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Phycosynthesis and characterization of silver nanoparticles produced by biological reduction method using *Ulva reticulata* Forssk

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Abstract
The eco-friendly synthesis of nanoparticles through various biological means helps us to explore various marine macro algae for the ability to synthesize silver nanoparticles (AgNPs). It was found that aqueous silver ions when exposed to aqueous extract of *Ulva reticulata* Forssk are reduced in solution, thereby leading to the formation of the AgNPs within 15 min. These AgNPs were characterized by means of several techniques. The nanoparticles show absorbance at 400 to 450 nm 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 XRD. The morphology of AgNPs was characterized by Scanning electron microscopy (SEM). The present analysis showed the presence and the size of the synthesized silver nanoparticles about 41-73 nm.

Keywords: Silver nanoparticles, *Ulva reticulate,* UV-Visible, FT-IR, EDS, SEM

1. Introduction
Particles having a diameter of less than 100 nm are termed nanoparticles. Nanoparticles exhibit novel and improved properties based on specific features such as better size, distribution, and morphology compared to their constituent larger particles of the bulk materials. Due to their small size, nanoparticles have a more excellent surface-to-volumeratio. The specific surface area of silver nanoparticles (AgNPs) is essential for their catalytic activity and other associated features such as antibacterial properties [1-2]. Nanoparticles can be synthesized in several ways, such as physical and various other chemical methods. These methods are expensive and use many different toxic substances, which makes them difficult to scale these methods for mass production. In recent years it has been found that plant molecules can perform the same reduction reactions necessary for the production of nanoparticles but in a much more efficient way. Here, green chemistry was employed to synthesize AgNPs using plant extracts. The synthesis of AgNPs is of much interest to the scientific community because of their wide applications. For almost 2000 years, the medicinal benefits of silver have been recognized. Since the nineteenth century, silver compounds have been employed in several antibacterial applications [3].
There are many physical, chemical, and biological methods depicted in various literatures on synthesizing AgNPs. The chemical processes include numerous ways that use toxic substances or are expensive and therefore are the 'not so favored' synthesis methods [4]. The physical methods include many pieces of high-end equipment, which are expensive and occupy a considerable amount of space. There have been tremendous developments in the field of nanobiotechnology in the recent past with numerous technologies formulated to synthesize nanoparticles with specific characteristics on morphology (shape and size) and distribution. Although, there are several methods for the synthesis of pure, well defined nanoparticles, they are very expensive and the use of toxic and hazardous chemicals which cause danger to environment, human and biological means. Nanocrystalline silver is a known noble metal and they have tremendous applications in the field of detection, diagnostics, therapeutics and antimicrobial activity. In the present study, it was aimed to design a protocol for eco-friendly synthesis and characterization of silver nanoparticles using the aqueous extract of *Ulva reticulata* Forssk.

2. Materials and Methods

2.1 Collection of Plant Materials

The present study area is Manapad located in the south east coast of Tamil Nadu, India. The collection of *Ulva reticulata* Forssk belonging to Chlorophyceae (Green 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 [5].

2.2. Synthesis of Silver Nanoparticles

2g dried algal 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 galss and store the nanoparticles [6].

2.3. UV-Visible Spectra Analysis

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

2.4. FTIR Analysis

Perkin-Elmer spectrometer FTIR Spectrum ONE in the range 4000 to 400 cm^{-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.

2.5. 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 30 mA with Cu Kα radiation.

2.6. EDX and SEM Analysis

The structure, composition, and average size of the synthesized silver nanoparticles were analyzed by Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray microanalysis spectroscopy (EDX). Scanning Electron Microscope analysis was made using Philips XL-30 SEM machine. After the preparation of the silver nanoparticles, thin films of the suspension of the silver nanoparticles were kept on a carbon coated copper grid by just dropping a very small amount of 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 minutes, followed by SEM and EDX observations were carried out.

3. Results and Discussion

3.1. Synthesis and Characterization 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 [7]. 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 exhibits striking colors (light yellow to dark brown) due to excitation of surface plasmon vibrations in the particles (Figure-1). It was reported that some amount of OH- groups tended to promote the reduction of silver ions in some chemical methods [8].

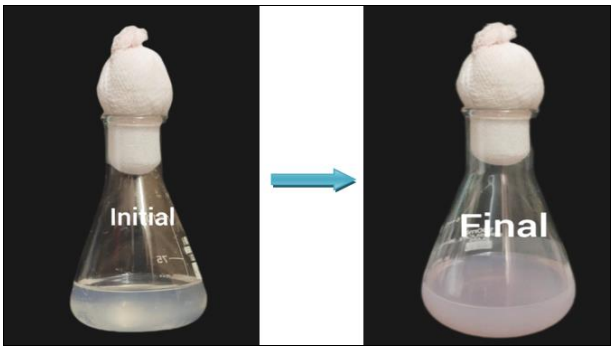

Fig 1: Plant extract and silver nitrate solution with initial and after the formation of silver nanoparticles using *Ulva reticulata* Forssk

3.2. UV-Visible Spectrum

UV-Visible spectra of the reaction media were taken at different time intervals. The Surface Plasmon Resonance (SPR) vibrations were found between 400 to 450 nm with λmax at 421 nm with absorption of 0.048 which was blue colour shifted at 15 min. The black colour shifted at 421 nm with absorption of 0.242 at 30 min, followed by pink colour at 420 nm with absorption of 0.976 at 1h, sky blue colour at 420 nm with absorption of 1.015 at 2 h and violet colour at 421 nm with absorption of 1.241 at 4h was related to an increase the amount of silver nanoparticles (Figure-2). In the present study, there is only one peak at the centre indicating the formation of silver nanoparticles in spherical shape [9].

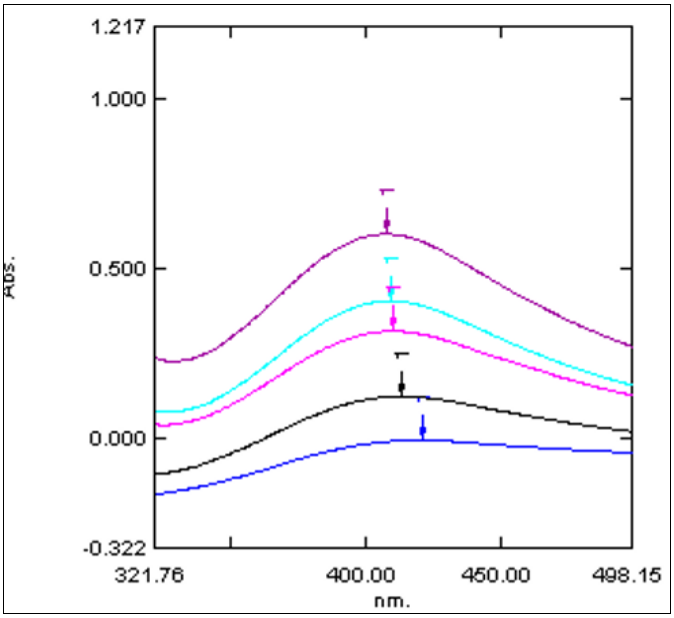

Fig 2: UV-Visible spectrum of silver nanoparticles using *Ulva reticulata* Forssk

3.3. FT-IR Spectrum

FT-IR spectrum of silver nanoparticles is shown in Figure-3 and Table-1. This spectrum shows the presence of bands at 2360.71, 1637.45, 1304.79, 1201.57, 1139.85, 1095.49 and 632.61. The bands at 2360.71 cm^{-1} corresponds to primary amine O-H band, 1637.45 cm^{-1} corresponds to primary amine N-H band, the band at 1304.79cm^{-1} is assigned to methylene scissoring vibration from the protein in the solution and the band at 1095.49cm^{-1} were assigned to C-N stretching vibration of the proteins [10]. The positions of these bands were close to that reported for native proteins. This evidence suggests that the protein molecules could possibly perform the function of the formation and stabilization of silver nanoparticles in the aqueous medium [11].

3.4. 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 silver nanoparticles at 2θ, scan axis: Gonio. A number of Bragg reflections corresponding to 32.37, 46.42, 27.47 and 54.65 sets of lattice planes are observed which can be indexed to face-centered cubic silver (Figure-4 and Table2). The peaks match with the Joint Committee on Powder Diffraction Standards (file No. 04-0783), which further proves the formation of crystal silver nanoparticles [12]. 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 [13].

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 Ag^2O 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 nano sized 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.

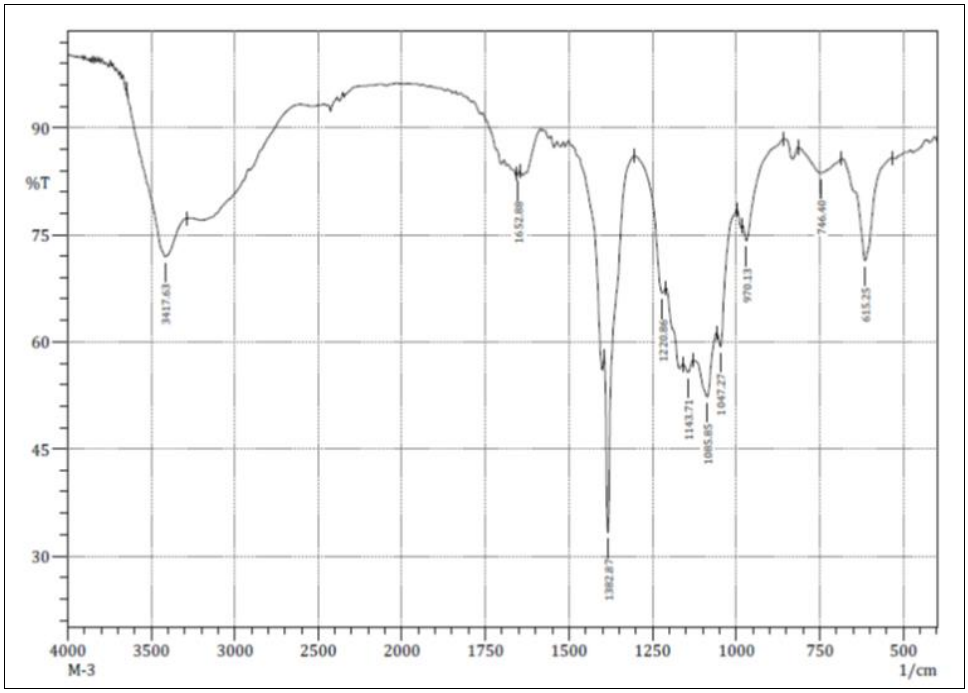

Fig 3: FTIR spectrum analysis of silver nanoparticles using *Ulva reticulata* Forssk

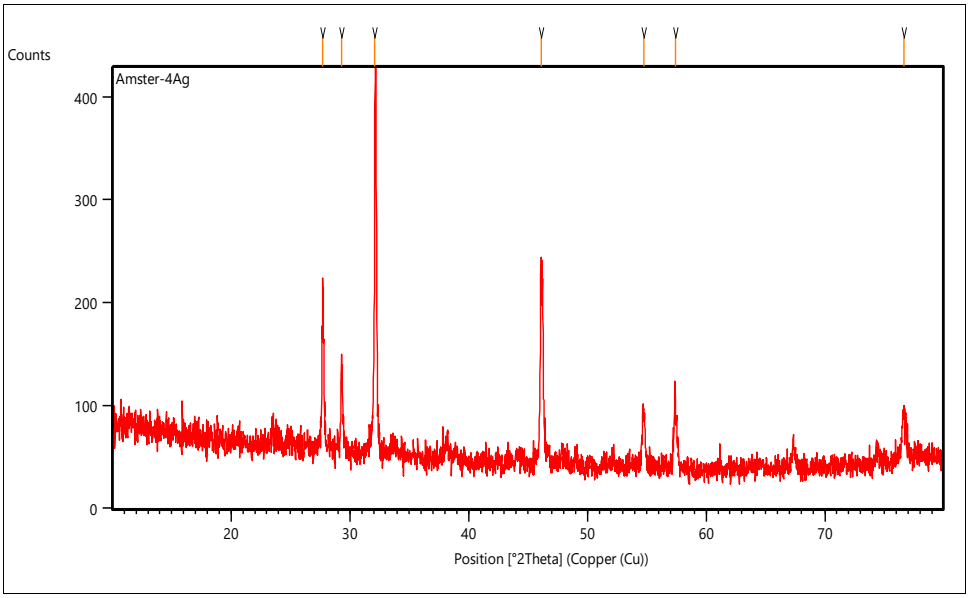

Fig 4: XRD analysis of silver nanoparticles using *Ulva reticulata* Forssk

Table 1: FTIR spectrum analysis of silver nanoparticles using *Ulva reticulata* Forssk

Peak value	Functional group	Assignment
3417	Amines and amides	N-H
1652	β-ketone esters	C=O
1382	Sufonlyl chloride	SO_2
1220	Vinyl ethers	C-O-C
1143	Alochols	C-O-H
1085	Siloxanes	C-O
1047	Organophosporous	P-O-C
970	Alkenes	=CH
746	Benzenes	CH
615	Phenols	OH

Table 2: XRD analysis of silver nanoparticles using *Ulva reticulata* Forssk

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
27.7351	145.16	0.1673	3.21655	42.64
29.3232	82.13	0.1338	3.04586	24.13
32.1104	340.44	0.1506	2.78756	100.00
46.1008	192.37	0.2342	1.96898	56.51
54.7315	57.77	0.2007	1.67716	16.97
57.3885	61.05	0.2676	1.60567	17.93
76.6752	48.81	0.4015	1.24286	14.34

3.5. SEM and EDX Analysis

To gain further insight into the features of the silver nanoparticles, the element analysis of the silver nanoparticles was performed using EDX on the SEM. The freeze dried silver nanoparticles were mounted on specimen stubs with double sided taps, coated with gold in a sputter coater and examined under a Philips XL-30 SEM at 12-16 kV with a tilt angle of 45°. Figure-5 shows the EDX spectrum of spherical nanoparticles prepared with this bioreduction method. The peaks around 3.40 keV correspond to the binding energies of AgL. Throughout the scanning range of binding energies, no peak belonging to impurity was detected. The results indicated that the reaction product was composed of high purity Ag nanoparticles [14]. A similar EDX spectrum was obtained for each sample analyzed. Scanning electron microscopy provided further insight into the morphology and size details of the silver nanoparticles. Comparison of experimental results showed that the diameter of prepared nanoparticles in the solution was about 41-73 nm. Figure-6 shows the scanning electron micrographs of silver nanoparticles obtained from the bioreduction method [15].

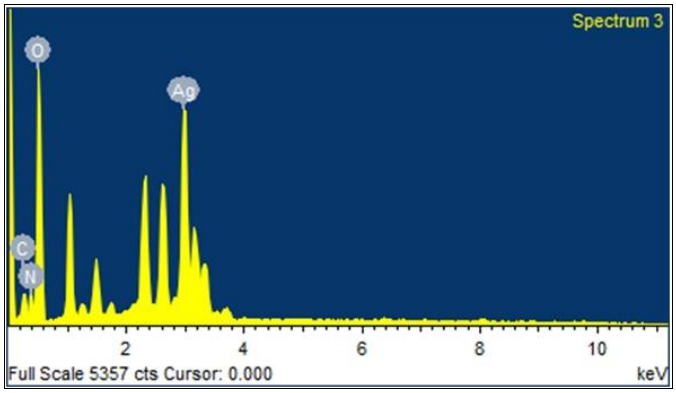

Fig 5: Energy Dispersive X-Ray (EDX) spectrum of silver nanoparticles using *Ulva reticulata* Forssk

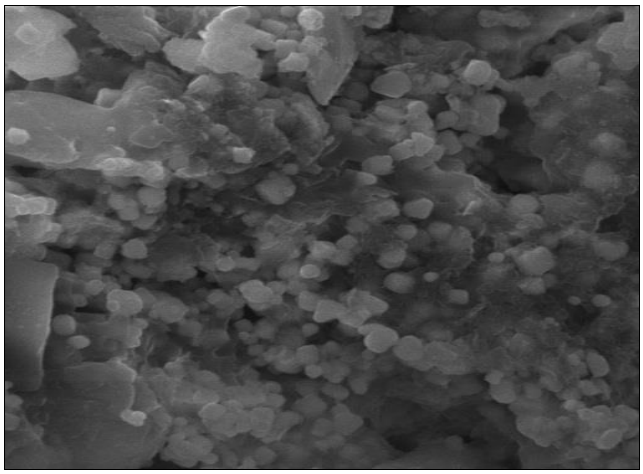

Fig 6: Scanning Electron Microscopic (SEM) image of silver nanoparticle using *Ulva reticulata* Forssk

4. Conclusion

The aqueous extract of *Ulva reticulata* Forssk used to synthesis silver nanoparticles. The silver nanoparticles were characterized by UV-Visible spectrophotometer, FT-IR, XRD, EDX and SEM. There is only one peak at the centre at different nanometer indicating the formation of silver

nanoparticles in spherical shape using UV-Visible spectrophotometer. FT-IR spectrum of silver nanoparticles shows the presence of bands at 632.61, 1095.49, 1139.85, 1201.57, 1304.79, 1637.45 and 2360.71cm^{-1}. XRD pattern of silver nanoparticles shows a number of Bragg reflections corresponding to ˚2θ of 32.37, 46.42, 27.47 and 54.65 sets of lattice planes of silver. EDX and SEM analysis showed the presence and the size of the synthesized silver nanoparticles about 41-73nm.

5. References

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