Bio inspired silver nanoparticle synthesis from fish liver oil and its antibacterial activity against shrimp pathogen

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Abstract

Fish liver oil is an excellent source of essential vitamins having nutritive supplements with effective medicinal importance. Reaction between the fish liver oil and silver nitrate is facilitated by the presence of nitrate salts, carboxylate ion and amine groups present in the fish liver oil during the nanoparticle synthesis. The silver nanoparticles formed after the biological interaction was characterized by the UV spectroscopic analysis for the confirmation of nanoparticles at 420nm, FTIR analysis confirms the presence of various functional groups in the sample. SEM analysis revealed the presence of silver nanoparticles at 46 nm with spherical in shape. Antibacterial efficacy of the nanoparticles was tested against the disease causing shrimp pathogen *Vibrio harveyi* by invitro method. A zone of inhibition of 13mm was seen. These synthesized nanoparticles were found to be effective.

Keywords: Biosynthesis; Fish oil; Nanoparticle; Nanoparticles Characterization; Antibacterial activity; Vibrio pathogen

1. Introduction

Nanotechnology is one of the emerging sectors. Biological mediators are being synthesized for the materialistic applications through the customized biomedical concepts. The minimalistic nanoparticle synthesis is achieved through the various biological processes. This process helps in better optimization through the specificity, ecofriendly and low-cost method. The biological process of nanoparticle synthesis using natural products such as plant extracts, microbial and various microorganisms attained great interest in the field of nanotechnology. The present investigation deals with the synthesis of silver nanoparticles (AgNps) using fish liver oil which has various biomedical properties and different biologically active compounds
responsible for the reduction of silver ions are catalyzing various intermediate reactions in the byproduct metabolism [1-4]. This study investigates the activity of the fish liver oil in the silver nitrate suspension during the synthesis. Fish liver oil containing omega fatty acid derivatives have bioactive constituents like various essential lipids, fatty acids, vitamins and esters possibly involved in the nanoparticle synthesis. The mechanism of synthesis of silver nanoparticles substantially differs from the various other methods of synthesis based on plants or microbial origin. The activity of AgNps prudently received attention for many reasons; especially the antimicrobial property of the nano silver which is still being investigated for its efficacy due to its low toxicity and versatility upon invitro and invivo administration [5-7]. The fish oil-mediated synthesis of silver nanoparticles has advantages in both invitro and invivo biological experiments. The known facts about the biological nanoparticle synthesis and formulations with effective antibacterial, antifungal, anti-inflammatory and antiviral properties helps in various bio medical surgical treatments [8]. It also exhibit the bio-inspired target specific drug delivery property for various cancer treatments. This study exploits the biological interaction between the essential fatty acids and other compounds present in fish liver oil for synthesis of AgNps. These particles were further characterized by various analytical methods like UV-Visible spectrophotometer and Dynamic Light Scattering (DLS) particles size analyzer for studying nanoparticle behavior in the colloidal suspension, Fourier infrared spectroscopic (FT-IR) analysis to determine the functional groups involved in the interaction enhanced by the fish oil during the synthesis. Further, scanning electron microscopic (SEM) analysis was performed to study the nanoparticle morphology at the nano size range. After carrying out all characterizations, the bioactivity of the silver nanoparticle was studied to determine the invitro antibacterial activity against shrimp pathogen Vibrio harveyi. Shrimp aquaculture has achieved worldwide growth due to the increasing demand. Vibrio pathogens are one of the major pathogen which substantially affect the small scale and large scale production by causing huge mortalities in the freshwater cultivation of Macrobrachium rosenbergii [9, 10].

2. Materials and Methods

2.1. Extraction of fish liver oil

The milk shark (Pups) fishes were collected from the Vellore fish market, Vellore, Tamil Nadu. Then whole fish liver was dissected out, chopped and cut into small pieces for the extraction of fish liver oil. Chopped pieces were dried for a week to eradicate the moisture content. Dried liver was heated for 10mins at 40-50°C in a hot air oven to extract the oil. The extracted fish liver oil was filtered and stored at room temperature.

2.2. Synthesis of AgNps

Biosynthesis of silver using fish liver oil was carried out by the modified method of [11]. 1mM concentration of Silver nitrate (AgNO₃) aqueous solution was used for the silver nanoparticles synthesis. 5ml of Fish liver oil was added with 90 ml of 1 mMAgNO₃solution after the time and temperature optimization process. This process involved continuous stirring for 15mins at 20°C for reduction of AgNO₃ into Ag⁺ ions at room temperature to obtain more yield of AgNps. Then product was centrifuged at 10,000 rpm for 10mins for the efficient separation of nanoparticles.
2.3. Characterizations

Characterization of silver nanoparticles was done using various techniques like UV-Vis (Systronics 2201, India) spectrophotometer to check the absorbance of the AgNps at range between 200-700nm. Dynamic Light Scattering (DLS) (90 Plus Particle Size Analyzer, Brookhaven Instruments Corporation, Holtsville, USA), was employed to check the particle size and its distribution at the colloidal surface. Its zeta potential was also studied to check the long term stability of nanoparticles. Scanning Electron Microscopic (SEM) was employed to measure the size and formation of silver nanoparticle at nanometer scale. FT-IR spectroscopy with the operated resolution of 1.0 cm⁻¹ by Perkin Elmer Spectrum with KBr press and Mylar beam splitter used for the screening of functional groups involved in the reduction of metal ions.

2.4. Comparative antibacterial study

The methodology of [12, 13] was monitored. The antibiotic sensitivity test was carried out. Tetracycline obtained from Hi-Media was used as control. The disc was carefully placed on to the surface of the Muller Hinton agar plate, after making a lawn culture of the bacterium, the nanoparticles loaded disc was kept on the lawn culture of the test organism. Two discs were placed apart from each other. The discs were then placed firmly on to the surface. The plates were incubated at 30 °C for 24 h. The diameter of inhibition was measured. The experiments were carried out in triplicates. Similarly the well diffusion study was also performed with addition synthesized nanoparticles loaded into the well and control which was added with silver nitrate.

2.5. Growth kinetics study

The potentiality of silver nanoparticles (AgNps) and their antibacterial activity was further confirmed by the time to kill assay study, which showed maximum inhibitory activity of bacterial pathogen. The bacterial inoculum of *Vibrio harveyi* 1ml was mixed with 25 µl of AgNps kept as a positive control; the negative control was maintained without the nanoparticle. The growth of the bacterial culture was measured by time kinetics by every hours at 600nm in calorimeter.

2.6. Bactericidal activity

Bactericidal effect of AgNps against *V. harveyi* was studied. 10⁴ colony forming units was spread on to the surface by spread plate method. AgNps at different concentrations ranging from 10–50 µg cm⁻³ were added. Nanoparticles free LB plates were used as a control. The plates were incubated for 24 h at 37°C, after which the colony counting was carried out in triplicates.

3. Results and discussion

Biologically synthesized AgNps using essential oil is an emerging method in the Nano technological research, especially with the fish liver oil which was known for its medicinal value and economic importance. In this study the synthesis of AgNps was achieved using the fish oil. It was confirmed by the presence of color change from light yellow to dark brown during the synthesis process. This showed the corresponding peak in the UV-Visible spectrophotometry at the wavelength of 420 nm which further confirms the presence of nanoparticle formation and this peak shift reveals the surface plasmon resonance of the prepared silver nanomaterial as shown in the (figure 1).
The biological synthesis was probably achieved through the secondary metabolites present in the reductant which helps in the synthesis process. These secondary metabolites exist in the form of various chemical compounds and phytocompounds. These catalyzed the reactions between the fish oil and silver nitrate possibly utilized the energy transition during the reaction and electron transfer between ion reductions for nanoparticle synthesis [14-16].

The formation of nanoparticles was attributed to the particles size which is in the nano range. Nanoparticles size range was measured by DLS system and SEM analysis. The DLS measurement detected the particles which are in the nanoscale range. This technique analyze the samples at the Brownian motion, where the countless number of particles behaves in the particular wave with respect to poly dispersed index, result of the study showed the nanoparticles size distribution range exhibits at 124 nm in an average which is shown in(figure 2).

This DLS size range partially confirms the nanoparticles behavior, and it ascertained the nano range property of the particles. SEM is one of the microscopic techniques which clearly illustrate the nanoparticle behavior, morphology and size range with an accurate measurement. SEM analysis revealed that the fish oil synthesized nanoparticles were in the range of 45.97nm and exhibited spherical shape as shown in (figure 3).
Figure 3. SEM micrograph of the spherical shaped AgNps at the size of average size of 45.97nm

Generally the particles in the shorter wavelength possess increased size of the nanoparticles, and vice versa [17-19]. [20] reported that the TEM analysis of their study revealed the nano agglomerated spherical shaped nanoparticles in a well scattered manner which was highly preferable for most of the biological applications especially for the antibacterial drug delivery applications.

The FTIR bands were expressed in the synthesized AgNps as follows; 1745.58 cm\(^{-1}\), 1641.42 cm\(^{-1}\) showed the presence of C=C stretching, Peak at 1462.4, 1402.25 cm\(^{-1}\) showed the C-O stretching, 1157.29, 1095.57 cm\(^{-1}\) exhibit C-O-C stretching and the peaks exhibited at 723.31 and 991.41 cm\(^{-1}\) are responsible for the C-H bending and C-C bending respectively as shown in the (figure 4). These peaks may be due to the presence of various phyto constituents expressed during the synthesis namely poly phenols, phenols, alcohols, esters and carboxylic groups and aliphatic, aromatic and alicyclic compounds. [20,21] reported the AgNps by laser ablation method which describes the functional groups present in coconut oil and castor oil.

Figure 4. FTIR Spectrum of fish oil interacted AgNps

This present study also focuses on the formation of AgNps and its antibacterial application against gram negative pathogens for controlling bacterial growth. The fish oil synthesized AgNps showed very good antibacterial activity against the pathogenic microorganism of \textit{Vibrio} which was responsible for causing severe disease outbreaks in
aquaculture farms. The in vitro antibacterial activity performed by well diffusion and disc diffusion methods revealed that it was found to be effective in controlling the pathogen. The zone of inhibition was found to be significantly high in the AgNps loaded disc and well (i.e. 14mm and 17mm respectively), whereas the control antibiotics showed 23mm of zone of inhibition. [10] reported the antibacterial activity of AgNps against the pathogenic *V. harveyi* using green tea leaf extracts and its protective efficacy on juvenile *Feneropenaeus indicus*. They also reported that AgNps based treatment diet through the in vitro and in vivo treatment was found to be a promising tool for controlling the *V. harveyi* infection in shrimps. [14] proposed the use of green leaves of *Leucasaspera* mediated silver synthesis for the control of *Aeromonas hydrophila* in *Catla catla* because of its antibacterial activity.

In addition to the antibacterial activity, antimicrobial assay of bacterial growth kinetic assay was performed to support the effectiveness of the AgNps by the time to kill assay. Freshly prepared bacterial culture of *V. harveyi* was studied for its growth performance. Bacterial growth was calorimetrically recorded in both control and test group. Control flask gradually attained the maximum of 2.8 OD for bacterial growth was observed, whereas in the test group supplemented with the highest concentration of the 25µg/ml of synthesized nanoparticles added to the flask, no growth was observed after the 10th hour as shown in (figure 5). This study revealed that the AgNps arrest the bacterial growth at the maximum concentration 25µg/ml. [10] reported the growth curve of *V. harveyi* with addition of different concentrations of AgNps in the culture containing Luria–Bertani (LB) broth, with the highest concentration of 35 µg/1ml arresting the complete growth of the test organism. They also reported the culture present in the media is directly related to the concentration of AgNps. This was corroborated with our present study. The results of our study indicate that synthesized AgNps were found to be effective in controlling shrimp pathogen *V. harveyi*.

![Figure 5. Bacterial growth kinetics study with the AgNps interacted bacterial culture](image)

Many studies have been carried out for testing the potency of the bacterial culture on the agar plates. Bactericidal activity is one of the most common methods to ascertain the bacterial growth inhibition on the agar plate. Our study aims to improve the antibacterial efficacy of the fish oil synthesized silver nanoparticles against the virulent, disease causing opportunistic *Vibrio* pathogen. The results of the present study indicate that bactericidal effect of nanoparticles exhibit a strong activity towards controlling the bacterial colony formation on the bacteria cultured nutrient broth. Different concentrations of the nanoparticles ranging from 10 µg to 40 µg were
supplemented to the broth by spread plate technique. Results shown that the increasing nanoparticles concentrations decrease the number of colony growth on the agar plate shown in the (figure 6). Similarly the study proposed by the [22-23] suggested the same concept that increasing concentration of AgNps will greatly influence the growth of the gram-negative bacterium *Escherichia coli*.

![Figure 6. Bactericidal activity of AgNps](image)

**Figure 6. Bactericidal activity of AgNps**

### 4. Conclusion

In conclusion, it is quite obvious that the synthesis of nanomaterial from the plant source demands a constant supply of plant materials for efficient synthesis. A similar method of synthesis with the effective biological properties can also be achieved with the same efficiency by essential oil mediated biological synthesis, oils extracted from the animal origins (especially liver) are known to have biomedical application in nanotechnology.

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**Conflict of Interest**

Authors have no conflict of interest to declare.

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