ENTOMOLOGY

Nilgirianthus ciliatus mediated environment friendly extracellular synthesis of AgNps to exact its potential against Dengue vector, *Aedes aegypti* and *Staphylococcus aureus*

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Abstract

Aedes aegypti, the dengue vector, is a menace continuing since 1780; it is due to development of resistant to synthetic insecticides and *Staphylococcus aureus,* the common microbial pathogen agent of food poisoning, skin infections and respiratory infections have developed multidrug resistant, which forced us to focus on novel agent for which the dengue vector and bacterial pathogen have not practiced to develop resistant and which cannot detoxify it using its usual enzyme activity as it did earlier. Silver nanoparticles a challenging insecticidal agents for the toxic degrading enzymes of both the insect and microbe was the target of our present study to excavate the potential in killing immature of dengue vector and bacterial pathogen. Green protocolled nanoparticles were successfully synthesized using leaf extract of *Nilgirianthus ciliatus* which act as reducing as well as capping agents. The UV-vis spectra observed at 441nm confirmed the presence of silver nanoparticles. XRD and FT-IR confirmed the crystalline nature and organic capping around the silver nanoparticles respectively. SEM and DLS showed the average size at 117 nm and the particle dispersion was at -17.2mV zeta potential.

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Introduction

Mosquitoes take the lead in causing human diseases and killing humans and other animals around the world, due to their ability to carrying pathogens from individual to individual. Millions of lives are lost and still being victims for her hunger (Benelli *et al*., 2017; Santhosh *et al*., 2015). Dengue and chikungunya killer arbovirus diseases transmitted by the adult female *Aedes aegypti* which are highly adaptable to biotic stimuli (Prado *et al*., 2017).The pathogen travel from human to human is mediated by *Aedes aegypti* (L.) as the primary vector (Li *et al*., 2012).This species is also a vector of few other diseases in human like yellow fever, Zika fever and Mayaro virus disease (Suganya *et al*., 2017). This species is a serious vector of infectious diseases threatening around 2.5 billion people around the globe (Hafeez *et al*., 2017). *Aedes aegypti* mediated infections affected 50-100 million cases every year worldwide with atleast half million victims have lost their lives with a five year period from 2010 to 2015 (Singla *et al*., 2016).

Management of this vector was failed since many generations due to human activity (providing breeding sites), population (providing maximum hosts), environmental factors (providing optimum condition for growth & development), resistant development (capable for detoxifying target insecticides) and many other (Ghosh *et al*., 2012). DDT, dieldrin, organophosphrous, fenithothion and propoxurare the insecticides used in India to control the vector mosquito, whereas, in very few generations they started metabolizing these foreign agents (Vasantha *et al*., 2016). Moreover, these mosquito control agents remind back as pollutant causing side effects and started reducing the non-target populations leading to an ecological imbalance (Mathew *et al*., 2015). Further bioaccumulation of these synthetic insecticides in aquatic

human feeds had few impacts on human health and other animals as well (Strode *et al*., 2012; Gambarra *et al*., 2013; Garcez *et al*., 2013; Pereira *et al*., 2014).

Staphylococcu aureus a widespread bacterial food-poisoning pathogen common in dairy industries worldwide (Zecconi *et al*., 2006; Persson *et al*., 2011). It also leads to pneumonia, bacteraemia, wound infections, surgical site infections and sepsis (Adibhesami *et al*., 2017). Nasopharyngeal colonization of *Staphylococcus aureus* was detected in 7.38% children (Chande *et al*., 2009). This bacterial pathogen is also a cause of respiratory, skin and tissue disease and infection in vascular grafts (Edwards *et al*., 2012; Shak *et al*., 2013). Problem with treating *Staphylococcus aureus* infections is development of resistance in it to all available antibiotics as well as to the evolving new antibiotics (Ha & Fowler, 2013). Indeed, Methicillin Resistant *Staphylococcus aureus* (MRSA) strains have long been a problem in hospital and community setting worldwide (Cuny *et al*., 2010)

In order to overcome the problem nanotechnology will be a best alternative. Development in novel instrumentations and reliable techniques progression of nanotechnology is being very fast leading to novel therapeutic interventions (Hamed *et al*., 2017). At present nanotechnology left no field behind becoming a promising research domain with broad range of application including antibacterial drugs and vector control programs (Muthukumaran *et al*., 2015).Eco-friendly approach for the synthesis of nanoparticles replacing synthetic chemicals as reducing and capping agents will use less energy, less or non-toxic for handling, no bioaccumulation and much effective as it targets the intracellular components (Kumar *et al*., 2017). Moreover, the organic capping of metal nanoparticles from the natural reducers reduces the toxicity of metal nanoparticles (Priya *et al*., 2016).

Bacteria, fungi and plant products are the important bio reducing agents in synthesis of nanoparticles. The active secondary metabolites play a major role in reduction and capping of metal ions forming nanoparticles (Nareshkumar *et al*., 2013).In the present study, *Nilgirianthus ciliatus* a shrub in Acanthaceae family with high potential medicinal compounds was used as a bioreductant in synthesis of silver nanoparticles.

Nilgirianthus ciliatus is an aromatic slender shrub of Western Ghats with extensive applications in Ayurveda and used in traditional culture to treat various diseases (Rameshkumar *et al*., 2015; Rani *et al*., 2013). Aquatic and solvent extracts of this plant also posses antibacterial and antifungal activities (Neethu *et al*., 2014). Terpenoids, flavonoids, phytosterols, phenolic compounds, fixed oils and carbohydrates were reported in this plants which has the capability to reduce the Ag ions and cap them to form a stable nanomaterial (Maria & Krishnan, 2016). This green synthesis provides an economic, eco-friendly and clean synthesis route to Ag nanoparticles.

Materials and Methods

Collection and preparation of leaves broth

Nilgirianthus ciliatus leaves were collected from ABS Garden, Kariyapatty, Salem, Tamil Nadu, India. The leaves were washed with tap water, shade dried in laboratory with a regular environmental condition. The shade dried leaves were powdered and further dried for 5 days at room temperature. Broth solution was prepared by taking 5 g of the dried powder in a 300 mL Erlenmeyer flask with 100 mL of sterile distilled water and then boiling the mixture for 5 min before finally decanting it. They were stored at 4°C and used within a week.

Synthesis of silver nanoparticles

10 mL of *Nilgirianthus ciliatus* leaves broth was added to 190 mL of 6mM aqueous $AgNO_3$ solution for reduction of Ag^+ ions. The synthesis rate and particle size of the silver nanoparticles was studied by carrying out the reactions in water bath at 90°C with reflux at a gradual increasing reaction time. The silver nanoparticle solution thus obtained at 120 min (maximum absorption at around 440 nm) was purified by repeated centrifugation at 8000 rpm for 20 min followed by redispersion of the pellet in deionized water.

Characterization of silver nanoparticles

UV-vis spectra were recorded as a function of reaction time on a UV-1800 Shimadzu spectrophotometer operated at resolution of 1 nm. After freeze drying of the purified silver particles, the structure and composition were analyzed by 10 kV Ultra High Resolution Scanning Electron Microscope (FEI QUANTA - 200 SEM) spectroscopy. The surface groups of the nanoparticles were qualitatively confirmed using FTIR spectroscopy (Perkin-Elmer spectrum 2000 FTIR spectrophotometer). X-ray diffraction using Cuk α radiation (PANanlytical X'pert Pro MPD diffractometer) was used to determine the crystalline structure of silver nanoparticles. The Particles size distribution of silver nanoparticles was evaluated using dynamic light scattering (DLS) measurement conducted with a Malvem Zetasizer Nano series compact Scattering Spectrometer. Data obtained were analyzed using Zetasizer software.

Mosquito culture

The eggs of *Aedes aegypti* were collected from water stored containers of local residential area in Salem, Tamil Nadu, India. These were returned to the laboratory and transferred (in approximately the same aliquot numbers of eggs) to 18 cm L × 13 cm W × 4 cm D enamel trays containing 500 mL of water where they were allowed to hatch.

Mosquito larvae were reared at 29±2°C and 75-85% RH in a 14:10 (L:D) photoperiod. Larvae were fed 5 g ground dog biscuit and brewer's yeast daily in a 3:1 ratio. Pupae were collected and transferred to plastic containers with 500 ml of water. The container was placed inside a screened cage (90 cm L × 90 cm H × 90 W) to retain emerging adults, for which 10% sucrose in water solution (v/v) was available *adlibitum.* On day 5 post emergence, the mosquitoes were provided access to a rabbit host for blood feeding. The shaved dorsal side of the rabbit was positioned on the top of the mosquito cage in contact with the cage screen (using a cloth sling to hold the rabbit) and held in this position overnight. Glass Petri dishes lined with filter paper and containing 50 mL of water were subsequently placed inside the cage for oviposition by female mosquitoes.

Larval toxicity test

Laboratory colonies of F3 mosquito larvae were used for larvicidal activity. The AgNps nanoparticle was evaluated at 1, 5, and 10 mg/L concentrations and untreated distilled water served as control. Twenty five actively swimming *Aedes aegypti* immature at different developmental stages were sieved out from the rearing trays to 250ml capacity experimental plastic containers containing 100ml distilled water with selected concentrations of Silver nanoparticle and untreated control setup in triplicate (WHO, 2005). The larvae were fed *adlabitum* on fine powdered liver and glucose at a ratio of 3:2 (wt: wt) as enhanced method of (Roberts, 1998) and as described by (Nareshkumar *et al*., 2013) and the larval mortality was assessed after 24h and 48h of exposure by probing the larvae with needle and moribund larvae were counted as dead (Azmi *et al*., 1998). The control mortalities were corrected by using Abbott formula (1925):

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

Anti-bacterial activity

The antibacterial activity of AgNPs against *Staphylococcus aureus* was experimented by the standard disc diffusion method (Diao *et al*., 2013). The Gram positive bacteria *Staphylococcus aureus* (MTCC 96), procured from Department of Microbiology, Periyar University, Salem, India was cultured using Agar agar type I (AA) and Nutrient Broth (NB). Filter paper discs (6 mm) were impregnated at concentrations from 25 µL to 100 µL AgNPs/disc. The AgNP impregnated filter paper discs were placed on the *Staphylococcus aureus* culture plates incubated overnight at 37°C. The diameters of the zones of inhibition around each filter paper disc were then recorded. The experiments were setup in triplicate.

Statistical analysis

Probit analysis was used to evaluate the median Lethal Concentration (LC_{50}), and the respective 95% fiducial limits for each stage of development in immature *Aedes aegypti*. Mortality data subjected to immature developmental stages were analyzed using Analysis of Variance (ANOVA) methods, where the 1st, 2nd, 3rd and 4th instars larvae were dependent variables and the concentrations used were independent variables. The level of significance used in all tests was 5%. Statistical significance of mean differences was assessed by Tukey's honestly significant difference (HSD) test. Analyses were made using SPSS Software version 16.0.

Results

Ultra-violet visible absorption spectroscopy studies

The silver nanoparticles synthesized mediated by *Nilgirianthus ciliatus* leaf extracts were confirmed visually by change of colour from yellow to dark brown. Initially, when leaf extract added to the $AgNO_3$ solution yellow colour appeared and turned dark brown as it was heated in a magnetic stirrer (80°C) for 2 hours. The appearance of dark brown colour is considered as visual technique showing the presence of Ag NPs. For further confirmation the dark brown solution is taken to UV absorption spectroscopy and the silver nanoparticles recorded from the reaction medium at 80°C using 5% *Nilgirianthus ciliatus* leaf extract with 6Mm AgNO3 exposures to 2h of reaction time is shown in Figure 1. The Surface Plasmon Resonance band of the silver nanoparticle mediated by *Nilgirianthus ciliatus* leaf extracts was recorded at 441 nm confirmed the presence of silver nanoparticles.

X-ray diffraction studies

Silver nanoparticles analyzed by UV-vis spectrophotometer was freeze dried to come out with a powder form for verification under XRD. The diffraction of X-rays travelling through the silver nanoparticles mediated by *Nilgirianthus ciliatus* leaf extract was recorded as peaks at 27.9°, 32.34°, 38.64°, 46.44°, 57.44° and 77.04°2θ corresponding to the facets 111, 220, 226, 200, 264 and 311 of lattice planes indexed as face centered-cubic crystals of silver (Figure 2). Hence, XRD pattern confirmed that the silver nanoparticles formed here are crystalline in nature. No additional diffraction peaks were observed other than the characteristic peak of the silver structure that reflects the purity of synthesized silver nanoparticles, which is comparable with the Joint Committee on Power Diffraction Standard (JCPDS) values.

Fourier transform infrared spectroscopy (FTIR) studies

FTIR spectra of silver nanoparticles were analyzed to identify the possible bio molecules responsible for the reduction of silver nitrate to silver nanoparticles and capping of the bio reduced silver nanoparticles synthesised by *Nilgirianthus ciliatus* leaf extract (Figure 3). FTIR spectrum recorded major peaks positioned at 3378.78, 1600.11, 1383.11 and 1098.47, 600.11 cm^{-1}. The peak recorded at 3378.78 cm^{-1} corresponds to the stretching vibrations of a hydroxyl (O–H) group and the spectral peak at 1600.11 cm^{-1} was assigned to stretching vibration (C=O) in carbonyl compounds characterized by the presence of major constituents of flavonoids

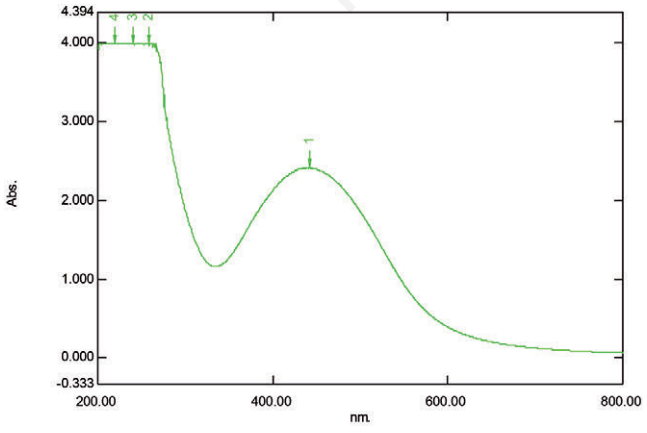

Figure 1. Ultraviolet – visible spectra recorded of silver nanoparticles synthesized using *Nilgirianthus ciliatus* leaf extract at 120 min reaction time.

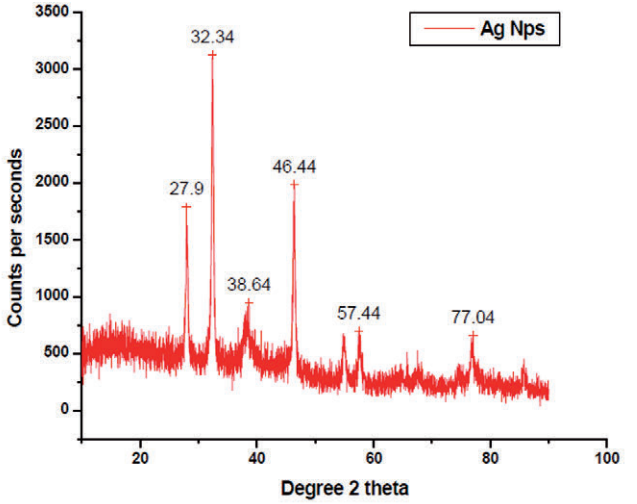

Figure 2. XRD pattern of silver nanoparticles synthesized using *Nilgirianthus ciliatus*.

and terpenoids. The band formed at 1383.11 cm^{-1} corresponds to the bending mode of alpha CH3 a possible methyl group in aldehydes and ketones of *Nilgirianthus ciliatus*. The peak at 1098.47 cm^{-1} matches with the C-N stretching vibration of aliphatic amines, alcohol or phenols characterizing the presence of polyphenols. A stretching vibration band which appears at 600.11 cm^{-1} may be due to the adsorption or interaction of O–H group on the surface of silver nanoparticles confirming the surface added stable silver nanoparticles.

Scanning electron microscopy (SEM) studies

SEM images enabled us to visualize the size and shape of Ag nanoparticles (Figure 4) obtained when exposed with *Nilgirianthus ciliatus* leaf extract at 90°C for 120 min. The morphology of biosynthesized Ag nanoparticles studied under Scanning electron micrographs revealed that they are irregular with ovoid, spherical, rod etc. SEM results provided us an average size range in between 100 nm to500 nm with interparticle distances.

Dynamic light scattering and zeta potential studies

Dynamic Light Scattering (DLS) technique was used in this study to determine the nanoparticles size distribution (Figure 5). The monochromatic laser diffraction collected by a photomultiplier recorded the poly dispersed particles with the size ranging from 100 nm to 500 nm with a Z-Average value of 116.9 nm. The Zetasizer's report on Zeta Potential of the biologically synthesized AgNPs revealed the stability of the metal nanoparticles in the aqueous medium (Figure 6). The Zeta Potential of the *Nilgirianthus ciliatus* mediated AgNPs was −17.2 mV confirms the repulsion among the particles and thereby increases the stability of the nanoparticles.

Bio-assay

The antibacterial activity of Silver nanoparticles formed after exposure to *Nilgirianthus ciliatus* leaf extract was investigated against the human pathogen, *Staphylococcus aureus* by disc diffusion method (Table 1). The antibacterial activity of the synthesized AgNps in 25 µL, 50 µL, 75 µL and 100 µL concentrations was quantitatively assessed on the basis of zone of inhibition. Silver nanoparticles synthesized using *Nilgirianthus ciliatus* leaf extract developed inhibitory zones of 0.36 mm, 0.53mm, 0.7 mm and 0.9 mm on the live culture plates of *Staphylococcus aureus* when treated with 25 µL, 50 µL, 75 µL and 100 µL concentrations respectively (Figure 7).

The selected developmental stages of *Aedes aegypti* (I, II, III

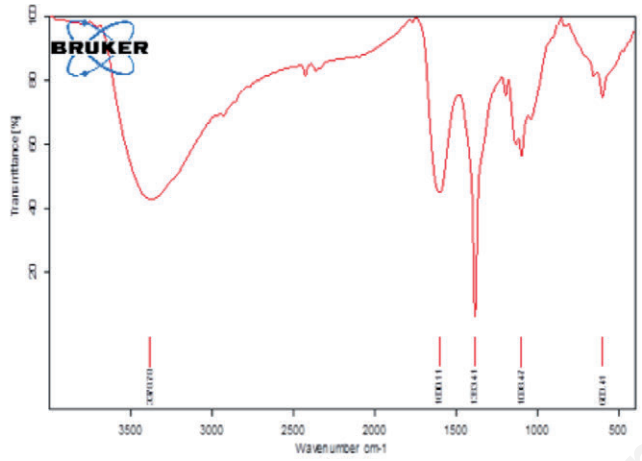

Figure 3. FT-IR pattern of silver nanoparticles synthesized using *Nilgirianthus ciliatus*.

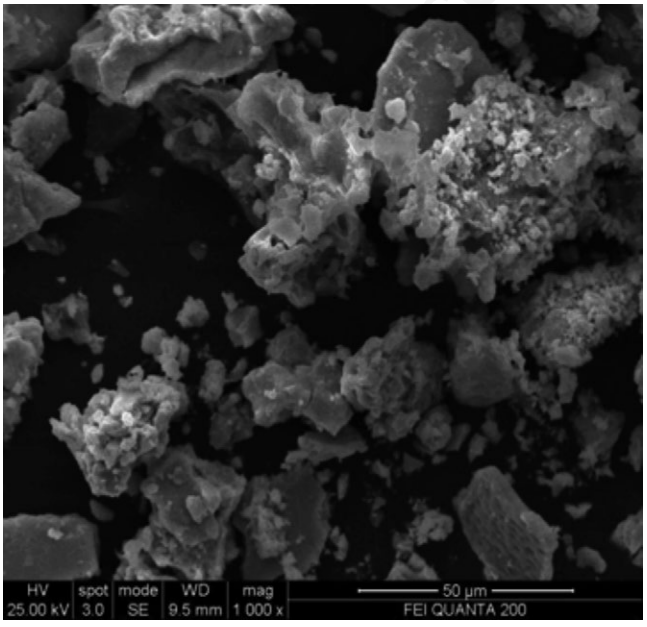

Figure 4. SEM image of silver nanoparticles synthesized using *Nilgirianthus ciliatus*.

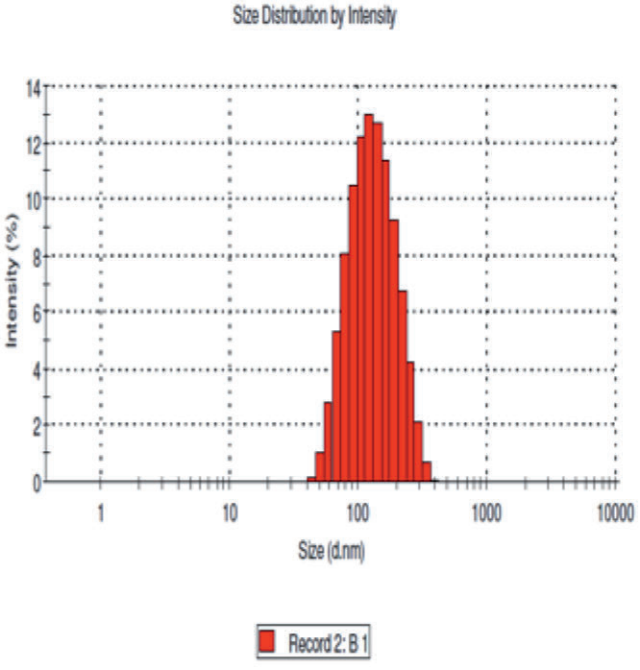

Figure 5. DLS of silver nanoparticles synthesized using *Nilgirianthus ciliatus*.

Table 1. Antibacterial activity of Silver nanoparticles on *Staphylococcus aureus*.

Concentration, g/L	Zone of inhibition, mm
Control	0.0
25	0.3
50	0.5
75	0.7
100	0.9

and IV instar) were not able to withstand the toxicity of biologically synthesized silver nanoparticles even at lower concentrations. 10mg/L of AgNps were capable for killing the mosquito immatures at all developmental stages. AgNps at 1mg/L was able kill more than 70% of the very early developmental stage of mosquitoes. LC_{50} for 1st to 4th developmental instars were 0.49, 1.25, 1.67 and 4.95 mg/L respectively at 24 hrs treatment (Table 2). The acquired chi-square values (1.16, 0.53, 0.61, and 1.31 for I, II, III, IV instars respectively) proves that the observed mortality is on a par with the expected mortality.

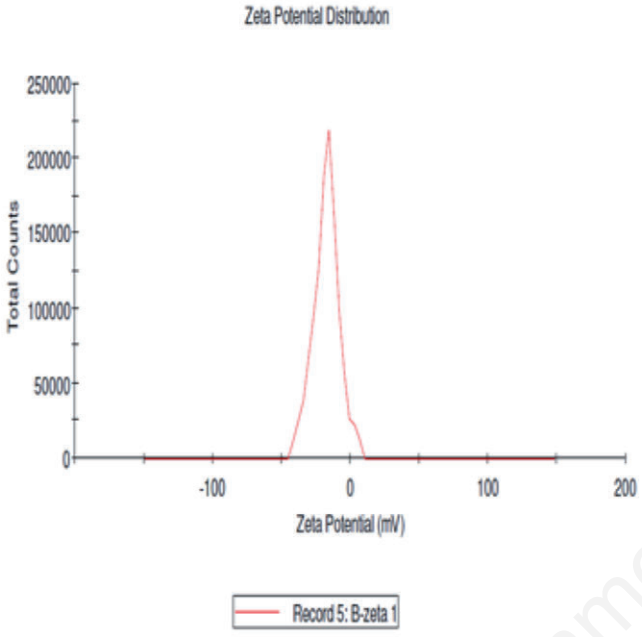

Figure 6. Zeta potential of silver nanoparticles synthesized using *Nilgirianthus ciliatus*.

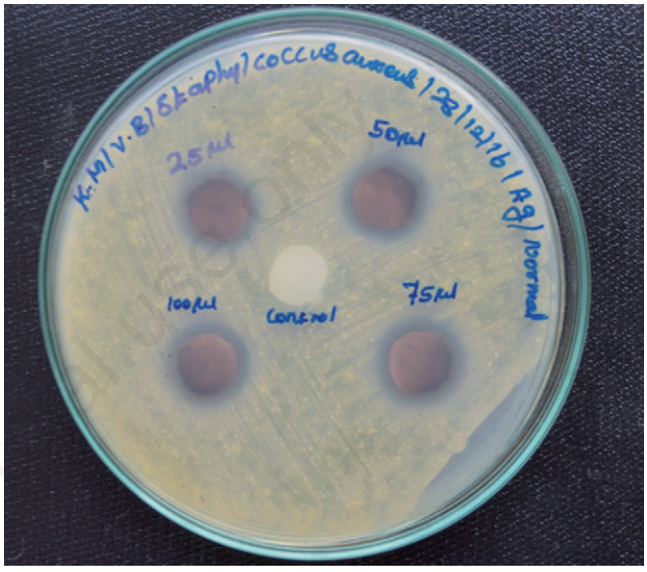

Figure 7. Antibacterial activity of Silver nanoparticles on *Staphylococcus aureus*.

Table 2. Efficacy of Silver nanoparticles treatment on *Aedes aegypti* larvae at 24 hours.

Developmental stages	Larvae introduced	Mortality/concentration Control	1 mg/L	5 mg/L	10 mg/L	L_{C50}	L_{C90}	X^2
I instar	100	0±0	58.66±6.11	69.33±8.32	81.33±2.30	0.49	61.63	1.16
II instar	100	0±0	48.00±4.00	64.00±4.00	76.00±10.58	1.25	75.83	0.53
III instar	100	0±0	45.33±6.11	58.66 ±8.32	70.66±2.30	1.67	188.09	0.61
IV instar	100	0±0	9.33±2.30	54.66±12.85	68.00±10.58	4.95	25.30	1.31

Table 3. Efficacy of Silver nanoparticles treatment on *Aedes aegypti* larvae at 48 hours.

Developmental stages	Larvae introduced	Mortality/concentration Control	1 mg/L	5 mg/L	10 mg/L	L_{C50}	L_{C90}	X^2
I instar	100	0±0	73.33±6.11	78.66±6.11	85.33±2.30	0.02	57.40	0.49
II instar	100	0±0	60.00±4.00	72.00±4.00	81.33±8.32	0.40	56.00	0.45
III instar	100	0±0	56.00±4.00	68.00±8.00	77.33±2.30	0.58	105.45	0.37
IV instar	100	0±0	22.66±2.30	64.00±10.58	72.00±10.58	3.28	27.99	1.16

The mortality rate of *Aedes aegypti* was increased at 48 hrs of treatment with Silver nanoparticles at 1 mg/L, 5 mg/L and 10 mg/L concentrations (Table 3). Maximum mortality (100%) was observed in all the developmental stages at treatment with 10 mg/L concentration of silver nanoparticles. Median Lethal Concentrations were reduced to 0.02 mg/L, 0.40 mg/L, 0.58 mg/L, and 3.28 mg/L for 1st to 4th instars respectively at 48 hrs treatment.

Discussion

Development of resistances in pathogens as well as vectors for maximum available drugs and insecticides are serious threat to human health. Good numbers of synthetic chemicals are being introduced every year for the treatment of pathogens and vectors but it is most important that environmental safety and non target organisms should be taken under consideration. In concern to environment safety and resistant development novel technologies with natural products will be a best alternative to overcome the problem. Therefore, many researchers started working on plant products and nanotechnology for the management of human disease causing pathogen and disease transmitting vectors (Mickymaray *et al*., 2016; Madhiyazhagan *et al*., 2016; Shanmugapriya *et al*., 2016 ; Elias *et al*., 2017).

In this study, we focussed on development of the antibacterial and antivectorial nanoparticles using the extracts of *Nilgirianthus ciliatus* leaves as reducing and capping agents. The formation of Nanoparticles in the experimental setup was initially confirmed by the colour change in reaction vessels, where, yellow turned to dark brown (Logeswari *et al*., 2015). Further for the liquid sample was taken to UV vis spectra to verify the Surface Plasmon Resonance band coincide with that of the silver nanoparticles. We observed the resonance peak at 441nm, which was a specific character of silver nanoparticles. The present result matches with the results of recent researches by (Gudikandula & Maringanti, 2016), (Ali *et al*., 2016) and many others.

The size and shape of the *Nilgirianthus ciliatus* mediated silver nanoparticles were similar to that of many reports of research done throughout the world (Sharma *et al*., 2017; Basavegowda *et al*., 2014). Reaction time plays a major role in the size and shape of the nanoparticles Verma *et al*. (2016) in our study we observed 120 min to be the optimum reaction time for nanoparticles in a range between 50 to 150nm. Zeta Potential record (−17.2mV) of the biosynthesized AgNPs using *Nilgirianthus ciliatus* in this study was up to mark proving the stability of the particles in the medium. Earlier reports on silver and other metal nanoparticles were in support to the Zeta Potential record of this study (Premasudha *et al*., 2016; Trinh *et al*., 2015).

The secondary metabolites of *Nilgirianthus ciliatus* leaves are the responsible agents for reducing, capping and stabilizing the metal nanoparticles. FTIR spectral bands confirmed the presence of hydroxyl group, carbonyl compounds, and methyl groups of polyphenols, flavonoids, terpenoids, aldehydes and ketones of *Nilgirianthus ciliatus* surrounding the silver nanoparticles or the reductants which disappeared later. Preceding results of many studies showed identical stretching vibrations confirming the presence of above mentioned organic compounds surrounding the metal nanoparticles (Trinh *et al*., 2015; Subbaiya *et al*., 2014). Further, XRD spectrum showed that the structure corresponds to face-centered cubic crystal of Silver. Pure Ag crystallite was obtained with the absence of unassigned peaks, weak peaks, oxide of Ag and incomplete peaks (Ghodsieh *et al*., 2017).

Silver is known for its antimicrobial activity from very ancient time. It has been an effective agent in wound healing, curing infections, reducing bacterial burden, post-operative incision dressings, blood and urinary catheter designs, endotracheal tubes, orthopedic devices, vascular prostheses, and the sewing ring of prosthetic heart valves (Politano *et al*., 2013).In this study, Silver nanoparticles synthesized using *Nilgirianthus ciliatus* leaf extract was tested for its efficacy in controlling the bacterial pathogen, *Staphylococcus aureus*. Silver nanoparticles were highly energetic in this case to control the bacterial pathogen as it required very low concentration for destroying maximum possible cells. Silver nanoparticles have a multiple mechanism of action on microbes reported since last two decades. Silver nanoparticles adheres to the bacterial cell wall and taking advantage of its size it penetrates the cell causing structural changes in the cell affecting permeability and finally leading to death of the cell(Sondi and Sondi, 2004).They form free radicals that damage the membrane and also interact with thiol groups in enzymes and phosphorus-containing bases, thus interact with DNA and prevent cell division leading to cell death Morones *et al*. (2005); Li *et al*. (2013) reported changes in protein, hypoxanthine, adenosine, and guanosine bands suggesting that Ag NPs have a significant impact on the protein and metabolic processes of purine.

Results obtained from the Larvicidal activity of silver nanoparticles mediated by *Nilgirianthus ciliatus*leaf extract shows that the nano silver will be a best alternative for synthetic larval control agents of *Aedes aegypti*. Silver Nanoparticles were much effective even at the lower concentration and for the late developmental stages of *Aedes aegypti*, which was failed by many synthetic chemicals. Effect of silver nanoparticles on *Aedes aegypti* in this study was greater that the effect reported earlier on *Culex quinquefasciatus* and *Anopheles stephensi* Chandrashekhar *et al*. (2012); Udaiyan *et al*. (2015) may be due to size of the particle or the biomolecules surrounding the nano silver reaching the target efficiently. Mechanism involved might be the penetration of Ag nanoparticles through the larval membrane targeting the phospholipids bilayer arresting the molting process which was seen rare in the treated larvae (Rawani, 2017; Murugan *et al*., 2015; Arjunan *et al*., 2012). Whereas, earlier Morsy *et al*. (2001) reported that Ag nanoparticles binds to the sulfur containing proteins and phosphorous containing DNA leads to inhibition of protein and DNA synthesis leading to decreased permeability and imbalanced proton motive force causing morphological deformation and death.

Conclusions

Silver nanoparticles synthesized using plant products are cost-effective environment friendly, target specific molecules and promising agents against the pathogenic microbes and disease transmitting vectors. From this study we conclude that the *Nilgirianthus ciliatus* leaf is hub of secondary metabolites that has the capability to act as reducing and capping agent in formation of silver nanoparticles. Studies needed to identify the exact molecules in *Nilgirianthus ciliatus* responsible for reducing as well as capping the silver ions and nanoparticles respectively to reduce the utilization of bio-resources. Further research on monodispersed nanoparticles can increase the biotoxicity against microbial pathogens and mosquito immature making it more suitable for commercialization. Present findings would prompt future research on nanotechnology in all aspects of life sciences.

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