Synthesis of silver nanoparticles using green and brown seaweeds


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Abstract

Currently, there is a growing need to develop environmentally benign nanoparticle synthesis process that does not use toxic chemicals in the synthesis protocols. Synthesis of nanoparticles by biological approach is innovative, cheaper and environmental friendly and requires less labor. In this regard, the present study focused on the synthesis of silver nanoparticles from the extracts of Sargassum plagiophyllum, Ulva reticulata and Enteromorpha compressa. Characterizations were performed by UV-Visible Spectroscopy (UV-Vis), Fourier-Transform Infra-red Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM) and X-ray Diffraction (XRD). It was found that spherical shaped nanoparticles of size 20-50nm were found in SEM. Altogether, extracts from seaweed were screened for phytochemicals followed by FT-IR prediction to reveal chemical functional groups present. The results showed that Sargassum plagiophyllum showed higher phytochemicals than the others. Therefore, the present study elucidates silver nanoparticles can play a vital role in nano-based therapy in future.

Keywords: Characterization, Enteromorpha compressa (Linnaeus) Nees, Phytochemicals, Sargassum plagiophyllum C. Agardh, Silver Nanoparticles, Ulva reticulata Forsskál.

Introduction

An important aspect of nanotechnology is the development and synthesis of metal nanoparticles is a big challenge. Nanoparticles are being viewed as fundamental building blocks of nanotechnology. The development of biologically inspired experimental process for synthesis of nanoparticles is evolving into an important branch of nanotechnology. Nanoparticles of Free metals have been extensively researched because of their unique physical properties, chemical reactivity and potential applications in catalysis, biological labeling, biosensing, drug delivery, antibacterial activity, antiviral activity, detection of genetic disorders, gene therapy and DNA sequencing (A. Thirumurugan et al., 2010).

Seaweeds or benthic marine algae are the group of plants that live either in marine or brackish water environment. The synthesis of nanoparticles using algae as source has been unexplored and underexploited. More recently, there are few, reported that algae being used as a biofactory for synthesis of metallic nanoparticles. In an isolated report, Singaravelu G et al., 2007 implemented an efficient approach for synthesis of stable gold nanoparticles by the reduction of aqueous AuCl4 by using Sargassum wightii. Interestingly, this was the first report for synthesis of stable metallic nanoparticles by the extract of marine algae, it results relatively very short period of incubation time requires compared with other biological materials. Recently Kumar et al., 2012 have showed that anti-bacterial activity of silver nanoparticles synthesized using Sargassum tenererrimum was comparably higher than the phytochemicals present.

Research is a crucial part of the response to new and emerging diseases. A sustained forward-thinking applied research programme would enable scientists to identify the weak links in the armour of emerging microbes, create novel ways to fight microbial foes, and evaluate the preventive power of new approaches. Seaweeds may be an answer to unsolved and growing problem of resistance, a novel untapped source to combat infectious diseases.

2. Materials and Methods

2.1. Collection of seaweeds

Seaweed samples (Sargassum plagiophyllum C. Agardh, Ulva reticulata Forsskál, Enteromorpha compressa (Linnaeus) Nees) were collected by hand picking method at a depth of 1 – 2 meter in Kovalam and Muttukadu, Tamilnadu, Chennai, India. Those seaweeds were surface sterilized with tap water to remove extraneous substances followed by distilled water. The seaweed is identified, shade dried for 15 days and powdered using mixer grinder.

2.2. Preparation of seaweed extracts and phytochemical analysis

Frozen seaweed samples (5g) were grinded using a mortar and pestle, then extracted by incubation for 24 hours with 50ml water and 60% methanol separately. The extraction was carried out in dark at room temperature using Ultrasonicator. The mixture was then centrifuged at 5000 rpm for 20 minutes at 4 °C and filter sterilized using 0.2 μm membrane syringe. A fraction of seaweed extract was subjected to phytochemical screening as described by Harborne, 1998. Natural chemical groups such as amino
acids, alkaloids, carbohydrates, flavonoids, saponins, sterols, tannins, terpenoids, proteins and phenolic compounds were probed. The water extract was further exemplified by FT-IR spectroscopic studies to reveal the characteristic functional group present.

2.3. Silver nanoparticles synthesis using seaweeds by various methods

2.3.1. Method – I: By using various concentrations

One mM AgNo3 solution was taken in a conical flask and increasing concentration of (200 mg) sample extracted using deionised water were added (1ml extract + 99ml AgNo3, 2ml extract + 98ml AgNo3, 3ml extract + 97ml AgNo3, 4ml extract + 96ml AgNo3 and 5ml extract + 95ml AgNo3). pH was checked before and after adding silver nitrate solution to the sample filtrate. Now the contents of the test tubes were mixed well and kept in dark at room temperature. After 12 hr the formation of pale yellow and dark red color for 3 different samples partly confirms the presence of silver nanoparticles. Now the sample was analyzed under UV-Visible spectrophotometer (Shimadzu - 2450) ranging from 200nm-800nm in order to confirm the presence of silver nanoparticles, 12 hours after centrifuged for 10-15min at 5000rpm. Stability was monitored periodically by spectral analysis.

2.3.2. Method – II: By boiling sample extract

Two hundred mg of every sample was dissolved in 100ml deionised water and kept in water bath at 60°C for 20 mins. 1mM AgNo3 solution was added to the boiled extracts and no immediate colour changes occurred.

2.3.3. Method III: By boiling sample extract with AgNo3

Five ml of aqueous extract (200 mg sample in 100ml deionised water) was dissolved in 95 ml of 1mM AgNo3 solution and were kept in water bath at 60°C for 15 mins (dark) (Kumar et al., 2012). Color change was monitored for 15 mins to 30 mins with constant pH maintenance. Stability was monitored periodically by spectral analysis.

2.4. Characterization of silver nanoparticles (AgNPs)

Characterization of silver nanoparticles was performed in sequence using UV-Visible Spectrophotometer (Shimadzu - 2450). Based upon colour change and UV-Visible spectral analysis, FT-IR (Spectrum RX-1 instrument), Scanning Electron Microscope (Hitachi S-4500 SEM) and X-ray diffraction (XRD) measurements were done (Siefert X-diffractometer instrument operating at a voltage of 40 kV and a current of 30 mA with Cu Ka radiation). Therefore from the analysis, samples processed by Method III showed outstanding results upon spectral analysis and were taken for further analysis.

3. Results

3.1. Phytochemical analysis

The preliminary phytochemical analysis of methanol extract revealed the presence of amino acids, alkaloids, carbohydrates, flavonoids, saponins, sterols, tannins, terpenoids, proteins, and phenolic compounds as shown in Table. 1. FT-IR predicts the molecular configuration of different functional group present in the seaweed extract. Considerable absorption peaks by Sargassum plagiophyllum (from Method – I) (Fig. 3) were found at 3935.44 cm-1 and 3828.20 cm-1 indicates the presence of N-H bond, 3435.10 cm-1 indicates the presence of hydrogen bonded alcohols (O-H), 2385.24 cm-1 (Dovbeshko et al., 1997) indicates the presence of carboxylic acids (O-H), 2076.11 cm-1 indicates the presence of benzene rings, 1638.10 cm-1 indicates the presence of C=O, 1371.88 cm-1 indicates the presence of C-H group, 1232.23 cm-1 indicates the presence of acetates, whereas for Ulva reticulata 3432.94 cm-1 indicates the presence of hydrogen bonded alcohols (O-H), 2777.28 cm-1 and 2676.19 cm-1 indicates the presence of carboxylic acids (O-H), 2071.75 cm-1 indicates the presence of benzene rings, 1637.58 cm-1 indicates the presence of C=N, 1121.56 cm-1 (Fujioka et al., 2004) indicates the presence of fluoroalkanes and for Enteromorpha compressa 3419.51 cm-1 indicates the presence of hydrogen bonded alcohols (O-H), 2073.47 cm-1 indicates the presence of benzene rings, 1638.53 cm-1 indicates the presence of C=N (Nyquist, 2001). Considerable absorption peaks by Sargassum plagiophyllum (from Method – III) (Fig. 4) were found at 3935.68 cm-1 indicates the presence of N-H bond (Krishnan et al., 2006), 3426.08 cm-1 indicates the presence of hydrogen bonded alcohols (O-H), 2075.13 cm-1 indicates the presence of benzene rings, 1637.02 cm-1 indicates the presence of C=O, 1261.50 cm-1 indicates the presence of benzene rings, 1371.88 cm-1 indicates the presence of C-N (Nyquist, 2001), 1069.43 cm-1 indicates the presence of fluoroalkanes and for Enteromorpha compressa 3428.57 cm-1 indicates the presence of hydrogen bonded alcohols (O-H), 2077.20 cm-1 indicates the presence of C=O respectively.
Table 1. The preliminary phytochemical analysis of methanol extract of seaweeds

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Sargassum plagiophyllum</th>
<th>Ulva reticulata</th>
<th>Enteromorpha compressa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Flavonoids</td>
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<td>+</td>
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<tr>
<td>Saponins</td>
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<tr>
<td>Sterols</td>
<td>+</td>
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<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Phenolic compounds</td>
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</tbody>
</table>

Preliminary Screening of Phytochemicals; (+) Presence (-) Absence

3.2. Synthesis and Characterization of silver nanoparticles

Several reports have been employed for the synthesis of silver nanoparticles for its beneficial applications. Recently, seaweeds have been identified as the potential source for synthesizing nanoparticles while Singaravelu et al have synthesized gold nanoparticles from Sargassum wightii extract within 24 h of incubation time. Similarly, we have synthesized silver nanoparticles using Method III Sargassum plagiophyllum rapidly within 15 min rather than Enteromorpha compressa and Ulva reticulata. Endpoint with prominent color change (Fig. 2) indicates the excitation of surface plasmon resonance due to reduction of silver nitrate (Harborne J. B et al., 1998). Longitudinal plasmon vibrations corresponding to silver nanoparticles were convincing with UV spectral peak at 420 nm and with no considerable peak in Method I (Fig. 1). This clearly indicates the interaction between silver ions and biomolecules present in the aqueous seaweed extract. Intensity of band increased upon varying time without any shift in peak position.
After interpretation of the results from the spectral analysis silver nanoparticles synthesized using method III were stable and thus analyzed for its shape in SEM. According to scanning electron microscope, the morphology of silver nanoparticles observed to be spherical with an average size of 20 nm approximately for Sargassum plagiophyllum, whereas Ulva reticulata and Enteromorpha compressa with sizes ranging between 40-50nm approximately (Fig. 5).

The dry powders of the silver nanoparticles were used for XRD analysis. The diffracted intensities were recorded from 20° to 80° at 2 theta angles. The diffraction pattern (Fig. 6) corresponds to pure silver metal powder. The XRD pattern indicates that the nanoparticles had a spherical structure. No peaks of the XRD pattern of Ag2O and other substances appear, and it can be stated that the obtained silver nanoparticles had a high purity. The observed peak broadening and noise were probably related to the effect of nanosized particles and the presence of various crystalline biological macromolecules in the plant extracts. The obtained results illustrate that silver ions had indeed been reduced to Ag0 by the extracts under reaction conditions.

4. Discussion

The results of the nanoparticles synthesized using three seaweeds and their phytochemical analysis suggested that only one method favored the synthesis and stabilization of silver nanoparticles. Silver nanoparticle were synthesized and characterized in ambient conditions with an average size of 20 nm. The presence of phytochemicals as reducing agents in synthesizing nanoparticle can be potent antimicrobials in near future. The result concluded that biosynthesized nanoparticles using method III showed evidence of more stability than the other methods. A further study is needed to find out the antimicrobial potential and actual inhibitory mechanism of silver nanoparticles.

5. Acknowledgement

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6. References

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