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Research Article

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Enhanced bactericidal effect of silver nanoparticles synthesized using marine brown macro algae

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ABSTRACT

In the present investigation silver nanoparticles of spherical shape was synthesized biologically by rapid biological drip method using marine brown macro algae, Sargassum wightii (S. Wightii). Seaweed extract act as a reducing agent for silver nitrate. Silver nanoparticles have been characterized by UV-Visible spectrophotometer, Fourier-Transform Infrared spectroscopy (FTIR), X-ray diffractometer (XRD) and High Resolution Transmission Electron Microscope (HR-TEM). Antibacterial activity of these nanoparticles was carried out by antibiotic disc diffusion method against pathogenic microorganisms such as Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae. Seaweed S. wightii mediated silver nanoparticles were found to be effective against these pathogens than silver nitrate.

Keywords*:* Brown algae, *Sargassum wightii*, Silver nanoparticles, Biosynthesis, antibacterial activity.

INTRODUCTION

Recently there is an increasing trend to develop environmentally benign synthetic methods to produce nanoparticles that do not utilize toxic chemicals in the synthesis procedure. In connection to this biological approach towards the synthesis of nanoparticles has gained importance as bioactive compounds responsible for synthesis and considered as the most active area in modern material science [1-3]. Biological production of nanoparticles possessing entirely new or enhanced properties based on specific characteristics like size, distribution and morphology has become a challenging task. In recent years scientists have proposed the microorganisms as possible eco-friendly method, for the synthesis of cadmium, gold and silver nanoparticles [4-9].

Few researchers support the idea that silver species release the Ag^+ ions and they interact with the thiol groups in bacterial proteins [10]. It is a well known fact that silver ions and silver based compounds inhibit the growth of pathogenic microorganisms[11, 12]. Silver is known for its antiseptic property due to its better anti bacterial property [13]. This unique property of silver makes it an excellent choice for multiple usages in the medical field. Silver nanoparticles can be synthesized in many ways depicted in various literature surveys which include physical, chemical and biological methods. The physical and chemical methods are cost intensive and involves the use of hazardous substances which are not environment friendly. Hence to synthesize silver nanoparticles an alternative feasible method is biological process using microbes and plants [14]. Seaweeds or sea vegetables are important sources of pharmaceuticals, nutraceuticals, food and plant growth regulators [15]. In the present study brown macro algae *Sargassum wightii* was collected as potential source and capsulating agent for the synthesis of silver nanoparticles using rapid drip method. Further these nanoparticles were tested for its antibacterial activity against clinical pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*.

EXPERIMENTAL SECTION

Materials

Silver nitrate of AR grade was obtained from Merck India and used for synthesis. The marine algae *Sargassum wightii* was collected from Rameswaram – Ramnad District (9˚16'54"N; 79˚11'8"E), TamilNadu, South East coast of India. Collected seaweeds were washed with fresh water, shade dried and pulverized to fine powder using mortar and pestle. Deionized water was used for all the aqueous preparations and synthesis.

Enrichment of biomass for extraction

One gram of seaweed biomass was added into 100 ml of deionized water in 250 ml conical flask and kept in a shaker incubator for 24 hours and later the extract was filtered using filter paper. The filtered extract was preserved under refrigeration condition for further process.

Synthesis of Silver nanoparticles using Seaweed extract

Seaweed extract was taken and added into drop wise to a beaker containing 100 ml of 10^{-3} M silver nitrate (Drip method). The beaker was kept over hot magnetic stirrer and the temperature was maintained at 45 ºC. The appearance of pale brown indicates formation of silver nanoparticles. Then the samples were analyzed for UV-vis spectral analysis (Schimadzu UV-Vis spectrophotometer) for determining Surface Plasmon Resonance in the wavelength range of 200 nm - 600 nm. The obtained silver nanoparticles were further characterized by FTIR, XRD and HR-TEM analysis.

Characterisation of biologically synthesized silver nanoparticles

Fourier Transform Infra Red spectroscopy

The biosynthesized nanoparticle was subjected to FTIR spectral analysis to identify the bio-molecules responsible for stabilizing and reducing the silver nitrate. For FTIR measurement the biologically synthesized silver nanoparticles solution was freeze-dried (Martin Christ). The dried powders were subjected to FTIR analysis in the diffuse reflectance mode at a resolution of 4 cm^{-1} using KBr pellets (Perkin Elmer).

X-ray diffractometry (XRD)

The x-ray powder diffraction patterns (XRD) were obtained using Rich Seifert P 300 instrument by operating at voltage 40 kV and with a current of 30 mA with Cu Kα radiation.

High Resolution – Transmission Electron Microscopy (HR-TEM)

The samples for HRTEM analysis were prepared by placing a drop of the suspensions on carbon coated copper grid and allowing water to evaporate. HRTEM instrument was operated at an accelerating voltage of 80 KV.

Antibacterial activity test

Antibacterial activity was performed using disc diffusion method in Mueller Hinton Agar plates. The bacterial pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeroginosa* were isolated from clinical samples and used for antibacterial studies. To the sterile solid Mueller Hinton agar plates the above clinical strains were swabbed uniformly onto the individual plates using sterile cotton swabs. Wells were created using a cork borer to about 5 mm diameter in size. Different concentrations of 10, 25, 50, 75 and 100 % of dry powder of biosynthesized silver nanoparticles dispersed in deionized water were prepared and used for ascertaining their antibacterial activity. The dispersed solution was amended into separate well using sterile micropipette tips. After incubation at 37°C for 24 to 36 hours, the different levels of zone of inhibition (mm) of bacteria were measured. Silver nitrate of the above concentrations was used as positive controls.

RESULTS AND DISCUSSION

Biosynthesis of Silver nanoparticles

Biosynthesis of noble metal nanoparticles especially silver nanoparticles are in great demand due to their unique physico-chemical properties which are not observed even in nanocomposites [16]. Even though there are many biological processes to synthesise nanoparticles a new area of macro algae has been utilized for silver nanoparticle synthesis for which *Sargassum wightii* has been utilized for one pot facile synthesis within 10 minutes.

Silver nanoparticles exhibit strong absorption of electromagnetic waves in the visible range due to Surface Plasmon Resonance (SPR) which is highly influenced by shape and size of the nanoparticles and in this study the maximum absorbance was noted at 438 nm (Fig. 1).

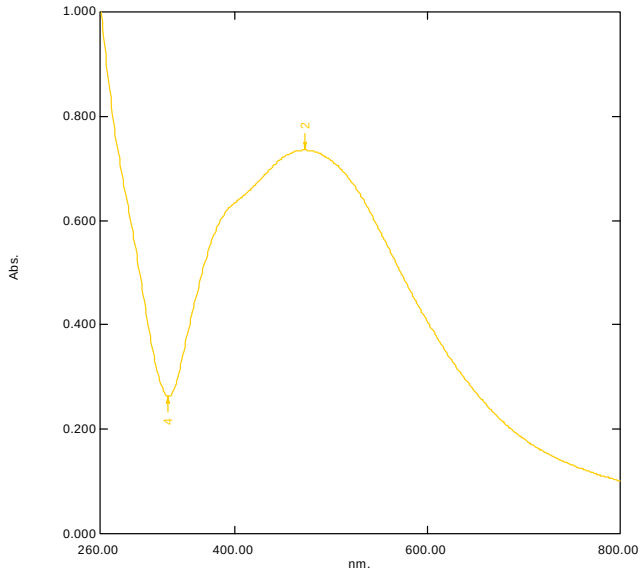

Fig.1. UV-Visible spectra of Silver nanoparticles synthesized by *S. wightii*

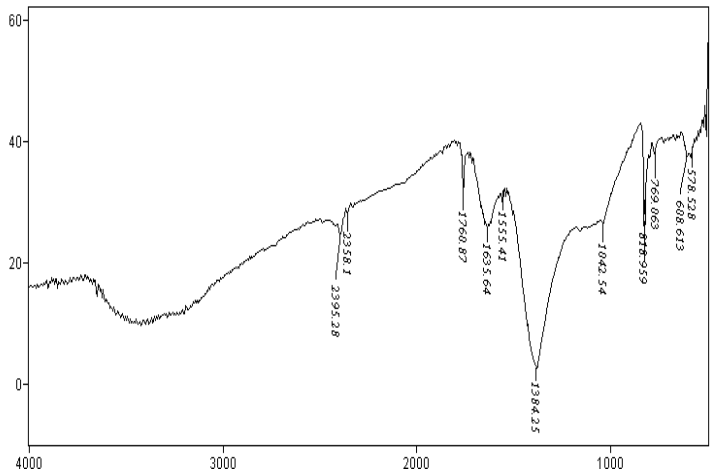

Fig. 2. FTIR spectra of Silver nanoparticles synthesized by *S. wightii*

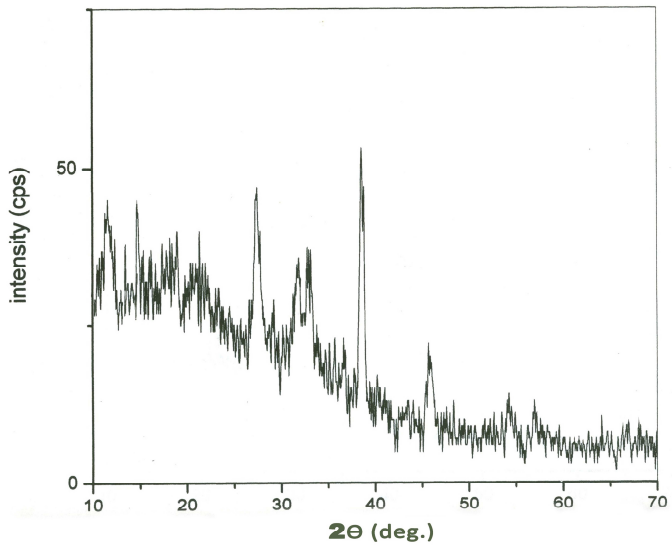

Fig. 3. XRD pattern of Silver nanoparticles synthesized by *S. wightii*

The biomolecules responsible for biofunctionalization of silver nanoparticles were elucidated in FTIR. Fig. 2,

represent the FTIR spectrum of the *S.wightii* and shows peaks at 2395,1635,1555,1384 cm^{-1} respestively. The peak at 2395 cm^{-1} shows stretching of OH group and peak at 1635 cm^{-1} corresponds to C=O stretching bands of the carboxylic acid group. The peak at 1555 cm^{-1} and 1384 cm^{-1} shows the presence of asymmetrical and symmetrical vibration of carboxylate ions. The peak at 1042 cm^{-1} show the presence of alcoholic group, inferring that presence of phenolic compounds in the seaweed extracts responsible for the reduction of the metallic salt silver nanoparticle (6-8).

X-ray difraction (Fig. 3) of biosynthesized nanosilver exhibits Bragg reflection due to (200), (220) and (311) corresponding to fcc crystal lattice. Diffraction peaks are broadened around their base indicating that the silver nanoparticles are in nanosizes. XRD analysis showed three distinct difraction peak at 38˚, 44˚ and 64˚, 2θ can be indexed to these pattern which corroborates with joint committee pattern on powder diffraction standard file number 04 -0783. The result reveals that the Ag^{+} reduced to Ag^{0} by *S. wightii* are crystalline in nature. The average crystallite was calculated using Debye Scherer formula,

$$d = \frac{K\lambda}{B Cos\theta_B}$$

Where K is 0.89 (Scherer's constant), λ is the wavelength of X-rays, θ_B is the Bragg diffraction angle and B is the full width at half-maximum (FWHM) of the highly intense diffraction peak. Using the Debye-Scherer formula, the average crystallite size of silver was found to be 35 nm respectively.

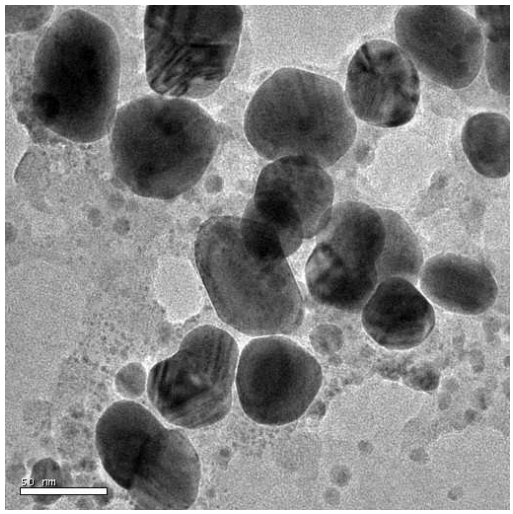

Fig. 4. HR-TEM image of silver nanoparticles synthesized by *S. wightii*

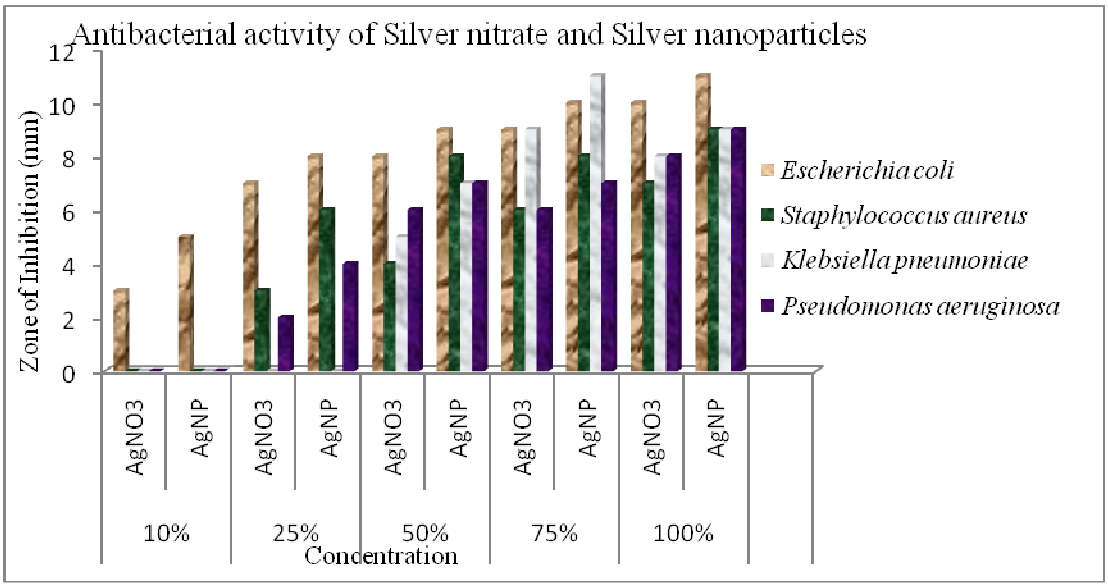

Fig. 5. Antibacterial activity of Silver nitrate and biologically synthesized Silver nanoparticles

Transmission Electron microscope was recorded from drop coated film of the silver nanoparticle synthesized by using *S.wightii* vividly describes the circular shapes. The micrograph showed nanoparticle with variable shape in the size of 40 nm (Fig .4). The strong interaction of biomolecules in the seaweed extract and surface of nanoparticles are found to be sufficient for formation of spherical shape (17).

The biosynthesized silver nanoparticles by *S. wightii* exhibit excellent antibacterial activity against the bacterial pathogens *Escherichia coli* even at 10% when compared to silver nitrate. Followed by *Staphylococcus aureus* (25 %), *Pseudomonas aeruginosa* (25%) and *Klebsiella pneumoniae* (50%) concentrations respectively (Fig. 5). Biologically synthesized nanoparticles interact with the proteins of cell surface and inhibit the permeability of bacterial cells would be a possible mechanism for inhibitory effect of nanoparticles against the above bacterial strains [18].

CONCLUSION

Silver nanoparticles were biologically synthesized using marine brown algae. The crystallite size was found to be 35 nm using XRD. FTIR studies revealed the presence of alcoholic group, inferring that presence of phenolic compounds in the seaweed extracts responsible for the reduction of the metallic salt silver nanoparticle. These silver nanoparticles were found to be effective against the clinical pathogens like *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia.* This study reveals that bio-chemical reducing agents extracted from the biomass of seaweeds are responsible for reduction of silver nitrate to silver nanoparticles. In this context the present drive found to be effective to develop greener technologies in material synthesis, as this drip method would be a rapid and alternative synthetic protocol than physical and chemical methods. This could be a preliminary study to explore the compounds from seaweeds responsible for synthesis of required shape and size of metal nanoparticles.

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