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Effect of silver nanoparticles synthesized from *Aristolochia bracteata* against IV instar larva and pupa of dengue and chikungunya vector, *Aedes aegypti*

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Abstract
In the present study the larvicidal and pupicidal activity of green synthesized silver nanoparticles by the aqueous leaf extract of *Aristolochia bracteata* against *Aedes aegypti* was carried out in laboratory condition. The silver nanoparticle synthesis was primarily confirmed by the of brown colour formation in the reaction mixture within 1 day incubation period. The silver nanoparticles synthesis in the reaction mixture was further confirmed by UV-Vis spectroscopy showed an absorption band at 371.50nm. SEM analysis provided the shape of sliver nanoparticle was mostly aggregated and spherical and the size of the green synthesized silver nanoparticle from *Aristolochia bracteata* was 18 to 352nm. FTIR analysis showed the synthesizes silver nanoparticles had many absorption bands. LC_{50} values were calculated. Larval mortality, pupal mortality and adult mortality of *Aedes aegypti* against *Aristolochia bracteata* were noted.

Keywords: Silver nanoparticles, *Aristolochia bracteata*, *Aedes aegypt*, UV-Vis spectroscopy, SEM, FTIR, Partcle size analysis

1. Introduction
Aedes aegypti is responsible for spreading Dengue and Chikungunya. Dengue is prevalent throughout the tropics and subtropics. *Aedes* mosquito on the other hand are also painful and persistent biters. The incidence of dengue has grown dramatically around the world in recent decades. Over 2.5 billion over 40% of the world's population are bow at risk from dengue. WHO currently estimates there may be 50-100 million dengue infections worldwide every year. Dengue is mainly vectored by *Aedes* mosquitoes (i.e. *Aedes aegypti* and, to a lesser extent, *Aedes albopictus*). The actual numbers of dengue cases are underreported and many cases are misclassified. 3900 million people, in 128 countries, are at risk of infection with dengue viruses (Bhatt *et al.,* 2013) [2]. Four distinct, but closely related, serotypes of the virus cause dengue (DEN-1, DEN-2, DEN-3, and DEN- 4). Recovery from infection by one provides lifelong immunity against that particular serotype. However, cross-immunity to the other serotypes after recovery is only partial and temporary. Currently, there is no specific treatment for dengue (WHO, 2015; Sujitha *et al.,* 2015) [9]. In this scenario, mosquito vector control is an important prevention tool. Indoors residual spraying and insecticide treated bed nets are also employed to reduce transmission of Dengue in tropical countries. However, these chemicals lead to high operational costs, strong negative effects on human health and the environment, and can select for resistance in a number of mosquito species (Benelli *et al.* 2015) [1]. Recently, eco-friendly control tools have been implemented to enhance mosquito control. Significant efforts have been carried out investigating the efficacy of botanical products, and many plant-borne compounds have been reported as excellent toxins against mosquitoes, acting as adulticidal, larvicidal, ovicidal, oviposition deterrent, growth and/or reproduction inhibitors and or adult repellents (Benelli *et al.,* 2015) [1] Botanicals can be used as alternative to synthetic insecticides or along with other insecticides under integrated vector control programs. The plant product of phytochemical, which is used as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites. Phytochemicals obtained from the whole plant or specific part of the plant by the extraction with different types of solvent such as aqueous, methanol, chloroform, benzene, ethyl acetate, acetone, etc., depending on the polarity of the phytochemical. Some phytochemicals act as toxicant (insecticide) both against adult as well as larval stages of mosquitoes, while others interfere with growth and growth inhibitor or with reproduction or produce an olfactory stimulus, thus acting as repellent or attractant **(**Krishnappa and Elumalai, 2013) [6].
In the present investigation *Aristolochea bracteata* was taken and silver nanoparticles were synthesaized in the leaf extract of selected plant. *Aristolochea bracteata* (Aristolochiaceae) commonly called as Worm killer in English and aaduteendapaalai in Tamil, widely distributed in Deccan Gujarat, Western and southern india, Bihar, Sindh, Bundelkhand and Bengal. The larvicidal activity of biosynthesized silver nanoparticles using the plant extracts has been tested against the dengue vector *Ae. albopictus and Ae.aegypti*. Hence the present investigation was aimed to synthesis of silver nanoparticles using a aqueous leaf extract of *Aristolochia bracteata* and evaluate its larvicidal activity against dengue vector.

2. Materials and methods
Experimental plant: *Aristolochia bracteata*
Preparation of Plant extract
The Dried leaves were grinded to fine powder using a blender. Ten grams of leaf powder was added into each three Erlenmeyer flasks containing 100 ml of double distilled water. This suspension was mixed well and left for 5 hours without disturbance, and the extracts obtained were

filtered through Whatman No. 1 filter paper. The filtrate was used to find out the lavicidal and pupicidal activity against the target vector

Silver nitrate preparation
Silver nitrate was used as precursor for the synthesis of silver nanoparticles. Analytical grade, silver nitrate (AgNO3) was prepared for 16.96 mg of silver nitrate was carefully weighed and dissolved in 90 ml of Milli-Q-water. This aqueous Silver nitrate solution was always prepared fresh.

Collection and maintenance of target vector
Different larval instars and pupae of *Ae.aegypti* were collected from the Indian Council for Medical Research, Madurai and were brought to the laboratory safely without disturbance. These larvae and pupae were maintained in enamel trays containing deionized water and allowed to feed on brewer's yeast, dog biscuits and sucrose in a 3:1:1 ratio in the laboratory at room temperature for 24 hours, before start of the experiment.

Synthesis of silver nanoparticles from leaf extract
Aqueous leaf extract of *Aristolochia bracteata* was prepared by placing 10 g of chopped fresh leaves in a 250 ml Erlenmeyar flask and boiled with 100 ml of sterile double distilled water up to 60 min at 600C in a water bath. The crude extract was passed through Whatmann filter paper (no.1), and the filtrates (aqueous leaf extract) were stored at 40C and used within 3 days. Ten millititre of aqueous leaf extract was treated with 90 ml of prepared 1mM aqueous Ag NO3Solution in an Erlenmeyer flask and incubated in dark at room temperature. The aqueous solution of 1mM of Ag NO3 was leading to change of pale yellow to dark brown resulting in synthesis of Ag NPs

Characterization of synthesized silver nanoparticle
The synthesized silver nanoparticles were characterized by carrying following analysis
UV-Visible spectral analysis
Particle size analysis
Scanning Electron Microscopy
Fourier Transform Infra Red Spectroscopy

Larvicidal and pupicidal activity
The larvicidal and pupicidal activity was evaluated using WHO method (1996) with slight modifications. Different test concentrations of leaf extract and AgNPs in 200 ml de-ionized water were prepared in 250 ml capacity autoclaved glass bottles. Bio – efficacy test was conducted against the larvae and pupa of target vector at ten different concentrations of aqueous leaf extract and synthesized AgNPs, 10 larvae were exposed to each test at different concentration. Similarly, each test included a set of control group(distilled water) with ten replicates for each individual concentration. Mortality rate was recorded after 24 h of exposure period. The dead larvae in ten replicates were combined expressed as a percentage of larval and pupal mortality for each concentration.

Statistical analysis
The results obtained were subjected to statistical analysis to ascertain their credibility. Standard deviation and mean separation statistical tools were employed for analysis of larval and pupal mortality obtained in the present investigation using computer software. The dose response mortality data were concerned to probit analysis for finding the LC50, upper and lower confidence limit at 95 % confidence, and values determined using the software.

3. Results and Discussion
The present study gives the evidences that *Aristolochia bracteata* aqueous leaf extract was found to be a successful agent for the synthersis of silver nanoparticles and can be used as a biological agent for the control of *Ae. aegypti* larvae.

Visible observation of silver nanoparticles synthesis
The fresh suspension of *Aristolochia bracteata* was yellowish-green in colour after addition of AgNO3 the yellowish-green colour transferred to dark brown within 5 hours incubation at room temperature.

Characterization of silver nanoparticles synthesis
UV- Visible Spectroscopy analysis
The UV-Visible spectra showed an absorption band at 371.50 nm which corresponds to the absorbance of silver nanoparticles. This absorption is unique property of metal nanoparticles called SPR (Surface Plasmon Resonance) that arise due to conduction of electrons on surface of silver nanoparticles. The Surface plasmon resonance (SPR) of the AgNPs produces a peak at 420nm Kamalakannan *et al.,* (2014) [4] for *Penecillium verucosum*.

Particle size analysis
The size of the silver nanoparticles synthesized from *Aristolochia bracteata* was predicted as 18 to 352 nm. Earlier study reported by Khan *et al.,* (2016) [5] should that the particles were analysed based on the mass median diameter which indicates the 50% diameter of the particle comprising smaller particles.

SEM analysis of silver nanoparticles
SEM analysis revealed that the particles were mostly aggregated and spherical in shape. Similar shape of nanoparicles was synthesized from Krishnappa *et al.,* 2013 [6]

FTIR Analysis
Fourier transform infrared (FTIR) spectroscopy measurements were obtained using dry powders of the nanoparticles. The FTIR spectrum produced silver nanoparticles had many absorption bands and the absorption bands seen at 601.75cm-1, 753.15cm-1, 1094.53 cm-1, 1122.49 cm-1, 1195.78 cm-1, 1400.22cm-1, 1638.42 cm-1, 2089.73 cm-1, 2828.41 cm-1, 2503.43 cm-1, 3579.64 cm-1, 3872.8 cm-1were assigned to the C-Cl stretching of alkyl halides, C-Cl stretching of alkyl halides, C-F stretch of alkyl halides, C-O stretch of alcohols, C-O stretch of alcohols and -C-H stretch of Alkanes, N-H bend of amides, N=C=S stretching of isothiocyanate, O-H stretch of carboxylic acids, O-H stretch of carboxylic acids, O-H bending of alcohols, N-H stretch of Amines. Similar results were reported by Kumar *et al.,* (2014) [7]. The spectrum exhibits the band at 1369 cm-1correspondinng to alkane group, 3435 cm-1 which' represents the hydroxyl group.

Larvicidal activity and pupicidal activity

In the present study, the leaf extract of *Aristolochia bracteata* was treated against the fourth instars larvae of mosquito of *Aedes aegypti*. The results are as follows:

LC50 Values for the aqueous leaf extract and synthesized silver nanoparticles were calculated against the IV instar larvae of *Ae. aegypti* using probit analysis. The LC50 the aqueous leaf extract against *Ae. aegypti* was 40.084ppm and LC50 of the synthesized silver nanoparticles against *Ae. aegypti* was 4.220ppm (Table 1). Similar results were observed *Elemike et al.,* (2017) [3] in larvicidal activity of synthesized silver nanoparticles utilizing an aqueous extract from *Eupatorium odoratum* against *Culex quinquefasciatus*.

The larval mortality of aqueous leaf extract increased from 1-9 larvae with the concentration from 10-100 ppm. The larval mortality by silver nanoparticles synthesized aqueous leaf extract increased from 1-10 larvae with the concentration from 1.0-10.0 ppm (Table 2 and 3). Similar results were observed by Mondal *et al.,* (2014) in the aqueous plant extract of *P. hysterophorus* against *Cx. quinquefasciatus.* The percentage mortality increased by increasing the dose and time of exposure of larvae. Two pupae were dead at 30 ppm and one pupa was dead in 20, 40, 50, 70, 80, 90 and 100 ppm. No pupal mortality in control The pupal mortality was recorded in the silver nanoparticles synthesized aqueous leaf extract. Two pupae were dead in 3.0 ppm and 7.0 ppm and one pupa were dead in 2.0, 4.0, 5.0, 6.0 and 8.0 ppm concentration. No pupal mortality in control (Table 2 and 3). Kamalakannan *et al.,* (2014) [4], for the pupae of *Ae. aegypti* that are susceptible to the *Penicillium verrucosum* synthesized silver nanoparticles than the pupae of *Culex quinquefasciatus.* The adult mortality was not found in aqueous leaf extract and silver nanoparticles synthesized aqueous leaf extract at various concentrations (Table 2 and 3). Similar results also observed in the acetone and aqueous leaf extract of *Ptaeroxylin obliquum* and *Pittosporum viridiflorum* against the malarial vector *Anopheles arabiensis* Maharaj *et al.,* (2011) [8].

The total mortality increased from 10-90% with the increasing concentrations of aqueous leaf extract from 10-100 ppm (Table 4). The total mortality increased from 10-100% with increasing concentration of silver nanoparticles synthesized from aqueous leaf extract of *Aristolochia bracteata* from 1.0-10.0 ppm

Adult emergence was found to be 70% in 10 ppm, 70% in 20 ppm, 50% in 30 ppm, 40% in 40 ppm, 30% in 50 ppm, 65% in 40 ppm, 60% in 50 and 60 ppm, 55% in 70 ppm, 50% in 80 ppm, 45% in 90 ppm and 30% in 100 ppm in aqueous leaf extract (Table 6; Fig 4). In silver nanoparticles synthesized from aqueous leaf extract of *Aristolochia bracteata*, the adult emergence was found to be 90% in 1.0 ppm, 8o% in 2.0 ppm, 50% in 3.0 ppm, 50% in 4.0 ppm, 40% in 5.0 ppm, 30% in 6.0 ppm, 10% in 7.0 ppm, 10% in 8.0 ppm.

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Table 1: LC_{50} values of the test solutions for aqueous leaf extract and silver nanoparticles synthesized aqueous leaf extract of *Aristolochia bracteata* against IV instar larvae of the mosquito *Aedes aegypti*.

Test solution	LC50 values in ppm
Aqueous extract of leaves *Aristolochia bracteata*	40.084
AgNPs synthesized extract of leaves of *Aristolochia bracteata*	4.220

Table 2: Effect of different concentrations of aqueous leaf extract on the larval and pupal period, pupal and adult mortality, percentage of total mortality and adult emergence on the IV instar larvae of *Aedes aegypti.*

S. No	Parametres	Control	Concentration of aqueous leaf extract in ppm									
			1	2	3	4	5	6	7	8	9	10
1	Larval period in days	4	4	4	4	4	4	4	4	4	4	4
2	Pupal period in days	2	2	2	2	2	2	2	2	2	2	2
3	Larval mortality	0	1	2	3	5	6	6	7	8	9	9
4	Pupal mortality	0	0	1	2	1	1	1	0	0	1	1
5	Adult mortality	0	0	0	0	0	0	0	0	0	0	0
6	Total mortality	0	30	30	50	60	70	70	70	80	100	100
7	Adult emergence %	100	70	70	50	40	30	30	30	20	0	0

Table 3: Effect of different concentrations of silver nanoparticles synthesized from aqueous leaf extract on the larval and pupal period, pupal and adult mortality, percentage of total mortality and adult emergence on the IV instar larvae of *Aedes aegypti.*

S. No	Parametres	Control	Concentration of silver nanoparticles synthesized from aqueous leaf extract in ppm									
			1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
1	Larval period in days	4	4	4	4	4	4	4	4	4	4	4
2	Pupal period in days	2	2	2	2	2	2	2	2	2	2	2
3	Larval mortality	0	1	1	3	4	5	6	7	8	10	10
4	Pupal mortality	0	0	1	2	1	1	1	2	1	0	0
5	Adult mortality	0	0	0	0	0	0	0	0	0	0	0
6	Total mortality	0	10	20	50	50	60	70	90	90	100	100
7	Adult emergence %	100	90	80	50	50	40	30	10	10	0	0

4. Conclusion

From the present investigation, it is observed that silver nanoparticles synthesized from the aqueous leaf extract of *Aristolochia bracteata* had a telling effect on the mortality of larvae and pupae of filarial mosquito *Aedes aegypti*. Since, the silver nanoparticles may become potential agents for the control of vector borne disease in future which will definitely become an eco friendly approach.

5. References

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