

# Green Synthesis of Silver Nanoparticles Using *Achyranthes bidentata* Leaf Extract and its Larvicidal Activity

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**Abstract:** Development of eco-friendly process for the synthesis of nanoparticles is one of the main steps in the area of nanotechnology research. One of the options to achieve this objective is to use natural biological processes. In the context of the current drive, this manuscript describes ways of synthesizing silver nanoparticles by using leaf extract of *Achyranthes bidentata*. The characteristics of the obtained silver nanoparticles were studied using Energy dispersive X-ray spectroscopy (EDX), Scanning electron microscopy (SEM) & Fourier transform Infrared spectroscopy (FT-IR). EDX analysis revealed the complete inorganic composition of the synthesized nanoparticles. SEM provided further insight into the morphology and size details of the silver nanoparticles. Furthermore, the synthesized nanoparticles were examined for toxic effects on the mortality of fourth instar larvae of *Aedes aegypti* and found to exhibit significant larvicidal activity. This method is considered as an innovative alternative approach using green nanochemistry technique to control vector parasites.

**Keywords:** Silver nanoparticles, Scanning electron microscopy, Energy dispersive X-ray spectroscopy, *Aedes aegypti*

## 1. Introduction

Nanobiotechnology is an emerging technical tool for development of eco-friendly and reliable methodology for synthesis of nanoscale materials. In modern nanoscience and technology, the interaction between inorganic nanoparticle and biological structures are one of the most exciting areas of research. The synthesis of nanomaterials is of current interest due to their wide variety of applications in fields such as electronics, photonics, catalysis, medicine etc. (Manorama *et al.*, 2012)

Silver nanoparticles play a profound role in the field of biology and medicine due to their attractive physiochemical properties. Silver products have long been known to have strong inhibitory and bactericidal effects, as well as a broad spectrum of antimicrobial activities, which has been used for centuries to prevent and treat various diseases (Shankar *et al.*, 2004). Silver nanoparticles are reported to possess anti-fungal (Wiley *et al.*, 2006), anti-inflammatory, anti-viral activity (Panacek *et al.*, 2009). There are many methods available for synthesis of silver nanoparticles but green synthesis have been reported to have many advantages such as eco-friendly, easy controlled economic viability and no need to use high pressure, energy, temperature and toxic chemicals in synthesis protocol. In literature, there are reports of the synthesis of silver nanoparticles using extracts of different plants (Rodriguez-Leon *et al.*, 2013).

*Achyranthes bidentata*, a member of Amaranthaceae (Tamil name: Sigappu Nayurivi) is an erect, annual herb distributed and grown in hilly districts of India. The plant is used in indigenous system of medicine as emmenagogue, antiarthritic, antispasmodic, antihelmenthic, diuretic and antitumor (Ratra & Misra, 1970). Also it is useful to treat cough, fistula, chronic malaria and snakebite (Selvanayagam *et al.*, 1994). Hence with respect to above stated information

synthesis of silver nanoparticles from the leaves of *Achyranthes bidentata* were carried out.

In spite of the wide usage of silver nanoparticles there are very few reports on the toxicity of silver nanoparticles (Hunt *et al.*, 2013). Singh *et al.* (2013) reported the toxicity and bioaccumulation of nanoparticles in living systems. The potential for adverse health effects due to prolonged exposure at various concentration levels in living organisms and their behavior has not yet been established. As need of study, larvae of *Aedes aegypti* (L) was taken as model organism to test the larvicidal activity of synthesized silver nanoparticles.

*Aedes aegypti* (L) is a fresh water breeding mosquito and is the vector of dengue. *Aedes aegypti* (L) is very closely associated with the human habitat. The geographical range of *Aedes aegypti* is increasing in part due to rapid urbanization and increased global movement of people (Manzoor *et al.*, 2013). The WHO currently estimates that there may be 50 million cases of dengue fever infection worldwide every year (WHO, 1996). One approach to decrease the mosquito population attempts to interrupt the mosquito life cycle at the larval stage. Larviciding is a successful way of reducing mosquito population in their breeding places before they emerge into adults. Here in, we report the green synthesis of silver nanoparticles from leaves of *Achyranthes bidentata* and its significant larvicidal activity.

## 2. Materials & Methods

### 2.1 Preparation of Plant Extract

Fresh leaves of *Achyranthes bidentata* were collected, washed and air dried for a week at room temperature. The dried leaves were ground into fine powder and stored in a

dry airtight container. The produced leaf samples were mixed with 10mL of distilled water. The mixture was ground using mortar and pestle, boiled in water bath for 10 mins at 60°C and filtered through Whatmann No.1 filter paper. The extract thus obtained was used for further analysis.

## 2.2 Biosynthesis of silver nanoparticles

1mM aqueous solution of silver nitrate was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Achyranthes bidentata* leaf extract was taken and added into 90 ml of aqueous solution of 1mM silver nitrate and incubated at room temperature. Brownish yellow solution was formed indicating the successful formation of silver nanoparticles. (Usha & Rachel, 2014)

## 2.3 UV-Vis Spectrum Analysis

The bioreduction of reaction mixture of pure silver ions was observed by observing the UV-Visible spectrum at different time intervals taking 1mL of the sample, compared with 1 mL of distilled water as blank. UV-Visible spectral analysis has been done from 200 to 700nm after diluting a small volume of aliquot of 100µl of the plant extract with 1 mL deionized water. (Jisha *et al.*, 2012)

## 2.4 Lyophilization of silver nanoparticles

Broths containing silver nanoparticles were centrifuged at 10,000 rpm for 15 mins. and the pellet was washed thrice with sterile distilled water (Usha & Rachel, 2014). The purified pellets thus formed were then freeze dried and lyophilized. The lyophilized silver nanoparticles were used for further analysis.

## 2.5 SEM Analysis

Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 mins (Prashanth & Balaji 2011).

## 2.6 EDX Analysis

EDX analysis was carried out for the detection and confirmation of elemental silver. Very small amount of the sample was drop coated onto carbon film and analysed for the composition of the synthesized nanoparticles (Vigneshwaran *et al.*, 2007)

## 2.7 FT-IR Analysis

Silver nanoparticles were characterized by FT-IR. The lyophilized silver nanoparticles were grinded with potassium bromide crystals and spectrum was recorded in the transmittance mode. The spectrum was obtained in the mid IR region of 400-4000cm<sup>-1</sup> (Geetha *et al.*, 2012)

## 2.8 Collection of Mosquito Larvae

The egg rafts of *Aedes aegypti* (L) (Diptera: Culicidae) mosquitoes were procured from Centre for Research in Medical Entomology, Madurai, Tamil Nadu. The egg rafts were kept in a tray containing tap water at laboratory condition (32±1°C). After 24 hrs of incubation, the eggs were observed to hatch out into first instar larvae. The fourth instar larvae were used in the present study.

## 2.9 Screening of Larvicidal Activity of Silver Nanoparticles

Larvicidal activity of silver nanoparticles were analysed as per the standard procedures recommended by World Health Organization (WHO, 1996). Randomly twenty, fourth instar larvae were placed in 200 ml sterilized double-distilled water with a photoperiod of 14-h light, 10-h dark cycle. The silver nanoparticle solutions were diluted using double distilled water according to the desired concentrations: 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, 50 mg/L respectively. A control (Plant extract) was maintained separately. At each test concentration, four trials were made and tested for anti-larval effects. The organisms were observed regularly with an interval of 4 hours for 2 days. The numbers of dead larvae were counted from the initial exposure to synthesized silver nanoparticles. (Singh *et al.*, 2013)

## 3. Results & Discussion

The appearance of brown color in the reaction vessels suggested the formation of silver nanoparticles. The brown color of the medium could be due to the excitation of surface plasmon vibrations, typical of silver nanoparticles. Silver nanoparticles are known to exhibit a UV-visible absorption maximum in the range of 400-500nm (Thangaraju *et al.*, 2012). In the present study, the surface plasmon resonance peak in the UV-Vis absorption spectra of the silver nanoparticles synthesized by biological reduction showed an absorption peak at 420 nm. The scanning electron microscope (SEM) has been employed to characterize the size, shape and morphology of synthesized silver nanoparticles. The SEM image showed high density nanoparticles of approximately 40nm (Fig.1)

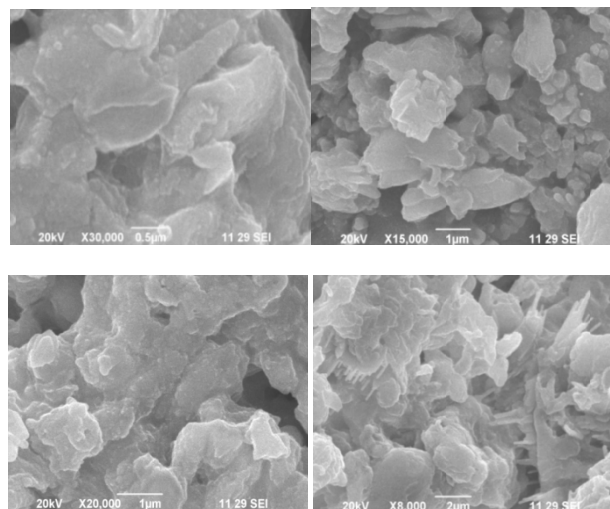
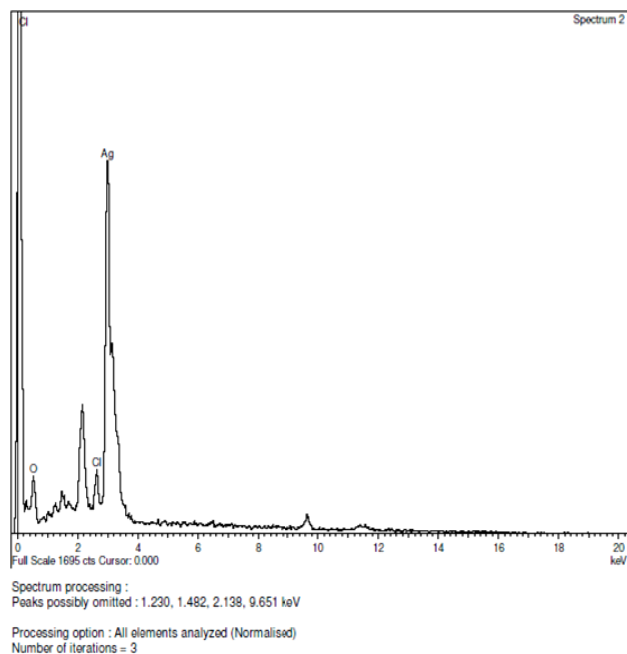


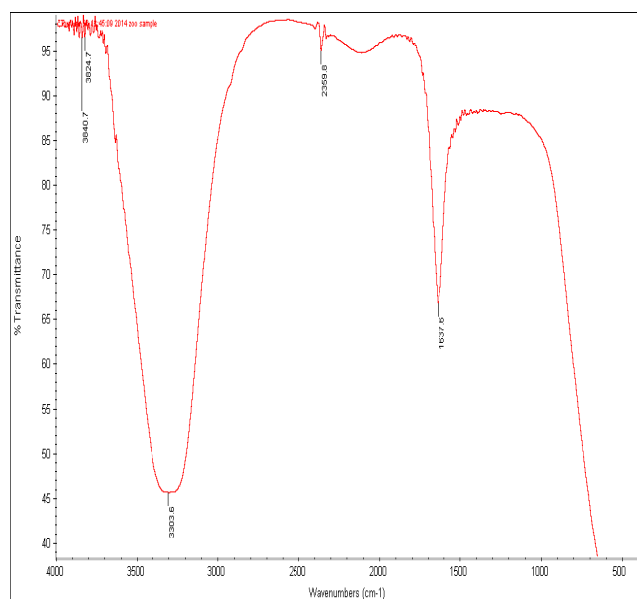
Figure 1: SEM image of silver nanoparticles

The elemental analysis of biologically synthesized nanoparticles was done by EDX (Fig. 2) where strong optical absorption peaks were observed approximately at 3keV, which is typical for absorption of metallic silver nanoparticles (Mouxing *et al.*, 2006).



**Figure 2:** EDX spectra of silver nanoparticles

FT-IR spectrum was carried out to recognize the possible biomolecules accountable for the reduction of silver ions and capping of the bioreduced silver nanoparticles synthesized by *A. bidentata* leaf extract. The FT-IR spectrum of silver nanoparticles is shown in Fig.3. The band at 3303.6  $\text{cm}^{-1}$  corresponds to O-H stretching H-bonded alcohols and phenols. The assignment at 1637.6  $\text{cm}^{-1}$  corresponds to the amide bond of proteins arising due to carbonyl stretch in protein. Carbonyl group from amino acid and proteins has the stronger ability to bind to metal indicating that proteins could possibly form the metal nanoparticles (Singh *et al.*, 2012).



**Figure 3:** FT-IR Spectra of silver nanoparticles

The larvicidal activity of silver nanoparticles against larvae of *Aedes aegypti* is presented in table 1. The silver nanoparticles at 10 mg/L slightly decreased the survival of larvae at 32 hrs of exposure, while 100% mortality of the larval population was observed in concentration of 50 mg/L at 16 hrs of exposure. The nanoparticle at 30 mg/l killed the larvae slowly and nearly 80% mortality was observed at 44 hrs of exposure. The data obtained from the present study clearly indicates that silver nanoparticles could provide an excellent larval control of *Aedes aegypti*. Major reduction on the bodily movements, especially the wriggling activity of larvae was found to decrease with increase in time of exposure to silver nanoparticles. This behavioral change is due to the binding of silver nanoparticles to proteins leading to the denaturation of cell organelles and enzymes. Subsequently, a loss in cellular function finally leads to cell death (Singh *et al.*, 2013)

**Table 1:** Screening of larvicidal activity against fourth instar *A. aegypti* larvae

Time (Hr)	Control	10 mg/L	20 mg/L	30 mg/L	40 mg/L	50 mg/L
0	0	0	0	0	0	0
4	0	0	0	0	2	4
8	0	0	0	0	7	8
12	0	0	0	0	13	16
16	0	0	0	1	16	20
20	0	0	0	3	18	20
24	0	0	1	4	20	20
28	0	0	2	6	20	20
32	0	0	4	10	20	20
36	0	1	5	12	20	20
40	0	2	6	14	20	20
44	0	3	8	16	20	20
48	0	4	10	18	20	20

#### 4. Conclusion

In the present investigation, environmentally benevolent biogenic method is used for the synthesis of silver nanoparticles from the leaf extract of *Achyranthes bidentata*. The biomolecules present in the leaves were responsible for the reduction of silver to silver nanoparticles. The synthesized nanoparticles ranged in size from 20-40 nm as shown by SEM. The results of larvicidal bioassay clearly indicate that silver nanoparticles could provide an excellent larval control of *Aedes aegypti*. Further research is required to gain insight into the bioactive principles and their mode of action in effectively using plant mediated silver nanoparticles as an anti-mosquito product.

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## Author Profile

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