Phycosynthesis of silver nanoparticles from extract of Gracilariafoliifera (Forsskal) Boergesen

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Abstract-*The present study deals with the synthesis of silver nanoparticles (Ag-NPs) using the aqueous extract of red seaweed Gracilariafoliifera extract. The complete reduction of silver ions was observed after 72 h of reaction at 300ºC under shaking conditions. The colour changes in reaction mixture (pale yellow to reddish brown) was observed during the incubation period, because of the formation of silver nanoparticles in the reaction mixture enables to produce particular colour due to their specific properties. The formation of Ag-NPs was confirmed by UV Visible Spectroscopy clearly indicates the interaction between silver ions and biomolecules present in the aqueous seaweed extract, X-Ray Diffraction (XRD) pattern showed crystalline nature of silver nanoparticles, Scanning Electron Microscopy (SEM) with EDS analysis patterns showed the synthesized of Ag-NPs were predominantly spherical in shape and polydispersed. Fourier Transform Infra-Red (FT-IR) spectroscopy analysis showed that the synthesized nano-Ag was capped with bimolecular compounds which are responsible for reduction of silver ions. The approach of seaweedmediated synthesis appears to be cost effective, eco-friendly and easy alternative to conventional methods of silver nanoparticles synthesis.*

Keywords- Gracilariafoliifera, Silver nanoparticles, Ecofriendly, Bioreduction.

I. INTRODUCTION

Nanoparticles with controlled size and composition are of fundamental and technological interest as they provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine, and water treatment. Thus, production and application of nanomaterials from 1 to 100 nanometers (nm) is an emerging field of research [1,2]]. Nanobiotechnology is a thrust area and currently, there is a growing need to use environmental friendly nanoparticles that do not produce toxic wastes in their process synthesis protocol. To achieve this, it is essential to begin synthesis processes, which happen to be mostly of biological nature [3].

Biological synthesized nanoparticles can have good control on the size distribution than the other methods. Nanoparticles could also be stabilized directly in the process by protein [4]. Biomolecules as reducing agents are found to have a significant advantage over their counterparts as protecting agents [5]. To date, metallic nanoparticles are mostly prepared from nobel metals, Silver (Ag) is the metal of choice in the field of biological systems, living organisms and medicine [6]. Silver nanoparticles have an advantage over other metal nanoparticles (e.g. gold and copper) because the surface plasmon resonance energy of Ag is located far from the interband transition energy. Biosynthesis of nanoparticles by plant extracts is currently under exploitation. The biosynthesis of gold and silver nanoparticles using seaweed Sargassumwightii have been achieved [7]. Nanoparticles are classified primarily into two types, viz organic and inorganic nanoparticles. The nanoparticles of carbon are called the organic nanoparticles. Magnetic nanoparticles, noble metal nanoparticles (platinum, gold and silver) and semiconductor nanoparticles (titanium dioxide, zinc oxide and zinc sulfide) are classified as inorganic nanoparticles [8].

Photocatalytic degradation of methyl orange was measured spectrophotometrically by using silver as nanocyst under visible light illumination. The result revealed that biosynthesized silver nanoparticles using Hypneamusciformis was found to be impressive in degrading methyl orange was reported by [9].

II. MATERIALS AND METHODS

Screening and selection of seaweed

Fresh seaweed of Gracilariafoliifera red seaweed was collected from Pudumadam coastal region (9.2770° N, 78.9938° E), in Gulf of Mannar, Tamil Nadu, South India. Samples were brought to laboratory in polythene bags and cleaned thoroughly with fresh water to remove adhering debris and associated biota. The algae were cleaned using brush for the removal of the epiphytes with distilled water. After cleaning, the algae were dried in shade at room temperature for a week.

Preparation of aqueous extract

The whole biomass of Gracilariafoliifera were initially rinsed thrice in distilled water and dried on paper toweling, and samples (25 g) were cut into fine pieces and boiled with 100 ml of sterile distilled water for 5 min. The crude extract was passed through Whatman No.1 filter paper and the filtrate was stored at 4°C for further use.

Synthesis of silver nanoparticles

Silver nitrate (AgNO3) was of analytical grade (AR) and purchased from E. Merck (India). In the typical synthesis of silver nanoparticles, 10 ml of the aqueous extract of Gracilariafoliifera was added to 90 ml of 1 mM aqueous AgNO3 solution in 250 ml conical flask and kept at room temperature for 72 h at 120 rpm. Suitable controls were maintained throughout the experiments.

UV-visible spectroscopy analysis

The colour change in reaction mixture (metal ion solution + seaweed extract) was recorded through visual observation. The bio reduction of silver ions in aqueous solution was monitored by periodic sampling of aliquots (0.5 ml) and subsequently measuring UV-Vis spectra of the solution. UV-vis spectra of these aliquots were monitored as a function of time of reaction on UV-Vis spectrophotometer UV-2450 (Shimadzu).

FTIR measurement

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 5000 rpm for 10 min. The supernatant was again centrifuged at 10000 rpm for 60 min and the pellet was obtained. This is followed by redispersion of the pellet of Ag-NPs into 1 ml of deionized water. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR Nicolet Avatar 660 (Nicolet, USA).

XRD measurement

The Ag-NPs solution thus obtained was purified by repeated centrifugation at 5000 rpm for 20 min followed by redispersion of the pellet of Ag-NPs in 10 ml of deionized water. After freeze drying of the purified Ag-NPs, the structure and composition were analyzed by XRD. The dried mixture of Ag-NPs was collected for the determination of the formation of Ag-NPs by an X'Pert Pro x-ray diffractometer (PAN analytical BV, The Netherlands) operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation in a θ - 2 θ configuration. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer's formula.

$$D = 0.94 \lambda / \beta \cos \theta$$

where D is the average crystallite domain size perpendicular to the reflecting planes, β is the X-ray wavelength, θ is the full width at half maximum (FWHM), and θ is the diffraction angle. To eliminate additional instrumental broadening the FWHM was corrected, using the FWHM from a large grained Si sample.

$$\beta \text{ corrected} = (\text{FWHM2 sample- FWHM2si})1/2$$

This modified formula is valid only when the crystallite size is smaller than 100 nm [24].

SEM analysis of silver nanoparticles

Scanning Electron Microscopy (SEM) analysis was examine in Joel JSM-56010 LV (INSA-EDS). Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the 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 min.

III. RESULTS AND DISCUSSION

Morphology of Gracilariafoliifera (Forsskal) Boergesen

Division : Rhodophyta Order : Gracilariales
Class : Rhodophyceae Family : Gracilariaceae

Plants are bushy, brownish-red 15 to 20 cm tall; polydichotomously, irregular and sometimes pinnately branched with thin and brittle fronds; margins proliferous. It grows very rarely in the intertidal zone and abundantly in shallow lagoon and submerged coral reefs. It occurs in all the months of the year but only in less quantities. It is a good source for production of agar along with other Gracilaria species (Fig. 1).

Scanning Electron microscopic observation

Agar is derived from the polysaccharides agarose, which form the supporting structure in the cell walls. The cell wall had two distinct layers; The outer amorphous electron dense layer 1-1.5 □m thick and the inner translucent layer. The outer cortical region was made of two layers of small and

rectangular cells cross wall 0.2-0.5 □m thick. Each cell with dense cytoplasm packed with spherical to ovaid in shape. Floridean starch present throughut the cytoplasm as small basin spherical and ovaid structure they are 2-4 □m long and 5-8 □m wide. Fragmented granules also recoded (Fig. 2).

Phyco-synthesis of Ag nanoparticles

The reduction of AgNO3 in aqueous seaweed extract by heating at 60°C showed visible color change whereas no color change was observed in Ag+ solution as shown in Fig. 3. The color of solution gradually intensified on heating, which indicates the synthesis of Ag nanoparticles. The change in color is an attribute to excitation of surface plasmon vibrations of Ag nanoparticles [10]. Notably, even after 24 h there is no remarkable deepening of color indicating the saturation of reaction (data not shown). This reflects that the particles may be well dispersed in the solution with mild agglomeration [11].

UV–visible spectroscopy

UV–vis spectroscopy is a convenient, preliminary and indirect method for characterization of Ag nanoparticles based on optical properties called surface plasmon resonance (SPR) [12]. The color of the seaweed extract becomes turbid after the addition of aqueous AgNO3 solution signifying the initiation of reaction.

The silver SPR band occurs at 420 nm with progressive increase in absorbance upon increasing time until 20 min as shown in Fig. 4. The observed band in this range has been associated with Ag nanoparticles confirming the synthesis of spherical Ag nanoparticles with narrow size distribution [13,14]. Elevation in temperature results in formation of spherical and octahedral shaped nanoparticles of size 5–200 nm [15]. Similarly, shape-controlled Ag nanoparticles can be also synthesized in biological route by varying temperature [16]. Hence, a possible mechanism to control particle size and shape with reference to SPR peak has been addressed.

FTIR spectrum

FTIR analysis was used for the characterization of the extract and the resulting nanoparticles. Absorbance bands seen at (3405.61), (2958.50), (2922.09 and 2851.91), (1639.40), (1528.93 and 1507.38), (1450.64 and 1383.23), (1228.82, 1158.34 and 1030.72) and (663.64) cm-1 were assigned to the NH stretching vibrations of free NH group, O-H Carboxylic acids broad stretching vibration, C-H Alkanes Strong stretching vibrations, N-H Amine medium bend, NO2 Nitro compounds stretching Asymmetrical bend,

C-H Alkanes Scissoring and bending, C-N Amines medium stretching vibrations and C-H Alkynes broad bend vibrations of non-conjugated group and CH bending vibrations of CH3 group respectively (Fig. 5). The results revealed that the capping ligand of the Ag-NPs may be an aromatic compound or alkanes or amines.

Several species of Gracilaria have been reported to contain abundant of amino acids, fatty acids, vitamins, minerals, phenolic compounds and carbohydrates [17]. Of which, phenolic compounds especially polyphenols and tannin have reported to have antimicrobial, anti-carcinogenic and anti-oxidant properties [18,19] have reported that silver ions may possibly bind to phenolic compounds with one or more aromatic ring resulting in the formation of Ag nanoparticles.

XRD analysis

The biosynthesized silver nanostructure by using Gracilariafoliifera extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image at $\theta = 28.09°$, marked with (220). The peaks matches with the Joint Committee on Powder Diffraction Standards (file No. 04-0783), which further proves the formation of crystal AgNPs [20]. A number of Bragg reflections corresponding to the (220) sets of lattice planes were observed which may be indexed based on the face-centred crystal structure of silver. The XRD pattern thus clearly showed that the Ag-NPs are crystalline in nature (Fig. 6). The X-ray diffraction results clearly show that the AgNPs formed by the reduction of silver ions by the extract of Gelidiella sp. are crystalline in nature [21].

Electron Microscopy Study (SEM)

The SEM image (Fig. 7) showing the high density Ag-NPs synthesized by the Gracilariafoliifera further confirmed the development of silver nanostructures. The SEM micrographs of nanoparticle obtained in the filtrate showed that Ag-NPs are spherical shaped, well distributed without aggregation in solution.

Analysis through Energy dispersive X-ray (EDS) spectrometers confirmed the presence of elemental silver signal of the silver nanoparticles (Fig. 7). The optical absorption peak is observed at 3KeV, which is typical for the absorption of metallic AgNPs [22]. The vertical axis displays the number of x-ray counts whilst the horizontal axis displays energy in KeV. Identification lines for the major emission

energies for silver (Ag) are displayed and these correspond with peaks in the spectrum, thus giving confidence that silver has been correctly identified. Similar phenomenon was reported by [23].

IV. CONCLUSION

To conclude the silver nanoparticles were characterized by Scanning electron microscope. The SEM images reveal that the particles are spherical in shape and their size varies from 10-100nm. The XRD result confirms that the particles have a face centered cubic crystalline structure. Gracilariafoliifera extract biologically synthesized nanomaterails are an innovative, efficient eco-friendly and pave way for ecological health and environmental bioremediation.

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Fig. 1. Morphology of *Gracilariafoliifera*

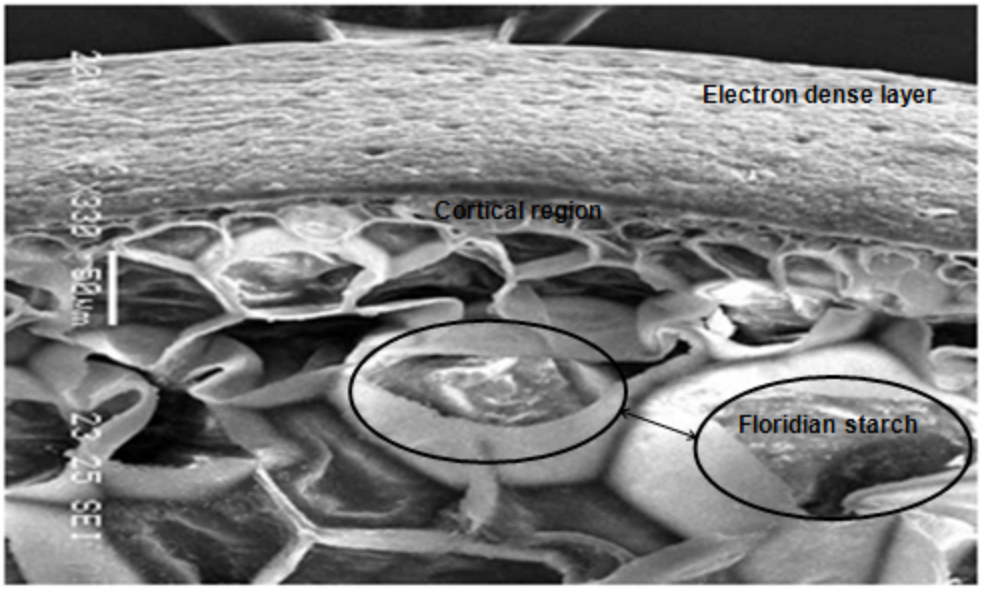


Fig. 2. Scanning Electron Micrograph of Internal Structure of *Gracilariafoliifera*

Fig. 3. Silver nitrate ($AgNO_3$) solution and colour changes during the reduction of $AgNO_3$ into AgNPs by the extract of *Gracilariafoliifera* after 24 h of incubation

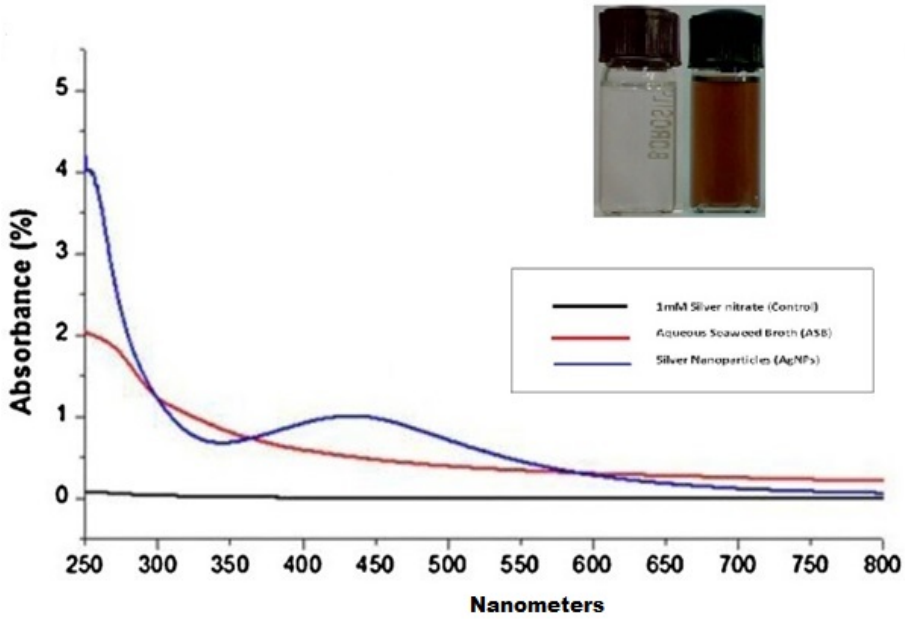


Fig. 4. UV–visible absorption spectra of silver nanoparticless

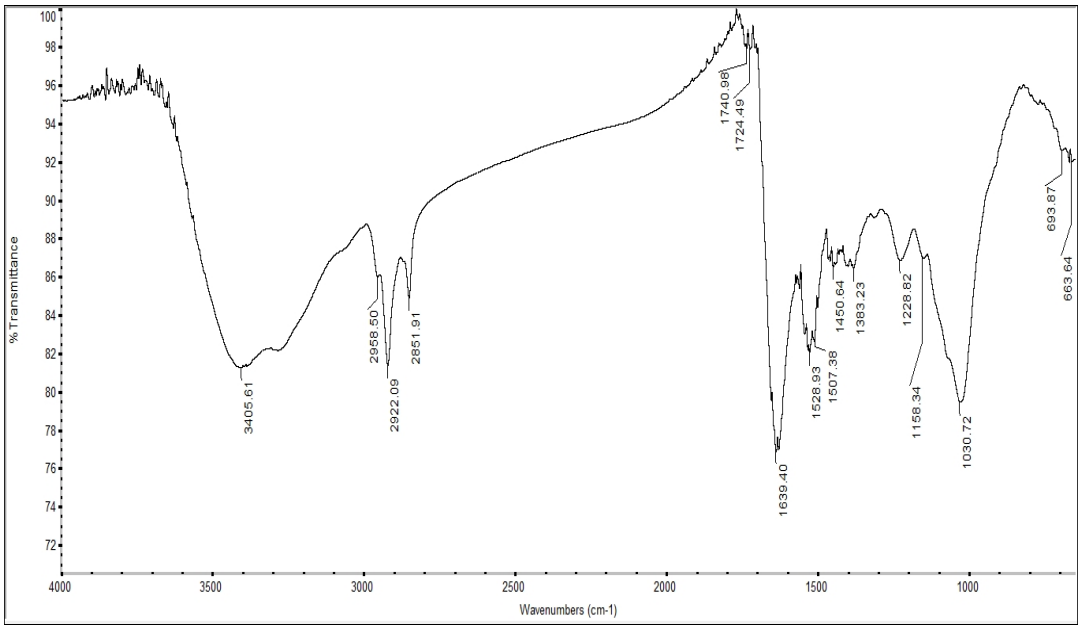


Fig. 5. FTIR spectra of silver nanoparticles synthesized by the reduction of 1mM silver nitrate with the *Gracilariafoliifera* extract

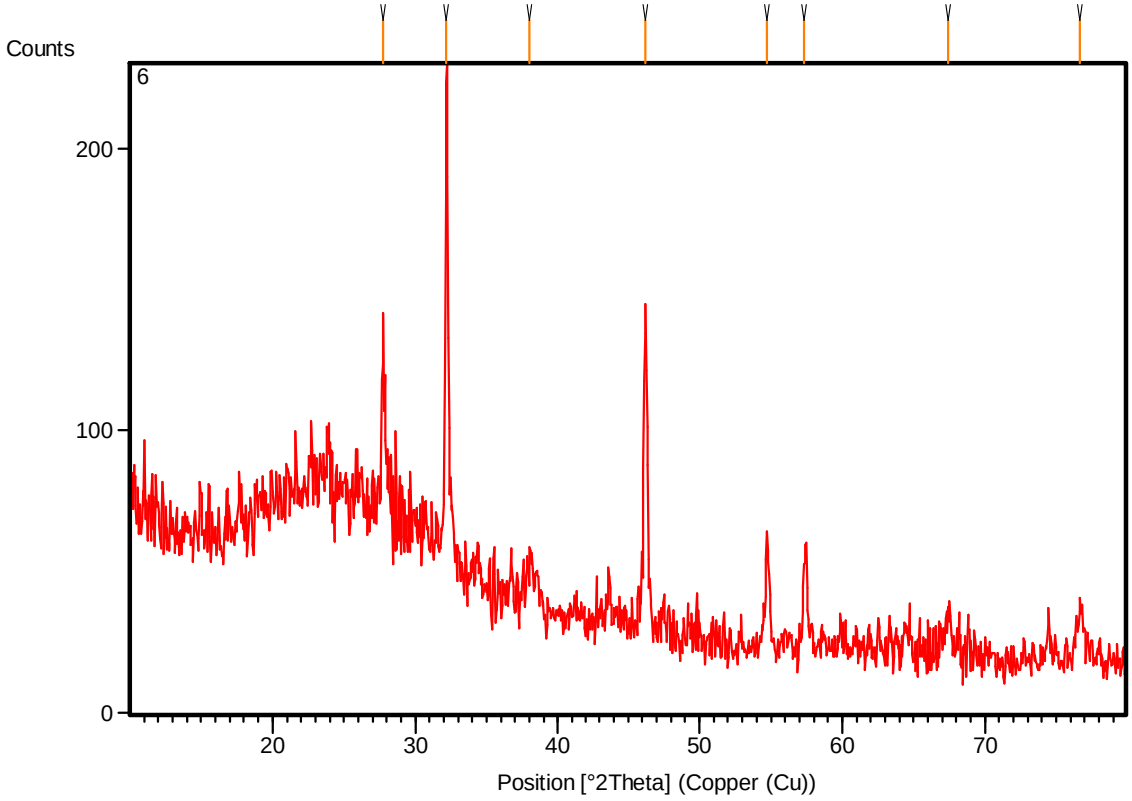
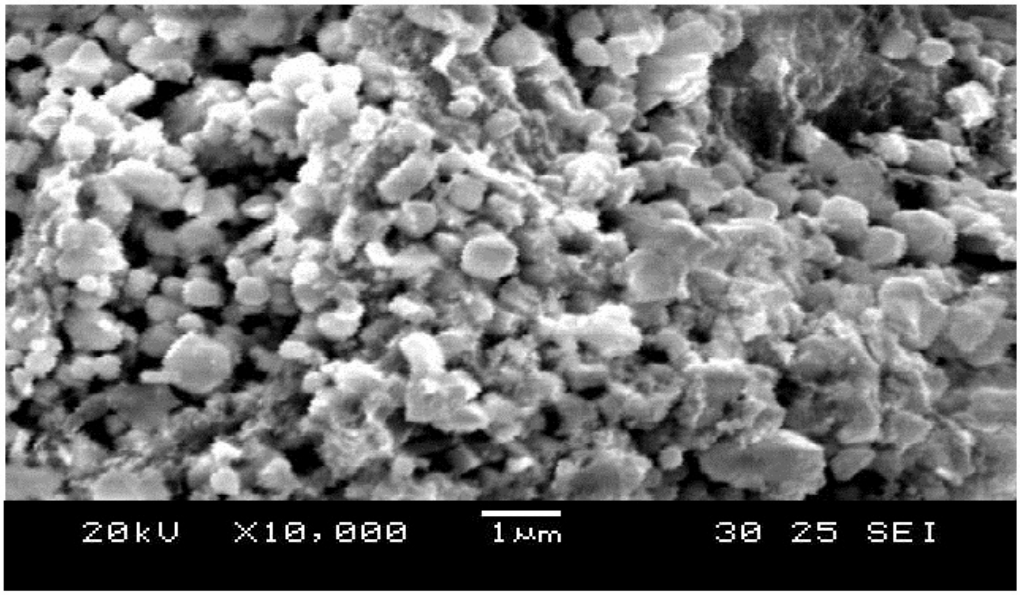


Fig. 6. XRD patterns of capped silver nanoparticles synthesized by treating *Gracilariafoliifera*extract with 1 mM silver nitrate



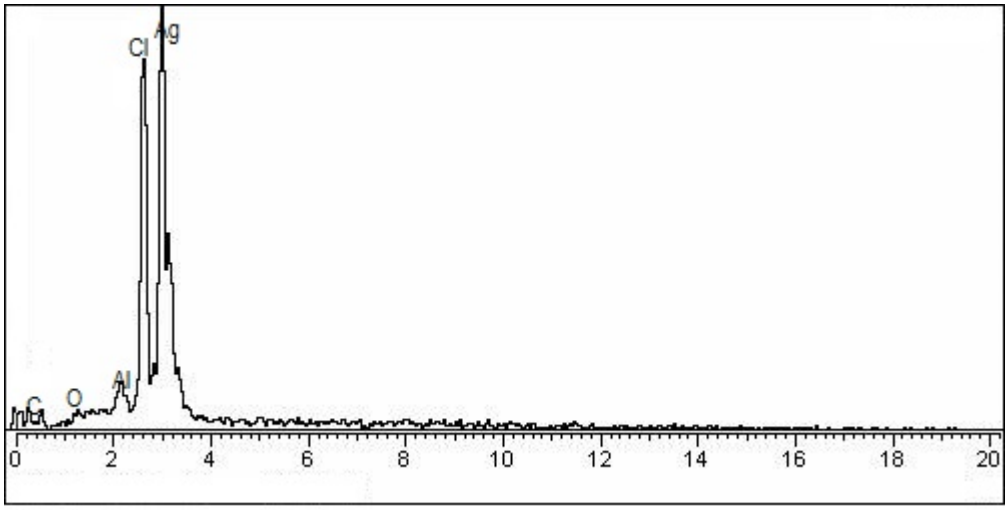


Fig. 7. SEM micrograph of silver nanoparticles synthesized by the reaction of 1 mM silver nitrate with *Gracilariafoliifera* extract and Energy Dispersive Spectroscopy analysis