Research Article

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Antibacterial greener silver nanoparticles synthesized using *Marsilea quadrifolia* extract and their eco-friendly evaluation against Zika virus vector, *Aedes aegypti*

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Abstract: Fabrication and use of nanoparticles have progressively enlarged within the last decade. Herein the silver nanoparticles (AgNps) were synthesized via the extract from *Marsilea quadrifolia* (*Mq*) as a decreasing and steadying mediator. The *Mq*-AgNPs demonstrated superior toxicity on Zika virus vector, *Aedes aegypti* with the LC₅₀ value of 10.69 µg·mL⁻¹. The *Mq*-AgNPs were established securely to non-target organisms *Artemia nauplii*

and *Ceriodaphnia cornuta*, and no structural or anatomical alterations confirm its security to the surroundings. The antibacterial activity exposed that *Mq*-AgNPs showed superior growth inhibition effect against the tested bacteria. Furthermore, the *Mq*-AgNPs showed strong antioxidant activities when compared to *Mq* leaf extract. Overall, our results highlight that *Mq*-AgNPs are hopeful and biodegradable devices against mosquito vectors of therapeutic significance, with moderate toxicity against non-target aquatic animals.

Keywords: *Marsilea quadrifolia*, Zika virus, *Artemia nauplii*, *Ceriodaphnia cornuta*, antioxidant assay

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1 Introduction

Mosquito vectors are exclusively liable for spreading pathogens and parasites of different infections like dengue, malaria, chikungunya, encephalitis, Zika virus infection, filariasis, and yellow fever [1–3]. World Health Organization (WHO) approximates that dengue is passed on from 50 to 400 million citizens yearly on the earth. The appearance of dengue-resistant strains is a significant challenge to hold; it has extended and impacted human health [4]. Since commercial vaccines for most diseases spread by *Aedes aegypti* do not exist, mosquito control remains a critical component of all prevention and control campaigns, which typically rely on pesticide spraying, biological control agents, and environmental management, among other things. As a result, developing novel, environmentally acceptable, and effective mosquito control techniques are critical to ensuring our future ability to prevent and manage diseases spread by these insects. Mosquito management is being improved in numerous regions; however, essential confronts and the rising mosquito resistances to insecticides require other

commercial and safe insecticides. The frequent use of chemical insecticides promotes numerous ecological risks and increases insecticide resistance and danger to non-target organisms and public health [5]. In this situation, ecological management devices against mosquitoes are precedence [6,7], and the progress of plant-borne pesticides with several action methods is perhaps the prosperity for managing disease-transmitting mosquitoes [8].

Nanotechnology is another innovative technology with considerable potential in the field of mosquito control. The synthesis of silver nanoparticles (AgNPs) with extracts of plants with insecticidal action has been proven to produce larvicidal chemicals that are effective against mosquitos at low concentrations. The good insecticidal resistance calls for a novel move towards a nanomaterial-based combinable system to extend the insecticidal exploit. Besides the plant-based biocontrol mediators, nanoparticles' formulation gives an enhanced approach for mosquito management [9]. AgNPs are the majority of hopeful nanoproducts with excellent physio-chemical properties, regularly occupied in various household and industrialized uses [10,11]. The AgNPs have potent inhibitory activity against a broad array of microorganisms, separately as their antimicrobial property; currently, these have been searching for differentiating their part in controlling the insect pests [12]. The AgNPs have several beneficial properties that could make them an effective bactericide in the future. However, the cost, preparation, and particle dispersion constraints must all be addressed.

Furthermore, before large-scale application, the environmental behavior of AgNPs and the toxicity on non-target creatures must be determined. *Marsilea quadrifolia* L. (family: Marsileaceae), a waterleaf, originates plentifully in India's eastern and southern areas. The *M. quadrifolia* (*Mq*) leaf aqueous extract contains phytosterols, flavonoids, alkaloids, tannins, reducing sugars, and phenolics compounds [13–15]. The *Mq* leaf juice is used to treat snakebite and applied to swellings; besides, it is anti-inflammatory and refrigerant [16,17]. Several studies have been published on the production of green AgNPs from diverse plant sources. However, for employing novel plant sources, there is still a need for an economically stable, commercially viable, and environmentally safe way to synthesize AgNPs. Herein the leaf extract of *Mq*, AgNO₃, and *Mq*-AgNPs were tested for their larvicidal activity and biotoxicity on Brine shrimp (*Artemia nauplii*) and *Ceriodaphnia cornuta* as non-target aquatic organisms.

Figure 1: (a) *Mq* leaf and (b) UV-Vis spectrum of *Mq*-AgNPs.

2 Materials and methods

2.1 *Mq* leaf extracts preparation

Mq leaves (Figure 1a) were collected from a local source, and they were rinsed with double distilled water (d.H₂O) and dehydrated at 37°C. Ten grams of thinly incised leaves were reserved in a beaker with 100 mL of d.H₂O and heated for 30 min and then stained and stocked up at 4°C for additional utilization.

2.2 *Mq*-AgNPs synthesis and characterization

In a sterile conical flask, 10 mL of leaf extract was combined with 90 mL of silver nitrate (10 mM) and kept at 37°C. To distinguish the AgNPs, it was examined via

Figure 2: XRD pattern of *Mq*-AgNPs.

Figure 3: FTIR analysis of *Mq*-AgNPs.

Figure 4: TEM images of *Mq*-AgNPs at different magnifications: (a) 9.6k $\times$, (b) 50k $\times$, and (c) 280k $\times$.

UV-Vis spectrum, XRD, FTIR spectroscopy, and transmission electron microscopy (TEM Technite 10 Philips).

2.3 Mosquito larvicidal activity and histology analysis

The *A. aegypti* larvae (III instar) were maintained for 1 day in a glass beaker (250 mL) full of 200 mL of dechlorinated

water with *Mq* leaf extract (30, 60, 90, 120, and 150 $\mu\text{g}\cdot\text{mL}^{-1}$) and *Mq*-AgNPs (5, 10, 15, 20, and 25 $\mu\text{g}\cdot\text{mL}^{-1}$) followed by the method suggested by Ishwarya et al. [18]. All concentrations were simulated 5 times, and larval mortality was documented 1 day (24 h) after exposure. In histology examination, the dead larvae were stocked up in formalin and mounded in paraffin blocks by 8 μm broad sectors of larval tissue, stained using eosin and hematoxylin, and examined under a stereomicroscope.

2.4 Biototoxicity on *Artemia nauplii*

Artemia cysts were first chlorinated for 20–30 s prior to the hatching procedure. At first, *Artemia* cysts that emerged in marine water were afforded incessant brightness coverage and energetic exposure to air for 1 day. Ten *Artemia nauplii* larvae were placed in each well of the 24-well plates and were experienced against different concentrations (2.5–10 $\mu\text{g}\cdot\text{mL}^{-1}$) of *Mq* extracts, AgNO_3 , and *Mq*-AgNPs. The death rate of *Artemia nauplii* was counted and the treated *Artemia nauplii* were viewed under a microscope to detect the nanoparticles' impairments. The investigation was conducted in triplicate.

2.5 Evaluation of the toxicity on *Ceriodaphnia cornuta*

The *Ceriodaphnia cornuta* employed in this study was a derivation of the store culture carried out in our laboratory by Vijayakumar et al. [19]. Acute toxicity tests for 1 day were executed based on USEPA procedure EPA-821-R-02-012. The neonates of *C. cornuta* were responsive to diverse concentrations (2.5–10 $\mu\text{g}\cdot\text{mL}^{-1}$) of *Mq* extract and *Mq*-AgNPs and this study was observed for 2 days. In brief, 10 neonates of *C. cornuta* were added to glass beakers having freshwater (60 mL) and continued throughout the test; the following day, the structural alterations of *C. cornuta* were noticed.

2.6 Antibacterial study

To assess the antibacterial activity of *Mq* extract and *Mq*-AgNPs against Gram-positive bacteria, *B. subtilis* and *E. faecalis*, and Gram-negative bacteria, *P. aeruginosa* and *P. vulgaris*, the agar well diffusion method carried out by Ishwarya et al. [20] was followed with slight modifications.

Table 1: Mosquito larvicidal efficacy of *Mq* extract and *Mq*-AgNPs on Zika virus vector, *Aedes aegypti*

Tested materials	Concentration ($\mu\text{g}\cdot\text{mL}^{-1}$)	% of mortality $\pm$ SD	LC ₅₀ (LCL-UCL)	LC ₉₀ (LCL-UCL)	Regression equation	χ^2
<i>Mq</i> extract	30	26 $\pm$ 0.6	69.68	140.41	$y = 8.200 + 0.586x$	3.064 ^a
	60	42 $\pm$ 0.8	(62.01–76.56)	(129.53–155.11)		
	90	63 $\pm$ 1.2				
	120	78 $\pm$ 0.6				
	150	96 $\pm$ 0.8				
<i>Mq</i> -AgNPs	5	29 $\pm$ 1.2	10.69 (9.41–11.82)	21.93 (20.25–24.18)	$y = 11.70 + 3.500x$	6.141 ^a
	10	46 $\pm$ 0.8				
	15	66 $\pm$ 1.4				
	20	81 $\pm$ 1.2				
	25	99 $\pm$ 0.8				

^a Not significant ($\alpha = 0.05$).

2.7 Antioxidant assay

To establish the antioxidant activity of *Mq* leaf extract and *Mq*-AgNPs for the 1,1-diphenyl-2-picrylhydrazil (DPPH) radical scavenging assay was carried out as per McDonald et al. [21] with some modifications. The scavenging activity of *Mq* leaf extract and *Mq*-AgNPs for the hydrogen peroxide was assayed according to the procedure followed by Halliwell et al. [22]. The nitric oxide radical inhibition was examined according to the procedure followed by Ebrahimzadeh et al. [23]. The reducing power of *Mq* leaf extract and *Mq*-AgNPs was determined according to the modified protocol of Chang et al. [24] and the absorbance was calculated spectrophotometrically at 700 nm.

2.8 Statistical analysis

All the studies were put through probit analysis, and the LC₅₀ and LC₉₀ values were calculated using SPSS software version 15.

3 Results

3.1 *Mq*-AgNPs characterization

The key indicator of AgNP production was an instantaneous color change after adding aqueous *Mq* extract to silver nitrate solution. AgNPs' surface plasmon resonance revealed a peak at 440 nm, corresponding to their absorbance (Figure 1b). XRD graph clearly shows peaks at 27.9, 32.3, 38.1, 46.3, 54.8, 57.6, 65.1, 67.6, and 76.8 and these peaks are corresponding to (210), (101), (112), (200),

(006), (105), (110), (112), and (201) planes, respectively. The peaks on planes lesser than 30 are due to the subsistence of phytochemical compounds in the leaf extracts (Figure 2). The X-ray pattern displayed the formation of AgNPs (JCPDS card no 41-1402). The FTIR analysis revealed numerous main peaks at 3,850, 3,417, 2,918, 1,633, 1,384, 1,030, and 467 cm^{-1} (Figure 3). The peaks in the range of 3,282 and 3,417 cm^{-1} could be due to the N–H, medium, broad and 2,918 cm^{-1} correspond to the 1,650 cm^{-1} alkene C–H group, medium to strong. The peak 1,384 cm^{-1} matched the OH bend of polyphenol and corroborated an aromatic group's incidence, even as the absorption peaks at 1,030 cm^{-1} were consigned for COC and secondary OH group. The TEM image of AgNPs displays that the particle sizes are nanoscale and uniform (Figure 4). Also, Figure 4a–c displays the shape and size of the produced NPs which has spherical AgNPs.

3.2 Analysis of histology and larvicidal assay

The *Mq*-AgNPs showed the best larvicidal activity against the Zika virus vector *A. aegypti* larvae with the LC₅₀ and LC₉₀ values of 10.69 and 21.93 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively (Table 1). The treated larvae's epithelial layer and surface cuticle had broken down, as seen by the stereomicroscope. Furthermore, the epithelium of *A. aegypti* larvae was damaged, and cells were vacuolated; the hindgut was exaggerated, and the epithelium showed severely vacuolated by spoiled intercellular membranes (Figure 5). Damage to the hindgut and larval muscles has been seen, and the deposition of *Mq*-AgNPs in the midgut, abdomen, and siphon suggests that larval disease may be caused by tissue injury.

3.3 Biototoxicity on *Artemia nauplii* and *C. cornuta*

The *Mq*-AgNPs were non-toxic to the non-target organisms *Artemia nauplii* and *C. cornuta*. The primary clarification hinted that the tested animals' long life and swimming action were not changed for 7 days following the experiment. A slight irregularity in swimming, heart rate, and thoracic limb progress was detected under a stereomicroscope in *Artemia nauplii* and *C. cornuta* treated with *Mq*-AgNPs (Figure 6). The confocal laser scanning microscopy

examination also evidenced the accretion and morphological irregularities of *Artemia nauplii* and *C. cornuta* treated with *Mq*-AgNPs (Figures 7 and 8).

3.4 Antibacterial activity

The *Mq* extract and *Mq*-AgNPs were assessed for the antibacterial activity against tested bacterial pathogens. The agar well diffusion method was used to investigate the

Figure 5: Stereomicroscopic images of third instar larvae of *Aedes aegypti*: control (a), *Mq* leaf extract (b), AgNO₃ (c), *Mq*-AgNPs (d). Histological studies of *A. aegypti* third instar larvae: control (e), *Mq* leaf extract (f), AgNO₃ (g), *Mq*-AgNPs (h). Red arrows indicate damages in head, abdomen, thorax region, and siphon.

Figure 6: *In vivo* toxicity analysis of *Artemia nauplii* and *C. cornuta*.

in vitro vulnerabilities of the favored bacterial pathogens toward the *Mq* extract and *Mq*-AgNPs at different concentrations of 25, 50, and 100 μL such as the width of the inhibition zone (mm) around discs with *Mq*-AgNPs (Figures 9 and 10). The antibacterial effect of inhibition was more for *Mq*-AgNPs compared to the *Mq* leaf extract.

3.5 Antioxidant assay

In vitro, the antioxidant activity of *Mq*-AgNPs was examined using DPPH, hydrogen peroxide, nitric oxide radical scavenging, and reducing power activity concerning the standard antioxidants butylated hydroxytoluene (BHT) (Figure 11). The DPPH reducing the activity of the *Mq*-AgNPs was assessed based on color change and revealed potent inhibition of *Mq*-AgNPs activity contrasted with standard BHT (Figure 11a). The varying concentration of the *Mq*-AgNPs (25, 50, and 100 $\mu\text{g}\cdot\text{mL}^{-1}$) significantly scavenged DPPH by 45.16%, 55.54%, and 65%, respectively. In hydrogen peroxide scavenging activity, concentrations at 100 $\mu\text{g}\cdot\text{mL}^{-1}$ inhibition were 70.25%, 30.12%, and 40.58% for the *Mq*-AgNPs, *Mq* leaf extract, and BHT, respectively (Figure 11b). In nitric oxide radical scavenging assay, at higher concentration of

100 $\mu\text{g}\cdot\text{mL}^{-1}$, the *Mq* leaf extract and *Mq*-AgNPs demonstrated the concentration-dependent NO scavenging activity of 48.12% and 62.16%, respectively (Figure 11c). The *Mq*-AgNPs were dose-dependent in reducing powers; the enlarged concentration of AgNPs constantly enlarged the reducing power activity. The *Mq*-AgNPs, *Mq* leaf extract, and BHT have exposed approximately equivalent reducing power activity 70.25%, 55.16%, and 65%, respectively (Figure 11d).

4 Discussion

The NPs synthesis is the lime glow of current nanotechnology. Ag ion's decline into AgNPs through disclosure to the plant extracts might be pursued via color modification. Biosynthesis of NPs using plant extracts is now well development and advanced into a significant division of nanotechnology. In the present study, the NPs were principally distinguished through UV spectrum, which was established to be a precious method for investigating NPs [25]. The maximum absorption peak observed in the AgNPs solution residues near 440 nm during the reaction time [26,27]. The XRD results of *Mq*-AgNPs correspond to

Figure 7: Confocal laser scanning microscopy analysis of *Artemia nauplii*.

the mass silver's FCC structure through the broad peaks at 32.4, 46.4, and 28.0, equivalent to 111, 200, and 311 planes, respectively. The line expansion of the peaks is mainly owing to minute particle size. The XRD results

display that the AgNPs produced by reducing Ag^+ ions through the *Mq* leaf extract are crystalline [28–31].

Nowadays, using poisonous chemicals to manage disease-spreading mosquito vectors are unsuccessful because

Figure 8: Confocal laser scanning microscopy analysis of *Mq*-AgNPs on micro-crustacean *C. cornuta*.

Figure 9: Antibacterial activity of *Mq* extract.

of ecological and health-associated troubles [32–36]. At present, outbursts of mosquito-borne diseases have been influenced to recognize efficient, constant, and ecological negotiators for the constant discharge of insecticides in mosquitoes propagation [37]. In our study, the *Mq* leaf extract illustrated larvicidal effectiveness on *A. aegypti* ($LC_{50} = 183.63 \mu\text{g}\cdot\text{mL}^{-1}$ and $LC_{90} = 167.19 \mu\text{g}\cdot\text{mL}^{-1}$) (Table 1). Earlier studies reported that numerous plant extracts, specifically *Clerodendrum chinense* [38], *Mukia maderaspatana* [39], *Chomelia asiatica* [40], and *Clausena dentata* [41], have been described as possible larvicidal agents against target mosquitoes, and these results carry the effect of the current investigation. The mortality outcome of AgNPs on larvae might be because of the toxic interior effects of

the minute particles within the cuticle [42]. The *Mq* leaf extract affecting the fourth instar larvae of *A. Aegypti* requires elevated concentrations to cause larvae mortality than AgNPs treatments, which emerged lethal consequence brightly on mosquito larvae tiny concentrations [43]. The stereomicroscopic results displayed the epithelial and external cuticle breakdowns in the treated larvae (Figure 3a). In agreement with our findings, numerous reports showed that plant-mediated AgNPs are conscientious of cruel histological modifies in larvae of *A. Aegypti* like *Hedychium coronarium* [44] and *Pedaliium murex*-mediated AgNPs [18].

Microscopic observations highlighted that the *Mq* leaf extract- and *Mq*-AgNPs-treated *Artemia nauplii*

Figure 10: Antibacterial activity of *Mq*-AgNPs.

and *C. cornuta* organisms have a long life and the study species' swimming action were not changed for a week following the experiment. At this time, a prudent understanding of the extreme toxicity of mosquitocidal NPs on non-target aquatic species is available [45]. Previously, much research was focused on the plant extract-mediated AgNPs on biotoxicity to the different non-target aquatic organisms such as *Poecilia reticulata* (*Plumeria rubra* and *Pergularia daemia*) [46], *C. cornuta* (garlic clove and sodium alginate) [19,47], *P. reticulata* (*Vinca rosea*) [48], mosquito predators (*Solanum nigrum*) [49], and copepods (*Melia azedarach*) [50]. Fascinatingly, the exposure to tiny doses of biosynthesized AgNPs did not harmfully affect the non-target organisms [51].

Mq-AgNPs show potent antibacterial properties against Gram-positive and Gram-negative bacterial strains. Our results were concurrent with the reports of Mani *et al.* [52] and Maji *et al.* [15], which found that AgNPs produced by *Mq* have excellent antibacterial activity. In the present study, the *Mq*-AgNPs have exposed more inhibition with 65%, 40.58%, 85.43%, and 65%, corresponding to scavenging of DPPH, hydrogen peroxide, nitric oxide, and reducing power activity, respectively. The antioxidant activity of *Mq*-AgNPs could be recognized to functional groups that remained to them, which were initiated from the *Mq* leaf extract. These results are in good agreement with the previous reports on the *in vitro* antioxidant effect of different plant extracts [53–58].

Figure 11: Antioxidant activity of *Mq* leaf extract and *Mq*-AgNPs: (a) DPPH method, (b) H₂O₂ assay, (c) NO scavenging assay, and (d) reducing power.

5 Conclusion

Overall, *Mq* leaf extract is used to synthesize the AgNPs. The biosynthesized *Mq*-AgNPs were typically spherical in shape and crystalline in nature. This study determined that *Mq*-AgNPs can be engaged in small quantities to powerfully decrease the populations of vector mosquitoes, which lacks harmful effects on predation rates of non-target aquatic organisms, like *Artemia* and *C. cornuta*. Moreover, the present research offers an insight into the usage of *Mq* leaf extract as an acceptable source of naturally occurring antioxidants and could have immense significance as a therapeutic agent in preventing oxidative stress-related diseases.

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