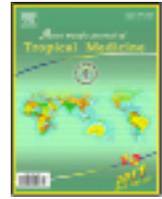




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Biosynthesis of silver nanoparticles by using mangrove plant extract and their potential mosquito larvicidal property

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ABSTRACT

Objective: To identify the larvicidal activities of silver nano particles synthesised with *Rhizophora mucronata* (*R. mucronata*) leaf extract against the larvae of *Aedes aegypti* (*Ae. aegypti*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*). **Methods:** *In vitro* larvicidal activities such as LC₅₀ and LC₉₀ were assessed for the *Ae. aegypti* and *Cx. quinquefasciatus* larval species. Further, characterisation such as UV, XRD, FTIR and AFM analysis were carried out for the synthesised silver nano particles. **Results:** The LC₅₀ value of the synthesised silver nano particle was identified as 0.585 and 0.891 mg/L for *Ae. aegypti* and *Cx. quinquefasciatus* larvae respectively. Further, the LC₉₀ values are also identified as 2.615 and 6.291 mg/L for *Ae. aegypti* and *Cx. quinquefasciatus* species respectively. The synthesised silver nanoparticles have maximum absorption at 420 nm with the average size of 60–95 nm. The XRD data showed 2θ intense values with various degrees such as 37.10°, 47.66°, 63.97° and 70.01°. The FTIR data showed prominent peaks in (3 426.89, 2 925.49, 2 869.56, 2 346.95, 1 631.49, 1 031.73, 669.18 and 455.12) different ranges. **Conclusions:** The biosynthesis of silver nanoparticles with leaf aqueous extract of *R. mucronata* provides potential source for the larvicidal activity against mosquito borne diseases.

1. Introduction

Deadly diseases such as malaria, dengue, chikunguniya and filariasis are mainly transmitted through the mosquito vectors. In India, two million malaria cases are being reported annually[1]. Application of chemical insecticides provoke undesirable effects of chemical resistance, toxicity to non–target organism and environmental and human health concerns[2,3]. In this regard, nanoparticles exhibits important role in the several aspects such as drug delivery, diagnostics, antimicrobial activities and

tissue engineering[4,5]. Synthesis of nanoparticles by using chemical and physical methods requires high pressure, energy, temperature and toxic chemicals. Plant extracts are suitably scaled up for large scale biosynthesis of silver nanoparticles in a controlled manner according to their size, shape and sensitivity. But the present study is the first attempt for the biosynthesis of nanoparticles by using mangrove plant extract. Among the mangrove plants *Rhizophora mucronata* (*R. mucronata*) is previously proved to have antibacterial[6], antiplasmodial[7] and antiviral[8] activities. Additionally variety of chemical constituents such as alkaloids, flavonoids, polyphenols and terpenoids are also reported[7]. In this connection the present study was made an attempt to identify the larvicidal activity of silver nanoparticles biosynthesised with *R. mucronata* leaf extract.

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2. Materials and methods

2.1. Collection of plant material

The fresh matured leaves of *R. mucronata* was collected from Karangadu mangrove forest (latitude 9° 38' N and longitude 78° 57'E) of South East coast of India, Ramanathapuram district, Tamilnadu, India. The authentication of the plant species was done by Prof. K. Kathiresan, Centre of Advanced Study in Marine Biology, Annamalai University, Porto Novo, Tamil Nadu, India. The voucher specimen (MKUCMS0023) was also maintained in the Department of Marine and Coastal Studies, Madurai Kamarajar University, Madurai, Tamilnadu, India.

2.2. Biosynthesis of silver nanoparticles

The collected leaf sample was washed thrice with tap water and twice with distilled water to remove the adhering salts and other associated animals. About 10 g of finely cut leaves was placed with 100 mL of double sterilized distilled water and then boiling the mixture for 5 minutes. The boiled extract was filtered with Whatmann no. 1 filter paper. A total of 10 mL of collected filtrate was treated with 90 mL of silver nitrate aqueous solution (21.2 g of AgNO₃ powder in 125 mL of Milli Q water) and incubated at room temperature for 10 min, resulting in the formation of brownish yellow solution indicating the synthesis of silver nanoparticles^[9]

2.3. Characterisation of biosynthesised nanoparticles

About 1 mL of solution (Diluted with 1:20 v/v Milli Q water) was monitored in UV–VIS spectrophotometer (Between 300–700 nm ranges with 5 nm intervals) with different time intervals (15 min, 30 min, 4 h, 6 h and 8 h). After 8 h of incubation, the solution was centrifuged with 12 000 rpm for 20 min and their pellets were redispersed in sterile distilled water. The centrifugation and redispersion was repeated three times to ensure the complete separation of nanoparticles. The purified pellet was dried and subjected to the FTIR spectroscopy measurement in the diffuse reflectance mode at a resolution of 4 cm⁻¹ in KBr pellets. The dried mixture of silver nanoparticles was further analysed with X–ray diffractometer (PAN alytical BV, The Netherlands) operated at a voltage of 40 kV and a current of 30 mA with Cu K_α radiation in a θ –2 θ configuration. Additionally, a thin film of sample was also prepared in the coverslip with the 100 μ L of the synthesised silver nanoparticles solution and allowed to dry for 5 min and the slides were analyzed with atomic force microscopy.

2.4. Larvicidal bioassay (preliminary screening)

The eggs and egg rafts of *Aedes aegypti* (*Ae. aegypti*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*) were procured from vector control research centre (VCRC), Puducherry, India. Filter paper with attached eggs was dipped into a plastic tray containing 500 mL of dechlorinated water for 30–40 min, to hatch out larvae. The reared larvae were

maintained for 5 d in standard environment (28±2) °C temperature and 14:10 light and dark period cycle; the larvae were fed with powdered mixture of dog biscuits and yeast powder in 3:1 ratio to attain IVth instar. The larvicidal activity was assessed by the procedure of WHO guidelines^[10] with some modification and as per the method of Rahuman *et al*^[11]. A total of 20 reared mosquito larvae was placed in 200 mL of double distilled sterilized water containing various concentrations (20.00, 10.00, 5.00, 2.50 and 1.25 mg/L) of biosynthesised nano particles. Sterile distilled water without nanoparticles served as control. Triplicates were maintained for each assay. Percentage of mortality was assessed after 24 h of incubation. The experimental media in which 100% mortality of larvae occurs alone were selected for dose response bioassay.

2.5. Dose dependant bio assay

Based on the preliminary larvicidal screening results different concentrations of biosynthesised silver nanoparticles (10.000, 5.000, 2.500, 1.250 and 0.625 mg/L) were prepared for dose dependant larvicidal bio assay. Briefly, a total of 20 reared mosquito larvae was placed in 200 mL of double distilled sterilized water containing various concentrations (10.000, 5.000, 2.500, 1.250, 0.625 and 0.313 mg/L) of synthesised nano particles. Sterile distilled water without nanoparticles was served as control. Triplicates were maintained for each assay. Percentage of mortality was assessed after 24 h incubation.

2.6. Statistical analysis

Statistical analysis such as LC₅₀, LC₉₀, 95% confidential limit and *chi* square values were calculated by using the StatPlus, 2009 programme. Results with *P*<0.05 were considered to be statistically significant.

3. Results

The results of the present study suggest that, the maximum (100%) percentage of larvicidal activity was identified with 5 mg/L and 10 mg/L for *Ae. aegypti* and *Cx. quinquefasciatus* fourth instar larvae (Table 1). The results of the dose dependant assay suggested that, the value LC₅₀ was identified as 0.585 and 0.891 mg/L for *Ae. aegypti* and *Cx. quinquefasciatus* respectively. Further, the LC₉₀ values were identified as 2.615 and 6.291 mg/L for *Ae. aegypti* and *Cx. quinquefasciatus* respectively. The results of upper confidential level (UCL), lower confidential level (LCL) and *chi* square (χ^2) values are mentioned in Table 2. The colour intensity of the synthesised silver nanoparticles were increased with increased time duration and the maximum intensity was observed with 420 nm wavelength (Figure 1). The AFM topographic image of the synthesised silver nanoparticles and the results are indicating the average size of the particle (60–95 nm), roughness of the particle (1.7 nm), maximum height of the roughness (11.7 nm) and average maximum height of the roughness (7.7 nm) (Figure

Table 1Larvicidal activity of the *R. mucronata* synthesised silver nanoparticles against *Ae. aegypti* and *Cx. quinquefasciatus* larvae.

Concentrations (mg/L)	Percentage of mortality in <i>Ae. aegypti</i>	Percentage of mortality in <i>Cx. quinquefasciatus</i>
20.00	100.00±0.00	100.00±0.00
10.00	100.00±0.00	100.00±0.00
5.00	100.00±0.00	82.37±10.98
2.50	87.65±7.87	67.35±8.54
1.25	65.36±6.98	54.38±3.65

Values are represented in Mean ± SD values.

Table 2Dose dependant larvicidal activity of silver nanoparticles synthesised by *R. mucronata* leaf extract against *Ae. aegypti* and *Cx. quinquefasciatus* larvae.

Species	Concentrations (mg/L)	Percentage of mortality	LC ₅₀ (mg/L)	UCL–LCL	LC ₉₀ (mg/L)	UCL–LCL	χ ²
<i>Ae. aegypti</i>	10.000	100.00±0.00					
	5.000	83.64±1.84					
	2.500	68.94±3.34	0.891	1.697–0.100	6.291	9.547–3.043	7.815*
	1.250	53.85±5.72					
	0.625	48.74±1.12					
<i>Cx. quinquefasciatus</i>	5.000	100.00±0.00					
	2.500	88.38±2.84					
	1.250	67.84±4.38	0.585	0.686–0.485	2.615	3.475–2.110	7.815*
	0.625	47.92±3.84					
	0.313	34.74±5.08					

Control–Nil mortality significant at * $P < 0.05$; LC₅₀–50% killing effect of the of the silver nanoparticles exposed larvae; LC₉₀–90% killing effect of the of the silver nanoparticles exposed larvae; UCL–Upper confidential limit; LCL–Lower confidential level; χ²–Chi square; values are represents as mean ± SD values.

2). Further the results of the XRD analysis showed 2θ intense values with various degrees (37.10°, 47.66°, 63.97° and 70.01°) and these results are corresponds to (111), (200), (220) and (311) Bragg's reflection^[12] based silver nanoparticles (Figure 3). The results of the FTIR spectra of the synthesised silver nanoparticles exhibited prominent peaks with (3 426.89, 2 925.49, 2 869.56, 2 346.95, 1 631.49, 1 031.73, 669.18 and 455.12) different values (Figure 4).

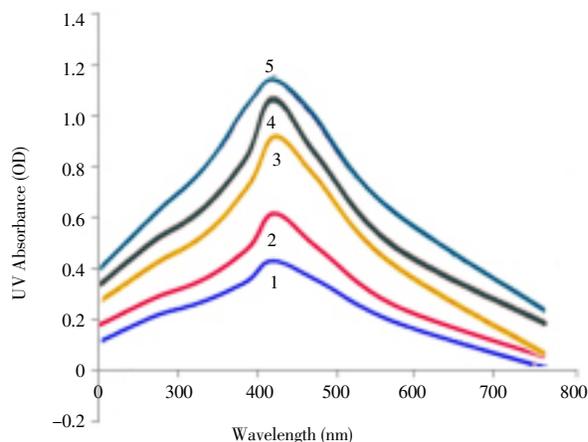


Figure 1. UV–VIS absorption spectra of silver nanoparticles synthesized by *R. mucronata* leaf extract with different time intervals. Number indicates different time intervals of the incubation period; 1= 15 min incubation; 2= 30 min incubation; 3= 4 h incubation; 4= 6 h incubation; 5= 8 h incubation.

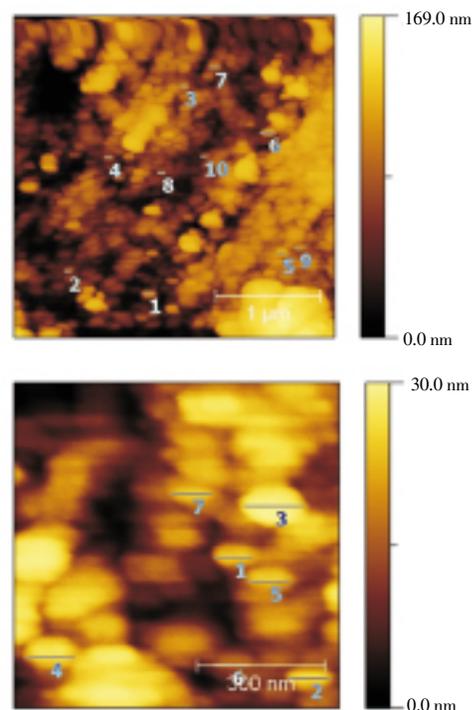


Figure 2. AFM topography of synthesised silver nanoparticles by *R. mucronata* leaf extract. Number indicates the different sizes of the synthesised nanoparticles with 60–95 nm ranges.

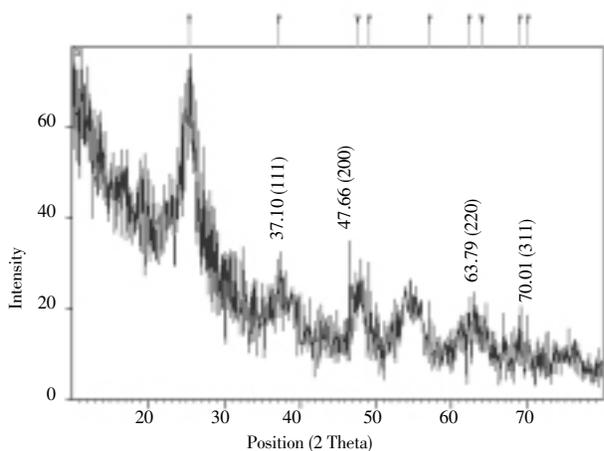


Figure 3. XRD analysis of the silver nanoparticles synthesised by *R. mucronata* leaf extract.

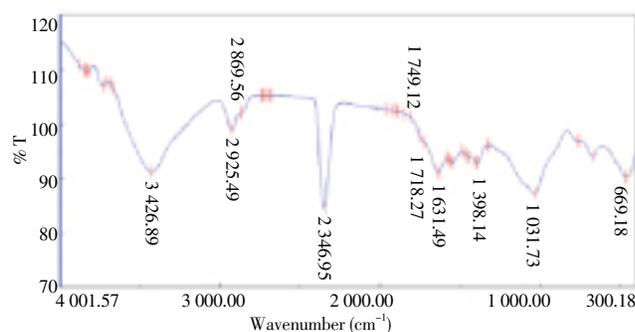


Figure 4. FTIR spectrum of silver nanoparticles synthesised by *R. mucronata* leaf extract.

4. Discussion

Generally, UV–VIS spectroscopy can be used to examine size and shape of the controlled nano particles in aqueous suspense. The results of the UV–VIS absorption showed increasing colour intensity with increased time intervals and this might be due to the production of the silver nanoparticles^[13] and the formation of the brownish yellow colour might be due to the excitation of the surface plasmon vibration of the synthesised silver nano particles^[14]. The result of the XRD pattern indicates the presence of sharp bands of Bragg peaks and this might be due to the stabilization of the synthesised nanoparticles by the leaf extract of the *R. mucronata* reducing agents, and thus confirming the crystallization of the bioorganic phase occurs on the surface of the silver nanoparticles^[15]. The results of the FTIR used to identify the possible bio molecules responsible for the stabilization of the synthesised silver nanoparticles. The prominent peaks of the FTIR results are showing the correspond values to the amide group (N–H stretching–3 426.89), aliphatic group (Cyclic CH₂–2 925.49), methyl group (CH₃–2 869.56), alkane group (CH–2 346.95) alkene (CC–1 631.49 and 669.178) and ether groups (COC–1 031.73). The observed peaks are considered as major functional groups in different chemical classes

such as flavonoids, triterpenoids and polyphenols^[16]. Hence, the terpenoids are proved to have good potential activity to convert the aldehyde groups to carboxylic acids in the metal ions. Further, amide groups are also responsible for the presence of the enzymes and these enzymes are responsible for the reduction synthesis and stabilization of the metal ions, further, polyphenols are also proved to have potential reducing agent in the synthesis of the silver nanoparticles^[16–18]. Previously, Baun *et al*^[19] reported that, the toxicity of C60–carbon nanotubes and titanium dioxide to an aquatic invertebrate *Daphnia magna*. Griffitt *et al*^[20] also reported that, the silver nanoparticles with embryonic injuries and reduced survivability in zebrafish and Sakulku *et al*^[21] also reported that, the encapsulated citronella oil nanoemulsion prepared by high pressure homogenization at varying amounts of surfactant and glycerol with *in vivo* mosquito protection. Mouchet *et al*^[22] reported the high mortality rate (85%) with doublewalled carbon nanotubes with the concentration of 500 mg/L against the larvae of *Xenopus laevis*. Similarly, Wang *et al*^[23] also reported that, the pesticidal activity with the nanoemulsion incorporated β –cypermethrin. In the present study, the biosynthesised silver nanoparticles from mangrove plant of *R. mucronata* leaf extract showed potential larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* larvae. Hence, the larvicidal activity of the silver nanoparticles might be due to the denaturation of the sulfur–containing proteins or phosphorous containing compound like DNA that, leads to the denaturation of organelles and enzymes^[24,25] and thus reduces the cellular membrane permeability and reduction in ATP synthesis which finally causes the lost of the cellular function and cell death^[26]. The size and shape of nanoparticles plays an important role in many of the pharmaceutical/industrial/biological applications. In this regard, the size of the synthesised nanoparticles was identified as 60–95 nm with various spherical shapes, which falls closer to many of the silver nanoparticles produced by other plant materials^[27,28]. It is concluded from the present findings that, the biosynthesised silver nanoparticles of leaf aqueous extract of *R. mucronata* provided potential killing effect of mosquito larvae's which could be used for prevention of several dreadful diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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