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Biosynthesis and Characterization of Silver Nanoparticles using *Gracilaria Dura* (AG.) J.AG. (Red Seaweed)

John Peter Paul, J[1], Shri Devi, S.D.K[2].

1.Research Department of Botany, St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India.

2. Department of Botany, Sri Sarada College for Women (Autonomous), Salem – 636 016, Tamil Nadu, India

ABSTRACT

The synthesis, characterization and application of biologically synthesized nanomaterials are an important aspect in nanotechnology. Biological synthesis of silver nanoparticles has received a tremendous attention and has been a focus of research due to their high chemical, thermal stability and promising applications in medicinal field due to its environmental friendly approach and also low cost techniques. In the present study silver nanoparticles were synthesized using the aqueous extract of red seaweed *Gracilaria dura* (Ag.) J.Ag. as the reducing agent. The formation of silver nanoparticles was confirmed by colour change, UV-Visible Spectroscopy, FT-IR and X-Ray Diffraction Method (XRD). The nanoparticles showed an absorbance at 446nm 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).These results not only provide a green approach for the synthesis of nanoparticles but also open a door for new pharmaceutical leads.

Keywords: Nanotechnology, Seaweeds, *Gracilaria dura*, silver nanoparticles.

*Corresponding Author Email: johnarock2008@yahoo.com

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INTRODUCTION

Nanotechnology involves synthesis of nanoparticles of size ranging from 1 to 100 nm which can be suitably manipulated for the desired applications in electronic and medicine. There have been impressive developments in the field of nanotechnology in the recent past with numerous methodologies formulated to synthesize nanoparticles of particular shape and size depending on specific requirements[1]. Nanoparticles have considerable utility as controlled drug delivery systems. When suitably encapsulated, a pharmaceutical can be delivered to the appropriate size, its concentration can be maintained at proper levels for long periods of time, and it can be prevented from undergoing premature degradation [2].

A new branch of nanotechnology is nanobiotechnology which combines biology principles with physical and chemical procedures to generate nanosized particles with specifics functions. Nanobiotechnology represents an economic alternative for chemical and physical methods of nanoparticles formation. One of the most important criteria of nanotechnology is that of the development of clean, nontoxic and ecofriendly green chemistry procedures [3]. Nanoparticles are synthesized by physical, chemical and biological methods. Metallic nanoparticles are most promising and remarkable biomedical agents. Due to their large surface volume ratio, they govern interest of researchers on microbial resistance. Silver, Aluminum, Gold, Zinc, Carbon, Titanium, Palladium, Iron, Fullerenes and Copper have been routinely used for the synthesis of nanoparticles. However, former three metals are most popular metals in bionanomaterial synthesis. Biosynthesis of nanoparticles is an exciting recent addition to the large repertoire of nanoparticles synthesis methods and now, nanoparticle has entered a commercial exploration period. Gold and silver nanoparticles are pertaining to have a wide range of application in the fields of physical, chemical and biological science [4].Silver nanoparticles are of great importance because of its high medicinal value that from ancient history it has been in use. So it is of high importance to manufacture these silver nanoparticles for use in medicine.

Seaweeds are the important natural resources that have in recent decades become fundamental for numerous industries. Some of the historical uses of marine plants in various parts of the world and to show how some simple uses of the wild vegetation have evolved into important industrial productions and marine agricultural practices that tough the lives of millions of people [5].The *Gracilaria* species are important for the industrial and biotechnological uses. In this report an attempt has been taken to synthesis silver nanoparticles using the red seaweed *Gracilaria dura* (Ag.) J.Ag.

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 *Gracilaria dura* (Ag.) J.Ag. (Figure-1) belonging to Rhodophyceae (Red 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.

Figure 1 Natural Habit of *Gracilaria dura* (Ag.) J.Ag.

Nanotechnological studies

Silver Nanoparticles Synthesis

2g dried seaweed powder was taken in a 100ml Erlenmeyer flask with 30ml of sterile distilled water and then boiled the mixture for 2 minutes [6]. 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.

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.

FTIR Analysis

Perkin-Elmer spectrometer FTIR Spectrum ONE in the range 4000 to 400cm^{-1} at a resolution of 4cm^{-1} was used. The sample was mixed with KCl procured from Sigma. Thin sample disc was prepared by pressing with the disc preparing machine and placed in Fourier Transform Infra Red (FTIR) for the analysis of the nanoparticles.

XRD Analysis

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 and X-ray diffractometer operated at a voltage of 40kv and a current of 30mA with Cu Kα radiation

RESULTS AND DISCUSSION

Synthesis and Characterization of Silver Nanoparticles

Synthesis of Silver Nanoparticles

Reduction of silver ion into silver particles during exposure to the seaweed 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 nanoparticle synthesis in the reaction mixture, as it is well known that silver nanoparticle 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 [7].

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 446nm, 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 [8]. 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 [9].

Table 1 UV-VIS Peak Values of synthesized Silver nanoparticles using *Gracilaria dura* (Ag.) J.Ag.

Peak No.	Nm	Abs
1	410	1.091
2	420	1.140
3	430	1.173
4	440	1.189
5	**446.5**	**1.191**
6	450	1.189
7	460	1.173
8	470	1.146
9	480	1.098
10	490	1.042
11	500	0.982
12	510	0.914
13	520	0.840
14	530	0.759
15	540	0.676
16	550	0.596
17	560	0.517
18	570	0.445
19	580	0.380
20	590	0.322
21	600	0.271

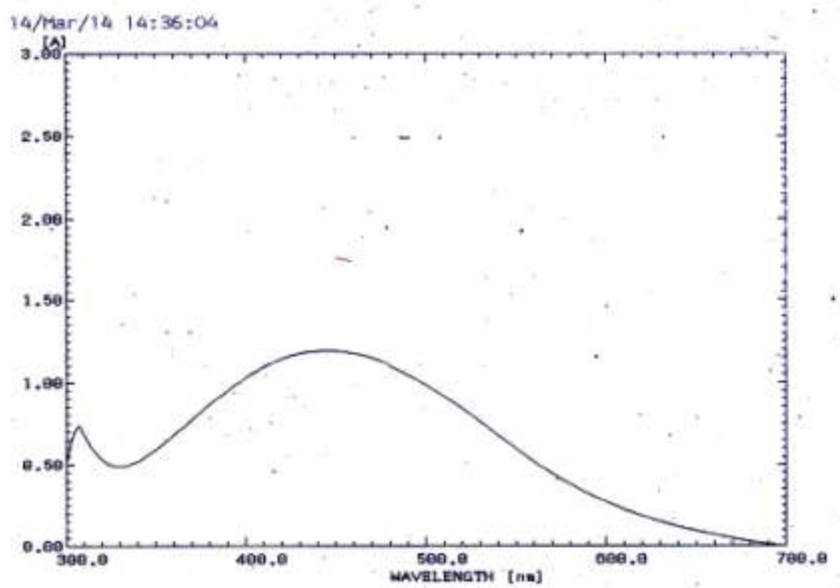

Figure 2 UV-VIS Peak Values of synthesized Silver nanoparticles using *Gracilaria dura* (Ag.) J.Ag.

FT-IR Spectrum

FT-IR spectrum of silver nanoparticles is presented in figure (Figure-3). This spectrum shows the presence of bands at 3402, 1629, 1452, 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 [10]. This evidence suggests that the protein molecules could possibly perform the function of the formation and stabilization of silver nanoparticles in the aqueous medium.

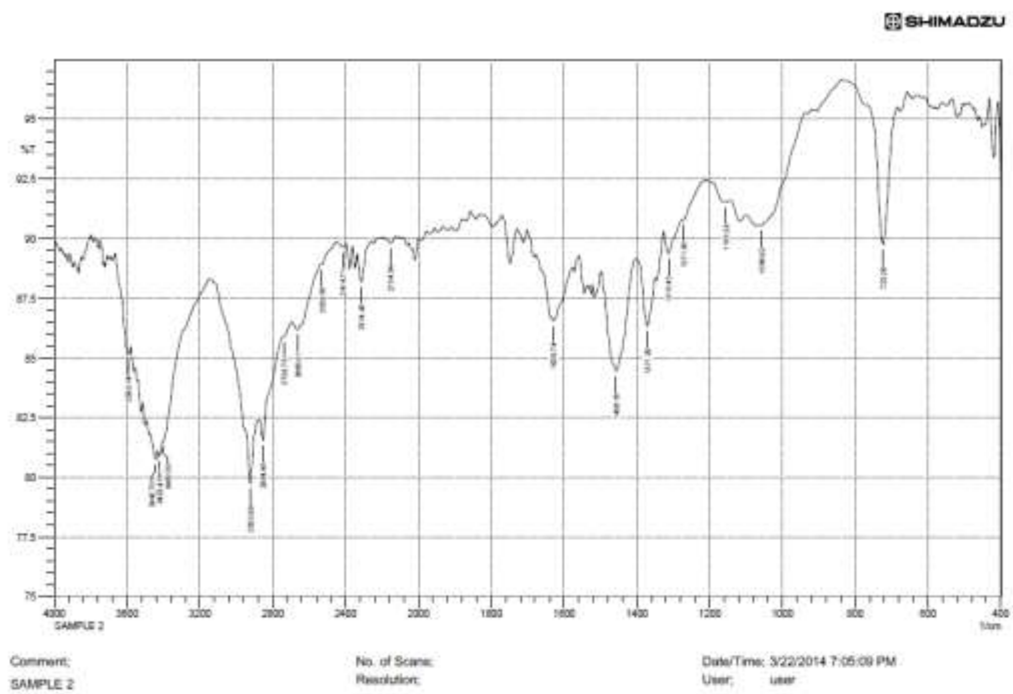

Figure 3 FT-IR Spectrum Peak values of synthesized Silver nanoparticles using *Gracilaria dura* (Ag.) J.Ag.

X-Ray Diffraction studies

XRD pattern taken using powder X-ray diffractometer instrument (XRDML) in the angle range of 10˚C-80˚C of the AgNPs at 2θ, scan axis: Gonio. A number of Bragg reflections corresponding to 28.87, 32.65, 46.68, 55.19, 57.84, 67.73 and 77.02 sets of lattice planes are observed which can be indexed to face-centered cubic silver (Table-2 and 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 [11]. The peaks were identified as AgNPs according to XPERT-PRO software (PDF#030921). The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature [12].

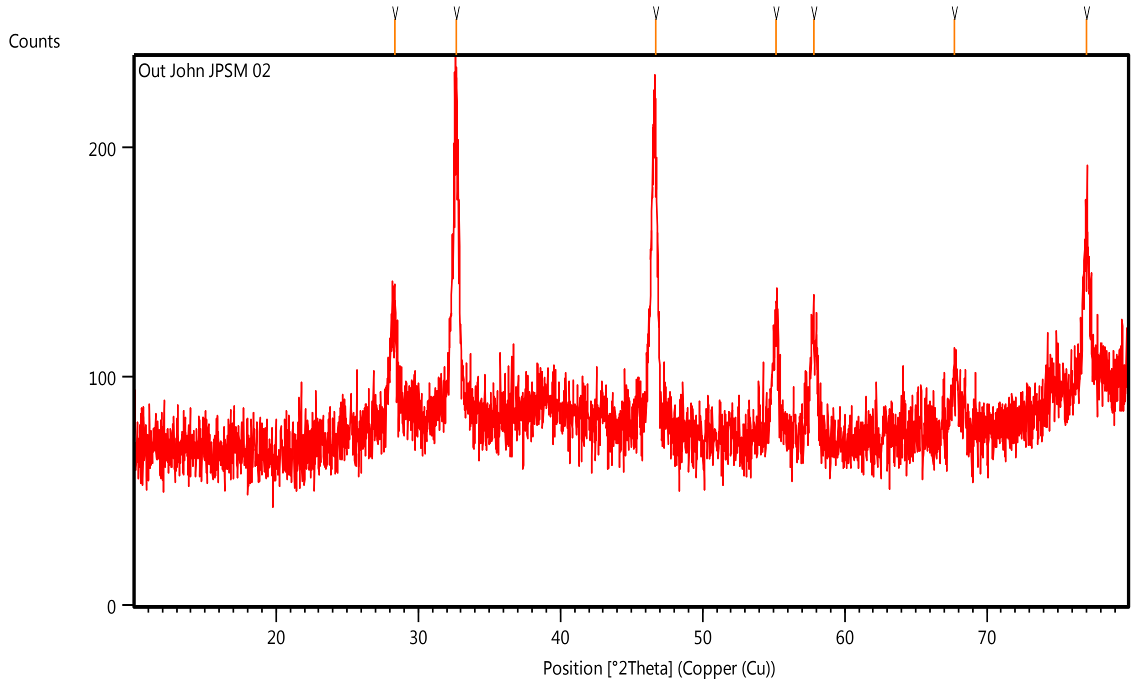

Figure 4 X-Ray Diffraction Peak values of synthesized Silver nanoparticles using *Gracilaria dura* (Ag.) J.Ag.

Table 2 X-Ray Diffraction Peak values of synthesized Silver nanoparticles using *Gracilaria dura* (Ag.) J.Ag.

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
28.3704	47.84	0.4015	3.14595	33.66
32.6506	142.14	0.2007	2.74267	100.00
46.6865	134.15	0.2342	1.94564	94.38
55.1906	49.84	0.4015	1.66429	35.06
57.8409	45.58	0.5353	1.59418	32.07
67.7379	24.48	0.4015	1.38335	17.22
77.0287	64.92	0.5353	1.23803	45.67

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 [13].

The medicinal and preservative properties of silver have been known for over 2,000 years. Silver is one of the basic element that makes up our planet. It is a rare, but naturally occurring element, slightly harder than gold and very ductile and malleable. Silver nanoparticles of many different shapes (spherical, rod-shaped, truncated, triangular nanoplates) were developed by various synthetic routes. Truncated triangular silver nanoplates were found to show the strongest anti-bacterial activity. The silver nanoparticles have excellent antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. Silver ions and nanoparticles are highly toxic and hazardous to microorganisms. Silver nonoparticles have many applications; for example, they might be used as spectrally selective coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, as catalysts in chemical reactions, for biolabelling and as antimicrobials [14,15,16]. Synthesis of silver nanoparticles using plants [17,18,19,20,21] fungal [22], Bacteria extracts [23]. Antimicrobial and cytotoxic effects of silver nanoparticles. Current research in inorganic nanomaterials having good antimicrobial properties has opened a new era in pharmaceutical and medical industries [24]. Silver is the metal of choice as they hold the promise to kill microbes effectively. Silver nanaoparticles have been recently known to be a promising antimicrobial agent that acts on a broad range of target sites both extracellular as well as intra-cellular on *Staphylococcus aureus* and *E. coli* [25,26].

CONCLUSION

It is concluded that plant mediated synthesis of silver nanoparticles possess potential antimicrobial, anticoagulant and anticancer activities. The characterization analysis proved that the particle so produced in nano dimensions would be equally effective as that of antibiotics and other drugs in pharmaceutical applications. The use of silver nanoparticles in drug delivery systems might be the future thrust in the field of medicine. The green chemistry synthetic route can be employed for silver nanoparticles synthesis. The on-going research efforts are focused on evaluating the safety of nano medicine and formulating the international regulatory guidelines for the same which is critical for further technology advancement. Recently, the researchers are looking into the development of cost-effective procedures for producing reproducible, stable and biocompatible silver nanoparticles from bioresources.

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