

BIOSYNTHESIS AND OPTIMIZATION OF GREEN SILVER NANOPARTICLES FROM A RED ALGA HALYMENIA PORPHYRAEFORMIS COLLECTED FROM THE COASTAL AREAS OF KARACHI, PAKISTAN

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Abstract

Current advances in the nanotechnology have a remarkable impact on everyday life. These advances have introduced the role of Plants in the nanotechnology for the synthesis of green stable nanoparticles. The current study employed the aqueous extracts of *Halymenia porphyraeformis* at varying concentrations (10%, 20%, and 30%) of Silver nitrate solution. The synthesized green AgNPs were characterized using UV-vis spectrophotometer, X-ray diffraction (XRD), scanning electron microscopy (SEM), and Fourier-transform infrared spectroscopy (FTIR). UV-Vis analysis revealed maximum absorption peaks at 480 nm, 520 nm, and 560 nm, corresponding to different concentrations. SEM imaging confirmed the spherical and oval shapes of the AgNPs with smooth outer surfaces. XRD analysis confirmed the crystalline nature of the nanoparticles, with characteristic peaks at 2θ values of 26.6° , 27.7° , 31.9° , 25.7° , 34.1° , and 32.3° . These peaks were indexed at 480, 440, 280, 140, 80, and 510 counts/s, respectively. FTIR spectroscopy identified functional groups such as O-H, carboxylic acids, C-H stretch, C-O bonds, N-H bonds, amine salts, C-F stretch, C-d stretch, C-I, and C-Br stretch, which were involved in the reduction of silver nitrate to AgNPs.

KEYWORDS: silver, nanoparticles, XRD, SEM, FTIR, functional groups

Introduction

Nanotechnology is a newly emerged field in the area of science, which is dealing with the formation of nanoparticles exhibiting various sizes and shapes (Elechiguerra et al. 2005).

Nanoparticles are classified on the basis of their composition i.e. organic and inorganic (online source 6). The particles containing carbon in their structure are organic nanoparticles, while the nanoparticles of metals like gold, silver, platinum, and palladium are all inorganic nanoparticles (Kathiresan & Asmathunisha 2013).

Moreover nanoparticles show remarkably different physical, chemical and biological properties. AgNPs are applicable in various fields around us. They are involved in the pharmaceutical field, electronics, cosmetics, textile industry as well. Moreover antibacterial (Venkatpurwar & Pokharkar, 2011), antimicrobial, antifungal, anti-HIV activities (Elechiguerra et al. 2005) catalytic, enzymatic properties are also included in the properties of silver nanoparticles. Silver nanoparticles play an essential role in the field of nanotechnology in the sense that it can control the drug resistant bacteria (Russel & Hugo 1994).

In previous times, different organisms like microorganisms, seaweeds and plants, were used to study the synthesis of AgNPs. Of them employment of plants to synthesize AgNPs is most valuable method (online source 3). The methods for synthesizing nanoparticles by using naturally occurring reducing agents like polysaccharides, other chemical substances, plant extracts, and use of microorganisms can be considered effective in the field of nanotechnology. (online source 2), But, green synthesis of nanoparticles is more attractive as it is simple, economically it costs less, moreover it is environmentally suitable and is more reproductive (Haefeli et al. 1984).

Marine life is always an important source of bioactive compounds with ominous impact in the pharmaceutical industry. Marine life has attained much attention in the last few years in the aspiration that it will be fruitful to nature and to mankind (Carneiro et al. 2014). From the marine life some biologically active compounds were obtained to see the pharmacological activities. Moreover, they also exhibit antimicrobial, antiviral, antifungal, and anticancer properties (Pereira & Lotufo 2012). The red seaweed *Halymenia* is comprising of almost 69 species which are accepted by the scientists, is considered as one of the largest genus in the line of

red seaweed for having species under the family Halymeniaceae (Smedt et al. 2001).

Once the Ag-NPs are obtained, their characteristics were studied using UV-Vis spectrophotometer, X-ray Diffraction pattern, Scanning Electron Microscope, Fourier Transform Infrared spectroscopy. These techniques will give the shape, size, absorbance, and presence of bio-molecular compounds in AgNPs which causes reduction of silver ions.

MATERIALS AND METHODS

Synthesis of Silver Nanoparticles using *Halymenia Porphyraeformis*:

Collection of plant Material:

The red seaweed *Halymenia porphyraeformis* was collected from the coast of Karachi and brought to Phycology lab, Government college university, Lahore for identification. It was then washed under tap water to remove the extraneous material mixed with it. Then the sample was dried under shade and a portion of the dried sample was ground to fine powder and stored in air tight containers.

Extract Preparation:

1g was weighed and mixed with 100ml of distilled water. This mixture was heated at hot plate till boiling. Then the mixture was filtered using Whatman's filter paper No 1. To get the extract of sample. For the synthesis of silver nanoparticles silver nitrate solution was prepared. 0.01M solution of silver nitrate was prepared.

Preparation of Silver Nitrate Solution:

To prepare 0.01M of 500ml solution, 0.8493 g of silver nitrate was mixed in some amount of water to homogenize it and then the final volume was raised to 500ml. Three concentrations 10%, 20% and 30% of reaction mixture were prepared. Then these different concentrations of sample were mixed with silver nitrate solution. The formation of silver nanoparticles was observed visually by noticing the change in color after 24 hours the color of the solution changed from light brown to dark brown.

Centrifugation of reaction mixture:

The reaction mixture was centrifuged and after centrifugation the reaction mixture was divided into

two phases i.e. supernatant phase and pellet phase. The supernatant was discarded and silver nanoparticles were assembled in the form of pellet, this pellet was used for the characterization of silver nanoparticles by UV-Vis spectrophotometer, XRD, SEM and FTIR.

Results

The synthesized silver nanoparticles were confirmed using UV-VIS Spectrophotometer. The reaction mixture was analysed by using wavelengths from 300-700nm. As there were three samples i.e. 10%, 20% and 30%. The 10% sample of *H. porphyraeformis* showed a highest peak at 520nm. The 20% sample showed a peak at 560nm. The 30% sample showed a peak at 480nm (Table 1.)

Table 1. Absorbance values of the reaction mixtures.

Wavelength (nm)	Absorbance		
	10% sample	20% sample	30% sample
320	1.009	0.569	1.256
360	1.112	0.882	1.559
400	1.372	1.183	1.567
440	1.282	1.553	1.675
480	1.551	1.559	2.546
520	2.98	2.320	2.091
560	2.11	2.885	2.001
600	1.819	2.183	1.498

640	1.551	1.49	1.319
680	1.183	1.004	1.256

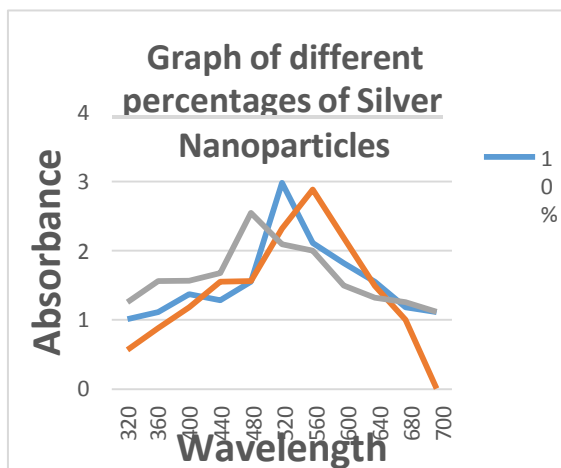


Fig 1. Graph showing the optimum peaks of different percentages of Silver Nanoparticles

The silver nanoparticles of three different percentages showed highest peaks at 520nm, 560nm and 480nm in Fig 1. These peaks were due to surface plasmon resonance property of silver nanoparticles which depend on the size and shape of the silver nanoparticles.

XRD (X RAY DIFFRACTION) Analysis:

The XRD analysis shown in fig. 2, 3 and 4 showed that the synthesized silver nanoparticles were crystalline, determining the nature of nanoparticles. The XRD displayed pattern of peaks at 2 theta such as 26.6, 27.7 and 31.9. These peaks are indexed at 480, 440 and 280 counts/s, presenting reflection peaks for crystalline structure of silver nanoparticles.

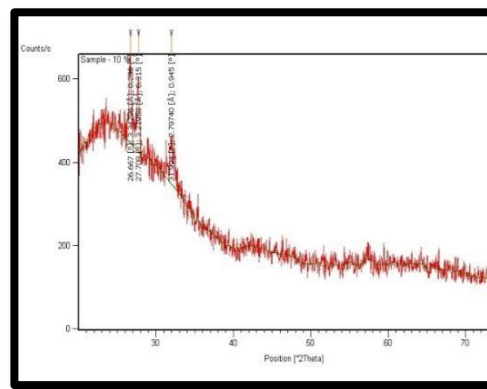


Fig. 2. XRD spectra of biosynthesized silver nanoparticles of 10% sample.

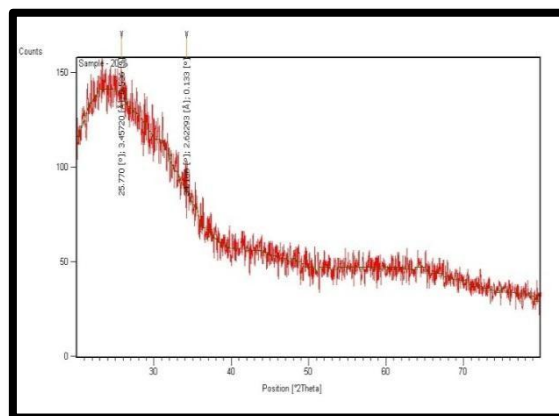


Fig. 3. XRD spectra of biosynthesized silver nanoparticles of 20% sample.

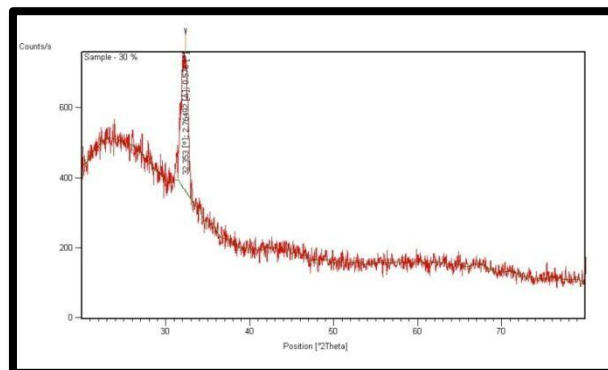


Fig. 4. XRD spectra of biosynthesized silver nanoparticles of 30% sample.

The sharp Bragg peaks of all the three samples have resulted due to crystalline properties of silver nanoparticles.

SEM (Scanning Electron Microscope):

The morphology and structure of synthesized silver nanoparticles was scanned by using scanning electron microscope at a magnification of 10 μ m as shown in Fig 5,6 and 7.

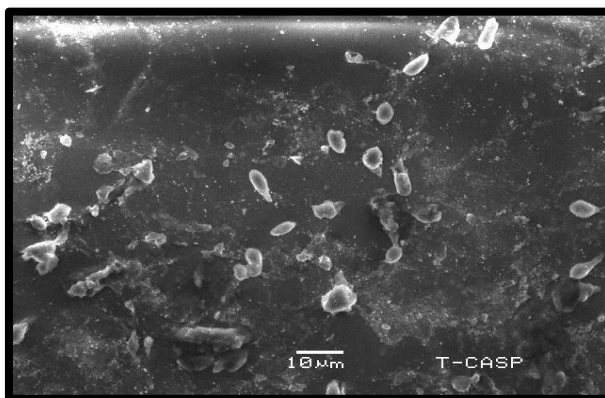


Fig. 5 SEM images of biosynthesized silver nanoparticles of 20%

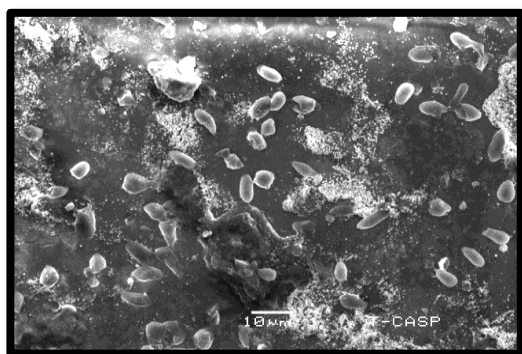


Fig 6. SEM images of biosynthesized silver nanoparticles of 30%

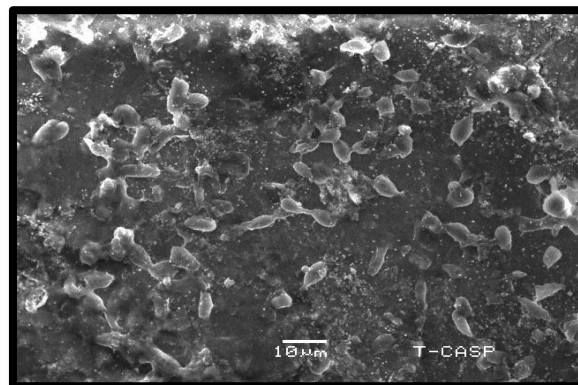


Fig 7. SEM images of biosynthesized silver nanoparticles of 10%

FTIR (Fourier Transform Infrared Spectroscopy):

SEM results showed that most of the silver nanoparticles were spherical, or elliptical in shape with smooth surfaces. All the nanoparticles were aggregated having some distance between them. SEM presented the size of silver nanoparticles in a range of 37-48nm

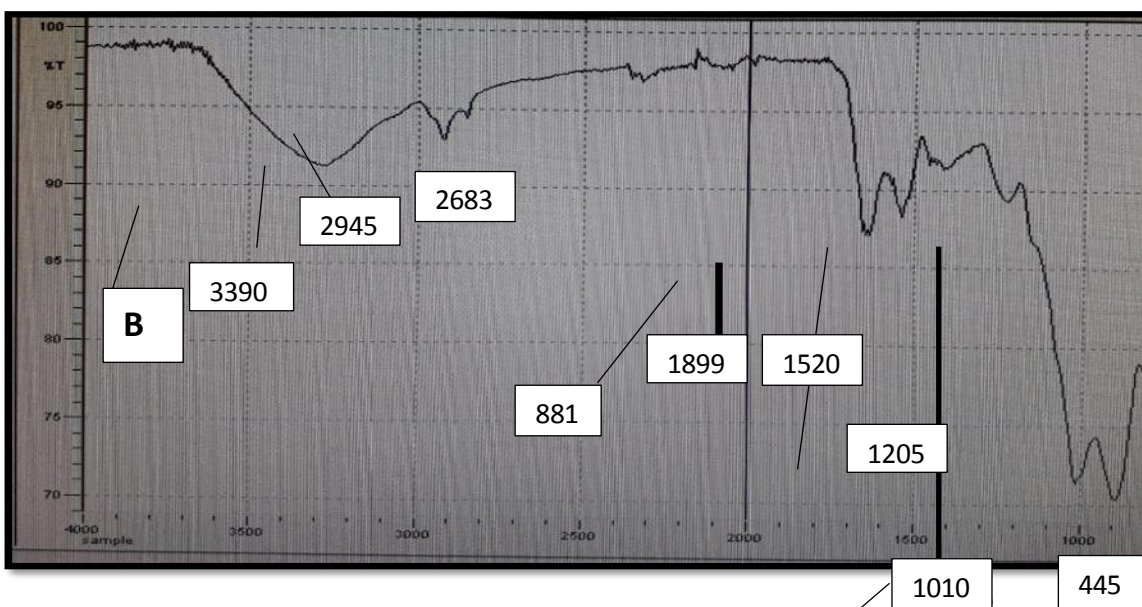


Fig. 8. FTIR spectra of 10% sample.

FTIR spectra of silver nanoparticles synthesized from *Halymeina porphyraeformis* (10% sample). This analysis revealed the bands for silver nanoparticles at 3390, 2945, 2683, 1899, 1520, 1205, 1010, 881 and

445cm⁻¹. These bands corresponds to O-H group, carboxylic acids, C-H stretch, C-O bonds, N-H bonds, amine salts, C-F stretch, C-d stretch, C-I and C-Br stretch.

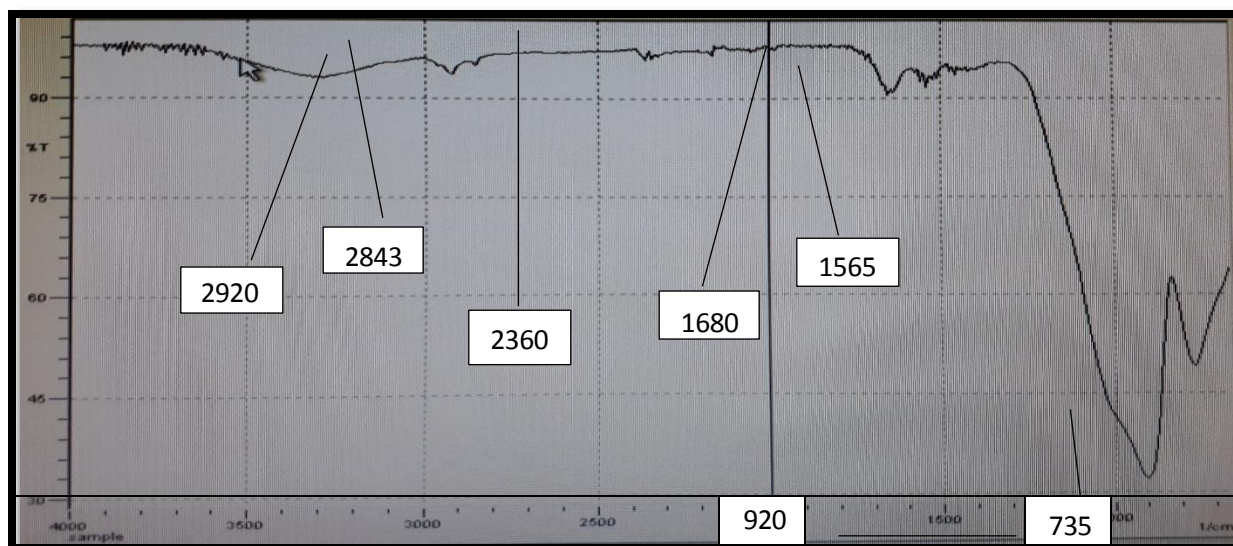


Fig. 9. FTIR spectra of 20% sample.

FTIR spectra of silver nanoparticles synthesized from *Halymeina porphyraeformis* (20% sample). This analysis revealed the bands for silver nanoparticles at 2920, 2843, 2360, 1680, 1565, 920, and 735cm⁻¹. These bands corresponds to O-H bond, carboxylic

acid, C-H bend, C-H stretch, Si-OR, CN₃H₅, COO⁻, COOH, N-H, Si-OR, and C-d stretch.

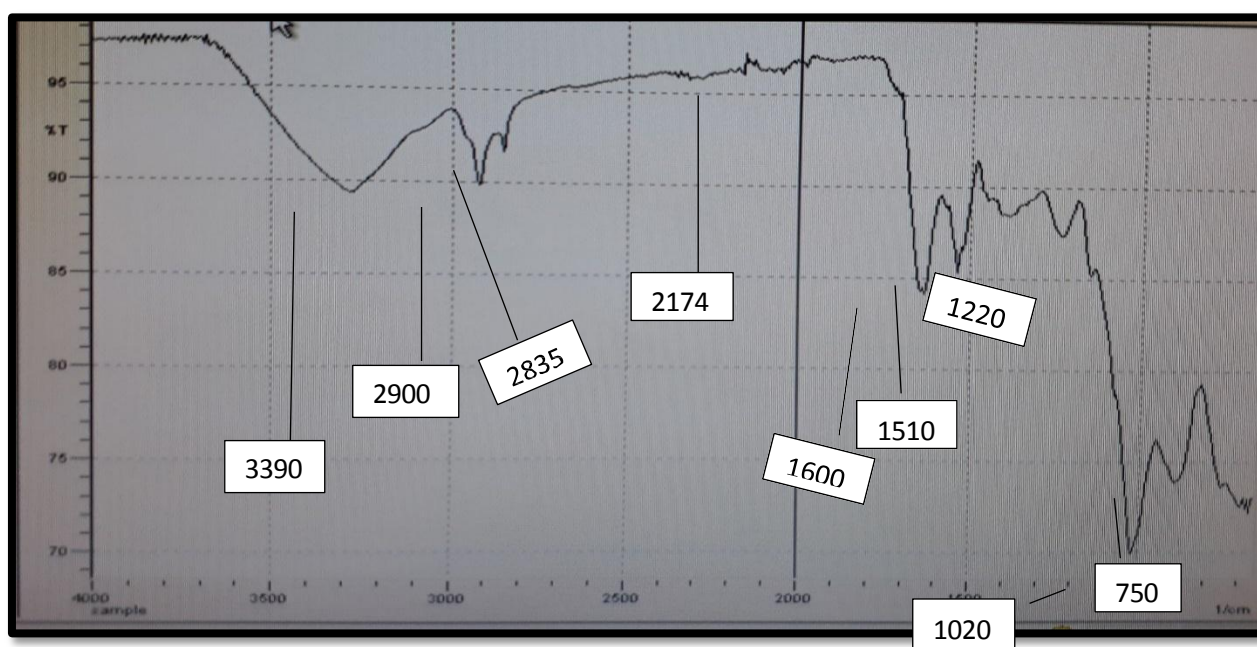


Fig. 10. FTIR spectra of 30% sample.

FTIR spectra of silver nanoparticles synthesized from *Halymenia porphyraeformis* (30% sample). This analysis revealed the bands for silver nanoparticles at 3390, 2900, 2835, 2174, 1600, 1510, 1220, 1020 and 750cm/1. These bands corresponds to O-H stretch, H-bond, N-H, Carboxylic acid, C-H bend, C-H stretch, Si-OR, NH₃, COO⁻, COOH, C-F stretch, C-d stretch.

Table 2. FTIR peak values and their assigned functional groups.

Band Position Cm/1	Functional Groups Assigned
2900 – 3500	O-H Stretch, Carboxylic acid and O-H
2500 – 2900	C-H Bend and C-H stretch
2000 – 2500	Si-OR Group
1500 – 2000	C-O, CN ₃ H ₅ , and NH ₃ Groups
1100 – 1500	COO ⁻ , COOH, N-H, and Amine salts
900 – 1100	Si-OR Group
700 – 900	C-d Stretch Group
400 – 500	C-I and C-Br stretch Group

Discussion

Employment of seaweeds to synthesize AgNPs is most valuable method. Seaweed extracts are most studied recently as they have ability to reduce metallic ions. The reducing capacity of seaweeds depends on the presence of various phenolic compounds, proteins, polysaccharides and other reducing agents (Kumar et al. 2016).

The updated studies of the seaweed *Halymenia porphyraeformis* was investigated to know the morphology, size, structure, and functional groups in the seaweed. The reaction mixtures were prepared by mixing silver nitrate solution and aqueous extracts of *Halymenia porphyraeformis*, for the synthesis of silver nanoparticles. These silver nanoparticles were characterized by using UV-Vis Spectrophotometer, XRD, SEM, and FTIR analysis. In this study, UV-Vis Spectrophotometric analysis showed the selective peaks for 10%, 20% and 30% sample of reaction mixtures at 480nm, 520nm and 560nm. This analysis is also reported by other scientists for seaweeds like *anthophora specifera*, *Codium capitatum*, *Sargassum wightii*, *Caulerpa racemosa* etc. their report confirmed the analysis for the synthesis of silver nanoparticles whose Surface Plasmon bands appear at a range of wavelength between 420nm-500nm (Kumar et al. 2012), (Kannan et al. 2012), (Kathiraven et al. 2015), (Shanmugam et al. 2014), (Venu et al. 2011).

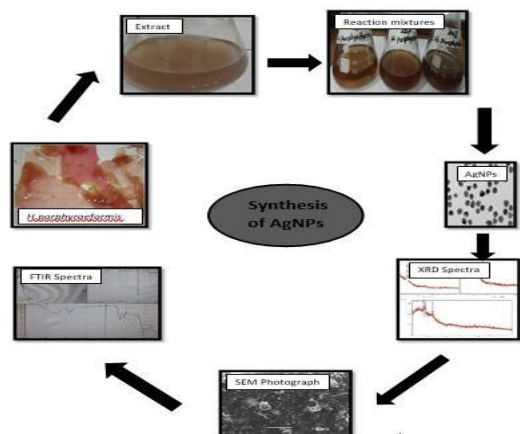


Fig. 4.11. Diagrammatic Chart of Synthesis of AgNPs.

XRD analysis of the samples of *Halymenia porphyraeformis* was done to predict the nature of silver nanoparticles. It was predicted that the silver nanoparticles are crystalline in nature. The XRD displayed pattern of peaks at 2 theta such as 26.6, 27.7, 31.9, 25.7, 34.1, and 32.3. These peaks are indexed at 480, 440, 280, 140, 80, and 510 counts/s, presenting reflection peaks for crystalline structure of silver nanoparticles for 10%, 20% and 30% samples of *Halymenia porphyraeformis*. They confirmed that the silver nanoparticles synthesized from seaweeds like *Acanthophora specifera*, *Codium capitatum*, *Sargassum wightii*, *Caulerpa racemosa* etc. are crystalline in nature (Kumar et al. 2012), (Kannan et al. 2012), (Kathiraven et al. 2015), (Shanmugam et al. 2014), (Venu et al. 2011) and the peaks of XRD analysis were almost similar to the seaweed used in present work also supported by (Khadija et al. 2022). Then the samples were characterized by Scanning Electron Microscope which gives the size and shapes of the silver nanoparticles. Under this analysis it was shown that silver nanoparticles synthesized from *Halymenia porphyraeformis* are spherical or oval in shape as like the silver nanoparticles synthesized from the seaweeds like *Halymenia porphyroides*, and *Gelidiella acerosa* (Marimuthu et al. 2011).

Then the samples were characterized by Scanning Electron Microscope which gives the size and shapes of the silver nanoparticles. Under this analysis it was shown that silver nanoparticles synthesized from *Halymenia porphyraeformis* were spherical or oval in shape with a size range of 37-48nm as like the silver nanoparticles synthesized from the seaweeds like *Halymenia porphyroides* (Vishnu & Murugesan 2013), and *Gelidiella acerosa* (Marimuthu et al., 2011).

The FTIR measurements were carried out to identify the possible functional groups. The FTIR bands for the 10% samples of *Halymenia porphyraeformis* were present at specific peaks of 3390, 2945, 2683, 1899, 1520, 1205, 1010, 881 and 445cm⁻¹, and the peaks for 20% sample were present at 2920, 2843, 2360, 1860, 1565, 920, and 735cm⁻¹, and the peaks for 30% sample appeared at 3390, 2900, 2835, 2174, 1600, 1510, 1220, 1020 and 750cm⁻¹. These peaks are assigned with specific functional groups like O-H group, carboxylic acids, C-H stretch, C-O bonds, N-H bonds, amine salts, C-F stretch, C-d stretch, C-I and C-Br stretch etc. (online source 8). The achieved peaks in this study are closely related to the peaks for *Gelidiella acerosa*, the peaks attained are at 3441, 1658, 1535 and 1400 cm⁻¹ (Marimuthu et al. 2011). The functional groups like C-O, C-N and N-H present in triterpenoids, flavavanoids or proteins in the plant's extract may be the causative agents of reduction of silver nitrate into silver nanoparticles (Vishnu & Murugesan 2013).

Conclusion

Silver nanoparticles were successfully synthesized from seaweed *Halymenia porphyraeformis*. Several types of analyses were required to study the characteristics of nanoparticles. The characteristics were studied through spectrophotometer, SEM, XRD and FTIR. Silver nanoparticles showed specific Surface Plasmon Resonance property which is shown in the form of peaks by taking absorbance of the three samples of *Halymenia porphyraeformis*. These samples show distinct peaks for absorbance at 480nm, 540nm, and 560nm. The structural study of nanoparticles were studied through SEM which presents the spherical and oval shape of nanoparticles with smooth outer surface, it also presents the size of nanoparticles in a range of 37-48nm. The chemical properties of nanoparticles were studied through XRD and FTIR. XRD analysis of samples presented the nature of silver nanoparticles. The nature of silver nanoparticles was confirmed to be crystalline. The XRD displayed pattern of peaks at 2 theta such as 26.6, 27.7, 31.9, 25.7, 34.1, and 32.3. These peaks are indexed at 480, 440, 280, 140, 80, and 510 counts/s, presenting reflection peaks for crystalline structure of silver nanoparticles for 10%, 20% and 30% samples of *Halymenia porphyraeformis*. The functional groups present in the biosynthesized nanoparticles were studied through FTIR analysis. The identified functional groups are O-H, carboxylic acids, C-H stretch, C-O bonds, N-H bonds, amine salts, C-F stretch, C-d stretch, C-I and C-Br stretch which are assigned to the specific band positions i.e. 3390, 2945, 2683, 1899, 1520, 1205, 1010, 881 and 445cm⁻¹. The green synthesis of silver nanoparticles using this

method proved to be successful by using red alga *Halymenia porphyraeformis*. It was confirmed from the present study that this seaweed has the potential to successfully synthesize nanoparticles of silver.

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