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BIOSYNTHESIS OF SILVER NANOPARTICLES USING BIVALVIA SHELL EXTRACT AND EVALUATION OF ITS LARVICIDAL ACTIVITY

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ABSTRACT

The aim of the present study is to use a metal-reducing source of marine Bivalvia shell extract, which belongs to the Mollusca phylum (Class: Bivalvia), to synthesize silver nanoparticles as a green synthesis approach. The collected Bivalvia shell was extracted with deionized water, and the extract was concentrated at 1/4th. That concentrated extract was used for the biosynthesis of silver nanoparticles. The Bivalvia shell extract was reduced with silver ions into the formation of silver nanoparticles, which confirmed the brown color formation after 24 hours in a dark place at room temperature. The collected AgNPs initially confirmed spectroscopy and SEM techniques. The FTIR spectrum revealed the presence of phenol, aromatice and aliphatic amine groups, and the UV-visible peak at 420 nm revealed the formation of AgNPs, while SEM agreed upon a particle size less than 100 nm (NPs<100 nm). The synthesized AgNPs and shell-extract showed promising larvicidal activity on mosquito larvae (LC50 = 47.58 and 71.25 ppm), based on concentration dependence (R2 = 0.99 and 0.99). Finding the present study evidenced that the chemical constituents of Bivalvia shell act as silver ion-reducing and capping agents. The current author recommended Bivalvia shell as an alternative to chemical reductants for metallic nanoparticle synthesis. Therefore, Bivalvia shell extracts eco-friendly metal ion reducing and capping agents..

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INTRODUCTION

The mosquito's canister spreads more diseases than any other group of arthropods and affects millions of people throughout the world. Mosquito-borne diseases are prevalent in more than 100 countries across the world, infecting several members of the Indian population every year. Dengue is a mosquito-borne viral infection found in tropical and subtropical regions around the world; its major vector is the mosquito *Aedes aegypti* (Velayutham and Ramanibai, 2016). The mosquito species *Aedes aegypti* is the primary vector of dengue, chikungunya, and Zika infections worldwide (Morejon et al., 2018). Recently, dengue transmission has strongly enlarged in urban and semi urban areas, becoming a major global public health concern, and over 2.5 billion people are now at risk from dengue (Velayutham and Ramanibai, 2016). The World Health Organization estimates that there may be 50–100 million dengue infections worldwide every year (WHO, 2012). The target species vector control is facing a threat due to the development of resistance to chemical insecticides, resulting in rebounding vectorial capacity (Liu et al., 2006). Insecticides have provoked undesirable effects, including toxicity to non-target organisms, and fostered environmental and human health concerns (Yang et al., 2002).

Silver nanoparticles are important materials that have been studied extensively. They can be synthesized by several physical, chemical, and biological methods (Sharma et al., 2009). Such nanoparticles possess unique electrical, optical, and biological properties and are thus applied in catalysis, biosensing, imaging, drug delivery, nanodevice fabrication, and medicine (Jain et al., 2008). In the past few years, there has been an increasing interest in silver nanoparticles on account of the antimicrobial properties that they display (Choi et al., 2008). Green synthesis provides advancement over chemical and physical methods as it is cost effective, environment-friendly, and easily scaled up for large scale synthesis. In this method, there is no need to use high pressure, energy, temperature, or toxic chemicals (Lok et al., 2007). AgNPs, which are less likely to cause ecological damage, have been identified as potential replacements for synthetic chemical insecticides, hence the need to use green synthesized AgNPs for the control of disease vectors (Velayutham et al., 2013). Green synthesis is an eco-friendly, cost efficient, rapid, and easy method for the synthesis of metallic nanoparticles using natural sources as reducing and capping agents, such as plants, microorganisms, and animals. The current study aimed to explore the larvicidal activity of bio-synthesized AgNPs using aqueous Bivalvia shell extract on mosquitoes' larvae.

MATERIALS AND METHOD

Collection and Extraction of Bivalvia shell extract

The collected dried specimens of Bivalvia shell, from the Bay of Bengal, south east coast of Mallipattinam, Thanjavur district, Tamil Nadu, India, in 2019. Take 10 grams of Bivalvia shell fine powder and add 100 ml of deionized water. Heat at 40 to 50°C for 20 minutes. After cooling, the extract was filtered with Whatman No. 1 filter paper and concentrated at 1/4th (100 ml to 25 ml) for Ag nanoparticle synthesis.

Figure 1: Bivalvia shell extraction and concentrated

Nanoparticles synthesis

Silver nanoparticle synthesized by the method of Arunachalam *et al*., (2012), with mild modification. In this method, 5 ml of Bivalvia shell extract was added to 45 ml of 1 mM aqueous AgNO3 solution in a 250 ml Erlenmeyer flask. The flask was then incubated in the dark at 24 hours (to minimize the photo activation of silver nitrate), at room temperature. A control setup was also maintained without extract. The AgNPs solution thus attained was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water. Then the silver nanoparticles were freeze dried for using characterization analysis.

Characterization of Nanoparticles

UV and FTIR Spectroscopic analysis

The silver nanoparticles were examined under UV and visible spectrophotometer analysis. The silver nanoparticles were scanned within the wavelength starting from 200-1000 nm using Perkin Elmer photometer and also the characteristic peaks were identified. FTIR analysis was performed using Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm-1 and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation

Electron microscopy (SEM) analysis of silver nanoparticles

The particle size and morphology of nanoparticles were analysed by ZEEISS-SEM machine. The dried form of silver nanoparticles were sonicated with distilled water, small droplet of silver nanoparticles were placed on glass slide and permitted to dry. The ZEEISS-SEM machine was worked at a vacuum of the order of 10-5 torr. The accelerating voltage is 10 kV. The particle size of nanoparticles can be analyzed by using image magnification software compatible with SEM.

Larvicidal Bioassay (WHO, 2005).

The bioassays were carried out using 20 early 4th instar mosquitoes' larvae at each concentration (20, 40, 60, 80, and 100 ppm) of Bivalvia shell extract and AgNPs. Probit was analyzed by SPSS, and the regression equation and r2 value were also calculated.

RESULTS AND DISCUSSION

5 ml of concentrated (1/4th) extract from Bivalvia shell was added to 45 ml of 1 mM aqueous AgNO3 solution and placed at room temperature for 24 hours in a dark room. After 24 hours, silver nanoparticles formed and a visibly observed brown color formation was observed (Figure 2). The synthesized AgNPs characterized by UV–vis spectrum, Fourier transform infrared (FTIR) and scanning electron microscopy (SEM). Similar findings were recently reported by Suresh et al. (2014) using aqueous root extract of Delphinium denudatum (natural source) by reduction of Ag+ ions. Rajakumar and Rahuman (2011) using leaf extracts of *Eclipta prostrate* (natural source). The aqueous silver nitrate solution was turned to a yellowish brown color within 1 hour with the addition of leaf extract. The intensity of the brown color increased in direct proportion to the incubation period. The current author recommended Bivalvia shell as an alternative to chemical reductants for metallic nanoparticle synthesis. Therefore, Bivalvia shell extracts eco-friendly metal ion reducing and capping agents.

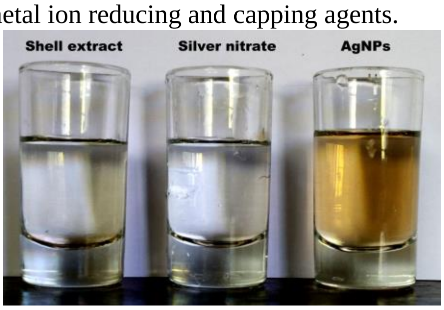

Figure 2: Synthesis of AgNPs using Bivalvia shell aqueous extract (Ag^+ into Ag^0)

UV-Vis spectroscopy is one of the most widely used techniques for the structural characterization of silver nanoparticles. The present synthesized AgNPs absorption spectrum showed a notable peak at 420 nm, indicating the presence of AgNPs (Figure 3). Similarly, Suresh et al. (2014) reported 416 nm of synthesized AgNPs using Delphinium denudatum extract. Jyoti et al. (2016) revealed a UV-Vis spectrum peak was observed at 414 nm that confirmed the synthesis of AgNPs using Urtica dioica Linn. leaves. The FTIR spectrum revealed the presence of functional groups of alcohols, phenols, and amines to indicate the reduction of silver ions in Bivalvia shell extract to the formation of AgNPs (Table 1 and Figure 4). Karthik et al. (2014) agreed that the FTIR analysis data confirms the presence of O-H stretching (around 3,417 cm-1), which may be responsible for reducing metal ions into their respective nanoparticles. The synthesized AgNPs revealed spherical-shaped morphology (Figure 5) and particle sizes less than 100 nm (67 to 79 nm). Jyoti et al. (2016) studied the synthesized AgNPs morphology and revealed the spherical nature of particles synthesized from silver metal using scanning electron microscopy (SEM) and TEM revealed at 50 nm to 20 nm range of synthesized AgNPs using Urtica dioica Linn. leaves.

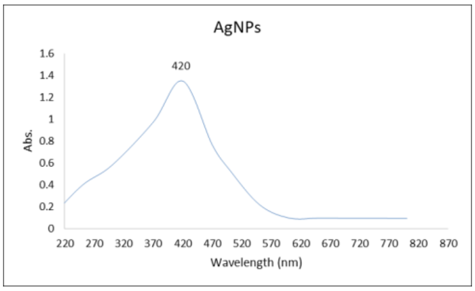

Figure 3: UV-Visible spectrum of synthesized AgNPs from Bivalvia shell extract

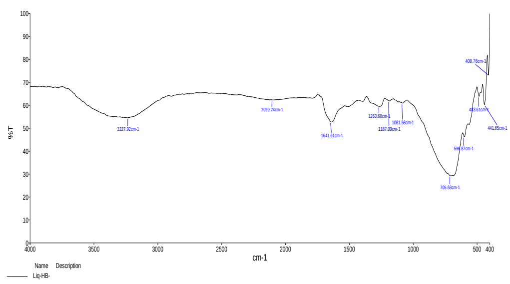

Figure 4: FTIR spetrum of synthesized AgNPs from Bivalvia shell extract

Table 1: FTIR analysis of synthesized AgNPs from Bivalvia shell extract

Frequency cm^{-1}	Bond	Functional group
3227.92	O-H stretch, H-bonded	Alcohols, phenols
1641.61	N-H bend	1° Amines
1263.68	C-N stretch	Aromatic amines
1187.09, 1081.58	C-N stretch	Aliphatic amines

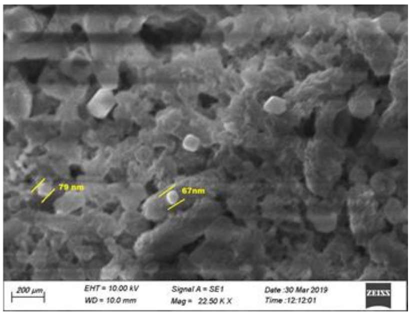

Figure 5: Morphology and particle size analysis of synthesized AgNPs from Bivalvia shell, using SEM techniques (67 to 79nm)

In the present study, the larvicidal aqueous crude Bivalvia shell extract and synthesized AgNPs were noted; however, the highest mortality was found in synthesized AgNPs against mosquitoes' larvae at a concentration of 100 ppm and in a dose-dependent manner. The larvicidal activity of aqueous crude Bivalvia shell extract and synthesized AgNPs, showed LC50 values of 71.25 ppm and 47.58 ppm; R2 values of 0.99 and 0.99 against mosquitoes' larvae (Aedes sp.), respectively (Figure 6 and Figure 7). Velayutham and Ramanibai (2016) described synthesized silver nanoparticles as having the potential to be utilized as a good, eco-friendly approach for the control of mosquito populations and reported that first to fourth instar mosquito larvae were exposed to varying concentrations of isoamyl acetate and synthesized Ag NPs for 24 h. The highest mortality was observed in synthesized Ag NPs against first to fourth instars of (LC50 = 2.50, 2.78, 3.02, 3.05 µg/ml; LC90 = 7.52, 8.34, 9.06, 9.15 µg/ml) and Culex quinquefasciatus (LC50 = 2.75, 3.00, 3.21, 3.48 µg/ml; LC90 = 8.25, 9.01, 9.63, 10.44 µg/ml), respectively. The colloidal solution of silver nanoparticles was found to exhibit mosquito larvicidal activity against dengue and filariasis vectors. Suresh et al. (2014) reported synthesized AgNPs showed potent larvicidal activity against second instar larvae of dengue vector Aedes aegypti with a LC50 value of 9.6 ppm. Overall, synthesized AgNPs showed promising larvicidal properties against mosquito larvae, and the dose-dependent manner of synthesized AgNPs and Bivalvia shell extract was statistically agreed upon using the correlation coefficient.

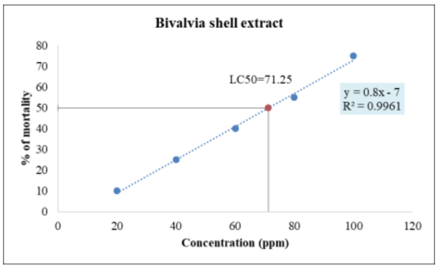

Figure 6: Larvicidal activity of Bivalvia shell extract on 4th instar mosquitoes' larvae

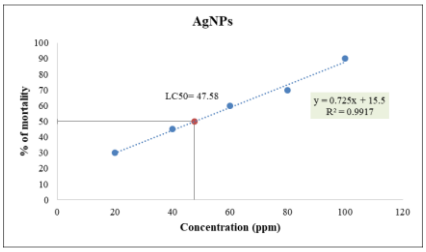

Figure 7: Larvicidal activity of synthesized AgNPs using Bivalvia shell extract on 4th instar mosquitoes' larvae

CONCLUSION

The Bivalvia shell extract evidenced a reduction of silver ions into the formation of silver nanoparticles, and spectroscopy and SEM techniques confirmed the formation of AgNPs from the shell extract. The synthesized AgNPs and shell extract showed promising larvicidal activity on mosquito larvae based on dose dependence. In the present study, Bivalvia shell extract was found to be involved in silver ion-reducing and capping agents with an effective nano-larvicide.

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