Asia and the Indian subcontinent. This challenge of

mosquito-extending diseases has become a huge concern due to the exponential urbanization and propulsive population along with the migration of humans from rural to urban areas. In our country, NVBDCP is a multifaceted, comprehensive public health activity that involves the avoidance and organization of mosquito disease [1]. Vector control is a challenge globally, as it is directed against the immature stages of mosquitoes. The problem of vector control varies across countries and varies according to the rural and urban parts of a country [1]. The mosquito-carrying disease causes increased mortality and morbidity while also greatly inflicting the economy, including labour and commercial output loss, mainly in subtropical and tropical countries. While every region of the world is affected by these diseases, the squirting of insecticides is the most common conventional method of vector control; however, the highlighted advantage of insecticides has become limited, and mosquitoes are allied with livestock and human diseases, particularly affecting human health. Mosquito-borne diseases reduce human productivity, cause chronic debilitation and early death, and exacerbate economic hardship by placing a burden on the health care system and resources. Low production is also caused by the enormous number of mosquitoes, which severely irritates animals and wildlife and causes significant blood loss. Mosquitoes are ubiquitous throughout the world, with the exception of Antarctica. From arctic tundra, salt marshes, boreal forests, and ditches to every tidal zone of oceans, they thrive in a broad range of extreme biotic communities. The greatest mosquito diversity occurs in tropical rainforests, but a dense population can be found in poor biomes such as tundra [2].

A. stephensi mosquitoes are the chief vector that transmits malaria parasites among humans in India and other regions of Asian countries. The trend of malaria being a huge health hazard continues globally, and nearly three billion people are affected annually in around 106 or more countries that are already prone to malaria [3]. In fact, 91% of the ~200 million cases occurred in 2010 owing to *Plasmodium falciparum*, making malaria one of the widespread ailments in the tropical world. In India, various ecological and disease vectors contribute to the prevalence of malaria. India accounts for ~80% of malaria incidence among southeast Asian countries, with the highest number of affected individuals in the world. India accounted for 753 attributable deaths and 1.3 million confirmed malaria cases; however, the expected deaths and cases were 20 times more in 2011. Numerous potentially fatal illnesses, including West Nile virus, filariasis, avian malaria, Japanese encephalitis, and St. Louis encephalitis, are mostly spread by *C. quinquefasciatus*. It is a vector for lymphatic filariasis in humans and has been assessed to afflict 120 million people worldwide. This mosquito species is the only vector of lymphatic filarial worms (*Wuchereria bancrofti*), according to the WHO. Filariasis remains a major public concern and a socioeconomic challenge in many countries in the tropics. The parasite remains endemic in tropical regions, with an estimated 120 million people affected in 81 countries, and about nearly one billion individuals are threatened by contracting the disease. Also, *A. aegypti* is generally notorious as a vector for dengue as well as chikungunya and is broadly situated in subtropical and tropical areas. Dengue virus (DENV) and chikungunya virus (CHIKV) are usually carried by *A. aegypti*, which generally breeds in peri-urban and urban regions. Breakthrough of dengue fever and chikungunya is a significant health concern in every part of rural India. An estimated 390 million cases of dengue fever are reported annually, making the re-emergence of arbovirus illness a concern. Among these, 100 million patients demand medical care, and >5 lakh patients require hospitalization. Regretfully, reports from India account for 34% of worldwide reports. DENV serotypes 1, 2, 3, and 4 are responsible for endemicity and spread throughout the year in different parts of the world. The main carrier of this virus is *A. aegypti*.

Over the years, controlling vector-causing diseases has spread widely, and hence, the ultimate motive to irradiate vectors has been a crucial demand in numerous studies. A few techniques that involve irradiation of diseases caused by mosquitoes include targeting adult mosquitoes using chemical insecticides or removal of the larvae themselves prior to their maturation by using insecticides, particularly synthetic and herbal larvicides, as a promising remedy [2]. It appears that pesticides and larvicides are quite dangerous to the environment and other living things, including people. Sometimes, the insecticides and other chemicals used will remain undesirably in the environment for longer periods, ending up as residues in food. The earth justifies the operational failure to control illness because of natural resistance. Chemical insecticides, such as DEET and DDT, which are used to kill pathogen-causing vectors, have gained widespread use since their discovery. Thus, to overcome the rules outlined by the Environment Protection Act, agents that are biocontrollable, biodegradable, and cost-effective, are safe and have been gaining more attention, thus bringing biosynthetic insecticides and larvicides.

Plants contain phytochemicals that can be used against insects and vectors without causing damage to the environment [4,5]. Plant-based materials have been used in human products to protect against insects. Secondary metabolites and phytochemicals available in plants provide protection against vectors and insects. Plants possess many bioactive chemicals that serve as growth inhibitors, anti-feed ants, insecticides, oviposition deterrents, moulting hormones,

repellents, and juvenile hormones. Also, phytochemicals sometimes cause delays in the advancement of resistance in vector bodies owing to their new structure and ease of biodegradability [1,6]. More than 2,000 plant species have been studied for their insecticidal properties, and 344 plant species have shown mosquitocidal activities. Essential plant oils and their extracts are known to be bioactive compounds with mosquito control potency, and previous investigations on plant material have shown several bioactive compounds with mosquito-killing ability. Plants with mosquitocidal activity include *Argemone Mexicana, Lantana camara, Azadirachtaindica, Atlantiamonophylla, Solanum xanthocarpum, Citrullus vulgaris, Artemisia annua, Ocimum sanctum, Solanum nigrum, Citrus sinensis, Morninga oleifera, Chrysanthemum indicum, Ocimum gratissimum,* and *Lantana camara*.

In particular, *Phyllanthus niruri* is an erect annual herb belonging to the *Euphorbiaceae* family and is extensively found in subtropical and tropical countries. They are commonly known as Keezhanelli in the vernacular tongue of Tamil. They grow to a height of 10–60 cm. It has a simple main stem, which could branch where the younger parts are usually terrete. The cataphylls and stipules are 1.5–1.9 mm high, with its deltoid acuminate blade subulate acuminate and of 1–1.5 mm length. The leaves are usually 3–11 mm × 1.5–6 mm longer minute apiculate at the apex or inequilateral at the base. The herb has white flowers that are usually minute, and the petioles could range in length from 0.3 to 0.5 mm of triangular acuminate. The flower has unisexual cymules, each consisting of 2–3 males and 2 female flowers for males that are millimetres long. The calyx had five subequal lobes. The lobes are toothed free, transmitting, and bifid shallow at the apex and divergent arms [7]. There were six triangular seeds of light brown shade, with seed capsules on stalks that were 1–2 mm high and exhibited 5–6 back ribs. It is more commonly used in Ayurvedic medicine in India for ailments related to the stomach, liver, genitourinary system, spleen, and kidney, and diseases such as jaundice, asthma, hepatitis, and malaria. The literature in Ayurvedic has shown its usage as an antidyspnoic, antispasmodic, and antitussive, which relieves polydipsia, Kapha Pitta Dosha, antianaemic, haemorrhagic disease, leprosy, jaundice, relieves burning sensation, refrigerant, urinary disorders, and trauma. Recently, *P. niruri* has been gaining exponential attention owing to its fresh start in the antiviral activity against the hepatitis B virus. The aim of this study is to evaluate the antibacterial and insecticidal toxicity of Pn-LME and Pn-silver nanoparticles (AgNPs) against the vector mosquitoes *A. stephensi, C. quinquefasciatus*, and *A. aegypti,* as shown in Figure 1.

2 Materials and methods

2.1 Sample collection

Phyllanthus niruri (Kizhanelli) was obtained from Puducherry, India (latitude 11°93′63″N; longitude 79°77′23″N). These leaves were identified taxonomically using common taxonomic keys. Once a consistent weight was reached, the leaves were gathered from the surrounding area, rinsed in water, and then dried at RT in the Zoology Department. The dried leaves were finely powdered using an electric blender.

2.2 Extraction of metabolic compounds from plants

In a conical flask, 50 g of each powdered dried leaf was added. Methanol (approximately) of the analytical grade was then added, and the mixture was shaken for 72 h while being shaken periodically with aluminium foil. The

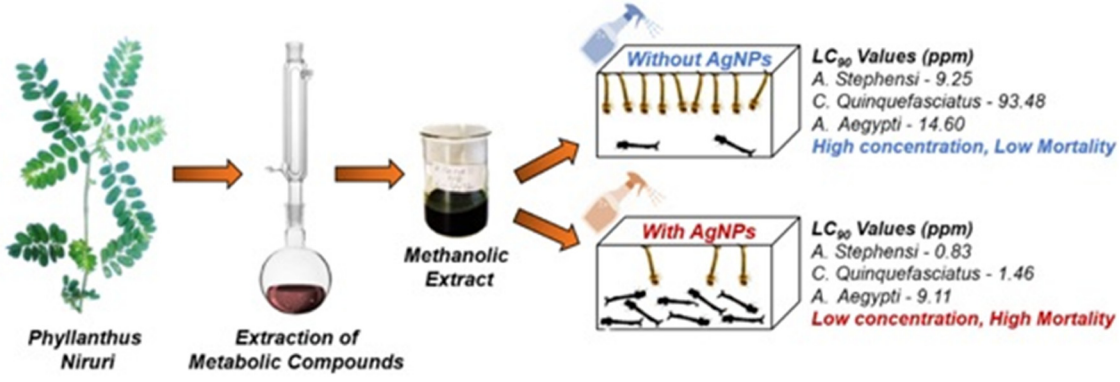

Figure 1: Schematic illustration of green synthesis of AgNPs using Pn-LME for effective vector control applications.

extracts were then filtered through "Whatman No. 1" filter paper after approximately 72 h. The remaining solvent was separated and removed from the extract using a vacuum in a rotary evaporator (Heidolph, Germany). The concentrated leaf extract (110 mg) was dried and stored at 4°C until use further phytochemical analysis and mosquito larvicidal assay studies.

2.3 Phytochemical analysis of Pn-LME

Pn-LME was subjected to phytochemical screening using typical conventional techniques in order to identify the presence of phytoconstituents [1], such as flavonoids, coumarin, terpenoids, glycosides, alkaloids, phenolic compounds, cardiac glycosides, and saponins.

2.4 Green synthesis of AgNPs from Pn-LME

About 10 ml of the leaf extract was mixed using aqueous solution (90 ml) and silver nitrate ($AgNO_3$) (1 mM). After centrifugation (15,000 rpm, 15 min) and lyophilization of AgNPs, this solution was allowed to form a dark yellowish-brown solution [8] for 3 h at room temperature (25 ± 3°C) in an orbital shaker (120 rpm).

2.5 Characterization of Pn-AgNPs

Pn-AgNPs were determined using a UV-vis spectrophotometer (OPTIGEN POP) with a scanning range of 200–700 nm [9]. AgNPs synthesized via green synthesis (Pn-AgNPs) were added for recording the intensity using 20 kV scanning electron microscopy (SEM) and transmission electron microscopy (TEM; FESEM Supra-55 Carlseiss, Germany) to determine their morphological and structural composition. By applying thin layers of AgNPs on a 40 µm × 4 µm carbon-coated grid, an extra solvent was used for blotting paper. Further, the film was dried using a mercury lamp for 5 min. The surfaces of the thin films of the AgNPs were scanned. The functional group changes on the AgNP surface were examined qualitatively using FT-IR spectroscopy (Thermo Nicolet spectrum 6700) [10]. The X'Pert Pro X-ray diffractometer (Philip, Netherlands) was used in the (2θ) mode to obtain the X-ray diffraction (XRD) patterns.

2.6 Mosquito larval collection

Using a 350 ml standard larval dipper, mosquito larvae samples were collected from breeding grounds and still water. Dips were taken gently with a 3–5 min pause to allow the larvae of mosquito to move freely in the air–water interface, and, therefore, a minimum of 8–15 dips were taken. Field-collected larvae were transferred to the laboratory for identification of *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti*. Using the identification of the US mosquito larvae manual as a guide, the collected larvae were examined under a microscope at 10× and 40× magnifications and classified into different genera based on their morphology [11].

2.7 Mosquito larvicidal bioassay of Pn-AgNPs

The WHO methodology was followed while performing the larvicidal experiments at room temperature (25 ± 3°C). Disposable paper cups coated in wax were utilized for the mosquito toxicity test (with a 350 ml capacity), and the dried leaf extract was utilized to create a uniform stock solution (100 mg/l) for the bioassays. The stock solution (25 mg/l) was diluted by dissolving 300 ml of distilled water. Into these, 25 early third instar larvae of different species of mosquito were separately introduced. The mosquito larvae were provided with food supplements such as yeast and dog biscuits at a ratio of 1:2. Additionally, the death rates were noted every 24 and 48 h, and the mortality percentage was computed using the following formula [12]. Further, the mortality rate was observed for every 24 and 48 h, and the mortality% was calculated as follows:

$$\text{Mortality\%} = \frac{\text{Number of dead larva}}{\text{Total number of larva exposed}} \times 100.$$

2.8 *Antimicrobial activity of* AgNPs

Gram-positive and Gram-negative bacteria, including *Bacillus subtilis*, *K. pneumoniae*, *E. coli*, and *S. aureus*, were used in the investigation of antibacterial activity. The well diffusion technique was used for the *in vitro* antimicrobial experiments [13]. The concentration of the bacterial suspension in Mueller Hinton agar was set at 108 cfu/ml. With 50, 100, 250, and 500 µg of the AgNPs synthesized, sterile filter paper discs of 6 mm in diameter were soaked and dried in air to evaporate the solvent. The dried discs were applied over seeded Mueller Hinton agar plates at uniform distances with sterile forceps. About 20 g of ciprofloxacin was added as a positive control.

The culture plates were incubated at 37°C for a full day. Using Vernier calipers to measure the inhibitory zone diameter in millimetres and record the results, antimicrobial activity was represented in terms of this diameter. The experiments were performed in triplicate.

2.9 Cell culture and MTT assay of AgNPs

At a concentration of 1×10^4 cells/well in DMEM with 1× antibiotic solution and 10% foetal bovine serum (Himedia, India), the fibroblast cell line was individually plated in 96-well plates in a controlled CO_2 incubator at 37°C. A 96-well plate is used for the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium (MTT) assay. After 4 h of incubation at various concentrations (25–500 µg/ml), the cells were treated with MTT. The absorbance at 570 nm was measured using a plate spectrophotometer to determine formazan formation.

2.10 Field trial

The procedure of the field trial was followed as prescribed, with some modifications [14]. By calculating the LC50 and LC90 values for each treatment, the amount of AgNPs transformed by Pn-LME was determined by measuring the surface area of the standing water bodies in each habitat. The required amounts of AgNPs were combined with water, and 0.05% teepol was used as an emulsifying agent. Knapsack sprayers (Sujatha Products Pvt Limited, India, 2010) were used to apply AgNPs to the surface of the water bodies. The density of larvae was measured 24, 48, and 72 h after treatment using dipper sampling and larval counting techniques. A control sample was used to calculate the composition of every larval habitat before 24 h of treatment as a reference. For the treatment, six trials were performed for AgNPs synthesized using *P. niruri* alone. The reduction percentage was estimated using the following formula:

$$\text{Reduction percentage} = \frac{C - T}{C} \times 100,$$

where T and C represent the total number of treated mosquitoes and the total number of mosquitoes under control, respectively.

2.11 Statistical analysis

The SPSS 25.0 version software was used to calculate the value of the regression equation, mean, standard (±SD), and lethal concentrations (LC50 and LC90, and other statistics with 95% confidence limits).

3 Results and discussion

3.1 Preliminary phytochemical screening

Pn-LME was prepared using methanol as the solvent, with an extract quantity of 0.6396 g and a yield percentage of 6.40%. Interestingly, Pn-LME revealed the presence of flavonoids, alkaloids, phenolic compounds, terpenoids, tannins, saponins, coumarins, and cardiac glycosides in the phytochemical screening test (Table 1). Similarly, PCs from indigenous medicinal plants could be different solvents extracted from various parts as well as species and are incredibly efficient bio-recourse phyto-pesticidal agents [12,15]. Similar investigations have demonstrated that secondary metabolites, such as tannins, steroids, phenols, and other bioactive metabolites, are the main players in the production of AgNPs [16].

3.2 AgNP characterization

The transition from translucent yellow to brown indicates the formation of the Pn-AgNP composite. The UV-vis spectrum of Pn-AgNPs exhibited a surface plasmon resonance peak at 422 nm (Figure 2). In accordance with the study of Elumalai et al. [17], the UV spectra of Am-AgNPs displayed a peak at 421 nm, indicating a direct band gap of approximately 2.25 eV.

As shown in Figure 3, AgNP generation from Pn-LE was characterized by XRD analysis. Using Bragg's formula, the 2θ values of the XRD patterns were determined to be 26.8°,

Table 1: Phytochemical screening of Pn-LME

Sl. no.	Phytoconstituents	Pn-LME
1	Alkaloids	✓
2	Flavonoids	✓
3	Terpenoids	✓
4	Phenolic compounds	✓
5	Saponins	✓
6	Tannins	✓
7	Glycosides	✓
8	Cardiac glycosides	✓
9	Coumarins	✓

✓: presence of phytoconstituents.

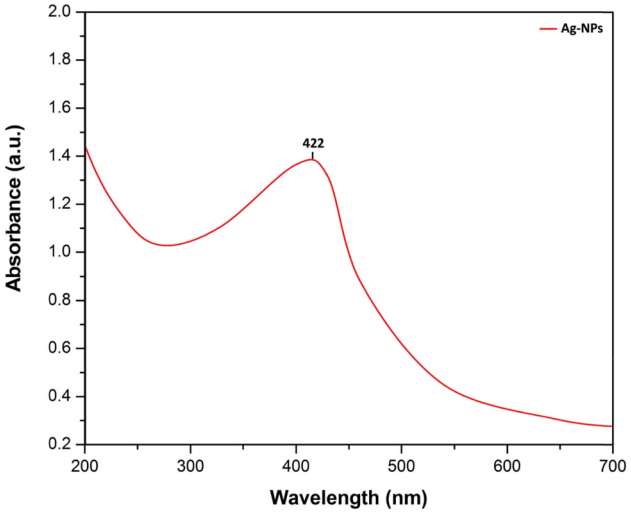

Figure 2: UV-vis spectra analysis of AgNPs synthesized using Pn-LE.

31.2°, 45.1°, 54.6°, 57.4°, and 76.6°, which correspond to 111, 200, 220, and 311. The lattice constant, which was also calculated from the pattern, was found to be α = 3.201 Å, which is in good agreement with the Joint Committee on Power Diffraction Standard data (JCPDS-PDF-04-0783). Analysis of Pn-AgNP crystals using XRD showed strong agreement with previously studied values. In line with previous studies, this study revealed a faint signal that validated the presence of certain chemical compounds [18].

3.3 SEM characterization

Because many reaction parameters must be considered and regulated, it is extremely difficult to repeatedly synthesize AgNPs with defined morphologies. Multiple analytical techniques should be used to analyse the particle size and shape. They should be isolated from synthetic byproducts that can disrupt biological systems. Colloidal stability in different media should be guaranteed at physiological temperatures throughout the experimental period, and the concentration should be high enough to have a discernible biological effect. According to these standards, we selected various synthesis methods from the literature and made necessary modifications where necessary.

In addition, we took into account the fact that each particle was stabilized with the same ligand to reduce the possible influence of the organic shell on the particle properties, as shown in Figures 1 and 2. It is impossible to make particles that are exactly the same size but have different shapes shown in Figure 2. The spherical particles produced by microwave synthesis were approximately twice as large as the particle size. An intact 3D surface plot for AgNPs is shown in Figure 3. The sizes and shapes of the different particles are shown in Figure 1. SEM showed that each particle was remarkably consistent in both size and shape (Figure 2). The core diameters of the metallic particles in the samples were summed up. The bases of the platelets were either triangular or circular, as described in the literature. The dice had sharp edges. The synthesis produced very small amounts of prisms and pentagonal decahedrons as byproducts. The rods were highly anisotropic with diameters in the range of 50–100 nm. Although it must be recognized that it is impossible to produce AgNPs with 100% identical size and shape, the differences between samples (Figure 1) are significantly greater than the variation within each sample.

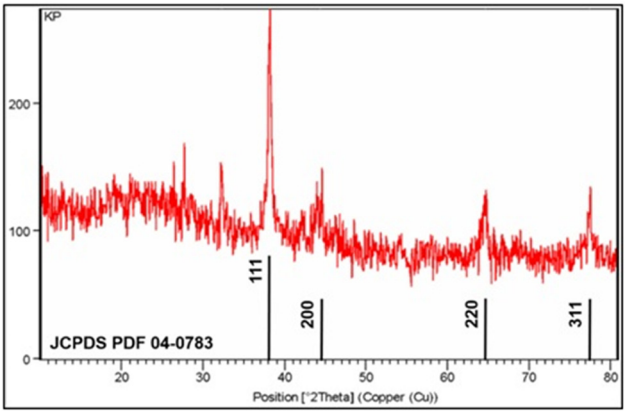

Figure 3: XRD analysis of AgNPs synthesized using Pn-LE.

3.4 TEM characterization

Figure 4 shows the shape and size of the different AgNPs obtained from the TEM images. The majority of the AgNPs were spherical and monodisperse, regardless of their size. The exact size and shape of the AgNPs can be seen in the high-resolution images obtained by TEM. If the particles are spherical or rod-shaped or have various morphologies, they are usually visible. Determining the consistency and distribution of particle sizes in a sample can be aided by precise size measurement. The properties and applications of nanoparticles can be influenced by their tendency to cluster or agglomerate, as revealed by TEM. Understanding the degree of aggregation can help determine whether the nanoparticle dispersion is stable. Size distribution histograms were constructed by measuring a significant number of nanoparticles in the TEM images. For applications

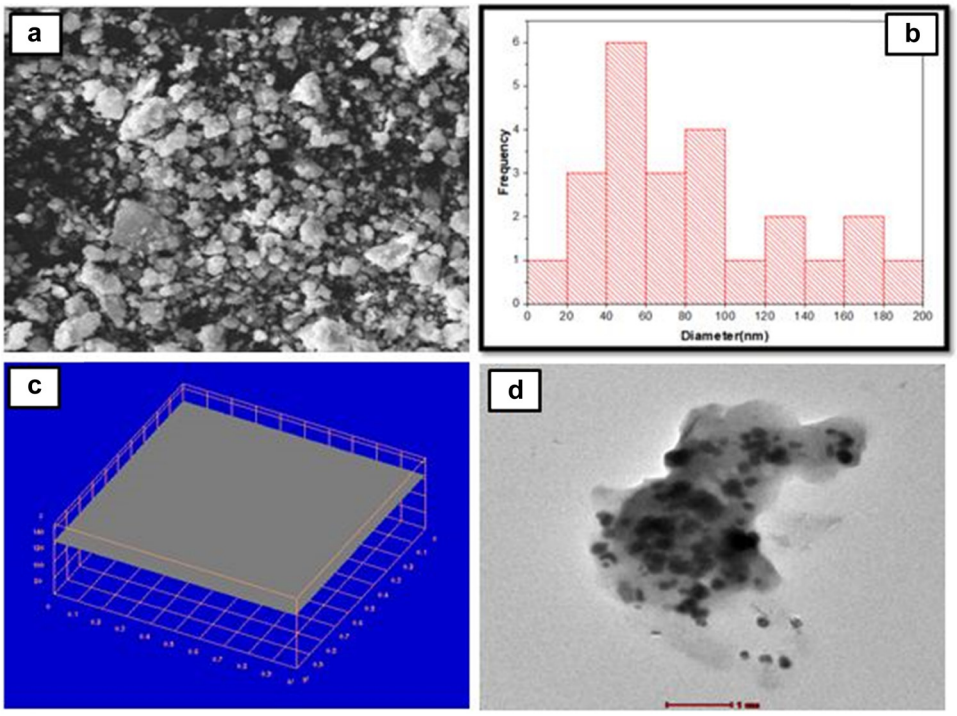

Figure 4: SEM (a)–(c) and TEM (d) images of spherical AgNPs synthesized using Pn-LE.

requiring specific size ranges, these data help understand the mean size and particle size range. When AgNPs are analysed using TEM, detailed information about their size, shape, distribution, and crystal structure becomes visible. These special features are important to ensure the effectiveness and durability of the nanoparticles and to adapt them for specific applications. By considering these features, it is possible to improve the synthesis techniques, predict the performance in real-world applications, and guarantee the consistency and quality of the nanoparticles produced. The SEM and TEM images verify the nanoscale size of the AgNPs. Propensity was noted in numerous earlier outcomes in previous investigations. The biosynthesized TiO_2NPs were well disseminated and spherical, with an average individual diameter of 25 nm, according to the SEM and TEM images [19]. SEM analysis of the *H. auriculata* AgNP cake extract currently available reports that it is primarily spherical or cubic. The results indicated above align with earlier research [20,21] that employed plant extract in the AgNP synthesis process.

The FTIR spectra of AgNPs obtained from *P. niruri* presented major peaks at 3901.25, 3883.06, 3851.44, 3836.20, 3818.85, 3799.14, 3709.28, 3687.69, 3673.52, 3626.85, 3585.70, 3565.27, 3445.19, 2952.01, 2919.70, 2359.51, 2342.03, 1791.17, 1771.06, 1748.66, 1732.82, 1715.95, 1698.60, 1682.89, 1652.17, 1646.00, 1635.10, 1557.94, 1540.17, 1520.76, 1506.91, 1488.88, 1471.89, 1456.45, 1435.48, 1417.61, 1384.44, 1338.60, 1278.98, 1032.75, 668.32, 649.13, 616.75, 578.81, 568.06, 546.86, 536.07, 525.64, 515.71, and 506.20 cm^{-1} (Figure 5), and the peaks were strong and sharp and corresponded to functional groups like alcohols, phenols (O–H stretch, free hydroxyl, 3626.85 cm^{-1}), alcohols, phenols (O–H stretching [strong and broad], H-bonded, 3445.19 cm^{-1}), alkanes (C–H stretch [medium], 2952.01 and 2919.70 cm^{-1}), carbonyls (general) (C═O stretching [strong], 1748.66, 1732.82, 1715.95, 1698.60 and 1682.89 cm^{-1}), alkenes (–C═C– stretching [medium], 1652.17 and 1646.00 cm^{-1}), 1* amines (N–H bending [medium], 1635.10 cm^{-1}), nitro compounds (N–O asymmetric stretching [strong], 1540.17, 1520.76, and 1506.91 cm^{-1}), aromatics (C–C stretching [in ring] [medium], 1488.88, 1471.89, 1456.45, 1435.48, and 1417.61 cm^{-1}), nitro compounds (N–O symmetric stretching [medium], 1338.60 cm^{-1}), alcohols, carboxylic acids, esters, ethers (C–O stretch [strong], 1278.98 and 1032.75 cm^{-1}), alkynes (–C≡C–H: C–H bending [broad, strong], 668.32, 649.13 and 616.75 cm^{-1}) and alkyl halides (C–Br stretching [medium], 578.81, 568.06, 546.86, 536.07, 525.64, and 515.71 cm^{-1}). FTIR studies revealed the presence of strong bonds, such as alcohols, phenols (O–H stretching), carbonyls (C═O stretching), nitro compounds (N–O asymmetric stretching), alcohols, carboxylic acids, and ester and ether (C–O stretching) groups present in Pn-LME are involved in the reduction of Ag^+ ions to Ag^0NPs. The maxima are almost the same, and the results agree with each other [22–24]. The functional groups

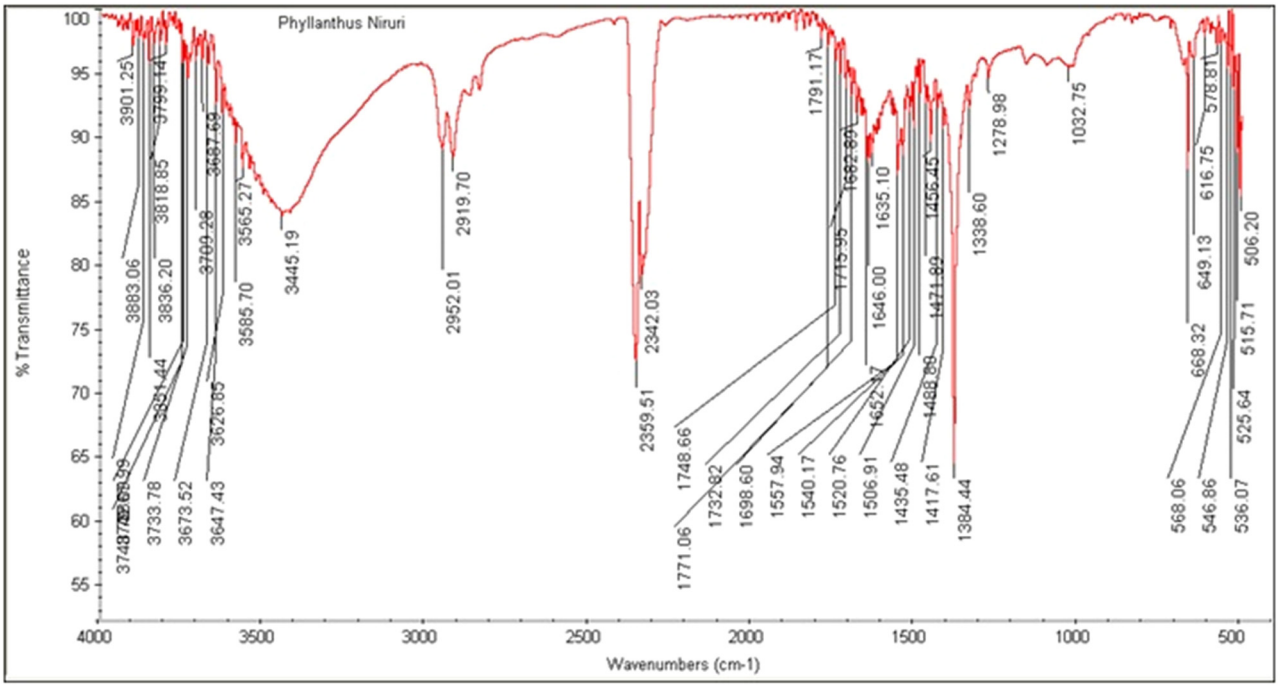

Figure 5: FTIR spectra of synthesized AgNPs from Pn-LE.

of the synthesized SP-AgNPs were determined by logging the FTIR spectrum signature. It is possible that these functional groups play a role in both Ag° capping and nanoparticle formation [25]. The positions of many amide I band spectra show that the secondary structure of the protein in the selenium nanoparticle capping layer is different from that of the biological proteins. Strong bands at 3,381 (primary amine), 2,916 (C–H stretching of the alkanes), 2,848 (C–H–O stretching of aldehyde), 1,607 (N–H bending of primary amines), and 1,207 cm^{-1} (C–N stretching of aliphatic amines) were detected in the FTIR spectra of AgNPs [26].

3.5 Larvicidal activity

The larvicidal activity of Pn-LME against mosquito larvae is shown in Table 2 and Figure 6. The extract more efficiently controlled the mosquito larvae, and the LC50 and LC90 values of 11.92 and 14.60 ppm (*A. aegypti*), 4.46 and 9.25 ppm (*A. stephensi*) were also moderately effective against the *C. quinquefasciatus* larvae (LC50 = 47.86 ppm and LC90 = 93.48 ppm). The results of larvicidal activity revealed that Pn-LME were more effective against the mosquito larvae of *A. stephensi, C. quinquefasciatus,* and *A. aegypti* that and are presented as LC50 and LC90 values. In addition, Table 3 and Figure 7 reveal the larval mortality of *A. stephensi, C. quinquefasciatus,* and *A. aegypti* after the treatment of AgNPs synthesized from *P. niruri*. The AgNPs have more efficiently controlled the larvae of *A. stephensi* (LC50 = 0.45 ppm and LC90 = 0.83 ppm), followed by *A. aegypti* (LC50 = 1.20 ppm and LC90 = 1.46 ppm) and *C. quinquefasciatus* (LC50 = 4.78 ppm and LC90 = 9.11 ppm). Similarly, the research provided the larval activity of green-synthesized AgNPs with the *Chrysanthemum indicum* extract against mosquito species [27]. Furthermore, the larval and pupal activity of *Acacia catechu*-AgNPs tested LC50 values of 71.04, 74.78, 85.33, and 88.91 ppm against insecticides [1]. Previous studies have reported the toxicity effects of *Vina rosa*-synthesized AgNPs from *P. reticulata* tested against *A. stephensi* and *C. quinquefasciatus* [28,29]. They found

Table 2: LC_{50}/LC_{90} values of Pn-LME against mosquito larvae

Mosquito species	Intercept	Slope	LC_{50} (PPM) (90%UCL-LCL)	LC_{90}(PPM) (90%UCL-LCL)	χ^2 (df)
A. stephensi	1.75	2.38 ± 0.06	4.46 (3.93–5.05)	9.25 (7.09–12.06)	11.1
C. quinquefasciatus	1.91	2.39 ± 0.06	47.86 (42.53–53.85)	93.48 (72.54–120.48)	16.1
A. aegypti	6.35	10.75 ± 0.02	11.92 (11.49–12.37)	14.60 (13.62–15.6)	1.3

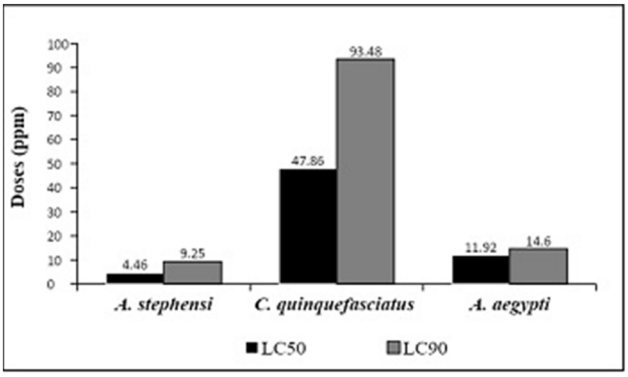

Figure 6: LC50/LC90 values of Pn-LME against mosquito larvae.

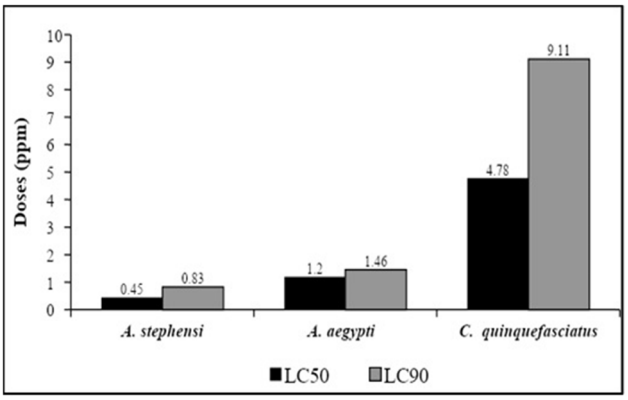

Figure 7: LC50/LC90 values of AgNPs synthesized using Pn-LE against mosquito larvae.

that after 24h of incubation, the greatest larvicidal effects of the LC50 and LD50 values were statistically significant ($p < 0.0267$). Based on an assessment of the biofabricated XC-SeNP larval killing activity against *A. aegypti* larvae in their fourth instar, the LC50 and LC90 values were found to be 79.4 and 722.4 ppm, respectively [30]. Additionally, *A. aegypti* (45.1 ppm) and *C. quinquefasciatus* (41.1 ppm) larvae in their fourth instar were suppressed by the synthesized nanoparticles [31]. About 4.287 µl/ml of AgNPs is needed to kill 50% of the larvae, while 10.795 µl/ml is needed to kill 90% of the larvae, according to the LC50 and LC90 analyses [32]. The *Blumeamollis*-AgNPs were tested against HVM larvae, and the predominant LC50/LC90 values of 18.17/39.56, 23.45/42.49, and 21.82/40.43 µg/ml were observed on *A. subpictus, C. vishnui,* and *A. vittatus*, respectively [17]. Damage to mosquito larvae treated with *Dillenia indica-mediated* selenium nanoparticles.

3.6 Microbial activity

Additionally, AgNPs synthesized with *P. niruri* extract were tested for antibacterial activity against four different bacterial species: *S. aureus*, *K. pneumoniae*, *E. coli*, and *B. subtilis*. The results are displayed in Figure 8 and presented in Table 4. AgNPs showed notable inhibitory zones against *S. aureus* and *E. coli* at all concentrations tested (50–500 µg). However, negligible inhibition of *B. subtilis* and *K. pneumoniae* was noted at 50 and 100 µg of AgNPs. A linear increase in the extent of the zone of inhibition was observed for each of the bacterial strains studied in relation to an increase in the concentration of AgNPs. Similarly, the biocompatibility by antimicrobials tested against Pn-AgNPs drug-resistant human pathogens like *S. aureus, E. coli, Pseudomonas* sp., *P. vulgaris,* and *S. typhi*. Furthermore, research has shown that AgNPs can inhibit pathogenic microorganisms such as *E. coli* and *S. coccus* [33,34]. The zones of inhibition for the antibacterial activity were 9.6 ± 0.58 and 10.67 ± 0.58 mm [35], while 3.33 ± 0.13 and 3.67 ± 0.11 mm for *S. aureus* and *E. coli* were the locations of the weak antibacterial activity. The findings also revealed the minimum inhibitory concentration and minimum bactericidal concentration values of SP-AgNPs against *B. subtilis* (45 and 60 g/ml) and *E. coli* (40 and 60 g/ml). According to the study of Liao et al. [36], AgNP damage to bacterial cell walls is most likely the cause of the zone of inhibition. AgNPs destroy enzymes and DNA and render cellular proteins inert; this could be a consequence of direct AgNP involvement. Moreover, AgNPs produced by plants can serve as a substitute for antibiotics to combat microbial pathogens [37]. The tetracycline-synergistic antibacterial activities of the characterized AgNPs were next investigated. The results show that while the nanoparticles are only somewhat effective against *B. cereus* and *K. pneumonia*, they are more active against *E. coli* and *S. aureus* [38]. The antibacterial activity of the biosynthesized *Myristica fragrans*-AgNPs was evaluated against multidrug-resistant *S. enterica and S. typhi* according to agar well diffusion,

Table 3: LC_{50}/LC_{90} values of AgNPs synthesized using Pn-LE against mosquito larvae

Sl. no.	Mosquito species	Intercept	Slope	LC_{50} (PPM) (90%UCL-LCL)	LC_{90} (PPM) (90%UCL-LCL)	χ^2 (df)
1	*A. stephensi*	2.14	6. 68 ± 0.05	0.45 (0.40–0.50)	0.83 (0.67–1.01)	15.2
2	*A. aegypti*	6.38	3.82 ± 0.02	1.20 (1.15 -1.24)	1.46 (1.36–1.57)	4.40
3	*C. quinquefasciatus*	1.98	1.89 ± 0.05	4.78 (4.26–5.35)	9.11 (7.15–11.68)	13.1

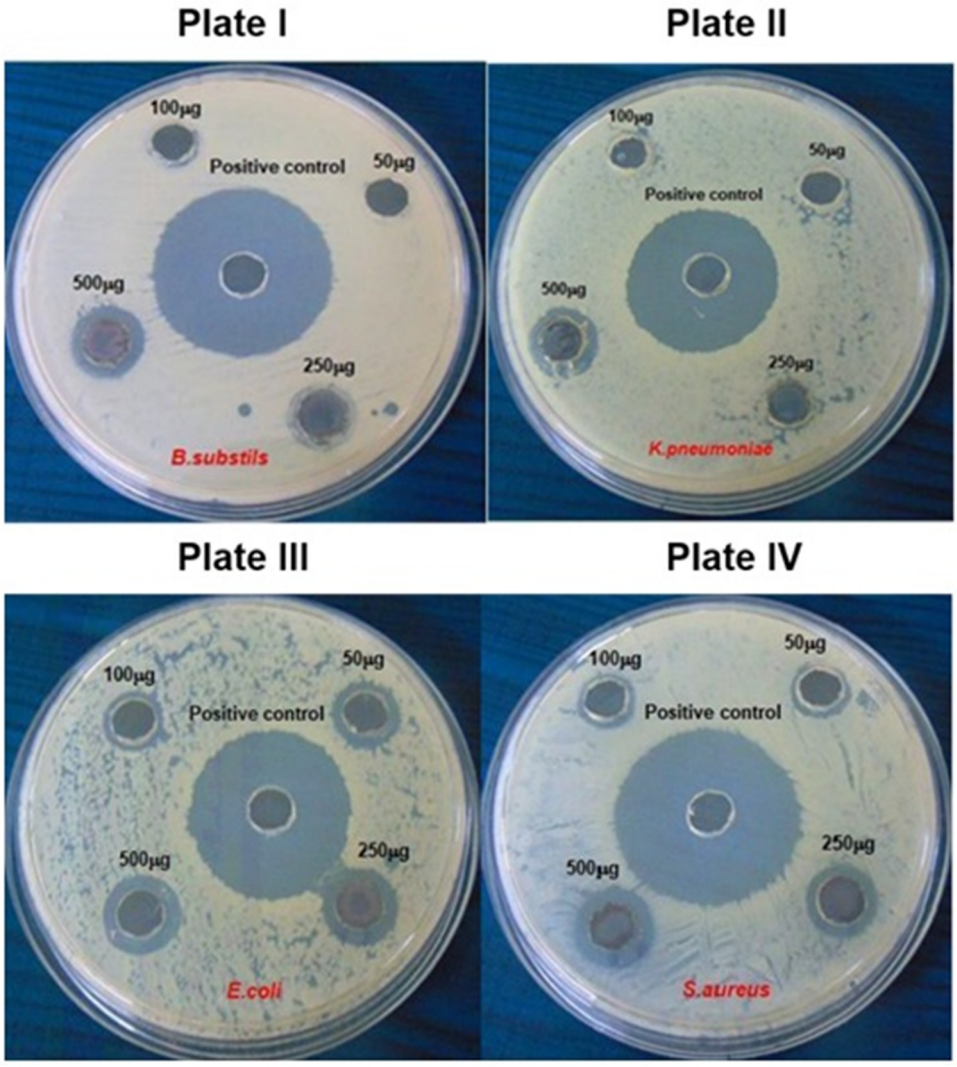

Figure 8: Disc diffusion assay of AgNPs synthesized using Pn-LE.

Table 4: Antibacterial activity of different concentrations of AgNPs synthesized using Pn-LME extract by disc diffusion assay

	50 µg	100 µg	250 µg	500 µg	+Control
B. subtilis	Nil	Nil	9	12	35
K. pneumoniae	Nil	Nil	8	13	36
E. coli	8	10	12	13	36
S .aureus	8	9	11	14	35

Values are the mean values of three independent analyses; zone of inhibition (mm) excluding the diameter of the disc (6 mm); Nil – no inhibition.

minimum inhibitory concentration, and IC50. The antibacterial activity of the synthesized AgNPs (2–10 mM) against the clinical pathogens *Klebsiella* sp. and *Staphylococcus* sp. was evaluated under *in vitro* conditions. The synthesized *Spirulina AgNPs* were evaluated against *Staphylococcus* sp. and *Klebsiella* sp. AgNPs (1–4 mM) extensively reduced the growth rate of pathogens. An effective method of utilizing market green vegetable waste for the synthesis of AgNPs with high antibacterial efficiency.

3.7 Cell culture and MTT activity

Fibroblast viability was evaluated by computing the viability percentage of treated cells compared with the untreated control. AgNPs showed a dose-dependent decrease in the viability of L929 cells. Viability dropped below 80% at concentrations above 500 µg/ml of AgNPs. The results revealed that AgNPs are bio-compatible up to the concentration of 250 µg/ml, as shown in Figure 9, and their respective

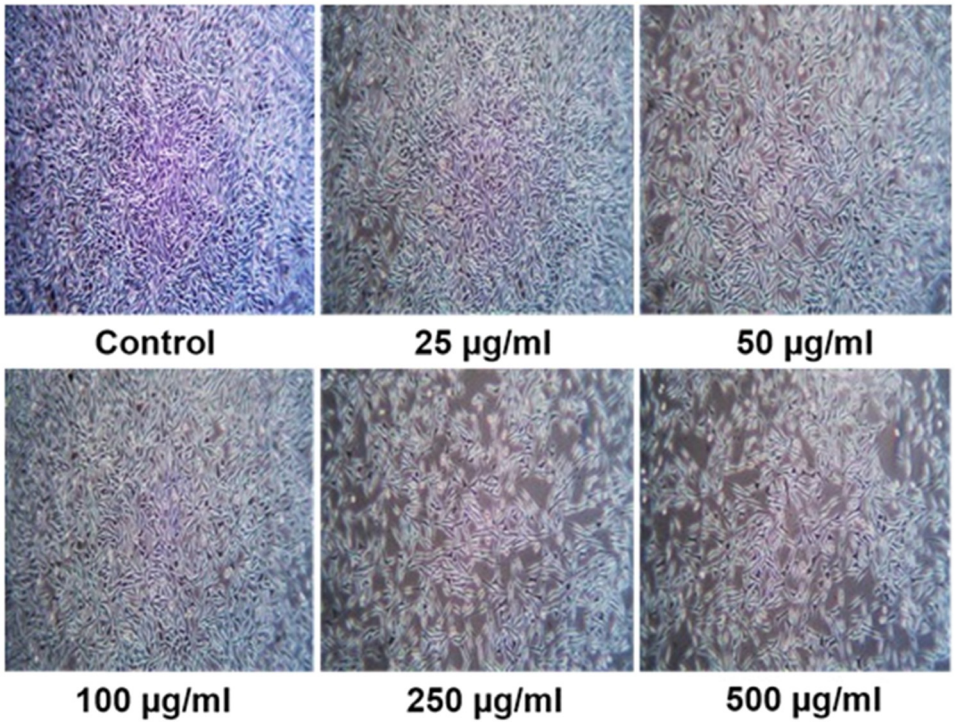

Figure 9: Photomicrographs of fibroblast cell line (L929) cells treated with various concentrations of AgNPs synthesized using Pn-LE.

viabilities are listed in Table 5. The behaviour and toxicity of AgNPs were greatly influenced by the composition of the CCM, especially when it contained a high number of amino acids, as evidenced by earlier studies conducted in the RTL-W1.

Likewise, we obtained consistent results from several cell lines, and fresh cell lines from different species are accessible [39].

3.8 Field trial

The field trial of AgNPs synthesized from Pn-LME was conducted in different water bodies like potable water bodies (breeding grounds of malarial vector *A. stephensi*), sewage water bodies (breeding grounds of filarial vector *C. quinquefasciatus*), and stagnant water bodies (breeding grounds of dengue vector *A. aegypti*), as shown in Tables 6–8. The selected six breeding grounds had the following average surface areas: 1.8 × 1.8 × 1.6 (*A. stephensi*), 0.6 × 1.6 × 0.3 (*C. quinquefasciatus*), and 0.7 × 0.8 × 0.5 (*A. aegypti*). The percentages of larvae density in *A. stephensi* drinkable water were 65.68, 85.40, and 98.70% at 24-, 48- and 72-h following treatment with bio-pesticide AgNPs synthesized from *P. niruri*. The sewage water contents of *C.*

Table 5: Percentage viability of fibroblast cell line (L929) treated with Pn-AgNPs

Tested concentration (µg/ml)	OD at 570 nm	% of cell viability
Control	0.306 ± 0.003	100 ± 0.00
25	0.271 ± 0.006	93.24 ± 1.89
50	0.241 ± 0.002	82.70 ± 0.69
100	0.220 ± 0.001	75.60 ± 0.41
250	0.195 ± 0.005	67.12 ± 1.64
500	0.173 ± 0.002	59.45 ± 0.80

Values are mean ± standard error.

Table 6: Effect of AgNPs synthesized using Pn-LE against the mosquito larval density at the breeding ground (potable water) of *A. stephensi*

Sl. no.	Before treatment	Larval density after treatment		
		24 h	48 h	72 h
1	57	19	8	1
2	62	22	11	2
3	45	16	9	0
4	39	17	7	0
5	64	18	6	1
6	41	14	4	0
Total	308	106	45	4
Average	51.33	17.67	7.50	0.67
Reduction %		65.58%	85.40%	98.70%

Table 7: Effect of AgNPs synthesized using Pn-LE against the mosquito larval density at the breeding ground (Sewage water) of *C. quinquefasciatus*

Sl. no.	Before treatment	Larval density after treatment		
		24 h	48 h	72 h
1	154	56	25	9
2	132	45	14	3
3	121	46	24	0
4	126	44	13	5
5	145	51	22	7
6	133	53	21	4
Total	811	295	119	28
Average	135.17	49.17	19.83	4.67
Reduction %		63.62%	85.33%	96.55%

Table 8: Effect of AgNPs synthesized using Pn-LE against the mosquito larval density at the breeding ground (stagnant water) of *A. aegypti*

Sl. no.	Before treatment	Larval density after treatment		
		24 h	48 h	72 h
1	84	35	12	3
2	68	28	11	2
3	89	25	8	0
4	74	29	10	1
5	87	32	14	4
6	64	26	7	0
Total	466	175	62	10
Average	77.67	29.17	10.33	1.67
Reduction %		62.45%	86.70%	97.85%

quinquefasciatus were 63.62, 85.33, and 96.55% (Table 7), and those of stagnant water were 62.45, 86.70, and 97.85% at 24, 48, and 72 h, respectively (Table 8). Field trials have corroborated that bio-pesticide AgNPs synthesized from *P. niruri* could be effective larvicidal agents against mosquito vectors [14].

4 Conclusions

The current study demonstrates that AgNPs synthesized with *P. niruri* exhibit significant lethality against *A. aegypti, A. stephensi*, and *C. quinquefasciatus*. The formation of AgNPs was characterized by XRD, HR-TEM, FE-SEM, and Fourier-transform infrared spectroscopy. These nanoparticles exhibited enhanced antibacterial activity against *B. subtilis, K. pneumoniae, S. aureus*, and *E. coli*. Additionally, the Pn-AgNPs synthesized showed enhanced antibacterial activity and low cytotoxicity in normal fibroblast cells (L929). Field trials revealed an increased larvicidal effect within 24–72 h, with the rate of reduction increasing over time. Therefore, our study presents an ideal eco-friendly bio-pesticide solution of AgNPs (3 mM) for controlling filarial, dengue, and malaria vectors. Further experiments are necessary to identify the active constituents and to elucidate their mechanisms of action against these species.

Acknowledgements: The authors are thankful to the Researchers Supporting Project (number RSPD2024R728), King Saud University, Riyadh, Saudi Arabia. The Government Arts College for Men (Autonomous), Chennai, India and Dr. K. Elumalai, Department of Advanced Zoology & Biotechnology supplied the laboratory facilities for this work. We thank the members of the Madurai-based Centre for Research in Medical Entomology (ICMR) for providing the mosquitoes.

Funding information: Authors state no funding involved.

Author contributions: K. Prabakaran and M. Mathiyazhagan: methodology, writing – original draft. C.N. Sumetha and Pinku Satnami: data curation and validation. P. Sasi, H. Irrusappan, and Shobana Sampath: formal analysis and resources. M. Baranitharan and Perumal Asaithambi: conceptualization, supervision, and writing – review. P. Surya, G. John James, and Mohammad Z. Ahmed: visualization and software.

Conflict of interest: The authors declare that there are no conflicts of interest regarding the publication of this paper.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: Data will be made available on request.

References

[1] Baranitharan M. Experimental investigations of Nagapattinam indigenous medicinal plant extracts against dengue, malaria and filarial disease. Int J Zool Appl Biosci. 2017;2(4):155–61.

[2] Dhanasekaran S, Prabhahar C, Gokulakrishnan J, Irussappan H, Elumalai K, Shanmugavel G, et al. Plants screening and laboratory investigation of Ageratina adenophora methanol extract against Anopheles stephensi. Int J Zool Appl Biosci. 2022 May;7(3):13–8. Department of Zoology, Annamalai University, Annamalai Nagar-608 002, Tamilnadu, India.

[3] Kavitha G, Gokulakrishnan J, Baranitharan M. Bio-efficacy of argemonemexicana leaf extract fractions against tobacco caterpillar, spodopteralitura. Int J Pharma Bio Sci. 2019 Apr; [cited 2024 Sep 13] 10(2):118–23. https://ijpbs.net/counter.php?aid = 6644.

[4] Rajaganesh K, Sumedha NC, Mathiyazhagan M, Basha SA, Angwanhu LA, Baranitharan M. Folklore claims of ethno-medicinal plants used by ethnic people of Kohima District, Nagaland, India. Indian J Sci Technol. 2023 Dec;16(45):4267–74. Department of Biotechnology, St. Joseph University, Dimapur, Nagaland, 797115, India.

[5] Amaku JF, Taziwa R. Sequestration of chromium by Ananas comosus extract–coated nanotubes: synthesis, characterization, optimization, thermodynamics, kinetics, and antioxidant activities. Biomass Convers Biorefin. 2024 Jan [cited 2024 Sep 13]; https://link.springer.com/10.1007/s13399-023-05210-9.

[6] Ustuner H, Nasircilar AG, Servi H, Demir Ü, Sen A, Gundogdu B, et al. Determination of total phenolic content and antidiabetic, antioxidant and antiproliferative activities of Gypsophila pilulifera extracts grown by in vitro culture. Biocatal Agric Biotechnol. 2024 Feb;56:103014.

[7] Mitra RL, Jain SK. Concept of *Phyllanthus niruri* (Euphorbiaceae) in Indian Floras. Nelumbo. 2024 May;27:161–76.

[8] Gnanadesigan M, Anand M, Ravikumar S, Maruthupandy M, Vijayakumar V, Selvam S, et al. Biosynthesis of silver nanoparticles by using mangrove plant extract and their potential mosquito larvicidal property. Asian Pac J Trop Med. 2011 Oct;4(10):799–803.

[9] Duraisamy S, Vijayakumar N, Rajendran J, Venkatesan A, Kartha B, Kandasamy SP, et al. Facile synthesis of silver nanoparticles using the Simarouba glauca leaf extract and their impact on biological outcomes: A novel perspective for nano-drug development. J Drug Deliv Sci Technol. 2022 Mar;69:103160.

[10] Stuart BH. Infrared spectroscopy: Fundamentals and applications. 1st edn. Amsterdam, Netherlands: Wiley; 2004. [cited 2024 Sep 13] (Analytical Techniques in the Sciences). https://onlinelibrary.wiley.com/doi/book/10.1002/0470011149.

[11] Zia A, Siddiqui BS, Begum S, Siddiqui S, Suria A. Studies on the constituents of the leaves of Nerium oleander on behavior pattern in mice. J Ethnopharmacol. 1995 Nov;49(1):33–9.

[12] Ahmed M, Peiwen Q, Gu Z, Liu Y, Sikandar A, Hussain D, et al. Insecticidal activity and biochemical composition of Citrullus colocynthis, Cannabis indica and Artemisia argyi extracts against cabbage aphid (Brevicoryne brassicae L). Sci Rep. 2020 Jan;10(1):522.

[13] Connolly M, Fernandez-Cruz ML, Quesada-Garcia A, Alte L, Segner H, Navas J. Comparative cytotoxicity study of silver nanoparticles (AgNPs) in a variety of rainbow trout cell lines (RTL-W1, RTH-149, RTG-2) and primary hepatocytes. Int J Environ Res Public Health. 2015 May;12(5):5386–405.

[14] Kovendan K, Murugan K, Vincent S. Evaluation of larvicidal activity of Acalypha alnifolia Klein ex Willd. (Euphorbiaceae) leaf extract against the malarial vector, Anopheles stephensi, dengue vector, Aedes aegypti and Bancroftian filariasis vector, Culex quinquefasciatus (Diptera: Culicidae). Parasitol Res. 2012 Feb;110(2):571–81.

[15] Mkindi AG, Tembo Y, Mbega ER, Medvecky B, Kendal-Smith A, Farrell IW, et al. Phytochemical Analysis of Tephrosia vogelii across East Africa Reveals Three Chemotypes that Influence Its Use as a Pesticidal Plant. Plants. 2019 Dec;8(12):597.

[16] Sowndarya P, Ramkumar G, Shivakumar MS. Green synthesis of selenium nanoparticles conjugated Clausena dentata plant leaf extract and their insecticidal potential against mosquito vectors. Artif Cells Nanomed Biotechnol. 2017 Nov;45(8):1490–5.

[17] Elumalai K, Kavipriya MR, Lakshmi Prabha A, Krishnappa K, Pandiyan J, Nicoletti M, et al. Green synthesis of silver nanoparticles using *Atalantia monophylla:* A potential eco-friendly agent for controlling blood-sucking vectors. Green Process Synth. 2022 Sep;11(1):915–30.

[18] Singh K, Panghal M, Kadyan S, Chaudhary U, Yadav JP. Green silver nanoparticles of Phyllanthus amarus: as an antibacterial agent against multi drug resistant clinical isolates of Pseudomonas aeruginosa. J Nanobiotechnol. 2014 Oct;12(1):40.

[19] Mathivanan D, Kamaraj C, Suseem SR, Gandhi PR, Malafaia G. Seaweed Sargassum wightii mediated preparation of TiO2 nanoparticles, larvicidal activity against malaria and filariasis vectors, and its effect on non-target organisms. Environ Res. 2023 May;225:115569.

[20] Alomar TS, AlMasoud N, Awad MA, El-Tohamy MF, Soliman DA. An eco-friendly plant-mediated synthesis of silver nanoparticles: Characterization, pharmaceutical and biomedical applications. Mater Chem Phys. 2020 Jul;249:123007.

[21] Sathiyaseelan A, Saravanakumar K, Mariadoss AVA, Wang MH. Biocompatible fungal chitosan encapsulated phytogenic silver nanoparticles enhanced antidiabetic, antioxidant and antibacterial activity. Int J Biol Macromol. 2020 Jun;153:63–71.

[22] Ahmed SF, Mofijur M, Rafa N, Chowdhury AT, Chowdhury S, Nahrin M, et al. Green approaches in synthesising nanomaterials for environmental nanobioremediation: Technological advancements, applications, benefits and challenges. Environ Res. 2022 Mar;204:111967.

[23] Balaraman P, Balasubramanian B, Liu WC, Kaliannan D, Durai M, Kamyab H, et al. Sargassum myriocystum-mediated TiO_2-nanoparticles and their antimicrobial, larvicidal activities and enhanced photocatalytic degradation of various dyes. Environ Res. 2022 Mar;204:112278.

[24] Imath M, Ragavendran C, Kamaraj C, Alrefaei AF, Almutairi MH, Raj M, et al. Fioria vitifolia-mediated silver nanoparticles: Eco-friendly synthesis and biomedical potential. J Water Process Eng. 2024 Sep;66:106020.

[25] Javed R, Zia M, Naz S, Aisida SO, Ain NU, Ao Q. Role of capping agents in the application of nanoparticles in biomedicine and environmental remediation: recent trends and future prospects. J Nanobiotechnol. 2020 Dec;18(1):172.

[26] Amiri A, Mousakhani-Ganjeh A, Amiri Z, Guo YG, Pratap Singh A, Esmaeilzadeh Kenari R. Fabrication of cumin loaded-chitosan particles: Characterized by molecular, morphological, thermal, antioxidant and anticancer properties as well as its utilization in food system. Food Chem. 2020 Apr;310:125821.

[27] Arokiyaraj S, Dinesh Kumar V, Elakya V, Kamala T, Park SK, Ragam M, et al. Biosynthesized silver nanoparticles using floral extract of Chrysanthemum indicum L.— potential for malaria vector control. Environ Sci Pollut Res. 2015 Jul;22(13):9759–65.

[28] Mondal A, Maity S, Mondal A, Mondal NK. Antibacterial, antibiofilm and larvicidal activity of silver nanoparticles synthesized from spider silk protein. Int J Biol Macromol. 2024 Feb;258:128775.

[29] Subarani S, Sabhanayakam S, Kamaraj C. Studies on the impact of biosynthesized silver nanoparticles (AgNPs) in relation to malaria and filariasis vector control against Anopheles stephensi Liston and Culex quinquefasciatus Say (Diptera: Culicidae). Parasitol Res. 2013 Feb;112(2):487–99.

[30] Raja RK, Kumar Seetharaman P, Kalidass B, Ananth S, Bo L, Kamaraj C, et al. Biosynthesis of selenium nanoparticles using cell-free extract of Xenorhabdus cabanillasii GU480990 and their potential mosquito larvicidal properties against yellow fever mosquito Aedes aegypti. J Invertebr Pathol. 2024 Mar;203:108045.

[31] Govindan L, Anbazhagan S, Altemimi AB, Lakshminarayanan K, Kuppan S, Pratap- Singh A, et al. Efficacy of antimicrobial and larvicidal activities of green synthesized silver nanoparticles using leaf extract of plumbago auriculata lam. Plants. 2020 Nov;9(11):1577.
[32] Wilson JJ, Mahendran S, Sivakumar T, Ponmanickam P, Thangaraj R. Mosquito larvicidal activity of silver nanoparticles synthesized using Azolla pinnata against Culex quinquefasciatus Say (Diptera: Culicidae). S Afr J Bot. 2023 Jun;157:380–6.
[33] Ajaykumar AP, Mathew A, Chandni AP, Varma SR, Jayaraj KN, Sabira O, et al. Green synthesis of silver nanoparticles using the leaf extract of the medicinal plant, uvaria narum and its antibacterial, antiangiogenic, anticancer and catalytic properties. Antibiotics. 2023 Mar 13;12(3):564.
[34] Pirtarighat S, Ghannadnia M, Baghshahi S. Green synthesis of silver nanoparticles using the plant extract of Salvia spinosa grown in vitro and their antibacterial activity assessment. J Nanostruct Chem. 2019 Mar;9(1):1–9.
[35] Durán N, Durán M, De Jesus MB, Seabra AB, Fávaro WJ, Nakazato G. Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. Nanomedicine. 2016 Apr;12(3):789–99.
[36] Liao C, Li Y, Tjong SC. Bactericidal and cytotoxic properties of silver nanoparticles. Int J Mol Sci. 2019 Jan;20(2):449.
[37] Arakha M, Saleem M, Mallick BC, Jha S. The effects of interfacial potential on antimicrobial propensity of ZnO nanoparticle. Sci Rep. 2015 Apr;5(1):9578.
[38] Palanisamy DS, Gounder BS, Selvaraj K, Kandhasamy S, Alqahtani T, Alqahtani A, et al. Synergistic antibacterial and mosquitocidal effect of Passiflora foetida synthesized silver nanoparticles. Braz J Biol. 2024;84:e263391.
[39] Thangaraj RS, Narendrakumar L, Prasannan Geetha P, Shanmuganathan AR, Dharmaratnam A, Nithianantham SR. Comprehensive update on inventory of finfish cell lines developed during the last decade (2010–2020). Rev Aquac. 2021 Sep;13(4):2248–88.