Original Article

Biogenic Silver Nanoparticles by *Gelidiella acerosa* Extract and their Antifungal Effects

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Abstract

The synthesis, characterization and application of biologically synthesized nanomaterials are an important aspect in nanotechnology. The present study deals with the synthesis of silver nanoparticles (Ag-NPs) using the aqueous extract of red seaweed *Gelidiella acerosa* as the reducing agent to study the antifungal activity. The formation of Ag-NPs was confirmed by UV-Visible Spectroscopy, X-Ray Diffraction (XRD) pattern, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The synthesized Ag-NPs was predominately spherical in shape and polydispersed. Fourier Transform Infra-Red (FT-IR) spectroscopy analysis showed that the synthesized nano-Ag was capped with bimolecular compounds which are responsible for reduction of silver ions. The antifungal effects of these nanoparticles were studied against *Humicola insolens* (MTCC 4520), *Fusarium dimerum* (MTCC 6583), *Mucor indicus* (MTCC 3318) and *Trichoderma reesei* (MTCC 3929). The present study indicates that Ag-NPs have considerable antifungal activity in comparison with standard antifungal drug, and hence further investigation for clinical applications is necessary.

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Introduction

Generally, metal nanoparticles are synthesized and stabilized through chemical and mechanical methods [1,2], electrochemical techniques [3], photochemical reactions in reverse micelles [4] and nowadays via green chemistry method [5]. Synthesis of nanoparticles through biological method is a good, environment friendly and economically alternative method. Synthesis of green nanomaterials and their characterization is an emerging field of nanotechnology from the past few decades, because of their applications in the fields of physics, chemistry, biology and medicine.

Application of green chemistry to the synthesis of nanomaterials has vital importance in medicinal and technological aspects [6,7]. Biologically synthesized silver nanoparticles (Ag-NPs) have wide range of applications because of their remarkable physical and chemical properties. There is a very little literature on the extra cellular biosynthesis of Ag-NPs using plants and pure compounds from plants [8-10]. Specifically, while there is relatively little or no literature on the extra cellular synthesis of Ag-NPs by using seaweeds [11].

In this article, we describe a simple one-step method for the synthesis of Ag-NPs by the reduction of aqueous Ag-ions using extracts of red sea weed, at direct sunlight condition. The amount of nanoparticles syn-

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thesized and qualitative differences between synthesized nanoparticles were also investigated by various analytical methods.

Gelidiella acerosa (Forsskal) (Class: Florideophyceae Order: Gelidiales, Family: Gelidiellaceae, Genus: Gelidiella) is an abundantly growing seaweed in Coastal areas of south India. It occurs in inter tidal region of Gulf of Mannar Southeast coast of India. *Gelidiella acerosa* has been used as gelling agent to make jellies, calorie free cookery ingredient, valuable antioxidant for treating ROS mediated diseases and useful post-coital contraceptive [12].

Present study is the first report on the synthesis of highly stable Ag-NPs using commonly available marine algae *Gelidiella acerosa.* The results reported here cover the biological synthesis of Ag-NPs and their antifungal activity.

Materials and Methods

Sample collection

In the present study, *Gelidiella acerosa* (Forsskal), a red seaweed was collected from Mandapam coastal region (78°8'E, 9°17'N), Gulf of Mannar, Tamilnadu, South India. Samples were brought to laboratory in polythene bags and cleaned thoroughly with fresh water to remove adhering debris and associated biota. The algae were cleaned using brush for the removal of the epiphytes with distilled water. After cleaning, algae were dried in shade at room temperature for one week.

Extraction

The whole plant of *Gelidiella acerosa* were initially rinsed thrice in distilled water and dried on paper toweling, and samples (25 *g*) were cut into fine pieces and boiled with 100 *ml* of sterile distilled water for 5 *min*. The crude extract was passed through Whatman No.1 filter paper and the filtrates were stored at 4°*C* for further use.

Synthesis of Ag-NPs

Silver nitrate ($AgNO_3$) was of analytical grade (AR) and purchased from E. Marck (India). In the typical synthesis of silver nanoparticles, 10 *ml* of the aqueous extract of *Gelidiella acerosa* was added to 90 *ml* of 1 *mM* aqueous $AgNO_3$ solution in 250 *ml* conical flask and kept at room temperature for 48 *hr* at 120 *rpm*. Suitable controls were maintained throughout the conduct of experiments.

UV-Vis spectral analysis

The colour change in reaction mixture (metal ion solution + seaweed extract) was recorded through visual observation. The bio reduction of silver ions in aqueous solution was monitored by periodic sampling of aliquots (0.5 *ml*) and subsequently measuring UV-Vis spectra of the solution. UV-Vis spectra of these aliquots was monitored as a function of time of reaction on UV-Vis spectrophotometer UV-2450 (Shimadzu).

XRD analysis

The Ag-NPs solution thus obtained was purified by repeated centrifugation at 5000 *rpm* for 20 *min* followed by redispersion of the pellet of Ag-NPs in 10 *ml* of deionized water. After freeze drying of the purified Ag-NPs, the structure and composition were analyzed by XRD and SEM. The dried mixture of Ag-NPs was collected for the determination of the formation of Ag-NPs by an X'Pert Pro x-ray diffractometer (PAN analyticalBV, The Netherlands) operated at a voltage of 40 *kV* and a current of 30 *mA* with Cu Kα radiation in a θ- 2 θ configuration. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer's formula:

$$D = 0.94 \lambda / \beta \cos \theta$$

1) where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle. To eliminate additional instrumental broadening the FWHM was corrected, using the FWHM from a large grained Si sample:

$$\beta \text{ corrected} = (FWHM^2 \text{sample} - FWHM^2 \text{si})1/2$$

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2) This modified formula is valid only when the crystallite size is smaller than 100 *nm* [13].

SEM analysis

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 *min*.

TEM analysis

The structural characterization of the silver nanoparticles was carried out by Transmission Electron Microscopy (TEM). The sample was prepared by air-drying drops of diluted solutions of the preparations on carbon films supported by copper grids.

FTIR analysis

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 *ml* after reaction was centrifuged at 5000 *rpm* for 10 *min*.

The supernatant was again centrifuged at 10000 *rpm* for 60 *min* and the pellet was obtained .This is followed by redispersion of the pellet of Ag-NPs into 1 *ml* of deionized water. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR Nicolet Avatar 660 (Nicolet, USA).

Antifungal activity

The antifungal activity of Ag-NPs was evaluated against the following human pathogenic strains: *Humicola insolens* (MTCC 4520), *Fusarium dimerum* (MTCC 6583), *Mucor indicus* (MTCC 3318) and *Trichoderma reesei* (MTCC 3929). These fungal strains were obtained from the Institute of Microbial Technology, Chandigarh, India. Cultures were maintained on potato dextrose agar (Hi Media, India) slants and they were subcultured before use. The fungal strains studied were clinically important, causing several infections and it is essential to overcome them through some active therapeutic agents.

The antifungal assay was performed by agar well diffusion method; a well was prepared in the plates with the help of cork-borer (0.85 *cm*). Using sterile micropipette, 50 *µl* (5 *mg/ml*) of the sample of nanoparticle solution was loaded in three plates along with positive control (containing 5 *mg/ml* Clotrimazol). After incubation at 37 ℃ for 48 *hr*, the different levels of zone of inhibition were measured using the Hi antibiotic zone scale.

Results

It is well known that Ag-NPs exhibit reddish-brown in water [14]. The formation of Ag-NPs by reduction of the aqueous Ag+ during exposure to the aqueous extract of *Gelidiella acerosa* showed reddish-brown colour, which suggested the formation of Ag-NPs in solution. The colour arises due to excitation of surface plasmon vibrations in the silver metal nanoparticles [15]. The reduction of silver was subjected to analysis by using the UV-Vis Spectrophotometer. Absorption spectra of Ag-NPs formed in the reaction media has absorbance peak at 408 *nm*, broadening of peak indicated that the particles are polydispersed (Figure 1). The frequency and width of the surface plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium [16-18].

The biosynthesised silver nanostructure by using *Gelidiella acerosa* extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image at $2\theta = 28.09^{o}$, marked with (220). A number of Bragg reflections corresponding to the (220) sets of lattice planes are observed which may be indexed based on the face-centred crystal structure of silver. The XRD pattern thus clearly shows that the Ag-NPs are crystalline in nature (Figure 2).

The SEM image (Figure 3A) showing the high density Ag-NPs synthesized by the

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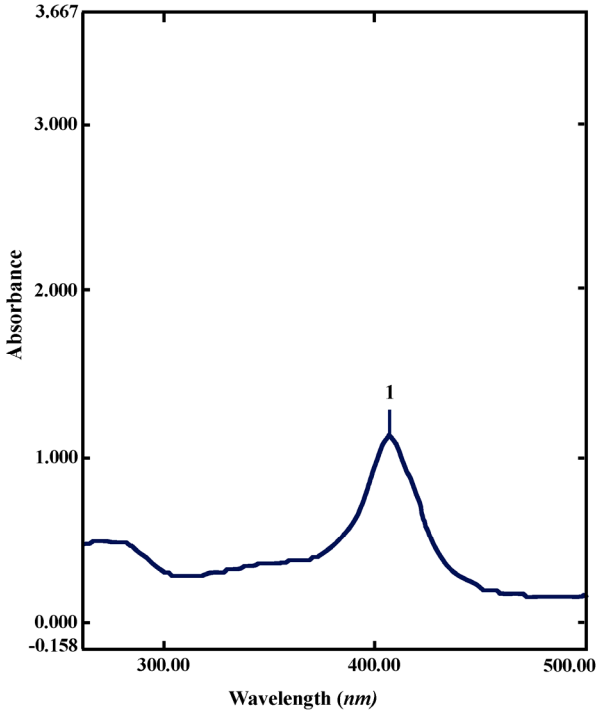

Figure 1. UV-Vis absorption spectra of silver nanoparticles synthesized from *Gelidiella acerosa* extract by treating 1*mM* silver nitrate after 48 *hr*

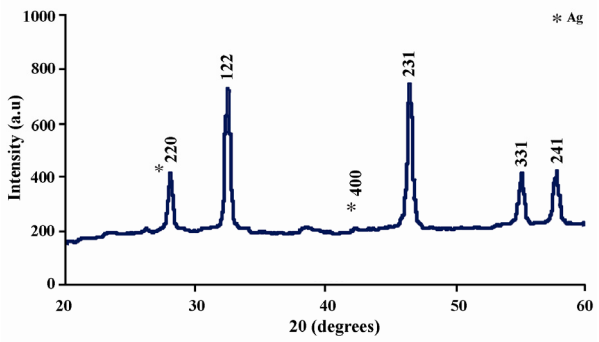

Figure 2. XRD patterns of capped silver nanoparticles synthesized by treating *Gelidiella acerosa* extract with 1 *mM* silver nitrate

Gelidiella acerosa further confirmed the development of silver nanostructures. The SEM micrographs of nanoparticle obtained in the filtrate showed that Ag-NPs are spherical shaped, well distributed without aggregation in solution. The silver nanoparticles synthesized by the help of *Gelidiella acerosa* extract were scanned using TEM from which the average mean size of the silver nanoparticles was 22 *nm* and seems to be spherical in morphology as shown in figure (Figure 3B).

FTIR analysis was used for the characterization of the extract and the resulting nanoparticles. Absorbance bands seen at 3441, 1658, 1535 and 1400 cm^{-1} were assigned to the stretching vibrations of primary and secondary amines, respectively (Figure 4). The result revealed that the capping ligand of the Ag-NPs may be an aromatic compound or alkanes or amines.

The evaluation of antibiotic resistant pathogenic fungi has stimulated the search for effective antifungal agent from alternative sources. Many studies have shown the antimicrobial effects of nano-Ag [19-22]. However, only limited literatures supports the effects of Ag-NPs against fungal pathogens**.** Further the nanoparticles synthesis by green route by

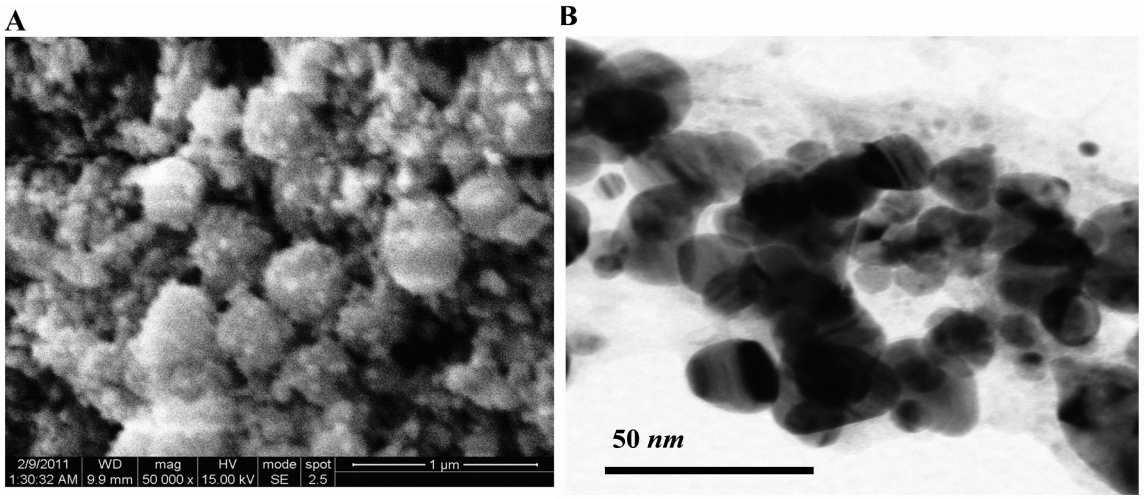

Figure 3. SEM (A) and TEM (B) micrograph of silver nanoparticles synthesised by the reaction of 1 *mM* silver nitrate with *Gelidiella acerosa* extract

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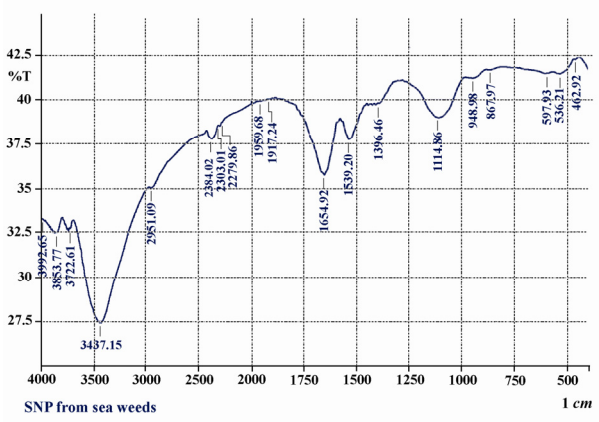

Figure 4. FTIR spectra *of* silver nanoparticles synthesized by the reduction of 1*mM* silver nitrate with the *Gelidiella acerosa* extract

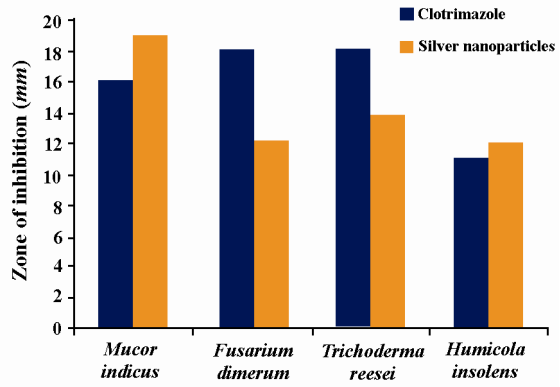

Figure 5. Antifungal activity of silver nanoparticles synthesized by the reduction of silver nitrate with the *Gelidiella acerosa* extract against some selected fungal pathogens

using *Gelidiella acerosa* extract was found highly active against tested fungal species at a concentration of 50 *µl* of synthesized Ag nanoparticles. The results showed higher antifungal activity against *Mucor indicus* (22.3 *vs.* 21.3) *and Trichoderma reesei* (17.2 *vs.* 14.3), whereas moderate activity was revealed against *Fusarium dimerum* (13.15 *vs.* 13.0), *Humicola insolens* (12.2 *vs.* 12.1) when compared with standard antifungal agent Clotrimazole (Figure 5).

Discussion

The present study showed a simple, rapid and economical route to synthesize Ag-NPs from red seaweeds. The zone of inhibition clearly showed that the fungal strains tested were susceptible to silver nanoparticles. Thus the present study proved that the silver nanoparticles synthesized from *Gelidiella acerosa* seem to be promising and effective antifungal agent against the pathogenic fungal strains.

Conclusion

In conclusion, the bio-reduction of aqueous Ag+ ions by the aqueous extract of *Gelidiella acerosa* has been demonstrated. This green chemistry approach towards the synthesis of silver nanoparticles has many advantages such as ease with which the process can be scaled up, economic viability, *etc*. Applications of such nanoparticles in medical and other applications makes this method potentially use for the large-scale synthesis of other inorganic nano materials. Toxicity studies of Ag-NPs on human pathogen opens a door for a new range of antibacterial and antifungal agents.

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