Balaraman Bala Baskar et al. IJRPS 2013, 3(3), 55-63

Research Article Available online www.ijrpsonline.com ISSN: 2249–3522

International Journal of Research in Pharmacy and Science

Biosynthesis of silver nanoparticles using Kappa phycus species

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ABSTRACT

In recent years, studies on the improvement of biological techniques for synthesis of nanoparticles have been extensively increased due to the harmful effects of chemical preparation methods, in this present work, a nontoxic, green and ecofriendly protocol for the synthesis of silver nanoparticles using *kappa phycus sp* were successfully synthesized and characterized. Maximum absorbance was measured at 200-800 nm scanning method, synthesis of silver nanoparticles was investigated by uv-vis spectroscopy, Atomic force microscopy and Fourier transform infrared spectroscopic analysis. Results indicates change in color from colorless to brown that is a clear indication of the formation of silver nanoparticles in reaction mixture, Uv-visible spectrum shows strong peak was absorbed between 400-420nm indicates silver the presence of silver nanoparticles.Afm images shows particle size of the silver nanoparticles ranges in size from 52-104nm.FT-IR peaks were in the extract ranging from 4000-450cm^{-1} at a resolution of 4cm^{-1} which confirmed for the synthesis and stabilization of silver nanoparticles.

KEYWORDS: Silver nanoparticles, alga *Kappa phycus*, Silver nitrate, Atomic force microscopy, Uv-visible spectroscopy.

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INTRODUCTION

Nanotechnology is emerging field of science which involves synthesis and development of various nanomaterials.At present, different types of metal nanomaterial's are being produced using copper, zinc, titanium, magnesium, gold,alginateand silver1.Nanomaterials are used in various purposes like nanovaccines, nanowires, surgical blades etc.

Silver is the metal of choice of preparation of Nps and his potential applications in the field of biological systems.synthesis of Nps from the above metals has reported using chemical, physical and biological methods.Among the methods involved, biological methods are currently gaining importance because they are eco-friendly, cost-effective, and don't involve the use of any toxic chemicals for the synthesis of Nps2.

Previous studies reported the biosynthesis of silver nanoparticles by plant such as Calotropis gigantean Leaf 3,Murraya Koengii (curry leaf) 4,Euphorbia hirta L 5,Vitex Negundo L6, and Microorganisms such as *Aspergillus fumigatus*[7], *Pencillium sp*[8] , *Trichoderma sp*[9], *candida albicans*[10],*Phoma glomerata*[10] ,*Phoma herbarum*[10],*Fusarium semitectum*[10],*Trichoderma sp* [10], *Pleurotus sajor caju*[11] , *Aspergillus flavus*[12],*Bacillus cerus*[13],*Aspergillus niger*[14], *Ostillatoria willei*[15], *Pestalotia sp*[16] , *Urospora sp*[17],*Xanthomonas campestris pv malvacearum*[18].Algae possess some advantages over bacteria and fungi, Algae having high tolerance wall-binding capacity and intracellular metal uptake [19]. This study involves the biological synthesis of silver nanoparticles using filamentous fungus and the characterization of the synthesized silver nanoparticles by UV-Spectroscopy, Atomic force microscopy (AFM) analysis and FTIR analysis 20. Further research is needed to develop the application of silver nanoparticles using various microbes.

MATERIALS AND METHODS

Source of Microorganisms

The *kappa phycus sp* was obtained from R&D department (Zeoes biotech, Aarakkonam) and maintained in natural water at 36'c.

Biosynthesis of silver nanoparticles

The *kappa phycus sp* was selected for the further studies for the production of silver nanoparticles. The*Kappa phycus sp*was inoculated in liquid media containing (g/l) KH_2PO_4, 7.0:K2HPO4, 2.0; MgSO4. $7H_20$, 0.1; $(NH_4)_2SO_4$, 1.0; Yeast extract, 0.6; and glucose, 10.0. The flasks were incubated at 25'c for 3 days in a rotary shaker at a speed of 150rpm. The biomass was harvested after 72 h of growth by sieving through a plastic sieve. The biomass was washed with sterilized distilled water to remove any medium component. 20 g of biomass (fresh weight) was mixed with 200 ml of deionized water in a 500 ml Erlenmeyer flask and agitated in the same condition for 72 h at 25^{o}C.After the incubation, the cell filtrate was obtained by passing it through Whatmann filter paper no.1.Filtrate was collected and used further from nanoparticles synthesis.

For the synthesis of silver nanoparticles, 50ml of 1mM $AgNO_3$solution was mixed with 50 ml of cell filtrate in a 250ml Erlenmeyer flask and agitated at 25^{o}C in dark .Control (without the silver ion.only biomass) was also run along with the experimental flask.

Characterization of synthesized silver nanoparticles

UV-visible spectroscopic analysis

The reduction of silver ions was confirmed by qualitative testing of supernatant by UV-visible spectrophotometer. 1ml of sample supernatant was withdrawn after 24hr and absorbance was measured by using UV-visible spectrophotometer between 400-600nm.

Fourier Transform Infrared (FTIR) Spectroscopy analysis

The lyophilized sample was subjected to FTIR Spectroscopy analysis. Two milligrams of the sample was mixed with 200 mg KBr (FT-IR grade) and pressed into a pellet. The sample pellet was placed into the sample holder and FT-IR spectra were recorded in the range 4000-450cm^{-1} in FT-IR spectroscopy at a resolution of 4 cm^{-1}.

Atomic force microscopy

A thin film of the sample was prepared on a glass slide by dropping 100 µl of the sample on the slide, and was allowed to dry for 5 min.The slides were then scanned with the AFM.

RESULTS AND DISCUSSION

Source of *Kappa phycus species*

Alga *kappa phycus sp* is obtained from R&D department (Zeoes biotech, Aarakonam, Vellore dist, Tamilnadu).The algal isolates were characterized on the basis of colony Characteristics and macroscopic appearance.

Figure 1: Macroscopic Appearance of *Kappa phycus sp*

Characterization of silver nanoparticles

Color change

Cell filtrate of *Kappa phycus sp* was mixed with silver nitrate solution and incubated in dark in rotary shaker.Samples showed changed in color from almost colorless and brown, this is a clear indication of the formation of silver nanoparticles in the reaction mixture. The intensity of the color was increased during the Period of incubation. The appearance of brown color was due to the excitation of surface plasmon vibrations. Control showed no change in color of the mixture when incubated in the same conditions. Results are reported in Figure 2.

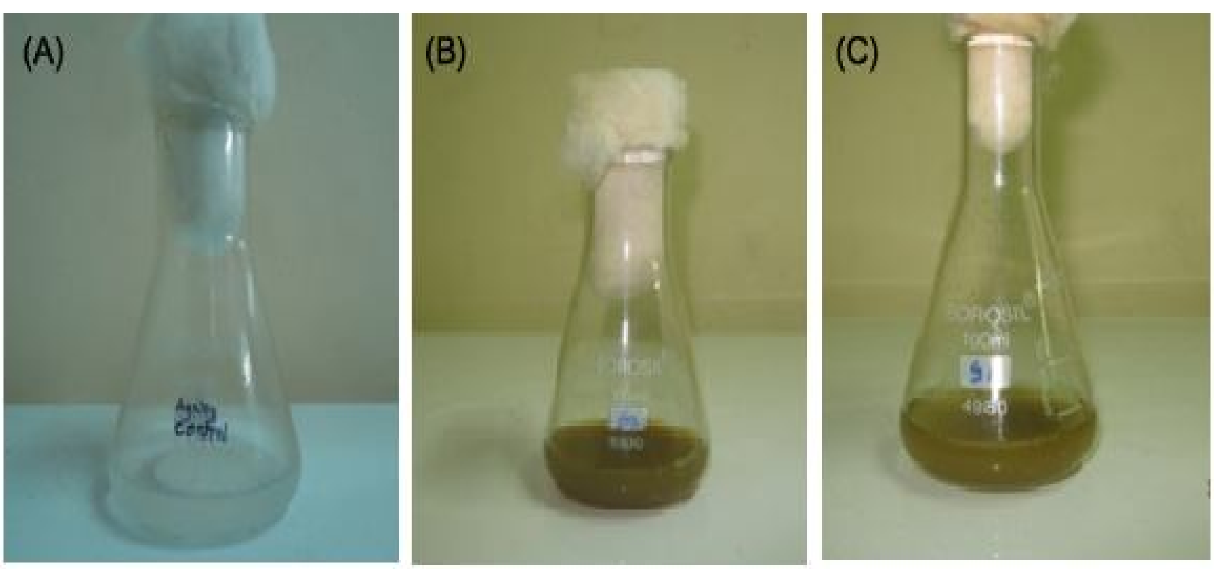

Figure 2: Biosynthesis of silver nanoparticles- color change reaction: conical flasks containing the extracellular filtrate of the kappa phycus sp.biomass (a) control (b) biomass after exposure of $Agno_3$ solution for 24 hr (c) after 72 hr.

UV – Visible spectroscopic analysis

Synthesis of colloidal silver nanoparticles was initially performed by UV-Visible Spectroscopic analysis.In UV-Visible spectrum, a strong peak was observed between 400-420nm,indicate the presence of silver nanoparticles. UV-visible spectra is reported in figure 3.

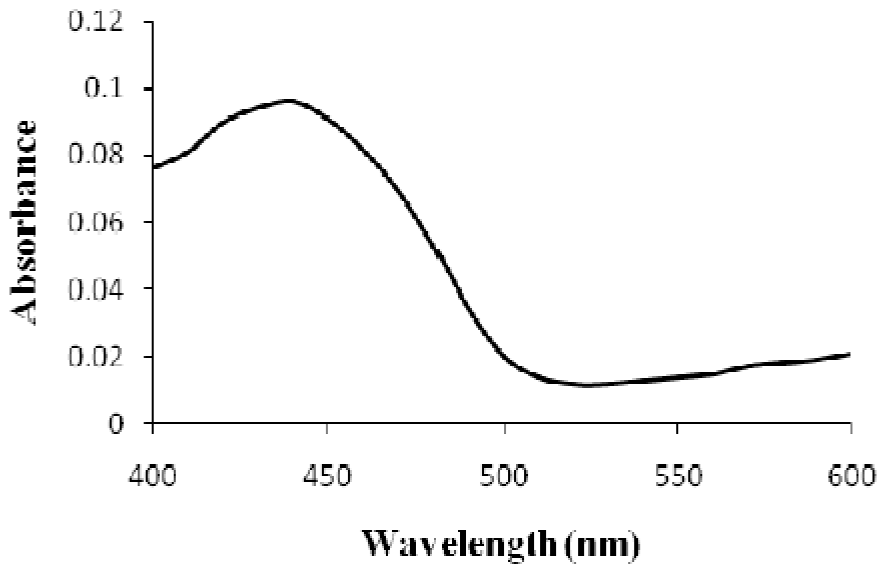

Figure 3: UV-visible spectrum of silver nanoparticles synthesized *using kappa phycus sp.*after 24 h

FTIR analysis

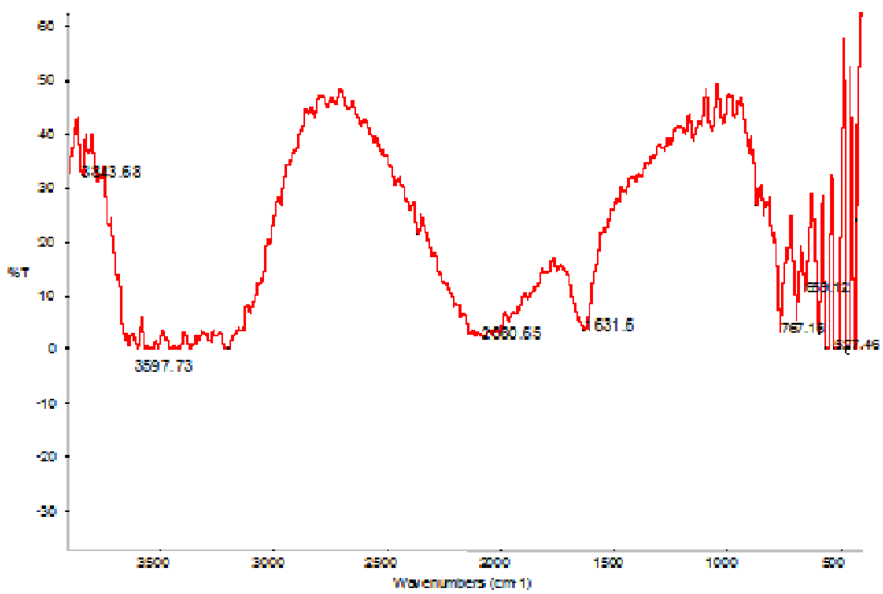

Figure 4: FTIR analysis of silver nanoparticles biosynthesis by using *kappa phycus sp.*

The lyophilized nanoparticle samples were analyzed in FTIR to identify the possible biomolecules responsible for the reduction of the Ag+ions by the cell filtrate.The FTIRspectrum is presented in figure 4. The representative spectra of nanoparticles obtained manifests absorption peaks located at about 3843.68cm^{-1} (-NH group of amines), 3597.73 cm^{-1} (-OH group of phenols), 2080.65 cm^{-1} (aromatic –CH stretching), 1631.66cm^{-1}(-NHCO of amide) and 767.16 cm^{-1} (C-C1).

Atomic force microscopic analysis

The silver nanoparticles were characterized by AFM for its detail size, morphology and

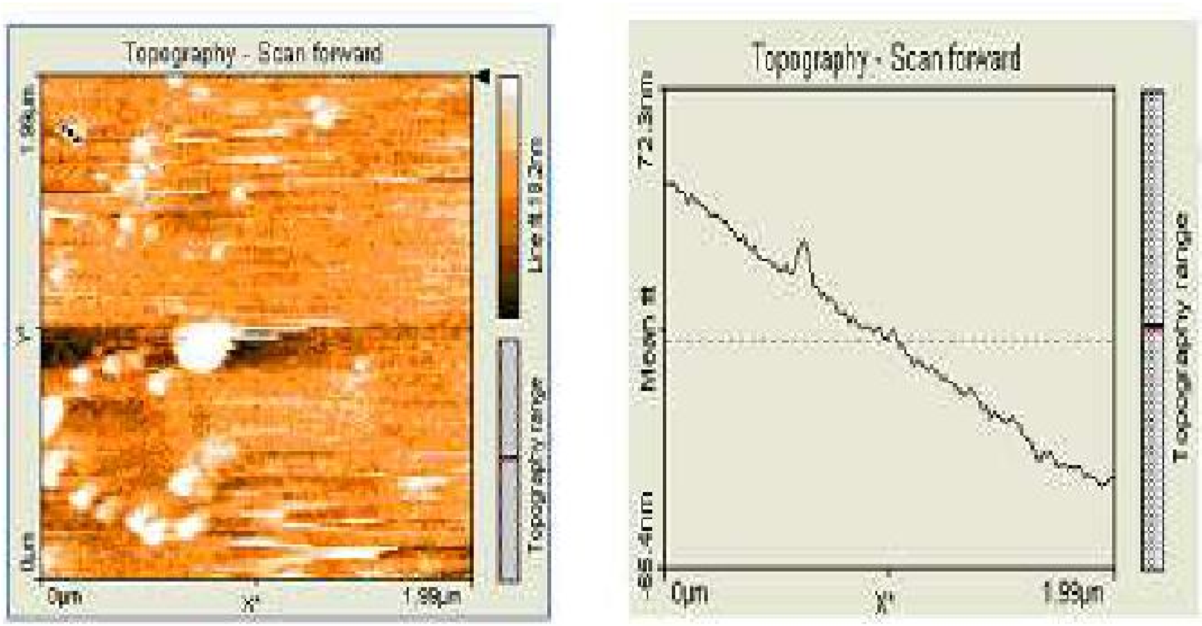

Figure 5: Atomic force microscopy image shows formation of nanoparticles by Kappa phycus sp.

0.02-0.77 N/m, tip height 10-15 nm, contact mode.it wad observed that the silver nanoparticles agglomerated and formed distinct nanostructures (nanoparticles). The topographical image of irregular silver nanoparticles is reported in figure 5.Formation of silver nanoparticles and its agglomeration was clearly observed in figure. The particle size of the silver nanoparticles ranges in size from 52-104nm.agglomeration of silver. AFM images were taken with silicon cantilevers with force constant.

CONCLUSION

In this study silver nanoparticles were biologically synthesized using alga *kappa phycussp* collected from sea water samples. The cell filtrate of kappa phycus sp.was challenged with 1Mm of silver nitrate, change of the mixture from colorless to orange brown indicate the synthesis of silver nanoparticles in the reaction mixture, size of the synthesized nanoparticles was measured 52-104 nm by AFM analysis. Results conclude that collected kappa phycus sp sample from marine water is prominent producer of silver nanoparticles.

ACKNOWLEDGEMENT

We great fully acknowledge the management of Vel's university for providing required facilities.

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