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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *ENTEROMORPHA LINZA* (L.) J.AG. (GREEN SEAWEED) FROM HARE ISLAND, THOOTHUKUDI, TAMIL NADU, INDIA

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ABSTRACT

The synthesis of silver nanoparticles is an expanding research area due to the potential applications for the development of novel technologies. In this work, a cost effective and environment friendly technique for green synthesis of silver nanoparticles were carried out from 3mM silver nitrate solution through the extract of *Enteromorpha linza* (L.) J.Ag. as reducing as well as capping agent. In the process of synthesizing silver nanoparticles, a rapid reduction of silver ions was observed leading to the formation of stable crystalline silver nanoparticles in the solution. The synthesis of silver nanoparticles was prepared by adding silver nitrate solution (3mM) to the plant extract. The formation of silver nanoparticles was characterized using colour change, UV-Visible Spectroscopy, FT-IR and X-Ray Diffraction Method (XRD). The nanoparticles showed an absorbance at 426nm on UV-Vis spectroscopy. The presence of proteins was identified by Fourier Transform Infra-Red spectroscopy (FT-IR). The presence of elemental silver was characterized by X-Ray Diffraction Method (XRD). The results recorded from the above techniques are support the green synthesis and characterization of silver nanoparticles.

INTRODUCTION

Nanotechnology concerns with the development of experimental processes for the synthesis of nanoparticles of different sizes, shapes and controlled disparity. This provides an efficient control over many of the physical and chemical properties and the potential application in optoelectronics, recording media, sensing devices, catalysis and medicine. To date, metallic nanoparticles are mostly prepared from noble metals including Ag, Pt, Au and Pd [1]. Among the noble metals, silver (Ag) is the metal of choice in the field of biological system, living organisms and medicine. Green synthesis of nanoparticles is an emerging branch of nanotechnology. The use of environmentally benign materials like plant leaf extract, bacteria and fungi for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and biomedical applications as they do not use toxic chemicals in the synthesis protocols [2]. Bio-inspired synthesis of nanoparticles provides advancement over chemical and physical methods as it is a cost effective and environment friendly and in this method there is no need to use high pressure, energy, temperature and toxic chemicals. Disease causing microbes that have become resistant to drug therapy are an increasing public health problem. Therefore there is an urgent need to develop new bactericides. Silver nanoparticles take advantages of the oligodynamic effect that silver has on microbes [3].

Nanotechnology is also emerging as rapidly growing field with application in science and technology for the purpose of manufacturing new materials at the nanoscale level. The field of Nanotechnology is one of the most active areas of research in modern material science [4]. The word "nano" is used to indicate one billionth of

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meter. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. In the field of nanotechnology many tools and machines are used. Nanotechnology is a growing field day by day making an impact in all spheres of human life [5]. Today, nano metal particles, especially silver have drawn the attention of scientists because of their extensive application in the development of new technologies in the areas of electronics, material sciences and medicine at the nanoscale [6]. Historically, silver has been found in applications ranging from traditional medicines to culinary items. It has been reported that silver nanoparticles (SNPs) are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organism at low concentrations and without any side effects [7].

Previous literature revealed that the nanoparticles synthesis using algae as source has been unexplored and underexploited. Recently there are a few reports that algae are being used as a biofactory for synthesis of metallic nanoparticles [8]. *Enteromorpha linza* (L.) J.Ag. is a predominant green seaweed (marine macro algae) species found in coastal regions of Indian subcontinent especially in the south east coast of Tamil Nadu [9]. With the above

Nanotechnological studies

Silver Nanoparticles Synthesis

2g dried *Enteromorpha linza* (L.) J.Ag. powder was taken in a 100ml Erlenmeyer flask with 30ml of sterile distilled water and then boiled the mixture for 2 minutes. After boiling, the mixture was filtered in the Whatmann No.1 filter paper. 3mM solution of silver nitrate was prepared. 5ml of plant extract was mixed with 25ml of 3mM silver nitrate. The formation of reddish brown colour was observed and λ max at different time intervals were taken for 8h using a UV-Visible spectroscopy. Then the solution is stored in room temperature for 24h for the complete settlement of nanoparticles. After 24h centrifuge the reaction mixture, discard the supernatant. Add 1ml of distilled water to the pellet and wash by using centrifugation. Collect the pellet by using acetone/ethyl acetate/alcohol. Dry in the watch glass and store the nanoparticles [10].

Characterization of Silver Nanoparticles

UV-Vis Spectra analysis

The reduction of pure silver ions was observed by measuring the UV-Vis spectrum of the reaction at different time intervals taking 1ml of the sample, compared with 1ml of 3mM silver nitrate used as blank. UV-Vis spectral analysis has been one by using An Elico spectrophotometer at a resolution of 1nm from 200 to 1100nm [11].

FTIR Analysis

The interaction between protein and silver nanoparticles were analyzed by Fourier Transform Infra-Red (FTIR) spectroscopy in the diffuse reflectance mode at

information, an attempt has been taken for the synthesis and characterization of silver nanoparticles using the green seaweed *Enteromorpha linza* (L.) J.Ag. in the present study.

MATERIALS AND METHODS

Collection of Plant Materials

The present study area is Thoothukudi (Lat 8° 48'N; Long 78° 11'E) located in the south east coast of Tamil Nadu, India. The collection of *Enteromorpha linza* (L.) J.Ag. (Figure 1) belonging to Chlorophyceae (Green marine macro algae) was made during the low tidal and subtidal regions (up to 1m depth) by hand picking. The collected materials were washed thoroughly with marine water in the field itself to remove the epiphytes and sediment particles. Then the samples were packed in polythene bags in wet conditions and brought to the laboratory, then thoroughly washed in tap water followed by distilled water to remove the salt on the surface of the thalli. The plant specimens were placed on blotting paper and spread out at room temperature in the shade condition for drying. The shade dried samples were grounded to fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use.

a resolution of $4cm^{-1}$ in the KBr pellets and the spectra were recorded in the wavelength interval of 4000 to $400nm^{-1}$. FTIR measurements were carried out to identify the possible biomolecules responsible for the reduction of the Ag^{+} ions and the capping of the bio reduced silver nanoparticles synthesized by *Enteromorpha linza* (L.) J.Ag. extract. For comparison, the green seaweed filtrate was mixed with KBr powder and pelletized after drying properly and subjected to measurement [12].

XRD Analysis

X-Ray Diffraction (XRD) measurement of *Enteromorpha linza* (L.) J.Ag. reduced silver nanoparticles was carried out using powder X-ray diffractometer instrument (PXRD-6000 SCHIMADZU) in the angle range of 10˚C-80˚C at 2θ, scan axis: 2:1 sym. The size of the silver nanoparticles was calculated from the PXRD peak positions using Bragg's law. X-Ray Diffraction (XRD) analysis of drop-coated films of silver nanoparticles in sample was prepared for the determination of the formation of silver nanoparticles by XPERT-PRO software [13].

RESULTS AND DISCUSSION

Synthesis and Characterization of Silver Nanoparticles

Synthesis of Silver Nanoparticles

Reduction of silver ions into silver particles during exposure to *Enteromorpha linza* (L.) J.Ag. extract could be followed by color change. Silver nanoparticles exhibit dark yellowish brown color in aqueous solution due to the surface Plasmon resonance phenomenon. The appearance of the yellowish brown color indicated the formation of silver nanoparticles synthesis in the reaction mixture, as it is well known that silver nanoparticles exhibit striking colors (light yellow to brown) due to excitation of surface plasmon vibrations in the particles. It was reported that some amount of OH- groups tended to promote the reduction of silver ions in some chemical methods [10].

UV-Vis Spectrum

UV-Vis spectrograph of silver nanoparticles has been recorded as a function of time (Table 1 and Figure 2). Absorption spectra of silver nanoparticles formed in the reaction media at 8h has absorbance peak at 426nm, broadening of peak. The position and the number of peaks in the absorption spectra are dependent on the shape of the particles. For an ellipsoidal particle there are two peaks whereas for spherical particle there is only one peak [14]. In the present study, there is only one peak at the centre at 426nm indicating the formation of silver nanoparticles in spherical shape. The absorption maximum at 426nm is attributed to the Mie scattering by silver metal [15].

FT-IR Spectrum

FT-IR spectrum of silver nanoparticles is presented in figure (Figure 3). The spectrum shows the presence of bands at $3402cm^{-1}$, $1629cm^{-1}$, $1452cm^{-1}$ and $1056cm^{-1}$. The bands at $3402cm^{-1}$ corresponds to primary amine O-H band, $1629cm^{-1}$ corresponds to primary amine N-H band, the band at $1452cm^{-1}$ is assigned to methylene scissoring vibration from the protein in the solution and the band at $1056cm^{-1}$ were assigned to C-N stretching vibration of the proteins. The positions of these bands were close to that reported for native proteins [16]. This evidence suggests that the protein molecules could possibly perform the function of the formation and stabilization of silver nanoparticles in the aqueous medium.

X-Ray Diffraction studies

XRD pattern was taken using powder X-Ray Diffracto Meter Instrument (XRDMI) in the angle range of 10˚C-80˚C of the silver nanoparticles at 2θ, Scan axis: Gonio (Table 2). A number of Bragg reflections corresponding to 27.83, 32.25, 46.19, 54.84, 57.55 and 76.74 sets of lattice planes are observed which can be indexed to face-centred cubic silver (Figure 4). The peaks matches with the Joint Committee on Powder Diffraction Standards (File No. 04-0783), which further proves the formation of crystal silver nanoparticles (Kumar et al. 2012). The peaks were identified as silver nanoparticles according to XPERT-PRO software (PDF#030921). The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature [17].The diffracted intensities were recorded from 10° to 80° at 2 theta angles. The diffraction pattern 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 [18].

Nanotechnology manipulates matter at 1-100 nm nanoscale [19] producing nanoproducts and nanomaterials that can have novel and size-related physicochemical properties differing significantly from those from larger particles. The novel properties of nanoparticles have been exploited widely for the use in medicine [20,21], cosmetics [22], renewable energies [23], environmental remediation [24] and electronic devices [25]. Silver is considered relatively harmless to humans. Indeed, silver's bactericidal properties have been exploited by certain groups commercialising colloidal silver suspensions as 'health supplements'. In addition, there is a potential impact on gut microflora affecting the population size of certain types of bacteria. Silver ions have a great propensity to bioconcentrate in organisms, since the chemical properties of the Ag ions make them compatible for uptake via cell membrane ion transporters, similar to those regulating Na+ and Cu+ ion transport into cells [26].

Silver nanoparticles have several characteristics that make it currently among the most widely used nanoparticles in science. One highly useful characteristic is its antimicrobial property [27]. Silver in its pure form was known as a great material to keep microbes at bay. If silver is transformed into nanoparticles, the antimicrobial property is intensified, making it useful in effectively eliminating fungus, bacteria and viruses. As natural material silver is known to be safe to man and produce little to no allergic reactions when tested for curing various diseases. Silver is an effective antibacterial agent with low toxicity which is important especially in the treatment of burn wounds. Given its broad spectrum activity, silver nanoparticles have been the focus of increasing interest and are being used as an excellent candidate for therapeutic purposes [28]. Cancer is an abnormal type of tissue growth in which the cells exhibit an uncontrolled division relatively in an autonomous fashion leading to a progressive increase in the number of dividing cell [29]. There is increasing demands for anticancer therapy [30]. *In vitro* cytotoxicity testing procedures reduces the use of laboratory animals [31] and hence use of cultured tissues and cells have increased [32]. The discovery and identification of new antitumor drug with low side effects on immune system has become an essential goal in many studies of immune-pharmacology [33]. With this aim, many attentions have been paid to natural compounds in plants, marine organism and microorganisms. Many medically relevant nanoparticles such as silver nanoparticles were investigated for different degrees of *in vitro* cytotoxicity [34]. Similarly the synthesized silver nanoparticles using *Enteromorpha linza* (L.) J.Ag. collected from Hare island, Thoothukudi, Tamil Nadu, India can be used for various purposes including as medicine, cosmetics etc.

Figure 1. Natural Habit of *Enteromorpha linza* (L.) J.Ag.

Figure 2. UV-VIS Peak Values of synthesized Silver nanoparticles using *Enteromorpha linza* (L.) J.Ag.

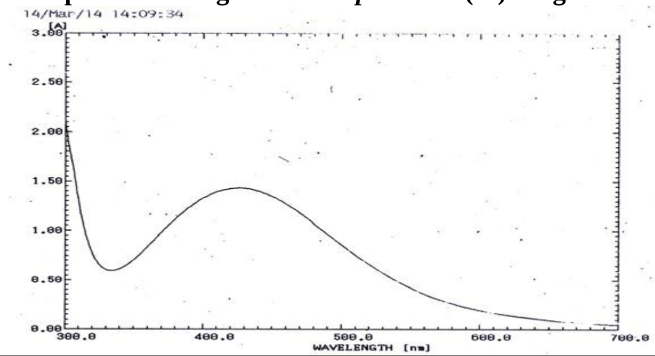

Figure 3. FTIR spectra of synthesized Silver nanoparticles using *Enteromorpha linza* (L.) J.Ag.

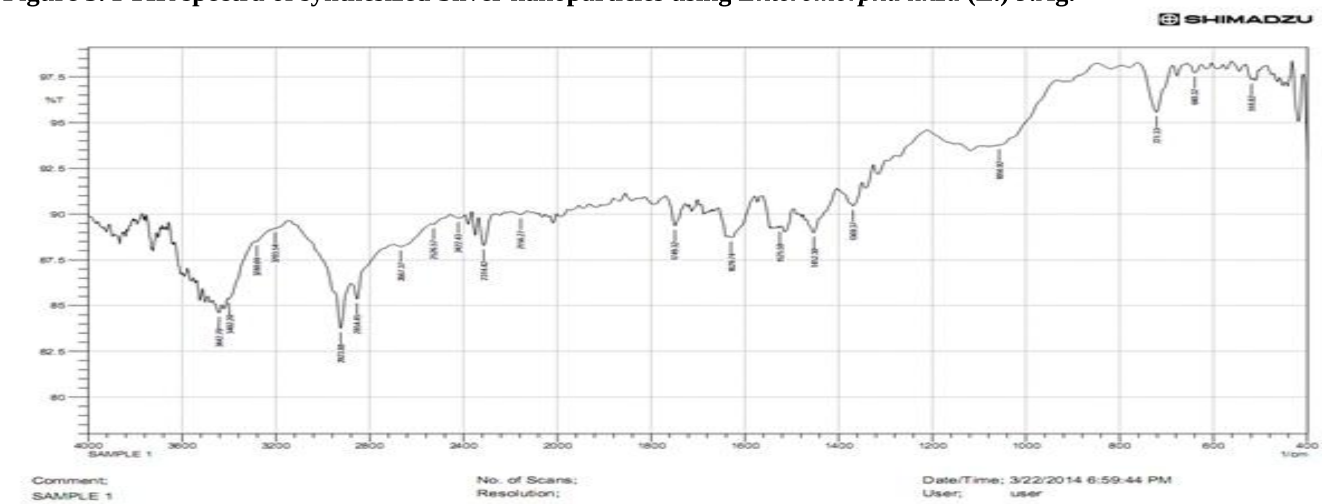

Figure 4. XRD pattern of synthesized Silver nanoparticles synthesized from *Enteromorpha linza* (L.) J. Ag.

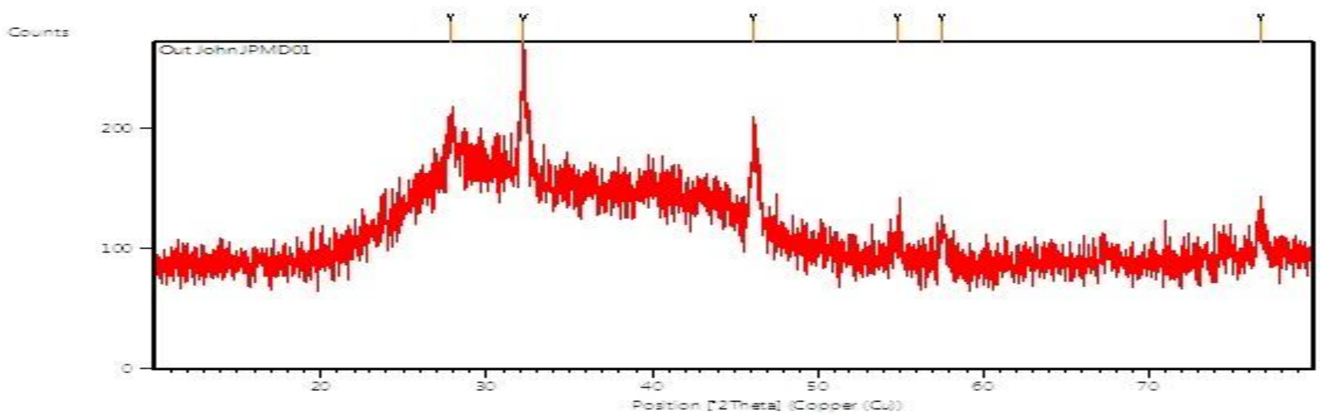

Table 1. UV-VIS Peak Values of synthesized Silver nanoparticles using *Enteromorpha linza* (L.) J.Ag.

Peak No.	Nanometres	Absorbency
1	410	1.469
2	420	1.426
3	430	1.414
4	440	1.401
5	426	1.434
6	450	1.313

7	460	1.269
8	470	1.107
9	480	1.068
10	490	0.925
11	500	0.858
12	510	0.747
13	520	0.662
14	530	0.578
15	540	0.491
16	550	0.409
17	560	0.356
18	570	0.308
19	580	0.262
20	590	0.214
21	600	0.194

Table 2. X-Ray Diffraction (XRD) Peak values of synthesized Silver nanoparticles using *Enteromorpha linza* (L.) J.Ag.

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
27.8381	36.73	0.4015	3.20488	39.00
32.2585	94.18	0.3346	2.77510	100.00
46.1999	75.80	0.3346	1.96499	80.48
54.8434	18.67	0.8029	1.67400	19.82
57.5550	30.37	0.4015	1.60142	32.25
76.7485	27.68	0.4015	1.24185	29.39

CONCLUSION

In conclusion, the bio-reduction of aqueous silver ions by the aqueous extract of *Enteromorpha linza* (L.) J.Ag. 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 and economic viability. Applications of such nanoparticles in medical and other applications make this method potentially use for the large-scale synthesis of other inorganic nano materials. Toxicity studies of silver nanoparticles open a door for a new range of antibacterial and antioxidant agents. Applications of the synthesized silver nanoparticles in bactericidal, fungicidal and cytotoxic applications make this method potentially for *in vivo* method.

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