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**Green synthesis of Silver nanoparticle by  
*Acanthus ilicifolius* mangrove plant against  
*Armigeressubalbatus* and *Aedesaegypti* mosquito larvae**

**ABSTRACT**

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To identify the larvicidal activities of silver nanoparticles synthesized with *Acanthus ilicifolius* (*A. ilicifolius*) leaf extract against the larvae of *Armigeressubalbatus* and *Aedesaegypti*. *In vitro* larvicidal activities such as LC<sub>50</sub> and LC<sub>90</sub> were assessed for the *A. subalbatus* and *Ae. aegypti* larval species. Further, characterization using different techniques such as UV, SEM, EDAX, XRD and FTIR analysis were carried out for the synthesized silver nanoparticles. The LC<sub>50</sub> value of the synthesized silver nanoparticle was determined as 0.532 and 0.754 mg/L for *A. subalbatus* and *Ae. aegypti* respectively. Further, the LC<sub>90</sub> values are also determined as 2.13 and 5.98 mg/L for *A. subalbatus* and *Ae. aegypti* respectively. The synthesized silver nanoparticles have maximum absorption at 420 nm with the average diameter  $\square$  180 nm. The Energy Dispersive Analysis of X-rays (EDAX) spectrum revealed the presence of silver metal in synthesized nanomaterials. The XRD data showed 2 $\theta$  intense values with various degrees such as 37.10°, 47.66°, 63.97° and 70.01°. The FTIR data showed prominent band at 3,400 cm<sup>-1</sup> and is assigned to the O–H stretching of H-bonded alcohols and phenols. The band at 2,925 cm<sup>-1</sup> is attributed to O–H stretching of carboxylic acids. The band at 1,616 cm<sup>-1</sup>, corresponds to the N–H bending of primary amines. The bands at 1,444 and 1,521 cm<sup>-1</sup> are related to the C–C stretching of aromatic ring structure and the characteristic peak at 1,360 cm<sup>-1</sup> corresponds to the C–N stretching of aromatic amine group whereas in the region of 1,150–1,282 cm<sup>-1</sup> are corresponding to the C–C stretching alcohols, carboxylic acids, ethers and esters. The significance of above results clearly indicate that no toxicity developed biosynthesis of silver nanoparticles with leaf aqueous extract of *A. ilicifolius* provides potential source for the larvicidal activity against mosquito borne diseases.

**Keywords:** *Acanthus ilicifolius*; Biosynthesis; Dengue; LC<sub>50</sub>, LC<sub>90</sub>, Mangrove; Silver nanoparticles.

## INTRODUCTION

Mosquito borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases [1]. Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. causing millions of deaths every year. *Aedes aegypti*, a vector of dengue is widely distributed in the tropical and subtropical zones. Dengue fever incidence has increased fourfold since 1970 and nearly half the world's population is now at risk. In 1990, almost 30% of the world population, 1.5 billion people, lived in regions where the estimated risk of dengue transmission was greater than 50% [2]. *Anopheles stephensi* is the major malarial vector in India. With an annual incidence of 300– 500 million clinically manifest cases and a death toll of 1.1–2.7 million; malaria is still one of the most important communicable diseases. Currently about 40% of the world's population lives in areas where malaria is endemic [3,4]. *Armigeressubalbatus* and *Culexquinquefasciatus*, vectors of lymphatic filariasis and is widely spreading tropical diseases with around 120 million people infected worldwide and 44 million people having common chronic manifestation [4]. Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in the development of resistance [3-5], undesirable effects on non-target organisms, and fostered environmental and human health concern. Chemical control methods using synthetic insecticides are in practice due to their speedy action and ease of application. Use of chemical agents however results in environmental degradation in addition to accumulation of toxicants as residual deposits in non-target species.

The Ag NPs which are less like lyto cause ecological damage have been identified as potential replacement of synthetic chemical insecticides, hence the need to use green synthesized silver nanoparticles for the control of disease vectors [6]. In this regard, nanoparticles exhibits important role

in the several aspects such as drug delivery, diagnostics, antimicrobial activities and tissue engineering [7]. 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. The use of plants for synthesise of nanoparticles are rapid low cost, eco-friendly and safe for human therapeutic use [8]. *A. ilicifolius* is a mangrove survives in the coastal environment with fluctuating saline conditions. Hence these plants considered to be a rich source of steroids, tannins, triterpenoids, saponins, flavonoids and alkaloids. This plant was traditionally used as medicine for dyspepsia, paralysis, asthma, headache, rheumatism, and skin diseases [9-11]. The present study aimed to explore the larvicidal activity of green synthesized silver nanoparticles by using a mangrove plant *A. ilicifolius* leaf extracts.

## EXPERIMENTAL

### *Collection of Plant materials*

The fresh matured mangrove leaves of *A. ilicifolius* was collected from Pitchavaram mangrove forest (latitude 11°27' N, longitudes 79° 47'E) South East coast of India, Tamil Nadu. 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 (MSCASB23) was also maintained in the PG& Research Department of Biotechnology, Mohamed Sathak College of Arts and Science, Chennai, Tamil Nadu, India.

### *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 the mixture was boiled 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 AgNO<sub>3</sub> 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 formation of silver nanoparticles [7].

#### **Characterization of biosynthesized 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 at 10000 X g 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<sup>-1</sup> in KBr pellets. The dried mixture of silver nanoparticles was further analyzed with X-ray diffractometer (PAN analytical BV, The Netherlands) operated at a voltage of 40 kV and a current of 30 mA with Cu K  $\alpha$  radiation in a  $\theta$ -2 $\theta$  configuration. Additionally, a thin film of sample was also prepared in the cover slip with the 100 ml of the synthesized silver nanoparticles solution and allowed to dry for 5 min and slide was analyzed with SEM [12].

#### **Larvicidal bioassay (preliminary screening)**

The eggs and egg rafts of *Armigeressubalbatus* (*A. subalbatus*) and *Aedes aegypti* (*Ae. aegypti*) 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 days in standard environment (28 $\pm$ 2)<sup>o</sup>C temperature and 14:10 light and dark cycle; the larvae were fed with powdered mixture of dog biscuit and yeast powder 3:1 ratio to attain IV<sup>th</sup> instar. The larvicidal activity was assessed by the procedure of WHO guidelines [13] with some modification and as per the method of Gnana desigan *et al.* 2011[12]. 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 biosynthesized nanoparticles. Sterile distilled water and aqueous

extract of *A. ilicifolious* without nanoparticles served as control. Triplicates were maintained for each assay. Percentage of mortality was assessed after 24h of incubation. The experimental media in which 100% mortality of larvae occurs alone were selected for dose response bioassay [2-4].

#### **Dose dependent bio assay**

Based on the preliminary larvicidal screening tests, different concentrations of biosynthesized silver nanoparticles (10.000, 5.000, 2.500, 1.250 and 0.625 mg/L) were prepared for dose dependent 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 synthesized nanoparticles. Sterile distilled water without nanoparticles was served as control. Triplicates were maintained for each assay. Percentage of mortality was assessed after 24h incubation [12].

#### **Statistical analysis**

Statistical analysis such as LC<sub>50</sub>, LC<sub>90</sub>, 95% confidential limit and chi square values were calculated by using the Stat Plus 2009 software. Results with P<0.05 were considered to be statistically significant.

## **RESULTS AND DISCUSSION**

The results of the UV-VIS absorption showed increasing OD (1=15min incubation; 2=30 min incubation; 3=4 h incubation; 4=6h incubation; 5=8h incubation) intensity values with the different time intervals and this confirms the production of silver nano-particles (Figure 1) [14]. The formation of the dark brownish color of the extract might be due to the excitation of the surface plasmon vibration with the synthesized silver nanoparticles [15]. The results of the larvicidal activities showed the maximum (100%) percentage of larvicidal activity was identified with 5 mg/L and 10 mg/L concentration with *A. subalbatus* and *Ae. aegypti* fourth instar larvae (Table 1).

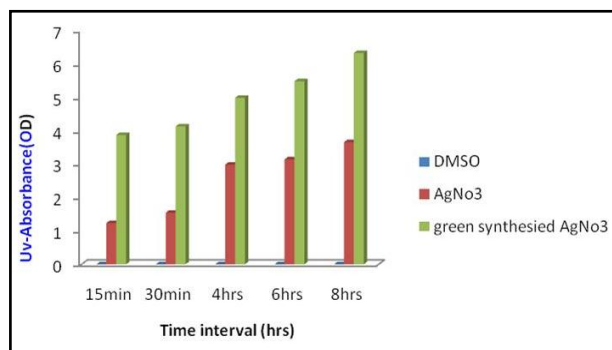


Fig. 1. UV-Vis analysis of silver nanoparticles from *A. ilicifolius*.

Table 1. Larvicidal activity of *A. ilicifolius* synthesized silver nanoparticles against *A. subalbatus* and *Ae. aegypti* larvae.

Concentration (mg/L)	Percentage of mortality in <i>A. subalbatus</i>	Percentage of mortality in <i>Ae. aegypti</i>	Control Extract of <i>A. ilicifolius</i>
20.00	100.00±0.00	100.00±0.00	38±2.1
10.00	100.00±0.00	100.00±0.00	21±0.87
5.00	100.00±0.00	79.34±9.31	11±1.1
2.50	92.43±6.87	68.12±6.90	-
1.25	85.92±5.46	59.63±3.76	-

Values are represented in Mean±SD

The results of the dose dependant assay suggested that, the LC<sub>50</sub> value was identified as 0.532 and 0.754mg/L for *A. subalbatus* and *Ae. aegypti*. Further, the LC<sub>90</sub> values were determined as 2.13 and 5.98mg/L for *A. subalbatus* and *Ae. aegypti*. The results of upper confidential level (UCL), lower confidential level (LCL) and chi square ( $\chi^2$ ) values are mentioned in Table 2. The larvicidal properties of the biosynthesized 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 and thus reduces the cellular membrane permeability and reduction in ATP synthesis which finally causes the loss of the cellular function and cell death [16]. The morphology of the silver nanoparticles was

determined by scanning electron microscopy. Figure 2 showed the SEM image of bio-synthesized silver nanoparticles. From the image it can be seen that the spherical morphology of silver nanoparticles is randomly distributed with average diameter 180 nm [17]. The Energy Dispersive Analysis of X-rays (EDAX) spectrum revealed the presence of silver metal in synthesized nanoparticles (Figure 3).

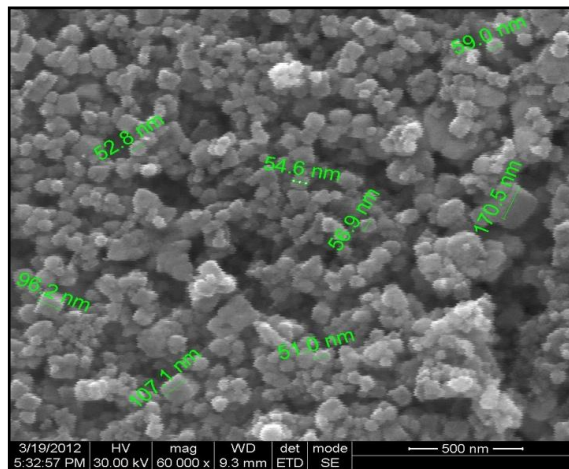


Fig. 2. Scanning electron microscopy (SEM) image of bio-synthesized silver nanoparticles from *A. ilicifolius* leaf extract.

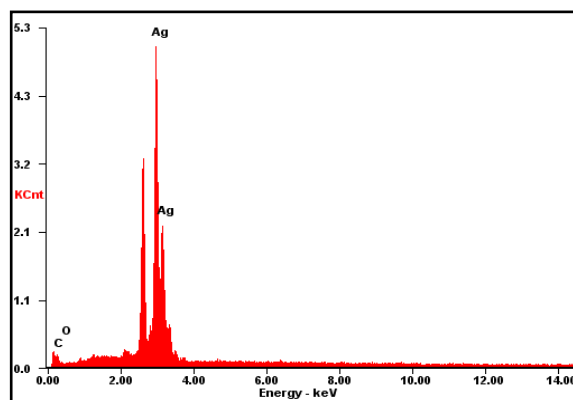


Fig. 3. EDAX spectrum revealed the presence of silver metal.

Figure 4 additionally, the results of the XRD analysis showed sharp peaks with various  $2\theta$  intense degree values ( $37.10^\circ$ ,  $47.66^\circ$ ,  $63.97^\circ$  and  $70.01^\circ$ ) and these results are corresponds to the standard [JCPDS No.89-3722 (111), (200) and (220)] planar values, and the formation of the sharp peaks might be due to the stabilization of the

synthesized nanoparticles by the reducing agents of the *A. ilicifoliosus* leaf extracts, and thus confirming the crystallization of the surface of the silver nanoparticles [18].

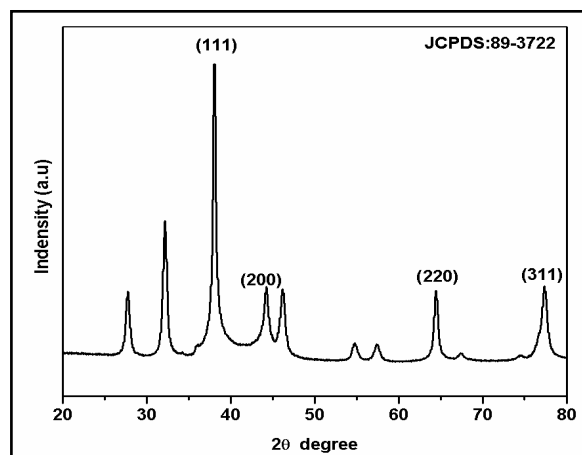


Fig. 4. XRD analysis of bio-synthesized silver nanoparticles from *A. ilicifoliosus* leaf extract

The FT-IR spectrum of silver nanoparticles is shown in Figure 5. The band at  $3,400\text{ cm}^{-1}$  is

assigned to the O–H stretching of H-bonded alcohols and phenols. The band at  $2,925\text{ cm}^{-1}$  is attributed to O–H stretching of carboxylic acids. The band at  $1,616\text{ cm}^{-1}$  corresponds to the N–H bending of primary amines. The bands at  $1,444$  and  $1,521\text{ cm}^{-1}$  are related to the C–C stretching of aromatic ring structure and the characteristic peak at  $1,360\text{ cm}^{-1}$  corresponds to the C–N stretching of aromatic amine group whereas in the region of  $1,150$ – $1,282\text{ cm}^{-1}$  are corresponding to the C–C stretching [11]. The results of FT-IR analysis showed the binding efficiency of several functional groups (alcohols, carboxylic acids, esters and ethers) with the metal to form the silver nanoparticles [19] and these groups are previously proved to have certain potential reducing agents with the major chemical classes [flavonoids, triterpenoids and polyphenols (20)] in the synthesis of silver nanoparticles [21]. The morphology of the silver nanoparticles was determined by scanning electron microscopy. Average size of the particle was identified as 50 nm with the Debye–Scherrer equation.

Table 2. Dose dependant larvicidal activity of silver nanoparticles synthesised by *A. ilicifoliosus* leaf extract Against *A. subalbatus* and *Ae. Aegypti*

Species	Concentration (mg/L)	Percentage of mortality	LC <sub>50</sub> (mg/L)	UCL-LCL	LC <sub>90</sub> (mg/L)	UCL-LCL	X <sup>2</sup>
<i>A. subalbatus</i>	10.000	100±0.00					
	5.000	88.3±4.56					
	2.500	66.56±3.09	0.754	1.98-0.65	5.98	6.12-4.32	6.815*
	1.250	50.34±2.12					
	0.625	46.19±1.19					
<i>Ae. aegypti</i>	5.000	100±0.00					
	2.500	76.87±4.19					
	1.250	61.87±3.01	0.532	0.684-0.41	2.13	1.92-3.65	6.815*
	0.625	47.12±2.14					

Control- Nil mortality significant at \*P<0.05; LC<sub>50</sub>-50% killing effect of the of the silver nanoparticles exposed larvae; LC<sub>90</sub>-90% killing effect of the of the silver nanoparticles exposed larvae; UCL-Upper confidential limit; LCL-Lower confidential level; X<sup>2</sup>-Chi square; values are represents as mean±SD values

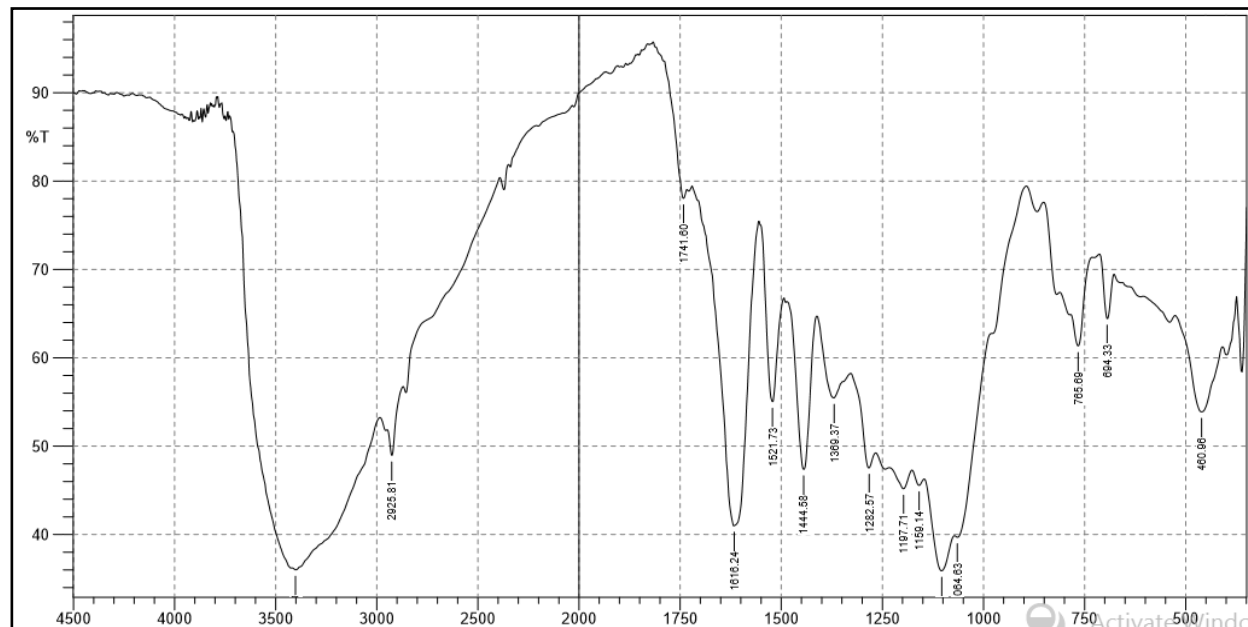


Fig. 5. FTIR spectrum analysis of bio-synthesised silver nanoparticles from *A. ilicifolius* leaf extract.

## CONCLUSIONS

It has concluded from the present findings that, the bio-synthesized silver nanoparticles of leaf aqueous extract of *A. ilicifolius* provided potential lethal effect on *A. subalbatus* and *Ae. Aegypti*. Which can be further analyzed for the lethal effect on other larvae too.

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