Green synthesis and cytotoxicity of silver nanoparticles from extracts of the marine macroalgae *Gracilaria corticata*

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The present study reports the synthesis of silver nanoparticles (AgNPs) using aqueous extract of a seaweed, *Gracilaria corticata*. Extremely stable AgNPs were characterized by UV-Vis spectrophotometer, FTIR, XRD, TEM and EDS analyses. The nanoparticles exhibited maximum absorbance at 424 nm in the UV spectrum. The presence of proteins was identified by FTIR. TEM micrograph revealed the formation of polydispersed and spherical shaped nanoparticles with the size range of 10-50 nm and the presence of elemental silver were confirmed by EDS analysis. These nanoparticles showed cytotoxic activity against MCF7 cells.

Keywords: EDS, FTIR, *Gracilaria corticata*, MCF7 cell lines, silver nanoparticles, TEM, XRD

Introduction

With the development of reliable technology to produce nanoparticles, several attempts to synthesize metal nanoparticles like AgNPs have been made. These particles play an indispensable role in the field of nanotechnology and nanomedicine due to their unique and superior size dependent physical, chemical and biological properties. Presently, there is a growing need to develop environmentally benign synthesis protocols that evade toxic chemicals. Biological methods that are considered for nanomaterial fabrication have been proposed by exploiting microorganisms as well as vascular plants.

Studies have indicated that biomolecules like protein, phenols and flavonoids not only play a role in reducing the ions to the nanosize, but also cap the nanoparticles. The reduction of Ag+ ions by combinations of biomolecules in plant extracts, such as, vitamins, enzymes/proteins, organic acids like citrates, amino acids and polysaccharides, is an environmentally benign process. The AgNPs are reported to be nontoxic to humans but are otherwise toxic to bacteria, viruses and other eukaryotic microorganisms even at very low concentrations posing no side effects.

Despite the number of available anticancer therapies, cancer still holds the reputation of a killer disease. Current trends designed to simplify and hasten the cytotoxicity tests implicate the reduction in the use of laboratory animals and to supplement totally or totally supplant the *in vivo* cytotoxicity studies with fast, simple, reliable and less expensive *in vitro* ones using cultured tissues and cells, thus responding to a humane societal call. The present study explores the possibility of synthesizing AgNPs using *Gracilaria corticata* and to analyze their cytotoxicity activity on MCF7 cells in addition to evaluating the morphology and the number of viable cells at different concentrations with respect to the duration of exposure.

Materials and Methods

Collection of Seaweeds

*Gracilaria corticata*, a seaweed, was collected in a cool bag from the intertidal regions of the Mandapam coastal regions (Lat 09° 17.417’ N; Long 079° 08.558’ E) of Gulf of Mannar and transferred to the laboratory. Samples were washed with freshwater to remove adhering debris and associated biota and authentically identified by CAS Botany, Madras University, Chennai. The processed samples were lyophilized, powdered and stored at −4°C.

Preparation of Seaweed Extract

The seaweed powder (5 g) was soaked for 24 h in 1 L of sterile water. Then the crude extract was blended thoroughly and filtered using a Whatman No.1 filter (42 µm) twice. The filtrate was used for further analysis.
Synthesis of Silver Nanoparticles

In the seaweed extract, 1 mM silver nitrate solution was added and the mixture was subjected to thermal treatment at 121°C. The reduction of silver nitrate occurred within 10 min, which resulted in colour change (dark brown), as noted by visual observation, indicating the formation of AgNPs. As per the absorption spectrum, this medium remained stable for more than 3 months. The absorbance of aliquots of the reaction solution was measured using a UV-1601 (Schimadzu Corp.) spectrophotometer operated at a resolution of 1 nm.

Fourier Transform Infrared Spectroscopy (FTIR)

The interaction between protein-silver nanoparticles was analyzed by FTIR in the diffuse reflectance mode at a resolution of 4 cm⁻¹, using KBr pellets (Perkin-Elmer Model 983). The spectra were recorded in the wavelength interval of 4000 to 400 nm⁻¹. FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction of the Ag⁺ ions and the capping of the bioreduced AgNPs. For comparison, the seaweed filtrate was mixed with KBr powder and pelletized after drying properly and subjected to measurement.

X-Ray Diffractometry

X-ray diffraction (XRD) measurement of the seaweed reduced AgNPs was carried out using powder X-ray diffractometer instrument (PXRD-6000, Schimadzu Corp.) in the angle range of 10°-80°C at 20; scan axis 2:1 sym. The size of the AgNPs was calculated from the PXRD (Powder X-Ray Diffraction) peak positions using Bragg’s law.

Transmission Electron Microscopy (TEM)

TEM was employed to visualize the size and shape of AgNPs. The silver nanoparticles were added on a carbon-coated copper grid and the examinations were operated at an accelerating voltage of 120 keV. TEM measurements were performed using Philips Tecnai 10 (FEI Company) instrument.

Energy Dispersive Analysis X-Ray Spectroscopy (EDS)

The presence of elemental silver was confirmed through EDS, which was carried out at the Sophisticated Test Instrumentation Centre, Cochin University of Science and Technology, Kerala, India, with the model JED-2300, JEOL Ltd.

Cytotoxic Activity of AgNPs on MCF7 Cell Line

The study was focused on the human breast cancer cell line MCF7, since it is a very common cancer. The cell line was seeded in 96-well tissue culture plates. Stock nanoparticle solutions (5 mg/mL) were prepared in sterile distilled water and diluted to the required concentrations by double dilution using the cell culture medium. Appropriate concentrations of AgNPs (1000-3.90 µg) were added and incubated for 48 h at 37°C. 5-Fluorouracil was added as positive control in volume of 1000 µg/mL. Each experiment was done in triplicates. Following AgNP treatment, the plates were observed under an inverted microscope to detect morphological changes and photographed. Incubated cultured cells were subjected to MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], a tetrazole colorimetric assay. Assay plates were read using a spectrophotometer at 520 nm. Data generated were used to plot a dose-response curve, of which the concentration of AgNPs required to kill 50% of cell population (IC₅₀) was determined.

Cell viability (%) = \( \frac{\text{Mean optical density}}{\text{Control optical density}} \times 100 \)

Results and Discussion

Biosynthesis of AgNPs

The present paper describe the simple synthesis of AgNPs utilizing extracts from the marine macroalgae G. corticata, which proceeded with the addition of 1 mM AgNO₃ at 121°C for 10 min. A control without AgNO₃ showed no colour formation, while the solutions with AgNO₃ changed colour to dark brown confirming the formation of AgNPs (Fig. 1). Mulvaney et al. reported that the colour change was due to excitation of surface plasmon vibrations in the metal nanoparticles, indicating the transition of silver nitrate to AgNPs. According to the report of Medina et al., the formation of AgNPs is attributed to hydrophilic-hydrophobic interactions resulting in
intermolecular forces. The use of this simple and ecofriendly synthetic procedure can help promote interest in the synthesis and application of metallic nanoparticles. The characterization of nanoparticles uses a number of following standard procedures.

UV-Visible Spectrum

According to Kreibig and Vollmer\(^\text{16}\), UV-visible spectroscopy is an important technique for confirmation of formation and stability of metal nanoparticles in aqueous solutions. A strong absorption peak originates from the surface plasmon absorption of the nano size silver particles\(^\text{17}\). Further, the UV-visible excitation spectra of AgNPs have been reported\(^\text{18}\). The absorption spectrum of AgNPs showed a well observable peak in visible range at 424 nm (Fig. 2), which is the characteristic wavelength range of AgNPs with the resolution of 1 nm between 200 to 800 nm\(^\text{19}\). Apart from this, one more absorption peak was observed in the UV region corresponding to 220 nm, maybe due to the presence of amide bond. The absorption strongly depends on the particle size, dielectric medium and chemical surroundings. The good symmetric absorption peak implies that the size distribution of the nanoparticles is narrow\(^\text{20}\). To detect the stability of the AgNPs the absorption spectra were rerecorded after 3 months. No obvious variance in the shape, position and symmetry of the absorption peak was observed, which indicates that the synthesized AgNPs can remain stable.

Fourier Transform Infrared Spectrum (FTIR)

FTIR measurements were carried out to identify the possible bio-molecule responsible for reduction of the Ag\(^+\) ions, and capping of the bio-reduced AgNPs synthesized by the seaweed extract. Curve of the extract (Fig. 3) resulted in the multiple broad peaks at 2921 cm\(^{-1}\) corresponding to N-H stretching of any ammonium ions; the medium band at 1630 cm\(^{-1}\) corresponding to stretching of C≡N; and the stronger band at 1455 cm\(^{-1}\) corresponding to N-O stretching of nitro compounds. The weaker band at 1244 cm\(^{-1}\) corresponding to N-O stretching of nitro compounds; the broad band at 1028 cm\(^{-1}\) corresponding to C-X stretching of fluoroalkanes; and the strong band at 797 cm\(^{-1}\) corresponding to C-H stretching of aromatic benzene (Fig. 3). Sathyavathi et al\(^\text{21}\) have suggested that the biological molecules could possibly perform dual functions of formation and stabilization of AgNPs in the aqueous medium\(^\text{22}\).

X-Ray Diffraction (XRD) Pattern

XRD patterns obtained for AgNPs synthesized using seaweed extract showed characteristic peaks (20=38.20°, 44.11°, 64.35° & 77.41°), marked with (111), (200), (220) and (311) (Fig. 4). A number of Bragg reflections corresponding to the (111) sets of lattice planes were observed, which may be indexed based on the face-centered cubic (FCC) structure of silver. The XRD pattern thus clearly shows that the AgNPs are crystalline in nature\(^\text{15}\). The XRD pattern of AgNPs\(^\text{22,23}\) is known to display peaks at 2θ=7.9°, 11.4°, 17.8°, 30°, 38° and 44°. The value of the pure silver

![Fig. 2—UV-Visible absorption spectra of AgNPs.](image)

![Fig. 3 (a & b)—FTIR spectra of biologically synthesized silver nanoparticles using the G. corticata extract (a). The seaweed extract used as control (b).](image)

![Fig. 4—XRD pattern of the synthesized silver nanoparticles.](image)
lattice constant has been estimated to be $a=4.081\,\text{Å}$, a value that is consistent with $a=4.0862\,\text{Å}$ reported by the JCPDS file no. 4-0783. This estimation confirmed the hypothesis of particle monocrystallinity.

**Transmission Electron Micrographs**

A morphological study of AgNPs was carried out using Transmission Electron Microscopy (TEM) (Fig. 5). The TEM micrograph shows the corresponding size distribution histogram of AgNPs. The AgNPs that got reduced with the help of extract of *G. corticata* had dimensions small enough to be electron transparent and imaged as polydisperse small and large spherical nanoparticles with variable diameter. The figure shows NPs having a particle size in the range of 10-35 nm, which can be related to the earlier report of Ahmad *et al.*

**Energy Dispersive Analysis X-Ray Spectroscopy (EDS)**

EDS confirmed the reduction of silver ions to elemental silver. The optical absorption peak observed approx at 3 keV is typical for the absorption of metallic silver nanocrystals due to surface plasmon resonance as per the report of Magudapathy *et al.*

**Cytotoxicity on MCF7 Cell Lines**

The cytotoxic activity of AgNPs on MCF7 cell lines was evaluated by the method of Daikoku *et al.* and the results were tabulated as the percentage of viable cells against different concentrations of samples (Table 1) as well as cellular changes observed under the microscope (Fig. 7). The microscopic observation gave indications

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**Table 1**—Cytotoxic activities of silver nanoparticles against MCF7 cell lines (MTT assay)

<table>
<thead>
<tr>
<th>No.</th>
<th>Concentration (µg/mL)</th>
<th>Cell viability for AgNPs (%)</th>
<th>Cell viability for STD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1000</td>
<td>19.72</td>
<td>4.16 ± 1.55</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>29.08</td>
<td>7.91 ± 1.98</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>34.17</td>
<td>10.31 ± 1.76</td>
</tr>
<tr>
<td>4</td>
<td>125</td>
<td>40.78</td>
<td>14.51 ± 1.54</td>
</tr>
<tr>
<td>5</td>
<td>62.50</td>
<td>51.49</td>
<td>18.90 ± 1.09</td>
</tr>
<tr>
<td>6</td>
<td>31.25</td>
<td>61.99</td>
<td>22.56 ± 2.98</td>
</tr>
<tr>
<td>7</td>
<td>15.62</td>
<td>73.21</td>
<td>47.71 ± 1.76</td>
</tr>
<tr>
<td>8</td>
<td>7.812</td>
<td>89.90</td>
<td>55.15 ± 1.87</td>
</tr>
<tr>
<td>9</td>
<td>3.90</td>
<td>91.67</td>
<td>71.92 ± 1.88</td>
</tr>
<tr>
<td>10</td>
<td>Cell control</td>
<td>100.00</td>
<td>100.00 ± 0.00</td>
</tr>
</tbody>
</table>
of an overall mitochondrial activity after short exposure time (4 h) of AgNPs and was also a measure of cell proliferation at longer exposure time (24–48 h) that allowed cell division. The AgNPs synthesized from seaweed extracts of G. corticata showed cytotoxic activity against MCF7 cell lines with an IC₅₀ value of 62.5 µg/mL.

Conclusion
In the present study, we developed a simple, green and efficient route to synthesize AgNPs by treating silver ions with aqueous extract of G. corticata. The extract plays an important role as the bio-friendly reducing and stabilizing agent and reduces the cost of production. The prepared AgNPs are spherical and single crystalline structures, with sizes in the range from 10-35 nm. The result concluded that biosynthesized nanoparticles using seaweed showed cytotoxicity against MCF7 cell line. However a further study is needed to find out the actual inhibitory mechanism of silver nanoparticles on these cell lines.

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References
the fungus *Fusarium oxysporum*, Colloid Surf (B) Biointerfaces, 28 (2003) 313-318.


