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Biosynthesis and Characterization of Silver Nanoparticles from Marine Macroscopic Red Seaweed *Halymenia porphyroides* Boergesen (Crypton)

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ABSTRACT

In the present study, economically scalable and energy efficient colloidal silver (Ag) nanoparticles were biosynthesized from marine red seaweed *Halymenia porphyroides* Boergesen (crypton) collected from southeast coast of Tamilnadu, India. The silver nanoparticles were biosynthesized as per green synthesis protocol. The rich presence of phytochemicals, bioactive compounds and secondary metabolites in marine macroscopic red seaweed *Halymenia porphyroides* Boergesen (crypton) play a major role since they possess reducing and capping agents for the biosynthesis of silver nanoparticles that may be environmentally acceptable and eco-friendly. Therefore, the red seaweed *Halymenia porphyroides* Boergesen was used in the experimental study for the biosynthesis of silver nanoparticle. The biosynthesized silver nanoparticles from marine macroscopic red seaweed *Halymenia porphyroides* Boergesen were characterized by UV-vis spectroscopy which confirmed the surface plasmon resonance of silver nanoparticles, Fourier transform infrared (FT-IR) spectroscopy to identify the presence of various functional groups in biomolecules responsible for the bio reduction of Ag⁺ and capping/stabilization of silver nanoparticles. X-ray diffraction (XRD) to observe face center cubic (fcc) and crystalline nature of silver nanoparticles, thermo gravimetric analysis (TGA) which revealed the thermal stability and purity of the silver nanoparticles. Particle size distribution and morphology were investigated by scanning electron microscope (SEM) which showed silver nanoparticles in the size range of 34.3-80.5 nm. The particle distribution under different nanometers was analyzed using transmission electron microscopy (TEM).

1. Introduction

Nanoscience and nanotechnology are the study and application of extremely small things and can be used across all the other science fields, such as chemistry, biology, physics, materials science, and engineering. Nanomaterials and nanoparticles are emerging as increasing scientific research due to their potential for achieving specific processes and selectivity, especially in biological and pharmaceutical industries [1-7]. The use of biological entities for the biosynthesis of nanoparticles has been of great interest in the past few decades due to their unusual optical [8], chemical [9], photo electrochemical [10] and electronic properties [11]. The size and shape of nanomaterials strongly influences their electromagnetic, optical and catalytic properties [12, 13]. The bacteria, fungi and seaweeds are used for the synthesis and assembly of nanoparticles which would result and benefit for the development of clean, nontoxic and environmentally acceptable "green chemistry" protocols [14, 15]. One of the promising nanoparticles in nanotechnology is the nano silver due to its potential to exhibit as strong antimicrobial, antiviral, superior catalytic activity and enhanced surface Raman spectroscopy [16-18].

Silver nitrate was used to treat ulcers during 17th and 18th century, whereas in 19th century silver nitrate pencils formed part of the standard surgical equipment [19]. Silver and its compounds are used as a biocide in the prevention of infections in burns, traumatic wounds and diabetic ulcers [20]. The reemergence and the use of silver have been used as a viable treatment for infections encountered in burns due to the stimulus publication by Moyer et al [21-24]. The other uses of silver would include as disinfectant in water treatment and as a coating in catheters as well as other medical devices implanted on/within the body [25, 26]. Silver nanoparticles remains to be one of the important scientific researches over past few decades and find their applications in the field of life sciences

especially in food chemistry [27], biomedicine [28], and agriculture [29]. Silver based nanoparticles have been synthesized by various synthetic methods involving physical, chemical [30] and biochemical techniques [31]. Currently with the increase on the focus of green chemistry the nanoparticles like silver, gold and CdS have been synthesized by biological methods using microorganisms [32-34], enzymes [35] and seaweeds or plant extracts [36-38]. Biomolecules have been used for nanomaterial synthesis/functionalization and in subsequent applications for decades [39]. The literature survey revealed that the nanoparticle synthesis using biological sources like plants/seaweeds has been unexplored and unexploited [40-43]. In the present study the green synthesis of silver nanoparticles from marine macroscopic red seaweed *Halymenia porphyroides* Boergesen (crypton) was biosynthesized and their characterization was studied by using UV-vis spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD), Thermo gravimetric analysis, scanning electron microscope (SEM) and transmission electron microscopy (TEM).

2. Experimental Methods

2.1 Collection and Preparation of Seaweed Extract

The marine red seaweed *Halymenia porphyroides* Boergesen (Crypton) was collected on summer season from 2.5-meter rapid intertidal regions of the Gulf of Mannar–Mandapam (latitude 9°17' N, longitude 79°11' E), Ramanathapuram District, South East Coast of Tamilnadu, South India. Collected seaweed was washed with sea water for eliminating impurities such as sand, rocks, epiphytes and epifauna. The washed samples were preserved with 5 -10% formaldehyde in sea water and transported to the laboratory in a box containing slush ice. The fumes of the formaldehyde would help to fix and preserve the seaweed material. In the laboratory, the samples were washed thoroughly in running tap water to remove salt and washed three times using distilled water which may remove metallic compounds and it was shade dried at room temperature (37 °C) for 10

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days. The dried seaweed materials were crushed by using mortar and pestle to get the powder form and it was stored in an air-tight container. About 1 g of crushed seaweed powder was added with 100 mL of distilled water in 250 mL conical flask and boiled for 5-10 minutes at 60-80 °C. The crude extract was then collected and stored at 4 °C for experimental use [44].

2.2 Biosynthesis of Silver Nanoparticles

The crude extract of the experimental marine red seaweed *Halymenia porphyroides* was used for the biosynthesis of silver nanoparticles. Silver nitrate (AgNO_3) (SD fine) was used for the synthesis of silver nanoparticles and double-distilled, deionized water was used for all the experiments. The silver nanoparticle formation was carried out by taking 500 mg of dry, shade dried powder samples of the seaweed *Halymenia porphyroides* in a 250 mL Erlenmeyer flask with 10^{-3} M aqueous AgNO_3 solution and was incubated at room temperature. The pH was checked during the course of reaction and it was found to be 5.09. Nearly 95% of bio reduction of AgNO_3 ions occurred within 24 hr at stirring condition. The biosynthesis of silver nanoparticles was characterized by UV Vis spectroscopy; size and morphology by employing SEM and TEM, structure from X-ray diffraction (XRD) technique, stability and purity of silver nanoparticles from Thermo gravimetric analysis (TGA) and biomolecules involved in the capping agent of silver nanoparticles from Fourier transform infrared (FT-IR) spectroscopy.

3. Result and Discussion

3.1 Visual Examination

The biosynthesis of silver nanoparticles was primarily identified by color change during exposure of crude seaweed extract of the *Halymenia porphyroides* into aqueous solution of silver ions is shown in Fig. 1. The shade dried powder preparations of the experimental seaweed *Halymenia porphyroides* were added in 10^{-3} M silver nitrate solution and allowed to react at 121 °C for 20 minutes. The colour of the reaction solution changed to dark reddish. The control (without seaweed powder) showed no colour formation. Formation of the colour arises due to the excitation of surface plasmon vibrations where the metabolites in the seaweed extract act as the capping agent. The colour of the solution gradually intensified on heating which clearly indicates and confirms the formation of silver nanoparticles. After 24 hr, there is no significant color change, indicating the saturation of the reaction of silver nanoparticle formation.



Fig. 1 Aqueous extract of *Halymenia porphyroides* before and after synthesis of silver nanoparticles

3.2 UV-Visible Spectroscopic Analysis

The silver nanoparticles synthesized by marine red seaweed *Halymenia porphyroides* were analyzed by using UV-Vis spectrophotometer (Labtron LUS-B16). The absorption spectra of silver nanoparticles formed in the reaction solution were characteristic of the nanoparticle from the seaweed and had specific absorption maxima at 430.5 nm is shown in Fig. 2. The characteristic absorption peaks of the silver nanoparticles are in the range between 410 to 440 nm, which confirms the synthesis and formation of silver nanoparticles [45, 46]. The frequency and width of the surface plasmon absorption depend on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium [47]. The broad peaks of the absorption spectra indicate the presence of biosynthesized silver nanoparticles from the experimental seaweed *Halymenia porphyroides* with large size distribution and are polydispersed. The interaction with the biomolecules presents in the aqueous part of the reaction solution by the biosynthesized silver nanoparticles from experimental seaweed *Halymenia porphyroides* has been indicated by UV Visible spectroscopic analysis. There were no little signs of aggregation with the biosynthesized silver nanoparticles solution which were stable for more than six months of observation.

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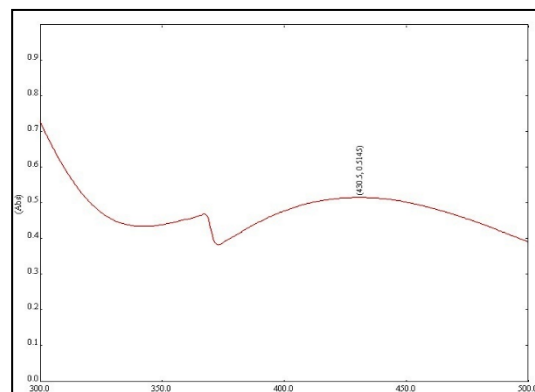


Fig. 2 UV Visible Spectral analysis of silver nanoparticles biosynthesized from *Halymenia porphyroides*

3.3 FT-IR Spectroscopic Analysis (FTIR)

The FTIR spectral measurements were carried out to identify the potential biomolecules in the crude extract of the seaweed *Halymenia porphyroides* which is responsible for reducing and capping the biologically reduced silver nanoparticles. Silver nanoparticles biosynthesized from experimental seaweed *Halymenia porphyroides* were analyzed using FT-IR spectroscopy is shown in Fig. 3 and Table 1.

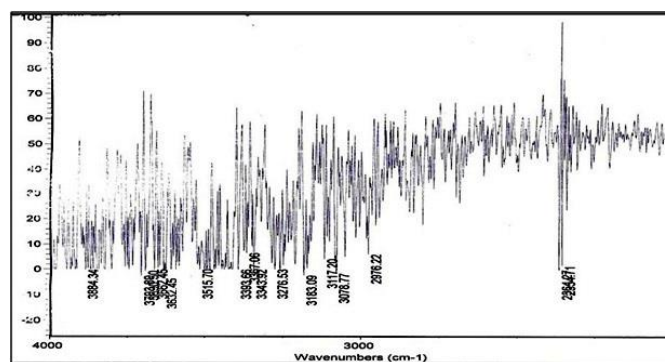


Fig. 3 FT-IR spectrum of *Halymenia porphyroides* mediated biosynthesized silver nanoparticles

Table 1 FT-IR Spectral interpretation of silver nanoparticles biosynthesized from *Halymenia porphyroides*

| Wave number (cm^{-1}) | Peak Assignments |
|----------------------------------|---|
| 3884.34 | O-H stretch free, alcohols |
| 3703.80 | O-H stretch free, alcohols |
| 3690.50 | O-H stretch free, alcohols |
| 3662.45 | O-H stretch free, alcohols |
| 3632.45 | O-H stretch free, hydroxyl, alcohols, phenols |
| 3515.70 | O-H stretch, H-bonded alcohol, phenols |
| 3393.66 | O-H stretch, H-bonded, alcohols, phenols |
| 3367.06 | O-H stretch, H-bonded, alcohols, phenols |
| 3343.92 | O-H stretch, H-bonded, alcohols, phenols |
| 3276.53 | N-H, H-bonded stretch, 1°, 2° amines, amides |
| 3183.09 | O-H stretch, carboxylic acids |
| 3117.20 | O-H stretch, carboxylic acids |
| 3078.77 | =C-H stretch, alkanes |
| 2976.22 | C-H stretch, alkanes |
| 2354.71 | C=N Stretch, nitrile |

The local molecular environment of the organic molecules on the surface of the nanoparticles was determined by the IR spectra. Fourier transform infrared spectroscopy (FTIR) is a technique which is used to analyze the chemical composition of many organic chemicals, semiconductor materials, gases, biological samples, inorganics, and minerals. FTIR analysis can give not only qualitative (identification) analysis of materials, but, with relevant standards, can be used for quantitative (amount) analysis. The FT-IR spectrum analysis of silver nanoparticles biosynthesized from red seaweed manifests an absorption peak at 3884.34 cm^{-1} (O-H stretch free, alcohols), 3703.80 cm^{-1} (O-H stretch free, alcohols), 3690.50 cm^{-1} (O-H stretch free, alcohols), 3662.45 cm^{-1} (O-H stretch free, alcohols), 3632.45 cm^{-1} (O-H stretch free, hydroxyl, alcohols, phenols), 3515.70 cm^{-1} (O-H stretch, H-bonded alcohol, phenols), 3393.66 cm^{-1} (O-H stretch, H-bonded, alcohols, phenols), 3367.06 cm^{-1} (O-H stretch, H-bonded, alcohols, phenols), 3343.92 cm^{-1} (O-H stretch, H-bonded, alcohols, phenols), 3276.53 cm^{-1} (N-H, H-bonded

stretch, primary, secondary amines, amides), 3183.09 cm^{-1} (O–H stretch, carboxylic acids), 3117.20 cm^{-1} (O–H stretch, carboxylic acids), 3078.77 cm^{-1} (=C–H stretch, alkanes), 2976.22 cm^{-1} (C–H stretch, alkanes) [48] and 2354.71 cm^{-1} (C=N stretch, nitrile) [49]. The results revealed that the capping ligand of the silver nanoparticles may be an aromatic compound or alkanes or amines [50]. The biological molecules such as secondary metabolites could possibly play a major role in the synthesis and stabilization of the metal nanoparticles [51, 52]. The Van-der-Waals forces between nitrogen and oxygen atoms as well as the release of protein molecules in bio compound of the experimental seaweed *Halymenia porphyroides* may be responsible for the biosynthesis and stabilization of silver nanoparticles in an aqueous solution. These results obtained are in good agreement with the literatures [53, 54].

3.4 X-Ray Diffraction Pattern (XRD)

XRD is a widely used to determine the size and crystal structure of silver nanoparticles. X-ray diffractogram of the biosynthesized silver nanoparticles by the experimental seaweed *Halymenia porphyroides* exhibits Bragg reflection corresponding to face centered cubic (fcc) type bulk silver. The broadened diffraction peaks around their base indicates that the silver nanoparticles are between nano sizes. XRD (Labtron LXRD-A10) analysis of biosynthesized silver nanoparticles from *Halymenia porphyroides* exhibited four distinct diffraction peaks is shown in Fig. 4. The XRD diffraction pattern of biosynthesized silver nanoparticles from *Halymenia porphyroides* showed four distinct diffraction peaks at 12.13°, 28.77°, 39.87° and 46.11° with 2 θ values corresponding to the lattice planes (1 0 0), (1 1 0), (1 1 1) and (2 1 1) which are indexed as crystalline silver face-centered cubic (fcc) phase [55]. The observed peak broadening and noise was probably related to the effect of nano sized particles and the presence of various crystalline biological macromolecules in the experimental seaweed extract. The results are in agreement with the findings of earlier investigations as reported by Paneerselvam *et al.* [56] and Alak and Swapan, [57]. The diffraction patterns of silver nanoparticles biosynthesized from the experimental seaweed *Halymenia porphyroides* indicate the uniqueness of the crystalline structure, phase purity, degree of crystallinity and the unit cell parameters. The presence of some unassigned peaks in the X-ray diffractogram of the biosynthesized silver nanoparticles from the experimental seaweed *Halymenia porphyroides* may be due to the biomolecules and proteins in the seaweed extract which acts as stabilizing agents [58]. The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature.

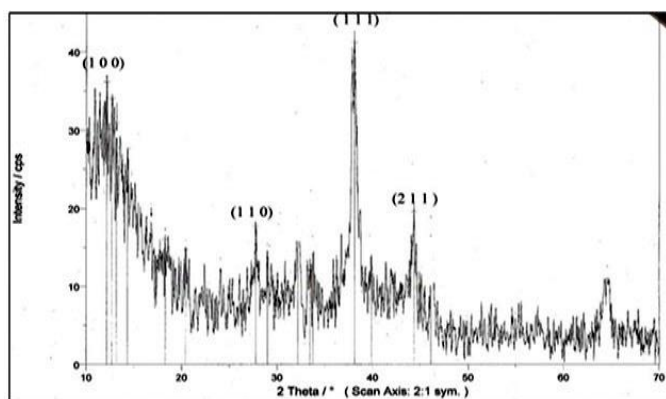


Fig. 4 X-ray diffraction analysis of silver nanoparticles biosynthesized from *Halymenia porphyroides*

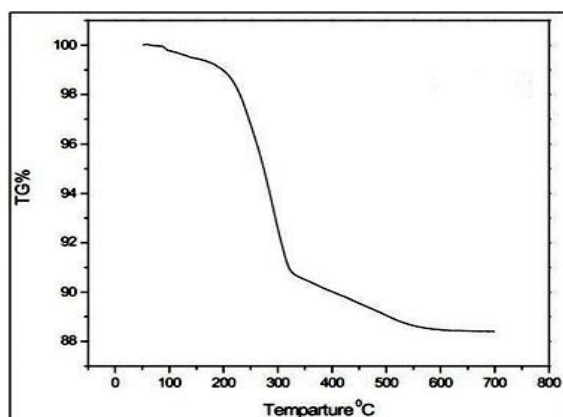


Fig. 5 TGA thermogram of silver nanoparticles biosynthesized from *Halymenia porphyroides*

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3.5 Thermo Gravimetric Analysis (TGA)

The biosynthesized silver nanoparticles from the experimental seaweed *Halymenia porphyroides* were subjected to thermo gravimetric analysis (TGA 4000 - PerkinElmer) is shown in Fig. 5. The thermal stability, purity and humidity of the particles were determined using thermo gravimetric analysis [59]. The TGA relies on a high degree precision in three measurements: weight, temperature and temperature change. It can be applied to silver nanoparticles to determine changes in weight in relation to the changes in temperature [60]. Thermo gravimetric analysis is commonly employed in research and testing to determine the characteristics of materials such as degradation temperature, absorbed moisture content of nanoparticles and polymers [61]. The silver nanoparticles biosynthesized from *Halymenia porphyroides* showed a weight loss in a two-step process, the first step indicated a weight loss of 9% at the temperature up to 340 °C which corresponds to the decomposition of bioorganic compounds present in the nano particle powder [62] and the second step involved weight loss of 3% at the temperature ranging from 340-650 °C which corresponds to the decomposition of amines [63]. Thermal decomposition of the Ag⁺ bioorganic complex at high temperature (200 °C) results in an Ag atom and organic molecules [64]. The crystallite growth of silver nanoparticles biosynthesized from the experimental seaweed *Halymenia porphyroides* appears to be consistent with “Ostwald ripening” where the stability gradient diffuses from the surfaces of small particles to the surfaces of larger particles in the Ag⁺-bioorganic solution [65]. The TGA results of the biosynthesized silver nanoparticles, from experimental seaweed *Halymenia porphyroides*, show 95% purity and their stabilization were observed at 200 °C which are in agreement with the findings of the earlier investigations reported by Forough and Farhadi [66] and Amjad *et al.* [67]. The 95% purity of the silver colloidal medium from the experimental seaweed *Halymenia porphyroides* was determined by an ultra-sonication method which eliminates and separates silver nanoparticles from bio organic complexes [68].

3.6 Scanning Electron Microscopy (SEM)

The morphology and shape of these silver nanoparticles were carried out using scanning electron microscopy (SEM Quanta – 400) shown in Fig. 6. The silver nanoparticles biosynthesized from *Halymenia porphyroides* biomass after exposure to 10⁻³ M aqueous silver nitrate solution for 2 hours showed the colloidal form of the particles in solution which micro precipitated on the surface of the biomass of the experimental seaweed.

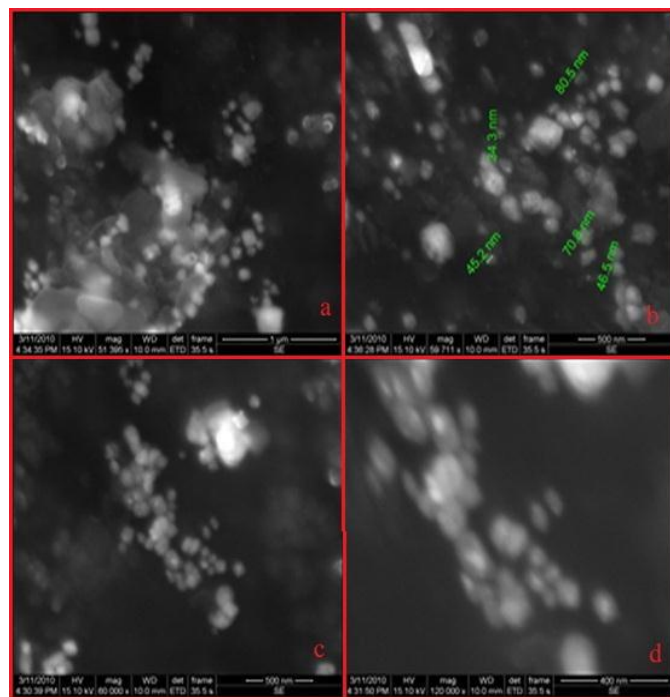


Fig. 6 Scanning electron micrograph of silver nanoparticles biosynthesized from *Halymenia porphyroides*

In the present study, the SEM analysis of silver nanoparticles, besides being present in colloidal form in solution was also micro precipitated on the surface of the biomass were clearly indicating that the nanoparticles formed by the reduction of Ag⁺ ions are bound to the surface of the cells. The silver nanoparticles biosynthesized from *Halymenia porphyroides* formed were predominate in cubical and spherical structures with

uniform shape as reported by Chandran *et al.* [69]. The brighter cubical and spherical area of the back scattered electron image corresponds to the silver nano particle indicating the cubic and spherical structure of silver. It is known that the shape of the metal nanoparticles has considerable changes their optical and electronic properties [70]. SEM pictures of silver nanoparticles from the experimental seaweed *Halymenia porphyroides* showed that they were intact after the reaction and immobilization. The experimental results showed that the diameter of prepared silver nanoparticles in the solution was about 34.3–80.5 nm for *Halymenia porphyroides*. Silver nanoparticles have a strong tendency to agglomerate which reduces the surface to volume ratio and thereby produce the catalytic effect. Therefore, a stabilizing agent is often used to prevent agglomeration. However, the agent is adsorbed on the surface of the nanoparticles, shielding them from the oxidant and reductant and thereby inhibiting the catalysis [71].

3.7 Transmission Electron Microscopy (TEM)

The transmission electron microscopy (HR-TEM) is the technique used to determine the size and particle distribution of silver nanoparticles [72]. The size and distribution of biosynthesized silver nanoparticles were observed by taking the micrograph from drop coated films from silver complex solution with the extract of the experimental seaweed *Halymenia porphyroides*. The TEM (HR-TEM JEOL 3010) images showed the formation of spherical shaped silver nanoparticles and are found in aggregates ranging from 5 to 50 nm shown in Fig. 7.

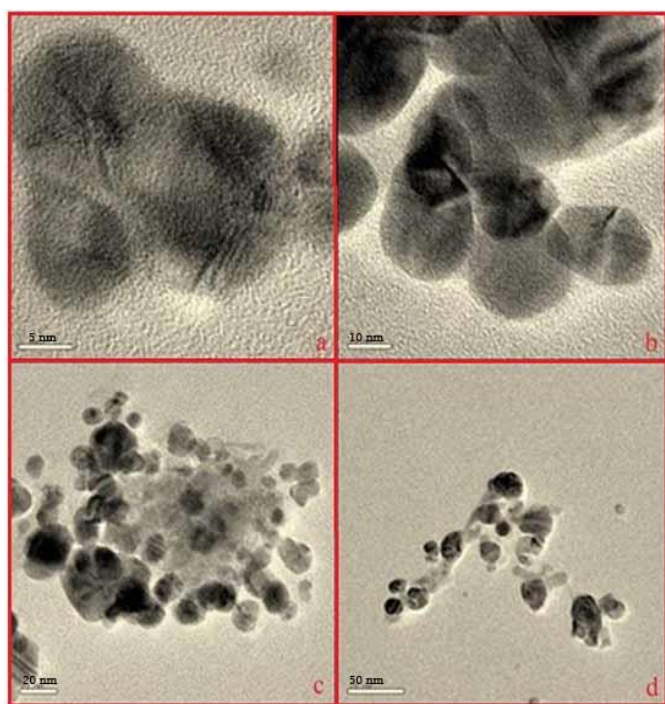


Fig. 7 HR-TEM images of silver nanoparticles biosynthesized from *Halymenia porphyroides*

The average mean size of silver nanoparticles from *Halymenia porphyroides* was 32 nm. Similar results were recorded by Murugesan *et al.* [73] using red alga *Gracilaria edulis* extract as a reducing and capping agent. The shape of the metal nanoparticles has considerably changed their optical and electronic properties [74]. The HR-TEM images do not confirm the presence of capping agents on the silver nanoparticles, but under careful observation, it is noted that the silver nanoparticles are surrounded by a faint thin layer of other material, which may be the capping organic material from the experimental seaweed *Halymenia porphyroides*. The silver nanoparticles of the TEM images are not in physical contact but are separated from each other by a uniform inter particle distance. The results of HR-TEM findings indicate that the seaweed mediated silver nano particle shape varies as cubical and spherical which are in agreement with earlier investigations reported by Raghunandan *et al.* [75] and Noruzi *et al.* [76].

4. Conclusion

In the current study silver nanoparticles were synthesized through the biological medium by the reduction of silver nitrate using the crude extract of marine red seaweed *Halymenia porphyroides*. The reaction mixture was <https://doi.org/10.30799/jnst.298.20060202>

successfully optimized to increase the yield of silver nanoparticles production using UV-Vis analysis results. The optimum conditions were as follows: 500 g extract concentration, 10^{-3} M aqueous AgNO_3 solution, 37 °C and pH 5.09. The *Halymenia porphyroides* seaweed extract acts as both reducing and stabilizing agents for the synthesis of silver nanoparticles, which were confirmed by FTIR. SEM analysis revealed that the silver nanoparticles ranged between 34.3 nm to 80.5 nm in size and were spherical with uniform distribution. TEM analysis revealed nearly spherical and hexagonal structures of the silver nanoparticles. The XRD pattern of silver nanoparticles showed a face-centred-cubic crystal structure. The purity and thermal stability of silver nanoparticles were detected by TGA analysis, which was closely related to that of bulk metallic silver, which indicates its purity and the thermal stability of the silver nanoparticle. The green protocol method used for the biosynthesis of silver nanoparticles using crude seaweed extract of *Halymenia porphyroides* makes the synthesis process energy efficient, economically scalable as well as non-toxic for its applications in food, pharmaceutical and nutraceutical industries. The seaweed extract contains bioactive compounds, secondary metabolites which act as strong capping and reducing agents in the synthesis process where 95 % of the bio reduction of silver nanoparticles was observed. Thus, the seaweed extract makes an ideal source for the biosynthesis of silver nanoparticles for various applications such as drug delivery, catalytic activity, disinfectant, medicine and biomedical applications.

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