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PHYTOSYNTHESIS OF SILVER NANOPARTICLES FROM THE EXTRACTS OF SEAWEED *ULVA LACTUCA* AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

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 catalysis, medicine, solar energy conversion and water treatment. Thus, production and application of nanomaterials is an emerging field of research. In this present study, silver nanoparticles were synthesized from the aqueous extract of seaweed *Ulva lactuca*. UV-visible spectroscopy studies were carried out to assess the formation of silver nanoparticles and further characterized by Fourier Transform Infrared spectroscopy (FTIR), X-ray Diffraction (XRD), Thermo Gravimetric Analysis (TGA), EDAX, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) analysis. Further, these synthesized nanoparticles were screened for anti-bacterial and antifungal activity.

KEY WORDS: **Ulva lactuca, Silver Nanoparticles, Antibacterial effects, Antifungal effects.**

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INTRODUCTION

Nanotechnology involves the tailoring of materials at atomic level to attain unique properties, which can be suitably manipulated for the desired applications[1]. Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like imaging[2], sensing[3], targeted drug delivery[4], and gene delivery systems[5] and artificial implants[6]. Hence, nanosized organic and inorganic particles are finding increasing attention in medical applications[7] due to their amenability to biological functionalization. Nanostructured materials have received considerable attention in recent years as a result of their optical, electronic, magnetic and chemical properties and their potential applications in subsequent technology development[8]. Silver nanoparticles can be used in areas such as integrated circuits[9,] cell electrodes[10], antimicrobial deodorant fibres[11], catalysis[12] and chemical analysis[13]. Their uniqueness arises from their high ratio of surface area to volume (aspect ratio), as these materials typically have diameters of 100 nm or less. There are several reports of physical/chemical processes for the production of nanoparticles in technical literature[14]. Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures. A bulk material has constant physical properties regardless of its size, but at the nanoscale this is often not the case. Several well characterized bulk materials have been found to possess most interesting properties when studied in the nanoscale. There are many reasons for this including the fact that nanoparticles possess a very high aspect ratio. In the case of silver nanoparticles (AgNPs), this allows them to easily interact with other particles and increases their antibacterial efficiency. This effect is extremely robust, and as little as 1g of AgNPs is known to impart antibacterial properties to hundreds of square meters of substrate material[15]. Marine Seaweeds draw an extraordinary wealth of mineral elements from the sea that can account for up to 36% of its dry mass. The mineral nutrients present in seaweeds are diverse and the main elements being iodine and calcium. The mineral macronutrients include sodium, calcium, magnesium, potassium, chlorine, sulphur and phosphorus and the micronutrients include iodine, iron, zinc, copper, selenium, molybdenum, fluoride, manganese, boron, nickel and cobalt. The marine seaweeds pose to grow almost exclusively in the shallow waters with diverse therapeutic activity. Because of the lack of a vascular system, the minerals/nutrients in seaweeds are in colloidal form, able to retain their molecular identity in liquid suspension. Seaweed also constitutes as a source of dietary fibre that differs chemically and physically from those of land plants and induces different physiological effects. Research also depicts the important functional activities of marine seaweeds, such as antioxidant, anti-mutagen and anticoagulant effect, anti-tumour activity, and an important role in the modification of lipid metabolism in the human body shown by the marine seaweeds. The chemical composition of seaweeds varies with species, habitat, maturity and environmental conditions[16]. In the present study, AgNPs were synthesized by using marine seaweed *Ulva lactuca.* It's a thin, flat green alga forms a discoid holdfast, also known by the common name sea lettuce. The algal extract reduces silver ions in silver nitrate solution and forms silver nanoparticles. The prepared silver nanoparticles were examined using UV-Vis, Fourier Transform Infrared spectroscopy (FTIR), X-ray Diffraction (XRD), Thermo Gravimetric Analysis (TGA), EDAX, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) analysis. Further silver nanoparticles were screened for antibacterial and antifungal activity.

MATERIALS AND METHODS

Seaweed sample was collected from Mandapam coastal regions lat. 09° 17.417N; long. 079° 08.558 of Gulf of Mannar. The collected samples were brought to laboratory and washed thoroughly with distilled water to remove the extraneous materials and salt on the surface of the sample. The sample was shade dried for 5 days and identified as *Ulva lactuca* (CAS Botany, Madras University).

Dried leaves were grind to fine powder using glass mortar and pestle. Then, they were stored in refrigerator at 4°c for further use. Silver Nitrate ($AgNO_3$) were obtained from Sigma Aldrich and used as such. Formation of Silver Nanoparticles was carried out by taking 2.5gms of seaweed leaf powder in a 500ml Erlenmeyer flask with 50ml of $AgNO_3$ solution (1mM). The bio- reduction occurred within 12hrs at stirring condition. Aliquots of the reaction, solution were removed and absorptions were measured using Cary-100 conc., Varian Spectrometer from 200-600 nm. X-Ray Diffraction (XRD) measurement of the marine algae reduced Ag Nanoparticles was carried out on films of the respective solutions drop coated on a grid operating at a voltage of at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation in θ- 2 θ configurations. Thermal decomposition behavior of the sample has been studied using Netzsch (SDT Q600). The TGA patterns were collected as a function of temperature up to 1000 °C. The heating rate was 0.1 to 100°C / min. The FTIR spectrum of the sample was recorded by a range from 1000 to 4000 cm^{-1} by making the sample into powder. The EDAX spectrum recorded in the spot-profile mode from one of the densely populated silver nanoparticles region on the surface of the film. The nanocrystallites were analyzed by using Quanta 200 FEG. 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 and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min. It was done using Quanta 200 FEG SEM machine. Sample for High Resolution Transmission Electron Microscopic (HR-TEM) analysis were prepared by coating Silver Nanoparticles (AgNPs) solutions onto carbon coated copper TEM grids. The film on the TEM grid was allowed to dry prior to the measurement. HR-TEM measurements were performed on a Phillips, TECHNAI 10 instrument operated at an accelerating voltage at 80KV. Further, antibacterial activity was screened by five test organisms such as *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Bacillus species* using agar well diffusion assay method. Sample solution contained 1mg/ml pure compound. Samples were prepared by taking 5 µg/ml of each solution pipetting it onto a sterile antibiotic filter disk, which was then placed onto the appropriate agar medium and sprayed with a suspension of the test organisms. Antifungal activity of the synthesized silver nanoparticles was determined, using the agar well diffusion assay method. Approximately 50 ml of molten and cooled media (SDA) was poured in sterilized petri dishes. The fungal test organism (*Aspergillus niger, Candida albicans and Candida parapsilosis*) was grown in sabouraud dextrose broth for 24 h. Agar wells of 5 mm diameter were prepared with the help of a sterilized stainless steel cork borer. Three wells were prepared in the agar plates. The wells were labeled as A,B,C. 'A' well was loaded with 5 µg/ml of silver nanoparticles, 'B' well was loaded with 5 µg/ml of silver nitrate and 'C' well loaded with 5 µg/ml of positive control drug (Nystatin) . The plates containing the fungal and silver nanoparticles were incubated at 37°C. The plates were examined for evidence of zones of inhibition.

RESULTS AND DISCUSSION

Detailed study of the marine algae (*Ulva lactuca*) biosynthesis of Silver Nanoparticles was carried out. Reduction of silver ions into Ag particles during exposure of the seaweed extracts could be followed by color change. Ag nanoparticles exhibit dark yellowish brown color (Fig.1a) in aqueous solution due to the excitation of surface plasmon vibration in metal nanoparticles[17]. Whereas the seaweeds incubated with deinonized water (Fig.1b) retained its original colour ie., yellow green. The result obtained in the investigation is very interesting in terms of identification of potential weeds for synthesizing the AgNPs. UV-Vis spectrum of the colloidal solutions of AgNPs has been recorded and the absorption spectrum was observed at 410 nm (Fig. 2) and this technique has been proved to be very useful for the analysis of nanoparticles[18]. The spectra of AgNP's obtained was followed the reported result published[19]. Several other investigators have observed absorption maxima of colloidal silver solution between 410 to 440 nm which is assigned to surface Plasmon of various metal nanoparticles[20, 21]. A remarkable broadening of peak at around 350

nm to 480 nm indicates that the particles are polydispersed. It was observed that the peak was blue shifted in the absorption spectrum from 350nm to 480 nm with increasing reaction time. The maximum absorption gave rise to a red shift from 404.2 to 418.4 nm which means the particle size increased[22, 23].

XRD

The biosynthesized silver nano structure by employing seaweed extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image (Fig. 3). The sharp diffraction patterns of the XRD spectra obtained by the annealing at 200°C indicates a pure crystalline silver structures, JCPDS card no: 04-0783. The figure shows 3 peaks at 2θ values of 37.77, 44.15 and 65.25 corresponding to 111, 200 and 220 planes of silver respectively. We observed no impurity peak in the X-ray diffraction pattern. All diffraction peaks correspond to the characteristic face centered cubic (FCC) phase[24].

TGA

TGA or thermo gravimetric analysis of AgNP's was carried out to observe characteristic weight loss with temperature. The TGA analysis of AgNP's was shown in Fig. 4. The synthesized AgNP's is subjected to a heating from room temperature to highly thermal temperature of 1000°C. The weight loss took place at around a temperature of 119°C. This property helped to characterize and confirm the formation of AgNP's. The peak indicates the evaporation of molecules formed by the decomposition of the Ag complex. Thermal decomposition of the Ag complex at high temperature (over 200°C) results in Ag atom molecules. The average size and size distribution of the crystallites in the sample are dependent on the growth temperature, consistent with surface-free energy considerations. The temperature necessary to maintain steady growth increases with the expansion of the crystallite's size. As the size distribution sharpens, the reaction temperature must be raised to maintain steady growth. Conversely, if the size distribution begins to spread, the temperature required for slow steady growth drops.

FTIR

FTIR measurements were carried out to identify the possible biomoloecules for the stabilization of the newly synthesized AgNPs. The Fig.5a shows the FT-IR spectrum of the seaweed extract as control that it did not contain $AgNO_3$, whereas Fig. 5b shows the spectrum of extract containing $AgNO_3$. Spectra Fig.5a showed transmission peaks at 3431.08, 2172.25 & 1747.83 Cm^{-1} respectively. Similarly, Fig.5b showed transmission peaks at 3661.86, 3539.98, 2083.96 & 1659.46 cm^{-1}. The band at 3661.86 is corresponds to alcohol, phenol O-H, free group. The band at 3539.98 corresponds to an amide N-H band; similarly, band at 2083.96 are corresponds to alkyne C≡C and the band 1659.46 corresponds to amide C=O group. This evidence suggests that the release of extracellular protein molecules could possibly perform the function for the formation and stabilization of AgNPs in aqueous solution.

EDAX

EDX analysis gives quantitative status of elements that may be involved in formation of nanoparticles. EDX (energy dispersive analysis of X-rays) spectrum recorded in the spot-profile mode from one of the densely populated silver nanoparticle regions on the surface of film. Fig. 6 shows the peak in silver region which is observed approximately at 3KeV which is typical for the absorption of metallic silver nanocrystalline due to surface plasmon resonance. From this we confirmed the presence of strong signals from silver (90.83%) atoms in the nanoparticles and weaker signals from phosphorous (9.17%) atoms. The P, O signals are likely to be due to Xray emission from proteins/enzymes present in the cell wall of the biomasss[25].

SEM

The SEM micrographs of nanoparticle obtained in the filtrate showed that silver nanoparticles are spherical shaped, well distributed in solution with an average size of about 50nm in Fig. 7. It is known that the shape of metal nanoparticles considerably change their optical and electronic properties[26].

TEM

A TEM micrograph recorded from the silver nanoparticles deposited on carbon coated copper TEM grid was shown in Fig. 8. This micrograph shows spherical silver nanoparticles. It was observed from the micrograph that most of silver nanoparticles are in the range of 20-50nm in size.

ANTIBACTERIAL ACTIVITY

Antibacterial activity of synthesized Ag nanoparticles were determined using the agar well diffusion method. AgNP's of *U.lactuca* were highly toxic to *Escherichia coli, Bacillus sp.* and *Staphylococcus aureus* with the inhibition zone of 22, 25 and 25 mm; low toxic against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* with the inhibition zone of 17 mm. Zone of inhibition for silver nanoparticles (S) aganist bacterial culture with standard antibiotic Amphicilin (+ve) of 5 µg/ml is shown in Fig. 9, while numerical value of inhibition zone is presented in Table 1. Dilute solution of silver nanoparticles have been used to treat various infections and burns[27]. A number of theory for antimicrobial actions of colloidal silver solution have been proposed for example, alteration of permeability of cell membrane[28], release of lipopolysaccharides and membrane proteins[29], generation of free radicals responsible for the damage of membrane[30], dissipation of the proton motive force resulting in the collapse of the membrane potential[31], however, exact mechanism has not been fully deciphered. the effect of silver nano balls on *E. coli*, *S. typhimurium*, *B. subtilis* and *P. aeruginosa* by colony forming unit (CFU) and growth curve at 40 µg/ml concentration[32].

ANTIFUNGAL ACTIVITY

AgNP's of *U.lactuca* were highly toxic against *Candida albicans* & *Aspergillus niger* with zone of 31 and 30 mm; showed low toxicity against *Candida parapsilosis* with 29mm of zone of inhibition. Zone of inhibition around silver nanoparticles of seaweed for individual fungal culture with standard antibiotic Nystatin is shown in fig. 10. The inhibitory activities in numerical values of the silver nanoparticles were reported in Table 2. Howerver, there has been considerable significant research in India in the field of biological synthesis of nanoparticles. More research has been found to be concentrated in the area of synthesis using terrestrial plants and marine medicinal plants. Recently stable gold nanoparticles have been synthesized using the marine alga, *Sargassum wightii*. Nanoparticles with a size range between 8 to 12 nm were obtained using the seaweed. An important potential benefit of the method of synthesis was that the nanoparticles were quite stable in solution[33,34]. The use of algae for the synthesis of nanoparticles is a largely unexplored area. There is very little literature supporting its use in nanoparticle formation.

Figure. 1
Silver nanoparticles synthesized by Ulva lactuca in an aqueous solution of silver nitrate (a) with control (b)

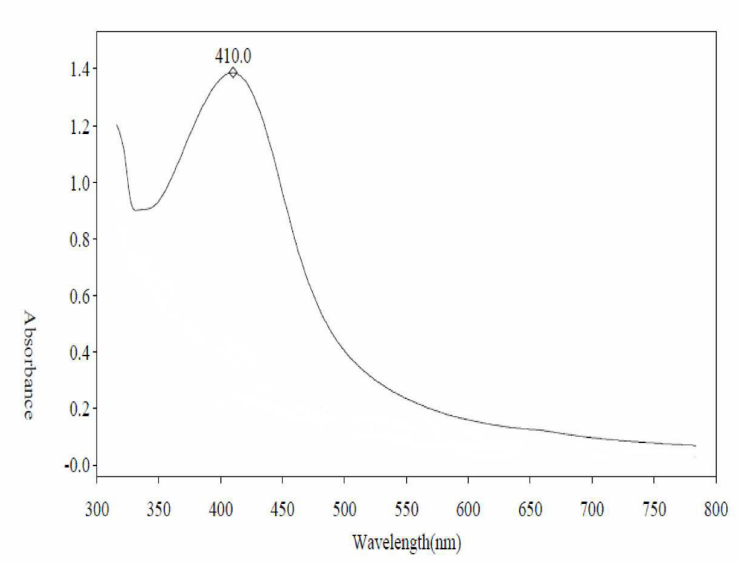

Figure. 2
UV-Vis spectra recorded

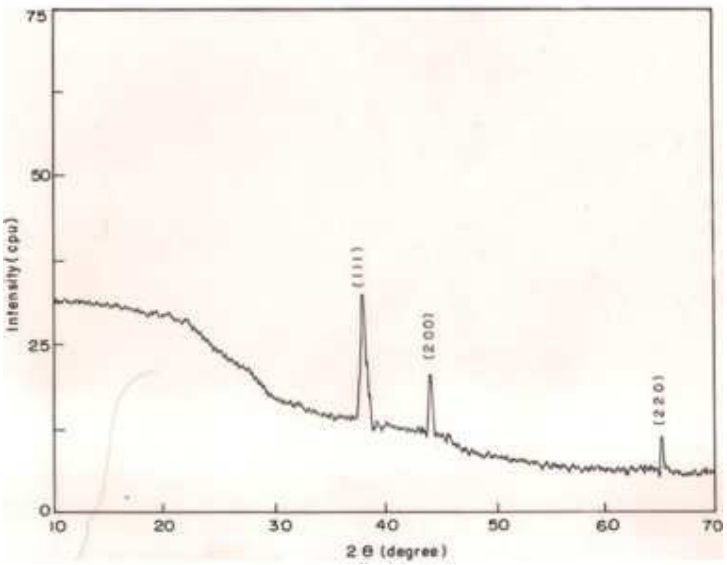

Figure. 3
XRD Image

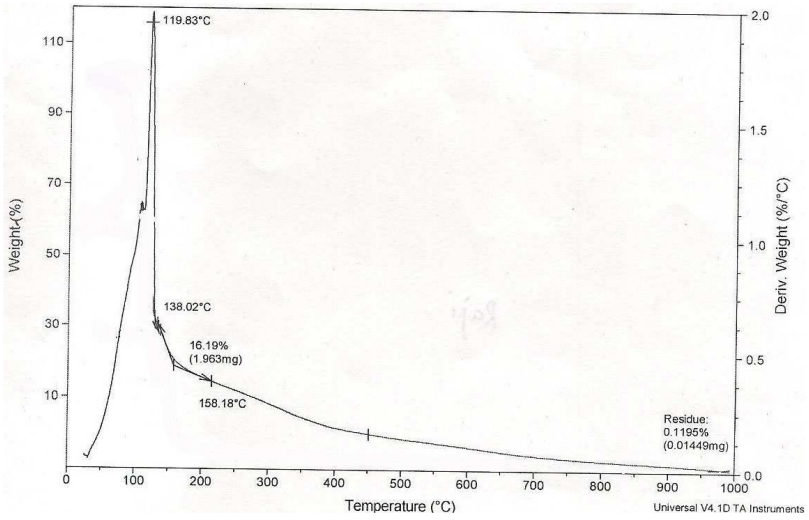

Figure. 4
TGA of Seaweed leaf reduced silver nanoparticle powder

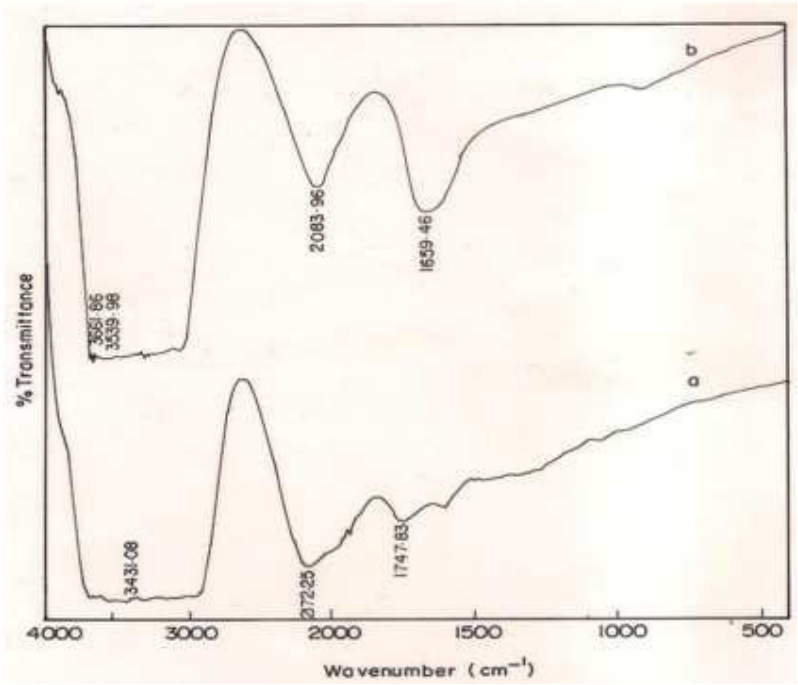

Figure. 5
FT-IR spectrum recorded for control (a) & silver nanoparticles (b)
Keys: (a) – Extract without AgNO3, (b) – Extract with AgNO3

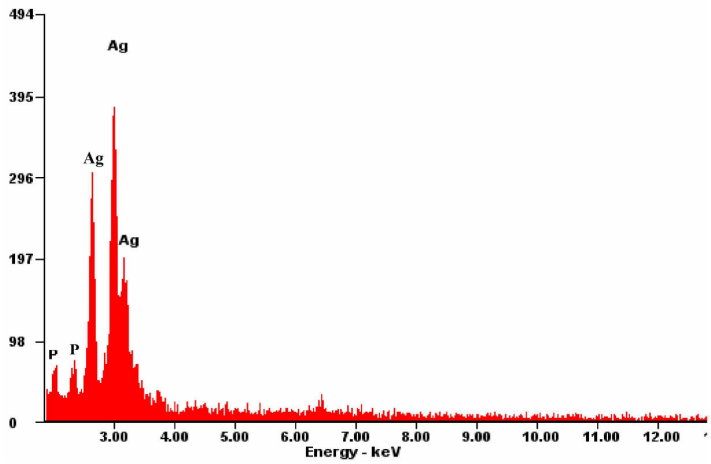

Figure. 6
EDAX spectrum recorded from drop coated film of silver nanoparticles

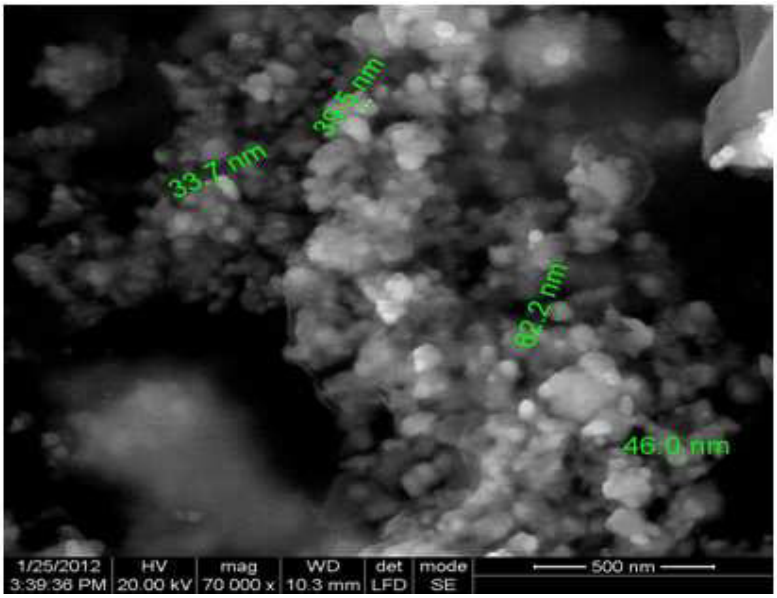

Figure. 7
SEM image of synthesized silver nanoparticles

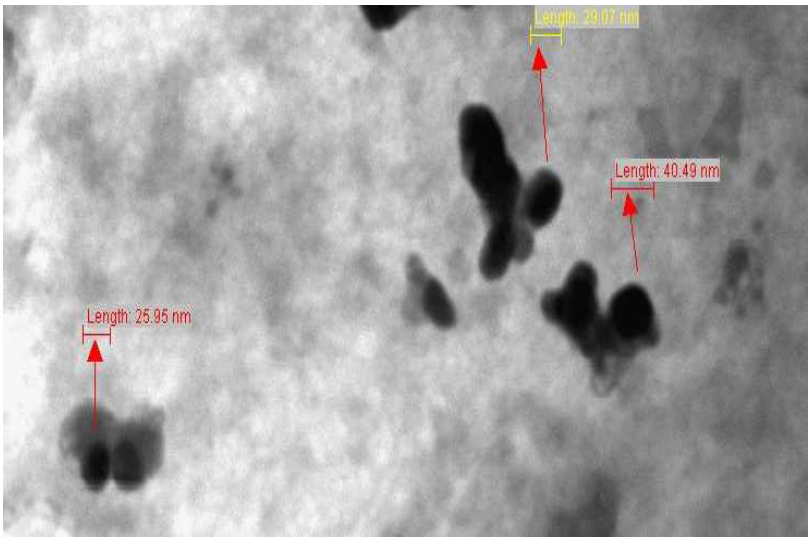

Figure. 8
TEM image of silver nanoparticles formed by reduction of silver ions

Figure. 9
Screening of Antibacterial activity of synthesized silver nanoparticles (S) against Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus sp, Escherichia coli & Staphylococcus aureus with positive control (+ve) and negative control (C) Keys: (+ve) – Amphicilin, (C) – Siver nitrate

Figure. 10
Screening of antifungal activity of synthesized silver nanoparticles (S) against Candida albicans, Aspergillus niger & Candida parapsilosis with positive control (+ve) and negative control (C) Keys: (+ve) – Nystatin, (C) – Silver nitrate

TABLE 1
SCREENING OF ANTIBACTERIAL ACTIVITY OF SYNTHESIZED SILVER NANOPARTICLE WITH POSITIVE CONTROL

Micro Organisms	Zone of inhibition (mm)	
	Ag Nanoparticle	Positive Control
	(5 µg/ml)	
Staphylococcus aureous	25±0.06	24±0.05
Bacillus	25±0.06	22±0.03
Klebseilla	17±0.02	18±0.15
Pseudomonas	17±0.02	-
E.coli	22±0.03	24±0.05

Keys: Positive Control - "Amphicilin"

TABLE 2
SCREENING OF ANTIFUNGAL ACTIVITY OF SYNTHESIZED SILVER NANOPARTICLE WITH POSITIVE CONTROL

Micro Organisms	Zone of inhibition (mm)	
	Ag Nanoparticle	Positive Control
	(5 µg/ml)	
Aspergillus niger	30±0.04	25±0.06
Candida albicans	31±0.02	33±0.03
Candida parapsilosis	29±0.03	30±0.04

Keys: Positive Control - "Nystatin"

CONCLUSION

Use of *Ulva lactuca* seaweed extract offers an affordable, environment friendly technique for synthesis of large scale silver nanoparticles which is simple, cost effective and the resultant nanoparticles are highly stable and reproducible.

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