Research Article

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Green synthesis of silver nanoparticles using *Atalantia monophylla*: A potential eco-friendly agent for controlling blood-sucking vectors

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Abstract: Developing floral-based replacement molecules might manage blood-sucking vectors in an eco-friendly way. *Atalantia monophylla* (*Am*) aqueous leaf extract (ALE) and silver nanoparticles (AgNPs) were evaluated against mosquitoes (*Aedes vittatus*, *Anopheles subpictus*, and *Culex vishnui*) and ticks (*Haemaphysalis bispinosa*, *Rhipicephalus microplus*, and *R. sanguineus*) at different concentrations. Phytochemical screening and AgNPs' synthesis were performed on ALE of *A. monophylla*. UV-visible spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, scanning electron microscope, and transmission electron microscope were used to examine the synthesized *Am*-AgNPs. *A. monophylla's* ALE included alkaloids, flavonoids, saponins, tannins, triterpenes, coumarins, anthraquinones, and phenolics. *Am*-AgNPs had a higher LC_{50} (22.19, 23.92, 26.09, 40.25, 51.87, and 60.53 $\mu g \cdot mL^{-1}$, respectively) than leaf aqueous extract (LAE) against *Ae. vittatus*, *An. subpictus*, *Cx. vishnui*, *H. bispinosa*, *R. microplus*, and *R. sanguineus* larvae. *A. monophylla* ALE and *Am*-AgNPs' bio-toxicity was investigated against aquatic and terrestrial non-target species (*Acilius sulcatus*, *Anisops bouvieri*, *Araneus mitificus*, and *Cyrtophora moluccensis*) with LC_{50} values ranging from 2,094.5 to 10,532.8 $\mu g \cdot mL^{-1}$, respectively. *A. monophylla* ALE and *Am*-AgNPs had little negative impacts on the chosen non-target fauna. Environmental protection is important nowadays. Green AgNPs are low-cost, readily accessible, environmentally safe, and effective pesticides. Am-AgNPs are effective alternative insecticides, requiring a considerable study on this plant to control blood-sucking vectors for worldwide human/animal health importance.

Keywords: greener nanoparticles, blood-sucking vectors, larval toxicity, environmental safety, non-target fauna

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1 Introduction

Blood-sucking vectors (BSVs) generating an abundance of newly developing illnesses substantially affect live-stock and public health [1]. As a vector, ticks disperse pathogens responsible for causing cardinal diseases among cattle and human beings [2]. Around 900 tick species were recently identified [3]. The life-threatening pathogenic viruses are conveyed to animals by infected ticks' bites, especially in the family Ixodidae [4]. Ticks are notorious vectors for around 38 pathogenic viral species transmitted in the animal kingdom [5]. Worldwide, US$ 7 billion is lost annually, and nearly 80% of farm animals are in high-risk because of ticks and tick-borne diseases (TTBDs). They are very serious BSVs that spread several arboviruses above 80% in lives-stock and have a positive infection of

TTBDs [6]. India alone estimated annually US$ 498.7 million in economic loss and around 1.1 million cattle deaths caused by TTBDs. They are significant ectoparasites and taste various vertebrates' blood [7,8]. Adult females taken more than 1 week to complete their feeding, consequently transmitting several pathogens to the host [9]. Nearly 7,000 eggs can be laid by a fully blood-fed female tick, an average of 4,000. The eggs' laying capacity depends on their size and the quantity of blood they ingest [10]. Tick bites highly injure the skin, cause severe weight losses in farm animals, lower quality/quantity of milk and meat production, and lead to poor markets of infected animal skin in the leather industry [11].

Dipterans are very dangerous and deadly vectors of vertebrates that may highly cause vector-borne epidemics and pandemics in the animal kingdom [12]. Mosquitoes transmit disease and cause death in both animals and humans [13]. Across the world, millions of human societies suffer from diseases spread by human vector mosquitos [14]. Vector-borne diseases (VBDs) are incredibly challenging and cause social-economic defeat worldwide [15]. In India alone, synthetic chemical pesticides (SCPs) are consumed above two million tons annually and unadvisable SCPs are common strategies for controlling diverse BSVs. Indiscriminate application of unadvisable SCPs increased worldwide as the results extremely irreversibly inhibit different bio-activities defects of enzymatic, hormonal, neural, metabolic, respiratory, reproductive, and genomic on faunal and floral communities [16]. These problems have been co-existing for several years in environmental compartments; around 355,000 human deaths associated with SCP poisoning have been recorded annually [17].

In this scenario, bio-relative eco-weapons are urgently needed as alternate remedies for SCPs. Biodegradable phytochemicals (PCs) are the most excellent alternate tool for BSVs' eradication and zero toxicity harm to the natural ecosystem [18]. Several countries turn towards PCs to kick the variety of VBDs and it has the potential entomotoxicity, which can be extracted from different plants. *Atalantia monophylla* Linn. (Rutaceae), commonly called wild lime, has been used as folk medicine in many countries. It has a wide variety of biotic potentials: antispasmodic, paralysis, chronic rheumatism, hemiplegia, leaf decoction applied for itching skin, pesticide/mosquitocidal activity, etc., [19]. The plant accumulates various PCs: alkaloids, benzoyltyramines, coumarins, flavonoids, furoquinoline, limonoids, steroids, triterpenoids, etc., [20]. *A. monophylla* woody climbers are abundant in wild tropic terrains with a versatile therapeutic usage and can cure hemiplegia, arthritis, skin diseases, and bacterial infections. Earlier, many works have been done on different pesticidal activities [21,22], mosquitocidal activities [23], antibacterial [24], antifungal [25], medicinal properties [26], antiproliferative [27], and stored product pests [19]. The nanomaterials were synthesized by plenty of methods. However, green synthesis has been a widely used prime method because it has fewer chemicals, negligible price, eco-safety, nontoxic, natural preparation, etc. Recently, nanoscience and nanotechnology are play vital roles in various fields: medicinal properties, bio-catalytic activities, antioxidant, antibacterial, anticancer, antibiotic, antileishmanial, antifungal, mosquitocidal, and pesticidal agents [28,29]. *Pistacia khinjuk* nanoparticles exhibited many outstanding bioactivities, including catalytic, antioxidant, antibacterial, and anticancer agents [30]. It has been found that *Crataegus monogyna* leaf extract is antibacterial and anticancer against human cancer cells and pathogenic microorganisms [31]. The leaf extract of *Convolvulus fruticosus* has catalytic and antibacterial properties [32]. Leaf extract of *Scrophularia striata*; antileishmanial and antibacterial properties [33]. *Medicago sativa* AgNPs revealed strong antioxidant activity [34]. Extract of *Sophora pachycarpa* has antibacterial, antioxidant, antifungal, and catalytic properties against tumor cell lines [35]. *Jujube* core extract showed remarkable industrial toxicant removal properties, *in vitro* antibacterial and anticanceros activities [36]. In this investigation, we evaluated the aqueous leaf extract (ALE) and *Am*-AgNPs for their potential to be toxic to BSV larvae (specifically, *Ae. vittatus, An. subpictus*, and *Cx. vishnui*, as well as *H. bispinosa, R. microplus,* and *R. sanguineus*), as well as non-target fauna (NTF) bio-toxicity potential at varying concentrations. Several spectroscopic and microscopic examinations were carried out to analyze *Am*-AgNPs.

2 Materials and methods

2.1 Materials

Silver nitrate ($AgNO_3$) of 99.9% pure analytical grade of was bought from Merck (Germany). All the glassware was cleaned with double distilled water and autoclaved.

2.2 *Atalantia monophylla* collection and processing

The clean, matured, and uninfected *A. monophylla* leaves were collected during the growing season (January–March) from in and around Cauvery Deltaic Zone, Koothur Village (11.7794°N, 78.2034°E), Nagapattinam District, Tamilnadu,

India (Figure 1a). Leaves were taken to the laboratory, washed, shade dried for a minimum of 10–15 days (27 ± 3°C), powdered using an electric blender, and extracted with different solvents using Soxhlet apparatus adapting a standard protocol [37]. A rotary evaporator (Sigma Scientific Glass Pvt. Ltd, India) was used for excess solvent evaporation at 40–45°C and the extract was stored in aluminum foiled glass vials in a cooled chamber maintained below 5°C (Figure 1b).

2.3 Phytochemical screening

The *A. monophylla* leaf extracts were analyzed for the presence of alkaloids, anthroquinnones, carbohydrates, coumarins, flavonoids, phenolics, resins, saponins, tannins, and triterpenes. Test for alkaloids: 5 mL of extract was added with 2 mL of HCL in which 1 mL of Drangendroff's reagent was evenly mixed and orange/red precipitation was immediately formed. Test for anthraquinones: 5 mL of extract was hydrolyzed with concentrated H_2SO_4 in which 1 mL of dilute NH_3 was added to create rose pink coloration. Test for carbohydrates: 1 mL of extract and 1 mL of Barfoed's reagent were added in a test tube and heated in a water bath for about 2 min to form red precipitation. Test for coumarin: To 10 mg extract a few drops of 10% NaOH was added to form yellow color. Test for flavonoids: 1 mL of extract was added with 1–2 drops of diluted NaOH; intense yellow color was produced in the plant extract, which became colorless with a few drops of dilute acid. Test for phenolics: 1 mL of extract was evenly mixed with 4 mL H_2O and 1 mL of 10% $FeCl_3$ solution was added to form a bluish black color. Test for resins: With the extract 1 mL of $C_4H_6O_3$ solution was added followed by 1 mL concentrated H_2SO_4 to create orange to yellow colour. Test for saponins: Extract was diluted with 20 mL of distilled H_2O, agitated in a measuring cylinder for 15 min, and formed a 1 cm layer of foam. Test for tannins: 5 mL of extract and a few drops of 1% $Pb(C_2H_3O_2)_2$ were added to form a yellow precipitate. Test for triterpenoids: 10 mg extract was dissolved with 1 mL of $CHCl_3$ in which 1 mL of $C_4H_6O_3$ has been added and subsequently 2 mL of concentrated H_2SO_4 of reddish-violet color was formed. The prescribed methodology screened the quality and effective crude extracts of PCs [38,39].

2.3.1 Thin layer chromatography (TLC), column chromatography (CC), and nuclear magnetic resonance (NMR) analysis

The ALE bio-effectiveness of phyto-compounds was analyzed through a run on a pre-coated (TLC) plate (Aluchrosep Silica

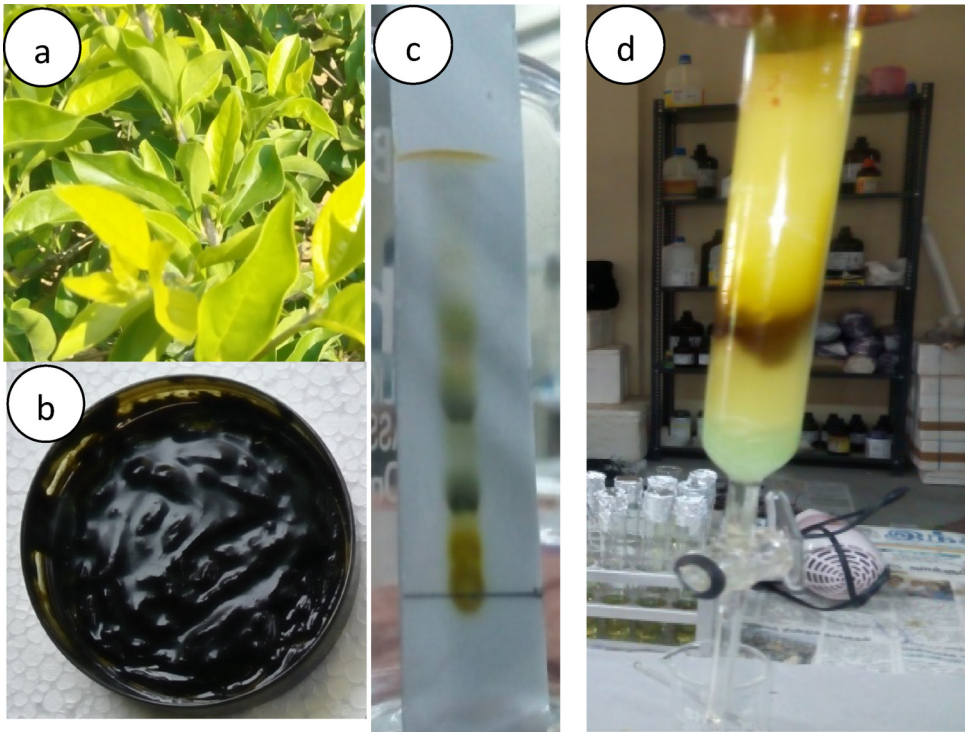

Figure 1: Phytochemical screening of LAE of *A. monophylla*. (a) *Atalantia monophylla* plant, (b) air-dried condensed LAE, (c) TLC, and (d) CC analysis.

Gel, 0.2 mm thick, Merck, India) and CC packed with silica gel (230–400 mesh, Merck, India) with the maximum height of 50 cm. It eluted successively with 50 mL of different aqueous solvent systems and ethyl acetate of 9:1 ratio. NMR spectroscopy can provide principles of additional information about peptides in solution. ^{1}H-NMR and ^{13}C NMR for *A. monophylla* spectra were recorded using Bruker DRX 300 spectrometers and $CDCl_3$ as the solvent.

2.4 Synthesis and characterization of silver nanoparticles (AgNPs)

The $AgNO_3$ solution of 90 and 10 mL of composite mixture ($AgNO_3$ + ALE) was prepared in 100 mL of Erlenmeyer flasks for reduction into Ag^+ ions and then kept at optimum temperature (28 ± 2°C) on the turntable of the microwave oven for 1 h for complete bio-reduction. Meanwhile, the initial finding of synthesized AgNPs composite mixture was examined, and the color change (transparent yellow to brown), the processing time, and periodic color change were recorded. At room temperature, the reactions were carried out in darkness (to avoid photoactivation of $AgNO_3$). All tests were carried out with appropriate controls in place. Complete reduction of $AgNO_3$ to Ag^+ ions was confirmed by the change in color from colorless to colloidal brown. After irradiation, the dilute colloidal solution was cooled to room temperature and kept aside for 24 h for complete bioreduction and saturation indicated by UV-visible (UV-Vis) spectrophotometric scanning. Then, the colloidal mixture was sealed and stored correctly for future use. The formation of AgNPs was furthermore confirmed by spectrophotometric analysis. AgNPs were purified by ultra-centrifugation and maintained above 4,000 rpm for 25 min. Ag^+ ions' bioreduction was monitored by using UV-Vis spectroscopy. Fourier transform infrared (FTIR) spectroscopy was used for examining the presence of bio-molecules in purified AgNPs. Crystalline pellet of AgNPs was dried at sixty degree Celsius and analyzed using X-ray diffraction (XRD) to recognize its accurate structure [40]. AgNP's morphometric parameters were examined through scanning electron microscope (SEM), energy dispersive X-ray (EDX), transmission electron microscope (TEM), and selected areas electron diffraction (SAED) analysis [41].

2.5 BSVs collection and rearing

The BSVs' (mosquito) eggs/larvae (*Ae. vittatus*, *An. subpictus*, and *Cx. vishnui*) were collected from Kodaikanal Wildlife Sanctuary (10.28°N, 77.46°E), Theni District, Tamilnadu, India and continuously reared in the laboratory. The collected mosquitoes were identified by ICMR, Madurai, Tamilnadu-625002. The larval feed was 3:1 ratio pedigree dog biscuits and yeast powder, and adults feed was 1:1% sucrose solution with natural honey. Mosquitoes were maintained at room temperature of 27 ± 2°C, relative humidity of 75 ± 5%, with a photoperiod of 12 h light:dark cycle. Adult BSVs (ticks) (*H. bispinosa*, *R. microplus*, and *R. sanguineus*) were directly collected from infested farm animals (cattle, horses, sheep, goats, and dogs), Cauvery Deltaic Zone, Koothur Village (11.7794°N, 78.2034°E), Mayiladuthurai District, Tamilnadu, India and BSVs were identified by Dr. A. Rathinakumar, Department of Veterinary and Animal Sciences, Tamilnadu, India. Engorged female ticks were brought to the laboratory and carefully transferred to a glass sealed, fully aerated container, allowing the Memmert oven to maintain above said temperature and relative humidity for egg-laying and hatching of larvae for assessing larval toxicity.

2.6 Mosquito larval toxicity

The standard method was followed to assess the larval toxicity of ALE and *Am*-AgNPs [42]. Five batches of 3rd instar larvae (0–6 h old, 20 number, well active, uniform size, and hale and healthy) of *Ae. vittatus, An. subpictus*, and *Cx. vishnui* were transferred to small transparent beakers with 250 mL capacity, each containing 200 mL of water, and an appropriate volume of *A. monophylla* ALE/*Am*-AgNPs was added to target BSVs. The toxic bioassay was tested on both a narrow and broad range of concentrations. Appropriate concentrations of ALE (25–150 $\mu g \cdot mL^{-1}$)/AgNPs (10–60 $\mu g \cdot mL^{-1}$) were added, and the mortality was counted every 6 h for 24 h, which was calculated by the prescribed method [43,44], probit analysis, for calculating LC_{50}/LC_{90} values after 24 h. Each concentration setup maintained five replications and the control setup was without PCs.

2.7 Tick larval toxicity

Larvicidal potentiality of BSVs were assessed against *A. onophylla* ALE (40–250 $\mu g \cdot mL^{-1}$)/*Am*-AgNPs (20–150 $\mu g \cdot mL^{-1}$) by standard protocol [45], a slight modification to improve the accuracy of practicality followed the method [46]. Different concentrations of *A. monophylla* ALE and *Am*-AgNPs were treated with Whatman filter paper envelopes (2 cm × 2 cm), uniformly treated with 3 mL of ALE and *Am*-AgNPs by using a pipette for internal surfaces.

The even-sized, 24 h old, hale and healthy larvae of 25 numbers were allowed into the treated envelope for 24 h and then released into the transparent glass cage (45 cm × 45 cm × 45 cm) with the closed opening. BSVs were identified by glass marking on the cage and envelopes were held in the biological oxygen demand incubator. Finally, the envelopes were opened at the end of their exposure period. The number of BSVs alive/dead were recorded with the help of fine brush and hand-lens. The technique [47] was used to determine mortality rates, and the precise replication and control procedures were followed in comparison to prior studies.

2.8 Bio-toxicity analysis on NTF

The acute toxic effect of *A. monophylla* ALE (1,200–15,000 µg·mL^{-1})/*Am*-AgNPs (600–7,500 µg·mL^{-1}) was examined on certain aquatic and terrestrial NTF (*A. sulcatus*, *A. bouvieri*, *A. mitificus*, and *C. moluccensis*) and evaluated by the procedure reported in refs [23,48]. The effect of ALE and *Am*-AgNPs potentiality tested against NTF reared as described [49]. The BSVs LC_{50} dose of ALE and *Am*-AgNPs was multiplied fifty times concentrations were evaluated against selected NTF. Each test was constantly replicated ten times, and the treated test without phytoconstituents was considered a control. The NTF mortality, as well as abnormalities activity, were observed after 2 days of exposure of phytoconstituents. The bio-toxic (survival and swimming) effects of NTF were monitored for 10 days to understand the post-treatment effect.

2.9 Statistical analysis

The BSV mosquito, larval tick toxicity, and bio-toxicity of NTF, as well as the suitability index (SI), were computed using the technique reported in ref. [50], and the data were entered into IBM-SPSS Statistics version 25.0. Different statistics were used to evaluate the larval toxicity of BSVs, including LC_{50}/LC_{90}, UCL, LCL, and Chi-Square.

3 Results

3.1 Phytochemical screening

A preliminary phytochemical analysis of *A. monophylla* ALE revealed that a high polar solvent, particularly aqueous extract, had the greatest PCs (Table A1 in Appendix). Alkaloids, flavonoids, saponins, tannins, triterpenes, coumarins, anthraquinones, and phenolics were observed from the ALE. Qualitative analysis of phytochemical screening in *A. monophylla* leaf extracts "+" denoted the presence of phytochemical group and "–" denoted the absence of phytochemical group.

3.1.1 TLC, CC, ^{1}H NMR, and ^{13}C NMR analysis

The ALE bio-effectiveness of phytocompounds was analyzed by TLC, which found a maximum of five fractions. (Figure 1c). The ALE of an active phytochemical group of *A. monophylla* was further separated by CC packed with silica gel and eluted successively with 50 mL of respective solvent systems. Five fractions were achieved and collected (Figure 1d). ^{1}H NMR and ^{13}C NMR spectral analysis of ALE of *A. monophylla* was executed and the spectral peaks that appeared are shown in Figure 2a and b. The following phytochemical compounds have been identified from the spectra in NIST chemical library. All the identified compounds showed more than 50% similarities with the available ones. They are as follows: benzofuran, 2,3-dihydro; 2-furancarboxaldehyde, 5-(hydroxymethyl)-; 2-methoxy-4-vinyl phenol; benzaldehyde, 2-hydroxy-6-methyl-[synonyms: 2,6-cresotaldehyde]; tetradecanoic acid; *n*-hexadecanoic acid; hexadecanoic acid, ethyl ester; 9,12-octadecadienoic acid (*Z*,*Z*)-; oleic acid; 13-docosenamide, (*z*)-; 9,12-octadecadienoic acid, methyl ester, (*e*,*e*)-; squalene; stigmasterol phytosterol; stigmastan-6,22-dien, 3,5-dedihydro-; and leupol.

3.2 Synthesis and characterization of *Am*-AgNPs

Am-AgNPs composite mixture was examined through color change from transparent yellow to brown (Figure 3a, inset). A UV-Vis spectrum of the *Am*-AgNPs showed the surface plasmon resonance (SPR) peak at 421 nm (Figure 3a). FTIR spectrum of *Am*-AgNPs is shown is shown in Figure 3b. The strong bands were recorded at 3,404–3,363, 2,924–2,856, 1,631, 1,321, and 1,060 cm^{-1} corresponding to OH stretching, aliphatic C–H stretching, C═H stretching, NO_2 stretching, and –C–O–C stretching. In addition, the weak bands recorded at 894 and 600 cm^{-1} are owing to the C–H bending and C–Cl stretching. The band at 520 cm^{-1} corresponds to the metal peak silver. These functional groups have confirmed the presence of phytocompound in leaf extracts, such as carbohydrates, alkaloids, flavonoids,

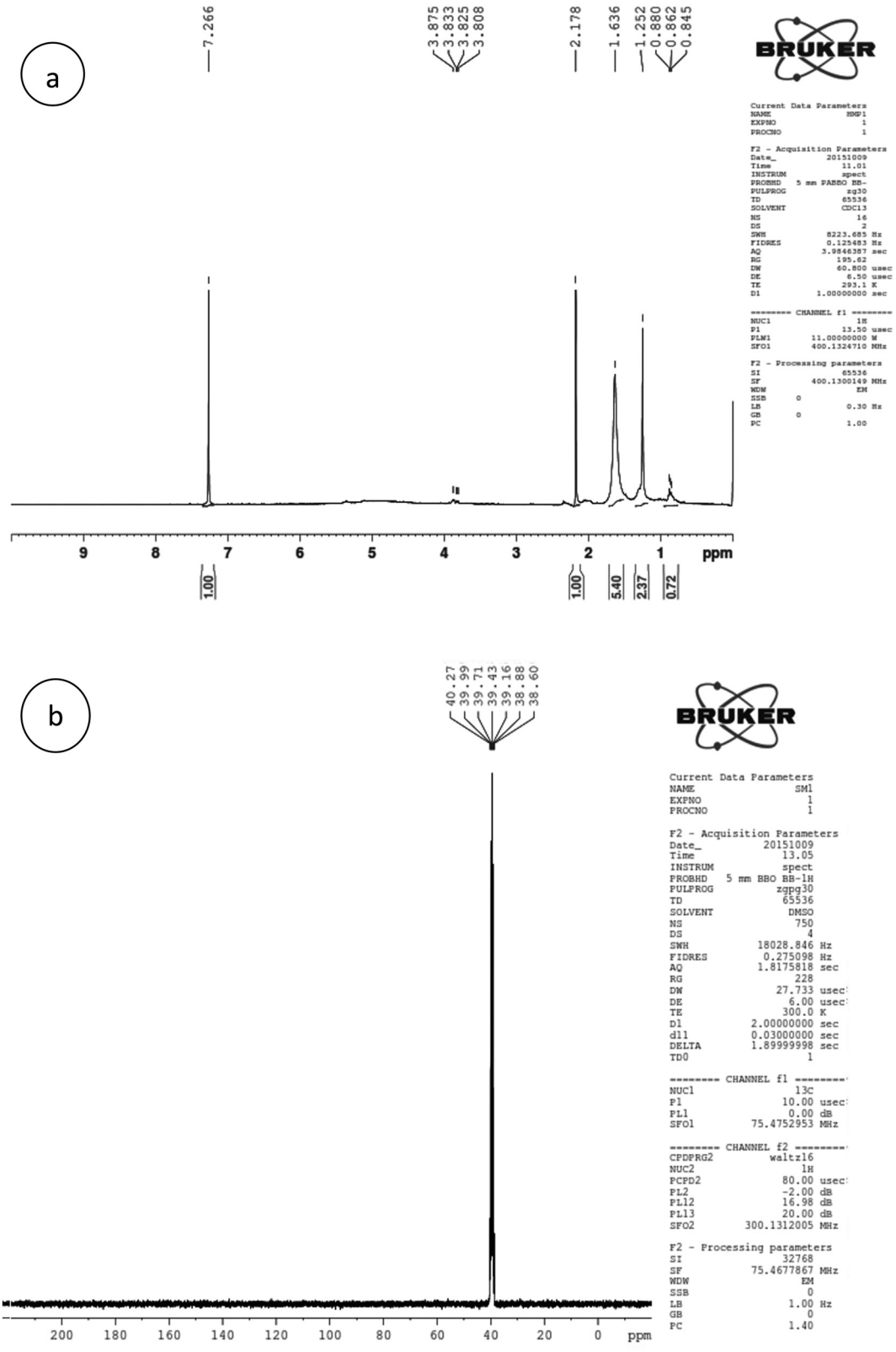

Figure 2: NMR spectrum obtained from aqueous leaf extract of *A. monophylla*: (a) ^{1}H NMR and (b) ^{13}C NMR.

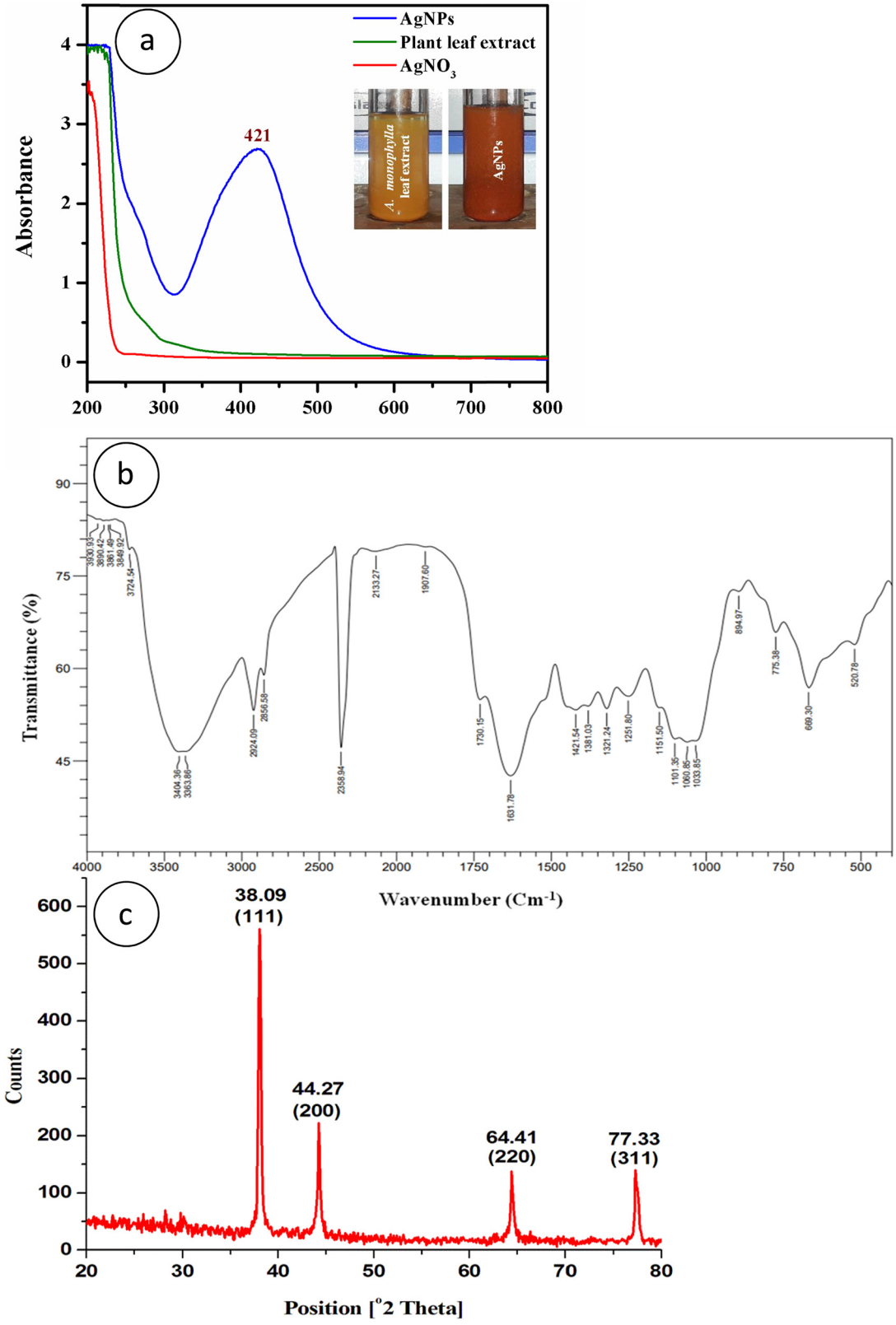

Figure 3: (a) UV-Vis, (b) FTIR, and (c) XRD analysis of AgNPs synthesized using *A. monophylla*.

saponins, tannins, triterpenes, coumarins, anthraquinones, and phenolics. These phytocompounds are responsible for the reduction of $AgNO_3$ to Ag NPs. The *Am*-AgNPs were estimated using XRD analysis, as shown in Figure 3c. The four-clear expression of reflections at 38.09° (111), 44.27° (200), 64.41° (220), and 77.33° (311) were recognized as a face-centered cubic (FCC) structure and point out the well crystalline nature of *Am*-AgNPs, according to the matching card no. 89-3722. SEM analysis of *Am*-AgNPs as shown in Figure 4a, plant extract mediated with AgNPs, showed the spherical morphology. EDX analysis examined the presence of metals in *Am*-AgNPs (Figure 4b). The accurate particle size and shape were measured by TEM analysis. Overall, synthesized *Am*-AgNPs spherical shape morphology ranges between 18 and 25 nm (Figure 4c), with average particle size of 20 nm. SAED analysis determined the crystal structure and simple spot patterns corresponding to single-crystal diffraction, as shown in Figure 4d. The four ring spots of d-spacing values attributed to the hkl planes of (111), (200), (220), and (311), respectively. These planes are well correlated with XRD results.

3.3 Larvicidal activity of aquous leaf extract of *A. monophylla* and synthesized silver nanoparticles tested against blood-sucking vectors

BSVs larval mortality values are presented in Table 1. Both ALE of *A. monophylla* and *Am*-AgNPs showed a dose-dependent toxic effect against selected BSVs. The *Am*-AgNPs provided maximum toxic potential than ALE with the LC_{50}/LC_{90} values of 22.19/43.63, 23.92/47.56, and 26.09/49.35 $\mu g \cdot mL^{-1}$ against BSVs of *Ae. vittatus*, *An. subpictus* and *Cx. vishnui*, respectively. Similarly, the LC_{50}/LC_{90} values of 40.25/81.69, 51.87/99.04, and 60.53/125.56 $\mu g \cdot mL^{-1}$ were noted against larvae of *H. bispinosa*, *R. microplus* and *R. sanguineus*, respectively. The other statistical units were obviously exhibited in Table 1.

3.4 Bio-toxicity analysis

The bio-toxicity of *A. monophylla* ALE and *Am*-AgNPs were tested against certain aquatic and terrestrial NTF

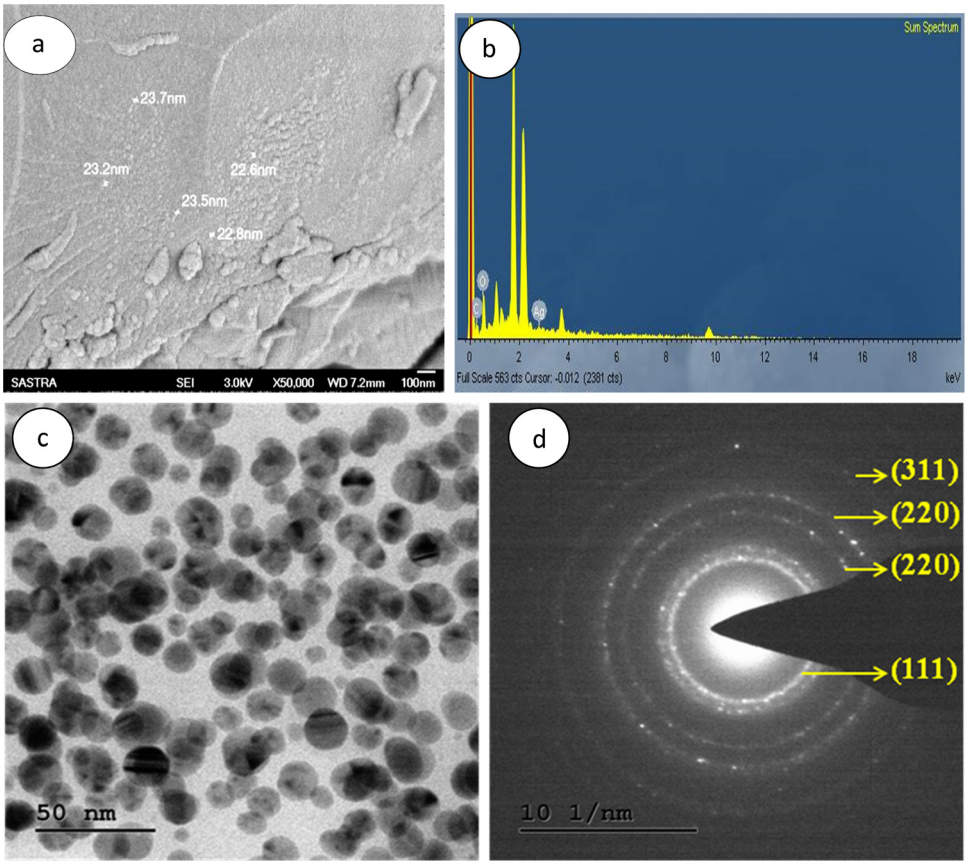

Figure 4: (a) SEM, (b) EDX, (c) TEM, and (d) SAED images of synthesized AgNPs using *A. monophylla*.

Table 1: Larvicidal activity of *A. monophylla* ALE and synthesized AgNPs against mosquitoes and ticks

Tested materials	Target organisms	LC_{50} (μg·mL^{-1})	95% fiducial limit (μg·mL^{-1})		LC_{90} (μg·mL^{-1})	95% fiducial limit (μg·mL^{-1})		Regression equation	χ^2
			LCL	UCL		LCL	UCL		
Mosquitoes									
Aqueous extract	*Ae. vittatus*	55.49	36.70	96.34	107.01	89.47	145.74	$y = 0.02 + 1.18x$	8.090
	An. subpictus	60.53	33.49	78.09	125.56	103.74	176.36	$y = 1.70 + 2.64x$	8.269
	Cx. vishnui	77.26	70.30	83.80	145.20	134.45	159.57	$y = 1.56 + 0.93x$	6.261
AgNPs	*Ae. vittatus*	22.19	13.78	28.19	43.63	35.83	61.27	$y = 1.14 + 0.05x$	9.044
	An. subpictus	23.92	13.81	30.71	47.56	39.35	66.33	$y = 1.17 + 0.05x$	8.813
	Cx. vishnui	26.09	23.73	28.32	49.35	45.63	54.36	$y = 1.48 + 0.06x$	4.246
Ticks									
Aqueous extract	*H. bispinosa*	94.69	84.18	104.14	193.07	177.64	214.17	$y = 2.30 + 18.06x$	5.831
	R. microplus	103.86	55.80	135.14	211.32	172.80	307.77	$y = 2.78 + 31.94x$	9.551
	R. sanguineus	127.03	114.81	138.39	246.04	227.07	271.72	$y = 2.75 + 8.02x$	3.338
AgNPs	*H. bispinosa*	40.25	35.31	44.57	81.69	75.61	89.73	$y = 0.03 + 1.09x$	6.040
	R. microplus	51.87	47.02	56.37	99.04	91.48	109.24	$y = 0.03 + 1.45x$	4.530
	R. sanguineus	60.53	33.49	78.09	125.56	103.74	176.36	$y = 2.64 + 1.70x$	8.269

Blood-sucking vectors larval toxicity experimentally exposed 24 h.
LC_{50}: BSVs 50% mortality representing concentration.
LC_{90}: BSVs 90% mortality representing concentration.
R value: regression value.
χ^2: Chi-square.

(*A. sulcatus*, *A. bouvieri*, *A. mitificus*, and *C. moluccensis*) and the results are presented in Table 2. The negligible toxicity effects were noted in the aquatic and terrestrial NTF compared to the target BSVs, with the LC_{50} values of aquatic NTF ranging from 4,422.4 to 10,532.8 μg·mL^{-1} and the terrestrial NTF from 2,094.5 to 5,382.7 μg·mL^{-1}, respectively. The SI of different aquatic and terrestrial NTF survive and share their habitats with selected BSVs. The chosen NTF were exposed to *A. monophylla* ALE and AgNPs and their SI indicate that this ALE and AgNPs have few detrimental effects on *A. sulcatus*, *A. bouvieri*, *A. mitificus*, and *C. moluccensis* (Table 3). Not only mortality, other suppressing activities like survival, swimming, and diving of NTF were not distorted while exposed to BSVs. The outcome of the present study reveals that the LAE and SNPs of *A. monophylla* has least or no impact on non-target fauna. The analysis of *A. monophylla* ALE and *Am*-AgNPs showed the best BSVs larval bio-toxic effects and eco-toxic potential against NTF.

4 Discussion

4.1 Preliminary phytochemical screening

The preliminary phytochemical screening was assessed in *A. monophylla* leaf extracts and its results were proven, and identified maximum groups of PCs were present in highly polar solvents (aqueous extract). Earlier, several PC types of research have been reported in various botanical sources and it also has an efficient agent for controlling different life threatening BSVs. Because the floral communities have a variety of PCs that can be extracted from various parts of flora, they are selective, highly biodegradable, non/less toxic bio-products, and alternate/replacement of synthetic pesticides [51].

4.1.1 NMR spectral analysis

Plants are enriched with complex mixtures of phytocompounds. It can be identified, quantified, and described through NMR spectroscopy. It is a uniquely powerful technique in the field of phytochemistry. NMR analysis confirmed the presence of a variety of phytocompounds identified from the ALE of *A. monophylla*. Different investigations in many plants have illustrated the NMR spectroscopy principle for identifying the potential phytocompounds/secondary metabolites in medicinal/toxic plants. Several research works have been done through NMR spectroscopy and present investigation of NMR spectral analysis results are comparable with some of the earlier reports. The NMR analysis confirmed the presence of 3,5-di-*t*-butyl-4-hydroxyanisole in *C. dactylon* leaf extract [52].

Table 2: The effect of *A. monophylla* ALE and synthesized AgNPs against NTF

Tested materials	NTF	LC_{50} (µg·mL^{-1})	95% fiducial limit (µg·mL^{-1})		LC_{90} (µg·mL^{-1})	95% fiducial limit (µg·mL^{-1})		*R*-value	χ^2
			LCL	UCL		LCL	UCL		
Aqueous extract	*A. sulcatus*	4,913.0	4,564.0	5,278.5	8,606.1	7,930.0	9,537.6	$y = 3.46 + 1.71x$	1.648
	A. bouvieri	4,422.4	4,116.3	4,772.3	7,618.6	6,953.2	8,568.8	$y = 3.99 + 1.76x$	1.443
	A. mitificus	10,532.8	9,725.7	11,433.2	19,241.1	17,439.5	2,1870.5	$y = 1.50 + 1.58x$	1.049
	C. moluccensis	9,644.9	8,946.7	10,372.8	17,037.3	15,689.9	18,894.7	$y = 1.75 + 1.67x$	2.201
AgNPs	*A. sulcatus*	2,227.0	2,066.2	2,412.7	3,923.1	3,561.0	4,450.4	$y = 7.71 + 1.72x$	2.488
	A. bouvieri	2,094.5	1,947.6	2,255.1	3,654.1	3,348.1	4,085.1	$y = 8.27 + 1.74x$	1.007
	A. mitificus	5,382.7	5,028.2	5,774.8	9,085.0	8,357.7	10,097.6	$y = 3.45 + 1.86x$	1.429
	C. moluccensis	3,850.69	3,570.38	4,141.73	6,817.96	6,279.84	7,559.24	$y = 4.30 + 1.66x$	0.518

NTF bio-toxicity experimentally exposed for 2 days.
NTF: non-target fauna.
LC_{50}: NTF 50% mortality representing concentration.
LC_{90}: NTF 90% mortality representing concentration.
R-value: regression value.
χ^2: Chi-square.

Phyto-compounds were compared on different tomato species [53]; *O. europaea* medicinal plant fruit and oil chemical composition [54]; *A. thaliana* metabolome changes in lettuce leaves by the influence of mancozeb pesticide [55]; Metabolomic analysis in *B. rapa* leaves [56]; geographic discrimination has been an essential factor for changing secondary metabolites in *P. vera* [57]; *H. lupulus* phytochemical analysis [58]; pea plant [59]; *A. sativum* phytochemical study [60].

4.2 Synthesis and characterization of AgNPs

The increasing number of applications of nanoparticles in biomedical devices, pharmaceuticals, and food has made them a promising future for many researchers. It has the ability to deliver drugs to specific tissues and enhance chemotherapeutic agents to kill tumors, in addition to its use in cosmetics, household products, and other health-related products. AgNPs, in particular, are widely used in different research fields due to their specific physical and chemical properties, which allow them to exert various activities, particularly in biomedical applications. AgNPs have antibacterial, antiviral, antifungal, antiangiogenic, antioxidant, and anticancer characteristics in addition to their roles as drug carriers, imaging devices, water treatment materials, biosensors, and antitumor chemicals. Additionally, AgNPs are helpful in the treatment of infections and burns. It would therefore be necessary to carry out further research to develop optimal methods to synthesize them. In an earlier study, Abdellatif et al. [61,62] synthesized silver nanoparticles (SNPs) using cellulosic polymer technology and reported that the encapsulated plant extract showed remarkable antioxidant, antibacterial, and apoptotic cancer cell death. In the present investigation, the selected plant extract was encapsulated with $AgNO_3$ and had a firm attachment with the plant extracts. Its characteristics were analyzed with various instrumental techniques. The color change (yellow to brown) of AgNPs was observed through SPR, which exhibited a peak at 421 nm (Figure 4). UV-Vis spectra analysis and similar trends were confirmed by earlier reports [63,64]. The observed peaks are considered such as flavonoids, triterpenoids, and polyphenols [65]. Hence, phytocompounds are proven to have potential activity and the present results indicate the presence of different functional groups involved in AgNPs synthesis. A similar result was reported earlier [66,67]. AgNPs sample obtained from the previous lyophilization step and acquisition of X-ray diffractograms the XRD pattern analyzed AgNPs.

Table 3: SI of aquatic and terrestrial NTF shared over selected BSVs exposed to ALE and synthesized AgNPs of *A. monophylla*

Tested materials	Aquatic NTF	*Ae. vittatus*	*An. subpictus*	*Cx. vishnui*	Terrestrial NTF	*H. bispinosa*	*R. microplus*	*R. sanguineus*
Aqueous extract	*A. sulcatus*	88.53	81.16	63.59	*A. mitificus*	111.23	101.41	82.91
	A. bouvieri	79.69	73.06	57.24	*C. moluccensis*	101.85	92.86	75.92
AgNPs	*A. sulcatus*	100.36	93.10	85.35	*A. mitificus*	133.73	103.77	88.92
	A. bouvieri	94.38	87.56	80.27	*C. moluccensis*	95.66	74.23	63.61

The XRD results of present investigation are in agreement with some of the earlier literature values of the crystal structure of AgNPs and earlier reports [68,69] are in general agreement with the just-cited results. AgNPs synthesized by *A. monophylla* ALE are evidenced by the SEM image magnified into a different range. NPs showed beads shape which belong to the category of nanoparticles. Similar trends were noticed in earlier published reports of other plants' synthesized NPs [70,71]. EDX attached with SEM examined and provided essential information on synthesized AgNPs of *A. monophylla* in the composite mixture's inner and outer surface. Earlier investigations on green synthesized NPs in several floral communities were conducted [72,73]. The different floral parts of AgNPs were evaluated by TEM and SAED analysis, proven by earlier published research [74,75].

4.3 BSVs larval toxicity of ALE and *Am*-AgNPs

The BSVs larval toxicity of *A. monophylla* ALE and *Am*-AgNPs were tested against larvae of selected BSVs (*Ae. vittatus, An. subpictus, Cx. vishnui*, *H. bispinosa, R. microplus*, and *R. sanguineus*), and the results showed that the *Am*-AgNPs provided the maximum toxic effects than ALE. The probable mechanism of larval toxicity could be due to the action of AgNPs associated with the plant extract causing blockings in the respiratory siphons of the larvae. Other possible cues are primarily SNPs that might have entered into the system where they cause an enzymatical imbalance in the larvae in general and acetylcholinesterase in particular. Secondarily, the AgNPs might alter the sodium-potassium channels in the larval nervous system and that could be the reason for the larvae exhibiting immobility when induced with a stimulus. Similar observations were recorded in earlier reports, and the different medicinal plants AgNPs were tested against larvae of different BSVs like *Cx. quinquefasciatus*, *An. subpictus, Ae. aegypti, An. stephensi*, and *R. microplus,* the highest larval toxicity was noticed in synthesized AgNPs and it could be used to control mosquitoes as an eco-friendly approach [76,77]. Earlier, a similar observation was also reported on other BSVs like ticks, the different medicinal plants AgNPs were tested on ticks and the maximum larval mortality occurred in the lowest concentration of *Am*-AgNPs, and it has highly stable and statically significant BSVs larvicidal potential on tick's community [78,79].

4.4 Bio-toxicity and SI of ALE and *Am*-AgNPs on NTF

Previously, many researchers reported different floral constituents against various aquatic and terrestrial NTF. It is first-hand information of selected BSVs and NTF. The SI of different aquatic and terrestrial NTF survive and share their habitats with BSVs, examined worldwide, and many studies identified similar outcomes [80–85].

5 Conclusion

For the management of several medical pests, natural products are crucial and substantial resources. Recent green AgNPs have advantages including eco-safety, target specificity, easy availability, low cost, and nanotechnology capabilities, making them an excellent material for pesticide characteristics. The naturally occurring phyto-properties have more benefits than any synthetic insecticide. Today, environmental safety is of the utmost importance, and the green synthesis of AgNPs was seen as a viable alternative treatment for SCPs. Several plants are utilized as traditional remedies and for pesticide/mosquitocidal purposes in India, a nation with rich plant biodiversity. Surprisingly, even though *A. monophylla* is an outstanding plant, little research has been conducted in various regions. There is an immediate need for intense research on this plant in order to use it to manage BSVs, which are of global relevance for human and animal health and pose fewer risks to NTF.

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Appendix

Table A1: Qualitative analysis of phytochemical screening in *A. monophylla* leaf extracts

S. no.	PCs	*A. monophylla* in different solvent systems				
		HEX	DEE	DCM	EA	AQU
1	Carbohydrates	–	–	–	–	+
2	Alkaloids	–	–	–	–	+
3	Flavonoids	+	+	+	–	+
4	Saponins	–	+	–	+	+
5	Tannins	+	+	+	+	+
6	Triterpenes	+	+	+	–	+
7	Resins	+	–	+	–	–
8	Coumarins	+	+	+	+	+
9	Anthroquinnones	+	–	–	+	+
10	Phenolics	+	+	+	–	+

HEX: hexane; DEE: diethyl ether; DCM: dichloromethane; EA: ethyl acetate; AQU: aqueous.
+ = indicates presence of phytochemical group.
– = indicates absence of phytochemical group.