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Original Research Article

Biosynthesis of silver nanoparticles from marine alga *Colpomenia sinuosa* and its antibacterial efficacy

M.Vishnu Kiran* and S.Murugesan

Unit of Algal Biotechnology and Bionanotechnology, PG and Research Department of Plant biology and Biotechnology, Pachaiyappa's College, Chennai 600 030, Tamilnadu, India
Corresponding author

Keywords

Silver nanoparticle, *Colpomenia sinuosa*, Antibacterial activity

A B S T R A C T

Silver nano particles are known to be good antibiotic agents. In this study biosynthesis of silver (Ag) nano particles of marine alga *Colopomenia sinuosa* were obtained by green synthesis method and their efficacy was studied against Gram-positive and Gram-negative bacteria. The efficacy was performed using Kirby – Bauer Method and MIC, MBC were also determined. The extra cellular mechanism of silver nano particle formation was characterized by UV-vis spectroscopy, Fourier Transform Infrared (FT-IR) Spectroscopy-ray Diffraction (XRD), and Scanning Electron Microscopic (SEM) study showed the formation of silver nano particle in the range of 54-65 nm in size. Silver nanoparticles showed greater efficacy towards Gram-positive and Gram-negative microorganisms compared to the standard antibiotics.

Introduction

Silver has been known for antibacterial activity since ancient times. The importance of silver has regained due to the increase of bacterial resistance to antibiotics, caused by their overuse. Silver nano particles and silver based compounds containing ionic silver (Ag^{+}) or metallic silver (Ag^{0}) which possess antimicrobial activity are of great importance in the field of medicine. Silver nano particles are attractive as these are non -toxic to human body at low concentrations and have broad spectrum anti-bacterial nature. Silver nano particles inhibit bacterial growth at low concentrations than standard antibiotics and have no side effects. Silver ions interact with a number of components of bacterial, protozoal and fungal cells. Studies have demonstrated that silver ions interact with sulfhydryl (-SH) groups of proteins as well as the bases of DNA leading either to the inhibition of respiratory process. Inhibition of cell division and damage to bacterial cell envelopes as well interaction with hydrogen bonding process has been demonstrated. In the current study green synthesis of silver nano particles from marine alga *Colpomenia sinuosa* were characterized and their efficacy was studied against bacterial pathogens.

Materials and Methods

Bio-synthesis of silver nanoparticles

Silver nano particle synthesis was carried out by taking 500 mg of dry seaweed powder of *Colpomenia sinuosa* in 250 ml Erlenmeyer flask with 10^{-3} M aqueous (Silver $AgNO_3^-$) solution and incubated at room temperature. The pH was checked during the course of reaction and it was found to be 5.09. The bio-synthesis of silver nanoparticles was characterized by UV–Vis nanophotometer; size and morphology by employing SEM, structure from X-ray diffraction (XRD) technique and Fourier transform infrared (FT-IR) spectroscopy.

Bacterial susceptibility to nanosilver

The bacterial cultures were obtained from IMTECH, Chandigarh, India. *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Klebsiella pneumoniae and Proteus vulgaris* are the strains used for the efficacy studies towards silver nano particles. Zone of inhibition test was performed in LB agar plates where 20 ml LB agar was poured in well rinsed, autoclaved petriplates, 1.0 ml of active bacterial culture was homogeneously spread in the agar plates and 30 µg/ml of silver nano particle was placed in the sterile disc which was placed on the LB agar along with the standard commercially available antibiotics of similar concentration. The plates were incubated at 37^0C for 24 hrs.

Minimum inhibitory concentration

The bactericidal activity of Ag Nano particles was determined by Minimal inhibitory concentration. Bacterial cells were grown in LB medium and 500 µl of 24 h - old bacterial culture (0.1 OD) was spreaded over the LB agar plates, supplemented with 5, 15, 30, 45 and 60 µg/ml of bared Ag nano particles. The plates were incubated at for 24h. Antimicrobial test compound below the MIC cannot inhibit microbial growth. The lowest concentration that inhibited the complete activity of microorganisms were recorded as minimum bactericidal concentration

Results and Discussion

Silver nano particles are formed by the reduction of Ag^+ during exposure to the extract of *Colpomenia sinuosa* followed by UV-Nano photometer. Pale yellow to brown colour formation indicates the presence of silver nano particles in solution. The change in colour formation arises due to the excitation of surface plasmon vibrations in the silver metal nano particles Fig 1 shows the UV – Nano photometer from the biosynthesized silver nanoparticles obtained from the extract of the marine brown alga *Colpomenia sinuosa*. It is observed that the silver surface plasmon resonance band occurs at 420 nm, the frequency and width of surface plasmon absorption depends upon the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding metal. It is generally recognized that UV – Nano photometer could be used to examine absorption peak of controlled nanoparticles in aqueous suspensions.

The FT-IR spectral measurements were carried out to identify the possible biomolecules form brown alga *Colpomenia sinuosa* which is responsible for reducing and capping the bio reduced silver nano particles. Fig 2 shows the FT-

IR spectrum analysis of silver nano particles which manifests absorption peaks. The absorption peak at 3435 cm^{-1} can be assigned as N-H stretching and that at 2923 cm^{-1} as CH2-C-H (Methyl) stretching, 2853 cm^{-1} as C-H (Methylene) stretching, 1633 cm^{-1} as C=C –stretching, 1469 cm^{-1} as C-C- stretching, 1103-1034 cm^{-1} as C-O stretching and 875-603 cm^{-1} as C-H out of plane bending respectively. The FT-IR spectrum provided information about the molecular environment of the organic molecules on the surface of nano particle.

Fig 3 shows the SEM images recorded from drop coated films of the silver nano particles synthesized by treating silver nitrate solution with the extract of the marine brown alga *Colpomenia sinuosa.* The silver nano particles formed were predominately cubical with uniform shape as reported by *Chandran et al*. It is known that shape of the metal nano particles considerably change their optical and electronic properties. The size of the silver nano particles was found *to* be 54-65 nm.

Figure 4 shows the X-ray Diffraction patterns of silver nanoparticle were recorded according to the description of Wang (2000). Samples were air dried, powdered and used for XRD analysis. Fig 4 shows the XRD patterns obtained from biosynthesized silver nano particles using *Colpomenia sinuosa* shows characteristics peaks shows characteristics peaks at (2^{o} =1), marked with {111}. A number of Bragg reflections corresponding to the {111} sets of lattice planes are observed which may be indexed based on the face- centered cubic structure of silver. The XRD pattern thus clearly shows that the silver nano particles are crystalline in nature. The XRD pattern of pure silver ions is known to display peaks at 2^{o} =1. The value of pure silver lattice constant has been estimated to be α = 4.081, a value that is consistent with α =4.0862 A0 reported by the JCPDS file no 4-0783.This estimation confirmed the hypothesis of particle monocrystallinity. The sharpening of the peaks clearly indicates that the particles are in nanoregime. The size of the silver nano crystallites as estimated from the FWHM of the {111} peak of silver using the Scherrer formula were reported.

Silver nano particles synthesized from marine alga *Halymenia poryphyroides* were tested against bacterial pathogens *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Klebsiella pneumoniae and Proteus vulgaris* and their efficacy were determined. The bacterial pathogens were susceptible to silver nanoparticles (30 µg/ml) in the range 20-22 mm in diameter as show in figure 5 compared to standard antibiotics Streptomycin, Erythromycin, polymyxin B and tetracycline. Minimum Inhibitory concentration was observed at a concentration of 5 µg/ml for all the organisms. The efficacy of silver nano particles may be attributed by the phosphorylation of various proteins in the bacterial pathogens and is found to influence the bacterial signal transduction. The gram negative bacteria have a layer of lipopolysaccharide at the exterior, followed underneath by a thin (about 7-8 nm) layer of peptidoglycan. They lack strength and rigidity although they are composed of covalently linked lipids and polysaccharides. Negative charges on the lipopolysaccharides are attracted towards weak positive charges available on silver nano particles. Gram positive cell wall is composed of a thick layer of peptidoglycan (20-80 nm) consisting of linear polysaccharide chains cross linked by short peptides to form three dimensional rigid structure. The rigidity

Fig.1 UV-Vis – Nano photometer of Silver Nanoparticles

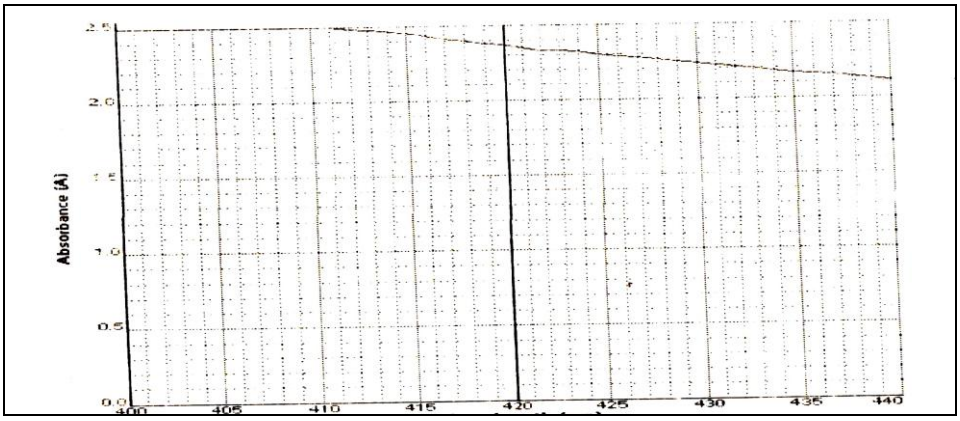

Figure.2 Fourier Transform Infra-Red analysis of Silver Nanoparticles

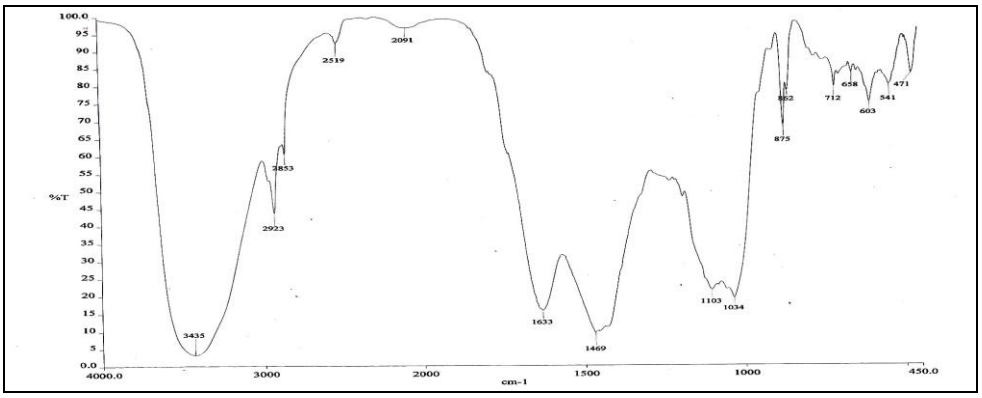

Table.1 FT-IR Spectrum of *C.sinuosa* mediated synthesized silver nano particles

Group	**Frequency Range (cm^{-1})**
N-H Stretching	3435
CH_2-C-H (Methyl) stretching	2923
C-H (Methylene) stretching	2853
C=C –stretching	1633
C-C- stretching	1469
C-O stretching	1034
C-H out of plane bending	875

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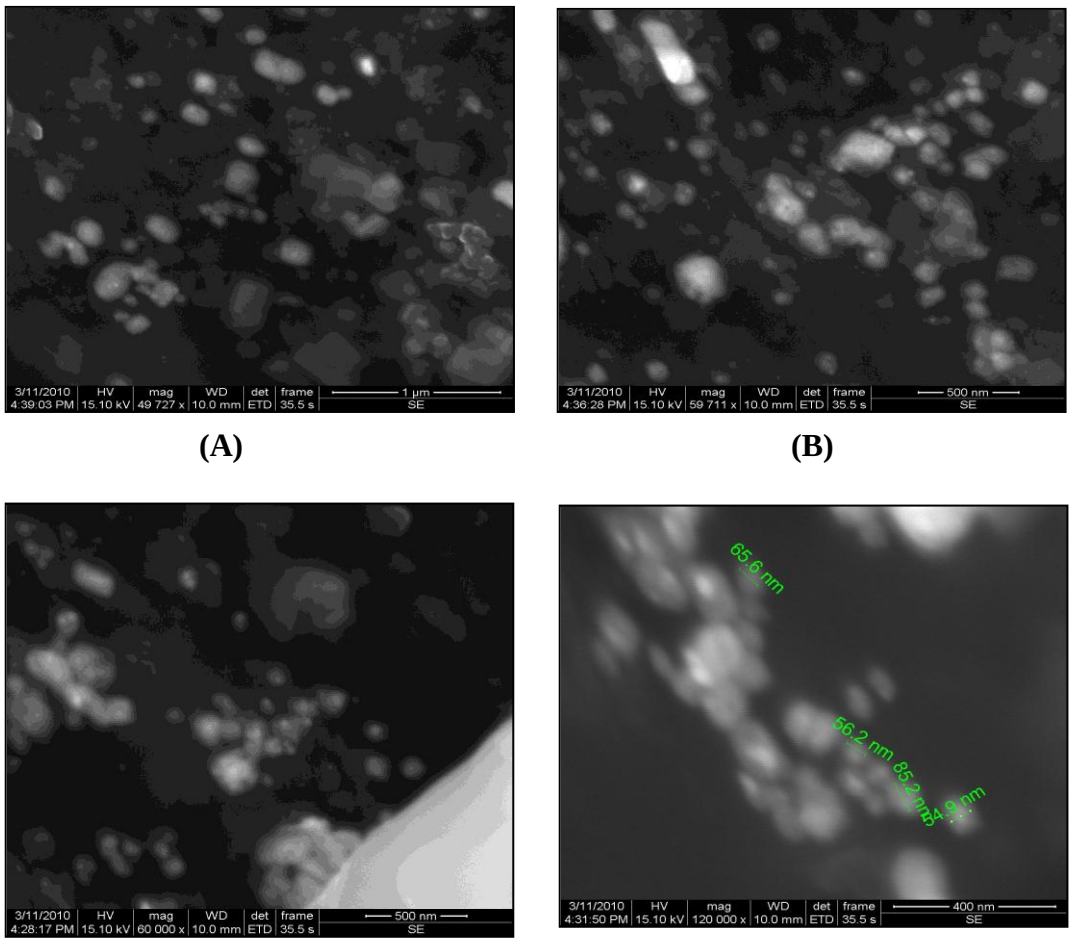
Figure.3 Scanning Electron Micrograph of silver nanoparticles



(A) **(B)**

(C) **(D)**

(A) 49727 X Magnification (B) 59711 X Magnification; (C) 60000 X Magnification (D) 12000 X Magnification

Figure.4 XRD studies of silver nano particles

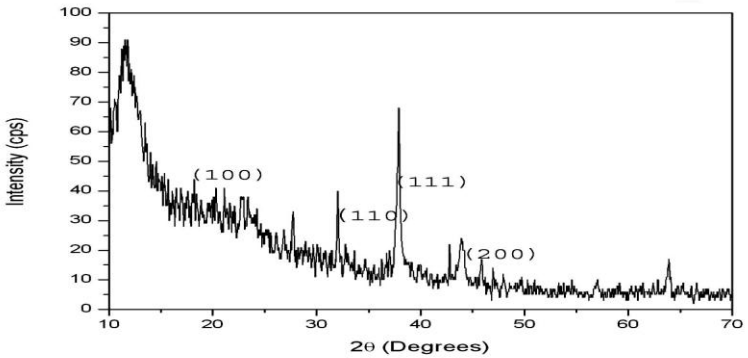


Table.2 Anti-bacterial efficacy of silver nano particles

Staphylococcus aureus	
Silver Nano particles	20 / mm
Streptomycin	10 / mm
Erythromycin	2 / mm
Polymyxin B	6 / mm
Tetracycline	6 /mm
Salmonella typhi	
Silver Nano particles	22 / mm
Streptomycin	10 / mm
Erythromycin	2 / mm
Polymyxin B	6 / mm
Tetracycline	4 / mm
Escherichia coli	
Silver Nano particles	20 / mm
Streptomycin	10 / mm
Erythromycin	2 / mm
Polymyxin B	6 / mm
Tetracycline	2 / mm
Klebsiella pneumoniae	
Silver Nano particles	20 / mm
Streptomycin	10 / mm
Erythromycin	2 / mm
PolymyxinB	4 / mm
Tetracycline	2 / mm
Proteus vulgaris	
Silver Nanoparticles	20 / mm
Streptomycin	10 / mm
Erythromycin	2 / mm
Polymyxin B	6 / mm
Tetracycline	4 / mm

and extended cross linking endow the cell walls with fewer anchoring sites and the efficacy varies depending upon the concentration of silver nanoparticles. Thus the silver nano particles showed a greater efficacy for bacterial pathogens as compared to commercially available antibiotics of the same concentration. This efficacy of silver nano particles may also be attributed to its use over the commercially available antibiotics which are less futile due to their overuse and becoming drug resistant. Therefore silver nano particles play an important role in antimicrobial activity.

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