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## BIOSYNTHESIS OF SILVER NANOPARTICLES USING *KAPPAPHYCUS SP* AND THEIR ANTIBACTERIAL ACTIVITY

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### Abstract

The systematic eco-based approach of silver nanoparticles (Ag-NPs) have developed using (*Kappaphycus alvarezii*) has tremendous advantages in drug discovery. The sponge was extracted for biosynthesis of Ag-NPs from the aqueous silver nitrate ( $\text{AgNO}_3$ ) solution. The Ag-NPs was characterized by UV-Vis, scanning electron microscopy (SEM). The Ag-NPs surface plasmon exhibited maximum at 445 nm. SEM show the surface morphology of Ag-NPs. The antibacterial activity was performed *Escherichia coli* and *Staphylococcus aureus*. This study concludes that the Ag-NPs synthesized from *K. alvarezii* has great potential compound to target against pathogenic microorganisms.

**Keywords:** Silver nanoparticles, *K. alvarezii*, Spectroscopic studies, Antibacterial Activity

### 1. Introduction

The development of biologically inspired process for the synthesis of silver nanoparticles is emerging into a significant field of nanotechnology. In this aspect metal nanoparticles have been the subjects of focused researches due to their unique electronic, optical, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials [1]. Their growing applications in various fields like biosensors [2] bioremediation of radioactive wastes [3], functional electrical coating[4], synthesis of enzyme electrodes[5] and particularly in medicine such as delivery of antigen for vaccination [6], gene delivery for treatment or prevention of genetic disorder[7], inspired the scientists to develop environment friendly procedures for the synthesis of nanoparticles and to avoid use of hazardous chemicals. In recent years, a significant number of novel metabolites with pharmacological properties have been discovered from the marine organisms [8].

Macroalgae have been widely recognised as producers of a broad range of bioactive metabolites [9, 10]. Numerous reports have described active compounds derived from macroalgae which has a broad range of biological activities,

such as antibiotics antifouling, anti-inflammatory, cytotoxic and antimitotic activity [11-17]. Harder [18] was the first who reported the antimicrobial substances secreted by algae.

A rapid reduction of the silver ions was observed when the silver nitrate solution was contacted with *Kappaphycus alvarezii* algal extract. Biological methods of nanoparticles synthesis using microorganism, enzyme, and plant or plant extract have been suggested as possible ecofriendly alternatives to chemical and physical methods [19] which have a possible outcome for drug delivery systems [20].

## **2. Materials and Methods**

### **2.1 Collection and Extract Preparation**

The algae, *K. alvarezii* were collected from subtidal areas of the sea at Rameswaram, India (latitude 18.55 N and longitude 72.54 E). After collection, the samples were rinsed with fresh seawater and distilled water to remove associated debris and epiphytes. The cleaned material was then air dried to dryness in the shade at 30°C. The dried samples were finely powdered and stored at -20°C until use. Approximately 10 g of *K. alvarezii* biomass was taken in a conical flask containing 100 mL of distilled water, kept for 24 hrs and then the aqueous solution components were separated by filtration. To this solution (liquid algal), AgNO<sub>3</sub> (10<sup>-3</sup> M) was added and kept for several hours at 24 hrs. Periodically, aliquots of the reaction solution were removed and the absorptions were measured in a Elico UV-Vis spectrophotometer.

### **2.2 Synthesis and Characterization**

For the synthesis of Ag- NPs 1ml of algal extracts as test solution were incubated at room temperature for 1-2 hours. The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 15,000 rpm for 20min. Supernatant is discarded and the pellet is dissolved in deionised water. The silver nanoparticles were confirmed by colour changes and qualitatively characterized by UV-visible spectrophotometer on a Elico UV- Vis spectrophotometer.

### **2.3 UV-Vis spectroscopy analysis**

UV- Vis spectral analysis was done by using Elico UV- Vis spectrophotometer. The reduction of pure Ag<sup>+</sup> ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 24 hrs, after diluting a small aliquot of the sample into distilled water. The UV-Vis spectra reveal the formation of silver nanoparticles by showing surface plasmon resonance at 440 nm.

## 2.4 SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) (Fig. 4) analysis was done using FEI QUANTA 200 FEG HR-SEM model. Thin films of the sample were prepared on a carbon coated, a very small amount of the specimen on the sample holder, extra solution was removed using a blotting paper, and then the film on the SEM allowed to dry by putting it under a mercury lamp for 5 min.

## 2.5 Results & Discussion

The colour change showed the presence of silver nanoparticles in the *K. alvarezii* extract and it was characterized by UV-visible spectrophotometer and monitored by taking readings at regular time intervals in a Elico UV- Vis spectrophotometer. The strong broad peak located at 455nm was observed for silver nanoparticles. UV-visible spectroscopy is one of the most widely used techniques for structural characterization of silver nanoparticles.

The absorption spectrum (Fig. 1) of the pale yellow-brown silver colloids prepared by hydrazine reduction showed a surface Plasmon absorption band with a maximum of 445 nm Scanning Electron Microscope (SEM) surface morphology image showed relatively spherical shape silver nanoparticles formed with diameter range 100-200 nm in Figure [2].

Finally, the antimicrobial susceptibility of silver nanoparticles synthesized was investigated. The disk diffusion method was used as antimicrobial susceptibility testing method. Disposable plates inoculated with the tested Gram-positive and Gram-negative bacteria, including highly multiresistant strains such as Chloramphenicol, Rifampicin, were used for the tests. Zones of inhibition were measured after 24 hr of incubation at 35 C.

The comparative stability of discs containing Chloramphenicol and Rifampicin was made. Figure [3] shows plates to which a bacterial suspension was applied. The presence of nanoparticles at a certain level inhibited bacterial growth by more than 90%. The diameter of inhibition zones (in millimeters) around the different silver nanoparticles against test strain are shown in Figure [3].

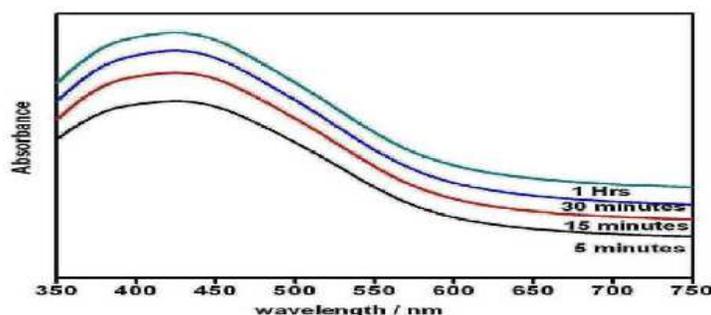


Figure 1, The UV-visible spectrum

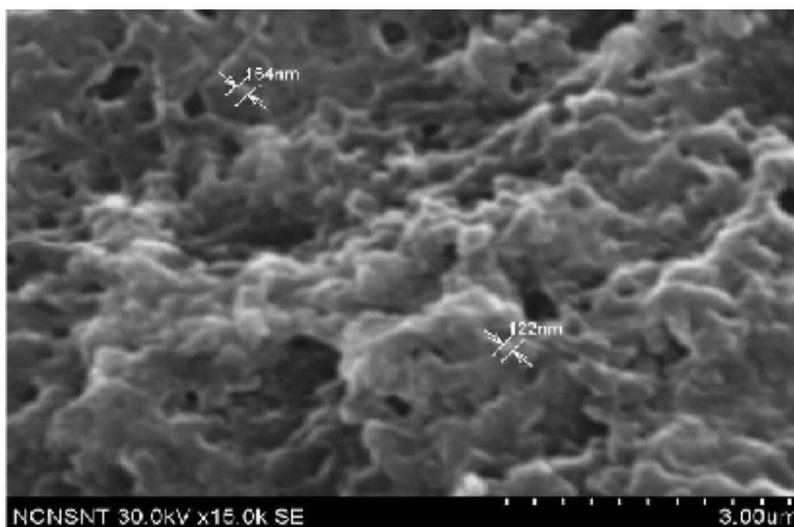


Figure 2. Scanning electron micrograph

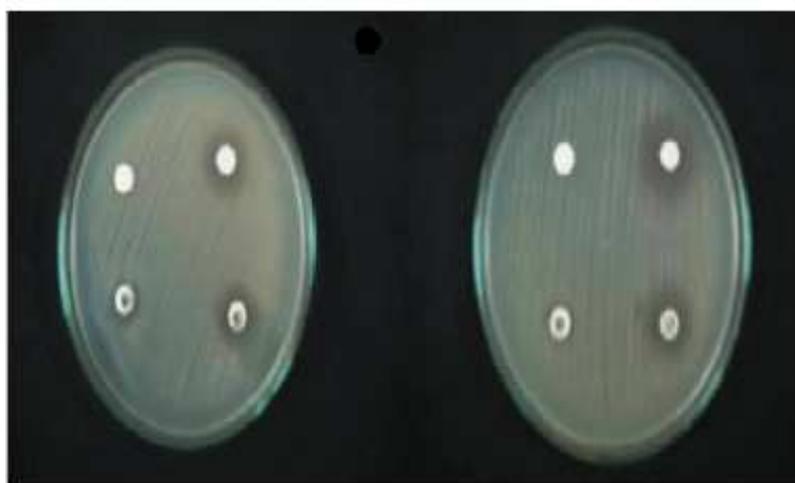


Figure 3. Antimicrobial activities of Ag nanoparticles

### 3. Conclusion

In summary, silver nanoparticles with mean diameters of 9,11, 24 and 30 nm were synthesized using silver nitrate solution. . The nanoparticles were characterized by UV/Vis, and SEM. UV/Vis spectra show the characteristic plasmon absorption peak for the silver nanoparticles ranging from 455 to 457 nm. Additionally, the antibacterial activity of the nanoparticles dispersion was measured by Disc diffusion method. The results of this study clearly demonstrated that the colloidal silver nanoparticles inhibited the growth and multiplication of the tested bacteria, including highly multiresistant bacteria such as Chloramphenicol and Rifampicin resistant against *Escherichia coli* and *Staphylococcus aureus*. Such high antibacterial activity was observed at *Staphylococcus aureus*, with Chloramphenicol.

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