Available online on 15.08.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Biosynthesis of Silver Nanoparticles from Seaweed *Caulerpa taxifolia* Against Vector Borne Disease *Culex quinquefasciatus*

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ABSTRACT

Globally, mosquitoes are transmitting agents for diseases like dengue, malaria, filaria and Japanese encephalitis. In this study, the larvicidal activities of silver nanoparticles (AgNPs) synthesized from seaweed *Caulerpa taxifolia* extract against the larvae of *Culex quinquefasciatus* was investigated *in vitro*. The synthesized AgNPs was further characterized using UV-Vis spectroscopy, FTIR, SEM, XRD, DLS and Zeta potential analysis. From the result, the LC_{50} value for AgNPs identified was 448.66. The synthesized silver nanoparticles have maximum absorption at 430 nm. The FTIR indicated a specific peak in 3275.11cm^{-1}, 2921.7cm^{-1} and 1244.28cm^{-1} range. Scanning electron microscopy resulted in spherical shaped approximately ranging from 1 µm to20 µm in size. The average size distributions of Ag nanoparticles were 72.99 nm and are fairly stable with a zeta potential value of -31.1 mV. The biosynthesis of silver nanoparticles with *Caulerpa taxifolia* extract provides potential source for the larvicidal activity against mosquito. The present study revealed that green synthesized silver nanoparticles can be used as an eco-friendly means for effective control of vector diseases.

Keywords: *Silver Nanoparticles, Caluerpa taxifolia, Culex quinquefasciatus, larvicidal activity Vector borne disease.*

Article Info: Received 12 June 2019; Review Completed 25 July 2019; Accepted 30 July 2019; Available online 15 August 2019

Cite this article as:

King SE, Coopoosamy RM, Olajuyigbe OO, Thiyagarajan P, Biosynthesis of Silver Nanoparticles from Seaweed *Caulerpa taxifolia* Against Vector Borne Disease *Culex quinquefasciatus*, Journal of Drug Delivery and Therapeutics. 2019; 9(4-s):240-243 **http://dx.doi.org/10.22270/jddt.v9i4-s.3222**

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1. INTRODUCTION

Advance of "Nano" was started over two decades ago as nanotechnology nonetheless increases impact in day to day life. While recent advancements in nanotechnology-based medicines have opened new horizons for combating multidrug resistance in microorganisms[1]. Nanoparticles possess unique properties than normal materials[2]. Silver nanoparticles (AgNPs) display a broad spectrum of anti-infective activities [3, 4]. It is less reactive than silver ions and well suited for its use in clinical and therapeutic applications[5].

One of the global issues in public health is vector borne diseases like malaria, filaria, dengue and chikungunya causing high mortality and morbidity in human. While mosquitoes are the best known disease vector, other species include ticks, flies, sandflies, fleas, bugs and freshwater snails[6]. *Culex quinquefasciatus* serves as the main vector of the filarial parasite *Wuchereria bancrofti* causing lymphatic filariasis[7]. Recently, more than 160 million peoples were diseased with filariasis and for that reason WHO initiated a process for targeting "Filariasis Elimination by 2020" [8]. Thus, there is an urgent need to develop a cheap, non-toxic and novel drug that can kill mosquitoes. In past, silver is used as several disease control agents because silver ions and silver based compounds were highly toxic towards microorganisms. However, its use was decreased or reduced after the discovery of penicillin against bacteria. Nevertheless, the use of silver for therapeutic purposes resurfaced as nanoparticles and fewer hazards to human health have been recorded[9].

Green nanoparticle synthesis is of concern in the recent times because of its several applications. Since the development of this concept, there has been increasing demand for synthesis of environmental-friendly metal-nanoparticles devoid of toxic chemicals[10,11]. Consequently, seaweeds which are benthic marine plants important as renewable marine life resources and having various biomedical effects such as antiviral, anti-inflammatory, anti-bacterial, anti-fungal and anti-cancer properties have been used in synthesis of nanoparticles[12]. For the past two decades, stated that there is an increase in the number of report on seaweed nano-synthesis to control mosquitoes than physical and chemical agents[13]. Although AgNPs prepared with seaweeds such as *Caulerpa scelpelliformis* and *Ulva lactuca*[14,15] as well as *Sargassum muticum*[16] possessed mosquitocidal activity, there is a paucity of information on the mosquito larvicidal activity of synthesized silver nanoparticles (AgNPs) from *Caulerpa taxifolia* seaweed extract against *Culex quinquefasciatus.* This study is therefore aimed at investigating the lavicidal effects of biosynthesized silever nanoparticles from the extract of *Caulerpa taxifolia* seaweed on *Culex quinquefasciatus* causing filariasis.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Seaweed Material: Fresh seaweed of *Caulerpa taxifolia* collected from Kuthenkuli, Thirunelveli district, Tamilnadu, India (Lat. 8.1901N and Long. 77.7002E). The collected seaweed was washed with double distilled water to remove the epiphytes and other associated marine organisms. The washed seaweed was shade dried before being pulverized. From the powder, 30 g was extracted successfully through maceration with six different solvents including acetone, benzene, chloroform, diethyl ether, methanol and petroleum ether subjected continuously into shaking at 200 rpm for 48 h. The contents were extracted in Whatmann No. 1 filter paper and the solvent was evaporated and stored in 4°C for further analysis.

2.2 Biosynthesis and Characterization of Silver Nanoparticles: From the seaweed extract, a total 10 mL was treated with 90 mL (2 mM $AgNO_3$) silver nitrate solution and incubated in dark at room temperature for up to 98 h. Resulting change in color indicate the synthesis of silver nanoparticles[17]. About 1 mL of biosynthesized nanoparticle solution was diluted 1:20 v/v and monitored in UV–vis spectrophotometer at wavelength between 300–700 nm for bioreduction of Ag^+ ions. The dried purified pellet was subjected to FTIR spectroscopy in the resolution of 4.0 cm^{-1} at room temperature using a KBr disc ranges from 400 to 4000 cm^{-1} for the presence of functional groups. The size, morphology and composition of AgNPs was determined by using Scanning Electron Microscopy (SEM) at a voltage of 10 Kv. The oxidation state of AgNPs as a function of time was determined in X-ray Diffraction assay (XRD) in X- ray diffractometer. Finally, the surface charge, particle size distribution and Zeta potential were characterized by dynamic light scattering (DLS).

2.3 Rearing of *Culex quinquefasciatus*: Eggs of *Culex quinquefasciatus* was procured from vector control laboratory, Bharathidasan University, Tiruchirappalli, TamilNadu, India. Mosquito larvae were kept in a plastic tray with sterilized double distilled water for 35- 45 min to hatch out larvae and the reared larvae were maintained in 27±2^{o}C, 70-85% relative humidity, with a photo period of 14 h light and 10 h dark.

2.4 Larvicidal toxicity of biosynthesized Silver nanoparticles: Synthesized AgNPs were tested for toxicity against *Culex quinquefasciatus* larvae. Twenty five larvae were introduced into a 250 mL beaker with 200 mL of sterilized double distilled water through different concentrations ranging between 100, 200, 300, 400 and 500 ppm. Experiment was carried out in five replicates with concurrent controls. At each concentration, three trials were made for all replicates. The mortality of larvae was calculated after 24 h by Abbott's formula [18].

2.5 Statistical analysis: Average *Culex quinquefasciatus* larval mortality information was analyzed through probit for calculating LC_{50} and 95% confidence. Fiducially, limits of lower and upper confidence limit were also calculated with SPSS 16.0 version software and represented in pie chart [19].

3. RESULTS AND DISCUSSION

This study determined the larvicidal activity of biosynthesized silver nanoparticles (AgNPs) of *Caulerpa taxifolia* extract against *Culex quinquefasciatus.* It showed the maximum ranges of larvicidal activity. The conformation of synthesized AgNPs as indicated through change in color to brownish yellow was attributed to the excitation of surface plasmon resonance of silver nanoparticles due to reduction of silver nitrate and it is directly proportional to the length of the incubation period. The preliminary indirect method of characterization of AgNPs was visual characteristics based. After mixing nanoparticles with the extract solution, the surface plasmon absorption peak was observed at 430 nm. The mixture indicated that bioreduction of Ag ions was due to presence of reducing agents like reductase enzyme and the excitation of plasma resonances of metallic nanoparticle dispersions. . This is similar to the homogenous AgNPs known to produce the surface plasmon resonance band at the range of 420 to 450nm[20]. The FTIR spectrum was examined for identification of biomolecules in biosynthesized surface of *Caluerpa taxifolia*-AgNPs extract and stabilization. The peaks observed at 3275.11cm^{-1} as vibration secondary amine N-H stretching band at 2921.7 cm^{-1} (C-H) stretching indicated the presence of alkanes and band at 1244.28 cm-1 (C-N) stretching denoted aliphatic amine. Almost all the indicated peaks were foremost functional groups in flavonoids[21]. The amide group in the extracts indicated the existence of enzymes responsible for reduction and stabilization of metal ions activity and carbonyl stretch in proteins. Most of the marine seaweeds were rich in flavonoids and polypheonlic compounds.

Scanning electron microscopic (SEM) was used to identify the size and shape of nanoparticles as a reduced form in silver nitrate solution[22]. Biosynthesized nanoparticles of *Caulerpa taxifolia* were spherical in nature, ranging in size and few particles were agglomerated as shown in Fig. 1. The agglomeration may be due to reduction and some chelation process. It may also be due to weak surface plasmon resonance vibrations in biosynthesized nanoparticles[23]. The X-ray diffraction pattern of biosynthesized nanoparticles of *Caulerpa taxifolia* showed that particles were small crystalline in nature with distinct indexed 2θ diffraction peaks at 111, 200 and 311 crystalline cubic Ag identified with the respective Bragg's angles of 37.95°, 45.84°, and 76.43° as shown in Fig. 2. The sharp peak formation may be due to reducing agents in the *Caulerpa taxifolia* extracts which stabilized biosynthesized nanoparticles and thus confirmed the crystalline shape[24]. Dynamic light scattering of biosynthesized *Caulerpa taxifolia* nanoparticles were found at average size distributions of 72.99 nm and well dispersed in the colloidal solution. Almost similar observation was recorded in *Sargassum tenerrimum* that attained maximum intensity at 48nm[25]. The zeta potential value, stability of the nanoparticles was -31.1 mV indicating that particles were highly stable in nature and this may be due to some forces like high attractive and repulsive forces between each nanoparticle[26]. The lavicidal activity showed that eighty eight percentage mortality was observed at maximum concentration of 500 ppm with LC_{50} value of 448.66 and their statistical probit analysis as 0.5269 when compared with other concentration 100 (0.1749), 200 (0.3074), 300 (0.4010) and 400 (0.4715) are presented in the pie chart (Fig. 3). The Lower Confidence Level (LCL) was 264.0797 and Upper Confidence Level (UPL) value was 602.86. Mortality may be due to silver nanoparticles penetrating through cuticle of insects and interfered with some physiological processes like binding to sulfur containing proteins and disturbance of proton motive force leading to changes in cellular activities. Earlier report indicated that biosynthesized AgNPs for larvicidal activity using *S. acuta* plant leaf extract against *Culex quinquefasciatus* and it showed LC_{50} value as 26.13 μg/mL[27]. Also, Indian marine plant extracts in previous study showed its potential larvicidal activity of *Culex quinquefasciatus*[28].

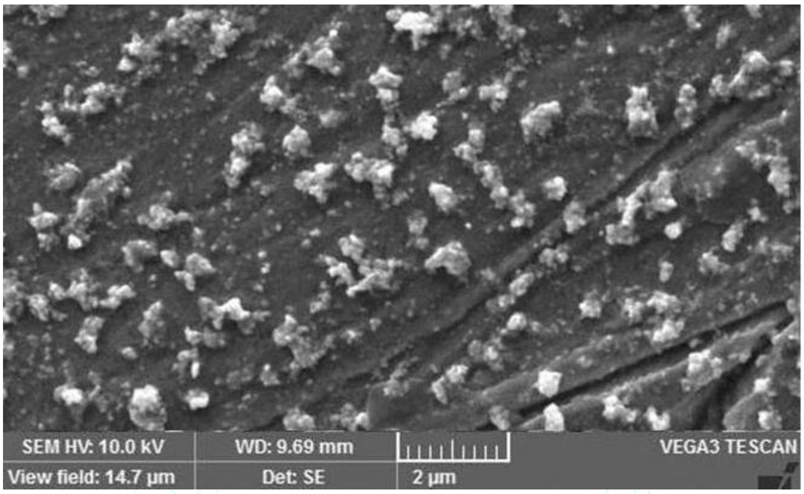

Fig. 1: Scanning Electron Micrograph for biosynthesized nanoparticles using *Caulerpa taxifolia* extract.

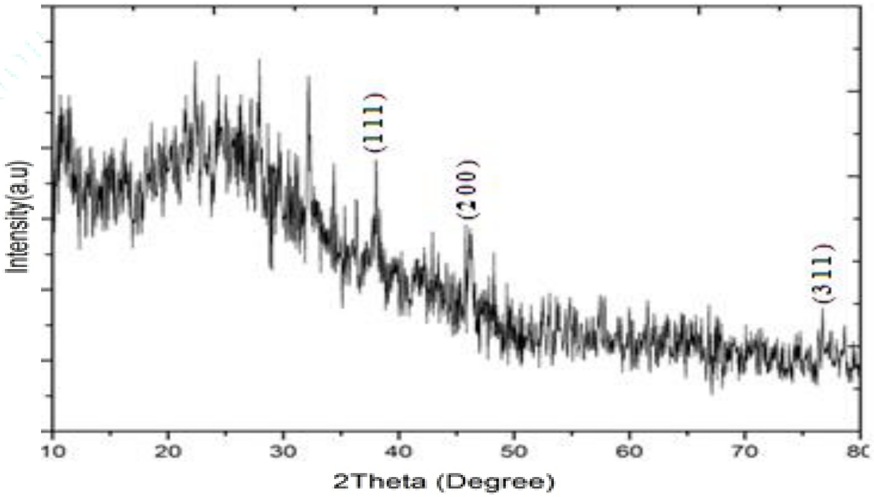

Fig. 2: X-ray Diffraction pattern of biosynthesized nanoparticles using *Caulerpa taxifolia* extract with 2θ index.

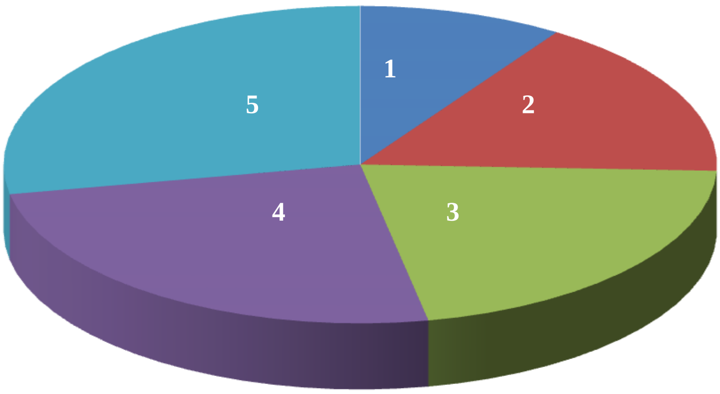

Fig. 3: Piechart for larvicidal activity of biosynthesized nanoparticles using *Caulerpa taxifolia* extract against *Culex quinquefasciatus* larvae. Represented with concentration in ppm and dose response statistically (probit analysis):1-100, 2-200, 3-300, 4-400 and 5-500 ppm.

4. CONCLUSION

In future, nanotechnology and their particles may be used as promising and remarkable alternative biomedical agents. The present research findings concluded that, biosynthesized silver nanoparticles of *Caulerpa taxifolia* extract inhibited *Culex quinquefasciatus* larvae and the nanoparticle of the extract of this seaweed could be used to control vector borne diseases.

ACKNOWLEDGEMENT

The authors would like to acknowledge Goel Cyrus Ebenezer, India for editorial assistance of the manuscript and deep sense of gratitude for all scientists for his research input.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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