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Biosynthesis of Gold Nano Particles Using Sargassum Myriocystum and Evaluation of their Antibacterial Activity

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ABSTRACT

The synthesis of gold nanoparticles (Au) using Sargassum myriocystum (powder or extract) is demonstrated here. The rapid formation of stable Au nanoparticles has been found using S. myriocystum extract in aqueous medium at normal atmospheric condition. Scanning electron microscopy (SEM) analysis revealed that the the size (diameter) of the synthesized gold nanoparticles lie within 40-85 nm and the average size of the nanoparticles is ~ 60 nm. Fourier transform infrared spectroscopy (FTIR) showed Representative spectra of obtained nanoparticles manifests absorption peaks located at about 1022.11, 1156.16 and 1550.68 cm^{-1} in the region 500–1600 cm^{-1}. The nanoparticles were also evaluated for their antibacterial activities using the agar diffusion method. Muller–Hinton agar plates were prepared. The inoculum suspension of (Pseudomonas, K. oxyctoca, E. facalis, K. pneumonia, V.parahaemolyticus, V. cholera, E. coli, S. typi, S. paratypi, P. vulgaris) were swabbed uniformly in different plates. The results were confirmed that gold nanoparticle has most of its activity against gram negative organism. In XRD analysis the bottom area of the peak is broad. The XRD patterns thus clearly showed that the gold nanoparticles formed by the bio reduction of $AuCl_4^-$ ions using S. myriocystum.

Key words: *S. myriocystum, gold nanoparticles, SEM, FTIR, XRD, Antibacterial activity, Gram (+) ve and (-) ve Bacteria.*

INTRODUCTION

The field of nanotechnology is one of the most active areas of research modern materials science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Nanotechnology mainly the study of nanoparticles of 1-100 nanometer size. The term Nano is adapted from the Greek word meaning “Dwarf”. A Nanometer (nm) is one millionth of a meter, or roughly the length of three atoms side by side. DNA molecule is 2.5nm wide. Protein approximately 50nm and flu virus about 100 nm in wide. Nanotechnology is the application of nano science, engineering and technology to produce novel materials and devices.

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The concept of nanotechnology though considered to be a modern science has its history dating to as back as the 9th century. The first use of the concept found in "nanotechnology" was in "There is plenty of room at the bottom", a talk given by physicist Richard Feynman at an American physical society meeting at calltech on December 29, 1959.

Synthesis of nanoparticles using biological entities has generated a great interest due to their unusual properties in optical, photoelectrochemical and electronic field. The synthesis of nanoparticles using plants can prove advantageous over other biological synthesis processes in terms of elaborate processes of maintaining microbial cultures[7]. The need for biosynthesis of nanoparticles rose as the physical and chemical processes were costly. So in the search of for cheaper pathways for nanoparticle synthesis, scientists used microorganisms and then plant extracts for synthesis[5].

Gold nanoparticles have attracted intensive research interest because of their important applications as antimicrobial, catalytic, and surface-enhanced Raman scattering effect[8]. Synthesis of Gold nanoparticles is usually achieved by chemical reduction method, thermal decomposition in organic solvent[4], chemical reduction and photo reduction in reverse micelles[6], and radiation chemical reduction[1]. Unfortunately, many of the nanoparticles synthesis or production methods involve use of hazardous chemicals, low material conversions, high energy requirements, difficult and wasteful purifications. Biosynthetic methods employing either biological microorganisms or plant extracts have merged as a simple and viable alternative to chemical synthesis procedure and physical methods. Nanoparticles of Gold, nickel, cobalt, zinc and copper have also been synthesized inside the live plants of *Brassica juncea* (Indian mustard), Medicago sativa (Alfa alfa) and *Heliantus annus* (Sunflower). Gold nanoparticles, have been synthesized using the live alfa alfa plants[10]. In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. Although biosynthesis of gold nanoparticles by plants such alfalfa[10-11], *aloe vera* Chandran *et al.*[2], *Cinnamomum camphora* Neem, *Emblica officianalis* biological materials for the synthesis of nanoparticles is yet to be fully explored.

The advantage of using plants for synthesis of nanoparticles is that they are easily available, safe to handle and possess a broad availability of metabolites that may aid in reduction. Large numbers of plants are being currently investigated for their role in the synthesis of nanoparticles. But still seaweeds are unexplored in the nanoparticle synthesis and very limited which trigger the interest to search seaweed for nanoparticle synthesis. Seaweed is primitive non flowering plant without true root, stem and leaves. They were classified into three broad groups based on their pigments *viz.*, Green (*Chlorophyceae)*, brown (*Phlaeophyceae*), and red algae (*Rhodophyceae*). Seaweeds are the excellent source of bioactive compounds such as carotenoids, dietary fibre, essential fatty acid, vitamins, minerals, trace elements, protein, iodine, bromine and bioactive substance with medicinal attributes. The present study describes an eco-friendly approach toward Gold nanoparticles biosynthesis by seaweeds.

MATERIAL AND METHODS

Reagents and chemicals

Gold chloride ($HAuCl_4$ XH_2O) was obtained from Hi – media. Freshly prepared double distilled water was used throughout the experimental work.

Collection of sample

Samples were collected from the Mandapam (9*16'47'N 79'7'12''e) region of Tamil Nadu, India. They were cleaned and washed in seawater to remove the exogenous material and brought to the laboratory in an ice box and again washed in distilled water to remove the salts. Then they were shade dried for few days and kept in oven at 60°C until constant weight obtained.

Preparation of algal biomass

The algae samples were crushed using mortar and pestle, sieved and filtered (mesh size 0.45 micro meter). The samples were stored in air tight container for future use.

Primary screening:

An elaborate screening process involving a number of seaweeds, *Chaetomorpha aerea* (Chlorophyta), *Sargassum myriocystum* (Phaeophyta) and *Kappahycus alvarezii* (Rhodophyta), were used for the screening process to identify the potential species for the synthesis of gold nanoparticles by Visual and Spectroscopic studies .

Biological Synthesis of gold nanoparticles:

The broth used for the reduction of Au^{3+} ions to Au^0 was prepared by taking 2.5 g of seaweed powder in a 500 ml Erlenmeyer flask with 100mL of 10^{-3} M aqueous $HAuCl_4$ solution.

Preliminary confirmation on synthesis of gold Nanoparticles

Visual inspection

The reduction of metal ions was roughly monitored by visual inspection of the solution. The conversion of turbid brown color reaction mixture to a blood red color clearly indicated that the formation of gold nanoparticles.

Characterization of gold nanoparticles

UV-Vis Spectral Analysis:

The bio-reduction of $AuCl_4^-$ was monitored using the UV-Vis spectroscopy of the solution according to Mie (1908) using Perkin Elmer λ 25 spectrophotometer. UV-Visible spectrometer with a resolution of 1nm between 300 and 800 nm possessing a scanning speed of 300 nm/min was used. The reduction of pure Au^+ ions was monitored by measuring the UV–Vis spectrum of reaction medium after diluting a small aliquot of the sample into de-ionized water.

Scale up process for characterization of gold nanoparticles:

2.0 g of *S. myriocystum* biomass was incubated with 200 ml of 10^{-3} M $HAuCl_4$ solution at room temperature.

Obtaining dry powder of gold nanoparticles:

After the desired reaction period, the algal biomass *S. myriocystum* $HAuCl_4$ mixture solution containing the gold nanoparticles were centrifuged at 12, 000 rpm for 15 minutes following which the pellets was redispersed in Millipore water to get rid of any uninteracted biological molecules. This process of centrifugation was repeated thrice to ensure better separation of the gold nanoparticles. The pellets were then frozen and dried using a Lyophilizer. The purified dried powders were then used for the subsequent characterization studies.

FTIR analysis of dried biomass after bioreduction

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 minutes and the resulting suspension was redispersed in 10ml sterile distilled water. The centrifuging and redispersing process was repeated thrice. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR (Nicolet A vatar 660 (Nicolet, USA) Edwards *et al*[3].

XRD observation of Gold Nanoparticles

The crystalline nature of Ag nanoparticles was confirmed from the x-ray diffraction analysis. X-ray diffraction pattern was recorded in the scanning mode on a (XPERT PAN ANALYTICAL INSTRUMENT) analytical instrument operated at 50 kV and a current of 30 mA with CuK radiation (λ = 1.5406 A°). The diffraction intensity was compared with the standard JCPDS files. The average particle size has been estimated by using Debye Scherrer formula.

$D = \lambda\ 0.9/ W \cos\theta$

Where'λ' is the wavelength of X-ray, 'W' is FWHM (full width at half maximum), 'θ' is the diffraction angle and 'D' is particle diameter (size).

Scanning Electron Microscopy (SEM) observation of Gold Nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-3400 N SEM machine. 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 minutes.

Particle size analysis:

Particle size distribution of gold nanoparticles size was analysed based on measuring the time dependent fluctuation of scattering of light by nanoparticles undergoing Brownian movement. Size (hydrodynamic diameter) of gold nanoparticles was determined by DLS (Dynamic Light Scattering) using Zetasizer Nano Series from Malvern Instruments with the detection angle of 173° in optically homogeneous square polystyrene cells. All measurements were performed at 25°C. Each value was obtained as average from three runs. The zeta potential of gold nanoparticles was measured by the microelectrophoretic method using Malvern Zetasizer Nano ZS apparatus. Each value was obtained as an average from three subsequent runs of the instrument Szcepanowicz *et al*[9].

Antibacterial activity of Gold nanoparticle

The gold nanoparticles were tested for their antibacterial activity by the agar diffusion method. Muller–Hinton agar plates were prepared. The inoculum suspension of (*Pseudomonas, Klebisella oxyctoca, Enterobacter facalis, Klebisella pneumonia, Vibrio parahaemolyticus, Vibrio cholera, Escherichia coli, Salmonella typi, Salmonella paratypi, Proteus vulgaris)* were swabbed uniformly in different plates. Cavities were made in each plate using a well-cutter and it was filled with gold nanoparticle solution (100ml) and then incubated at 370C. The formation of a clear zone around the cavity is an indication of antibacterial activity.

RESULTS

Primary Screening

An elaborate screening process involving a number of seaweeds, *Chaetomorpha aerea* (Chlorophyta), *Sargassum myriocystum* (Phaeophyta) and *Kappaphyus alvarezii* (Rhodophyta), has led to the species *Sargassum myriocystum* as a potential species for the synthesis of gold nanoparticles. Bioreduction of gold nanoparticles were carried out by incubating 0.100 mg of powdered seaweeds (*Chaetomorpha aerea*, *Sargassum myriocystum* and *Kappaphycus alvarezii*) sample with 10 ml of 10^{-3} M aqueous $HAuCl_4$ solution by incubating in water bath and they were observed by visual inspection and Spectroscopic studies. (Plate: 6).

Plate 6: The screening reaction of green, brown and red seaweeds

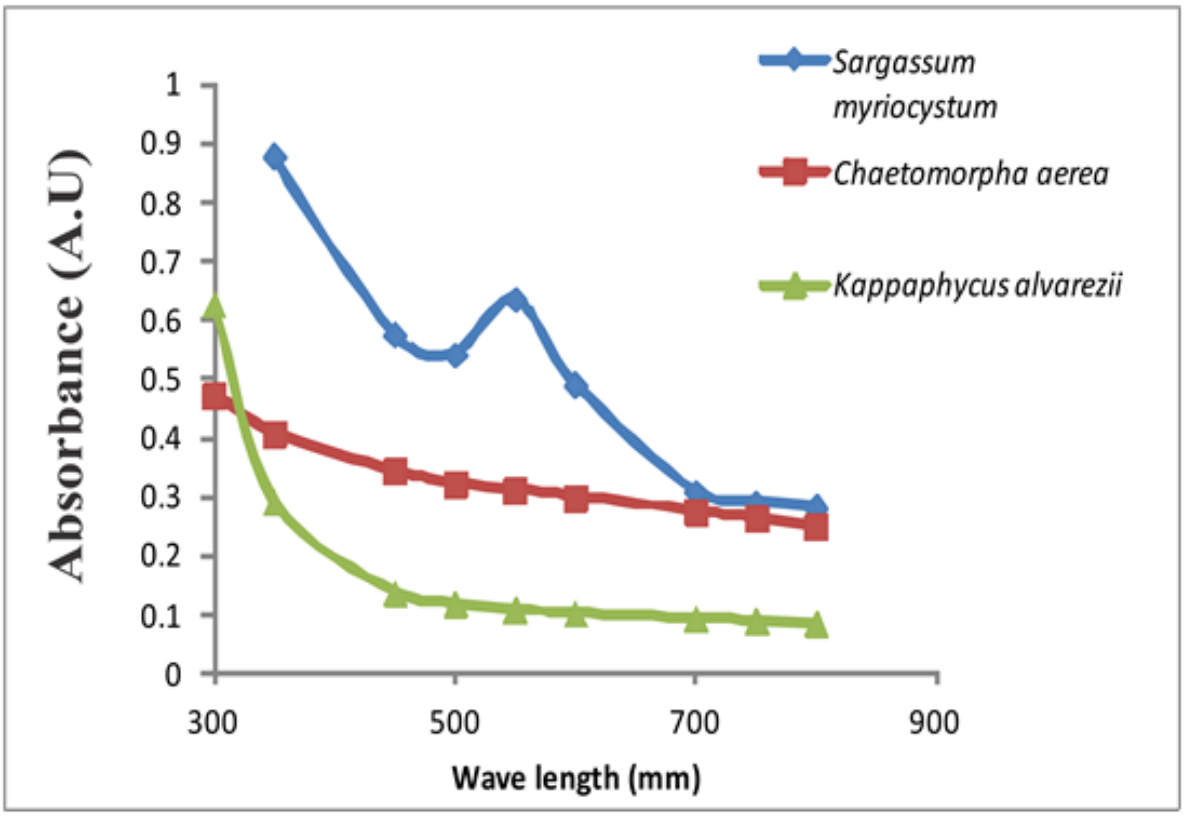


Fig. 1: The UV-Vis spectrum of green, brown and red seaweeds used for primary screening

Visual Inspection

The potential species for the synthesis of gold nanoparticles was primarily confirmed by visual inspection. The colour change of the reaction mixture (biomass extract and Gold chloride solution) on formation of gold nanoparticles was quite evident from the conversion of turbid brown colour reaction mixture to a blood red colour. This visible colour change confirms the formation of gold nanoparticles in the solution and that $AuCl_4^-$ ions have been reduced to Au^- ions (Plate: 7).

Plate 7: (A) The biomass of *S. myriocystum* with gold ions at the beginning of the reaction
(B) The colour change of the medium to Ruby red color after incubation

UV-Vis Spectral analysis

An UV–Vis spectrum is one of the important techniques to ascertain the formation of metal nanoparticle, provided Surface Plasmon Resonance (SPR) exists for the metal. In the present study formation and stability of the reduced gold nanoparticles in the colloidal solution was monitored by using UV–Vis spectral analysis. The light absorption pattern of the algal biomass was kinetically monitored in the range of 300–700 nm. UV–Vis spectra were recorded from the aqueous chloroauric acid and algae (*S. myriocystum)* reaction medium. In the present investigation it was observed that the sharp peaks in the visible region of the electromagnetic spectrum bands corresponding to the Surface Plasmon Resonance vibration of the gold nanoparticles were recorded at about 550 nm with in 60 min.

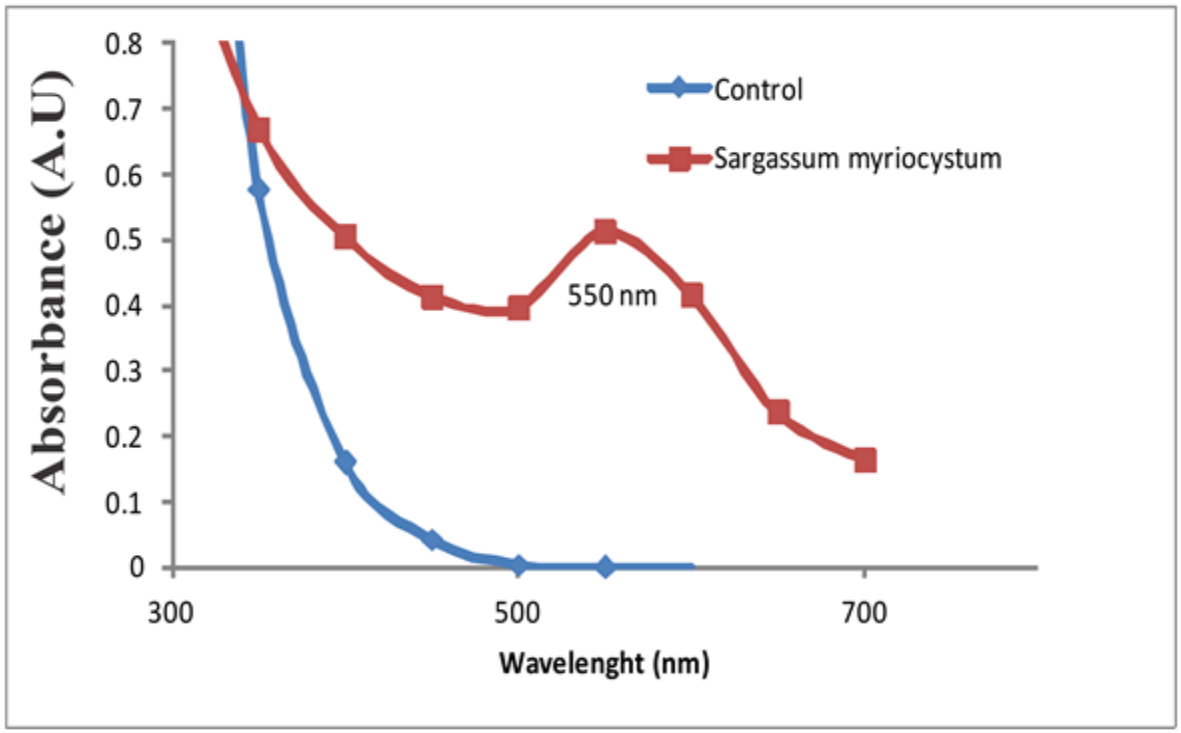


Fig. 2: The UV-Vis spectrum of synthesized gold nanoparticles using *S. myriocystum* with gold chloride as control

Fourier Transform Infrared spectroscopy (FT-IR) measurements:

In the present investigation FTIR measurements were carried out to identify the potential biomolecules present in dried seaweed *S. myriocystum* responsible for the reduction and efficient stabilization of the bio-reduced gold nanoparticles. The FTIR spectra reveal the presence of functional groups. Typical FTIR absorption spectra of seaweed extract showing before and after bioreduction are given in Fig (Plate: 8). Representative spectra of obtained nanoparticles manifests absorption peaks located at about 1022.11, 1156.16 and 1550.68 cm^{-1} in the region 500–1600 cm^{-1}. The FTIR spectra reveal the presence of different functional groups like – NH_2 and CH-O-H in cyclic alcohol and C-OH in alcohol of C-O-H in synthesized gold nanoparticles, which give rise to the well-known signatures in the infrared region of the electromagnetic spectrum. The appearance of new peaks at 1550 cm^{-1} confirms the reduction has been carried out by hydroxyl groups present in the diterpinoids of the brown alga. (Venkateswarlu and Biabani, 1995) reports confirmed the presence of diterpene in *S.myriocystum*.

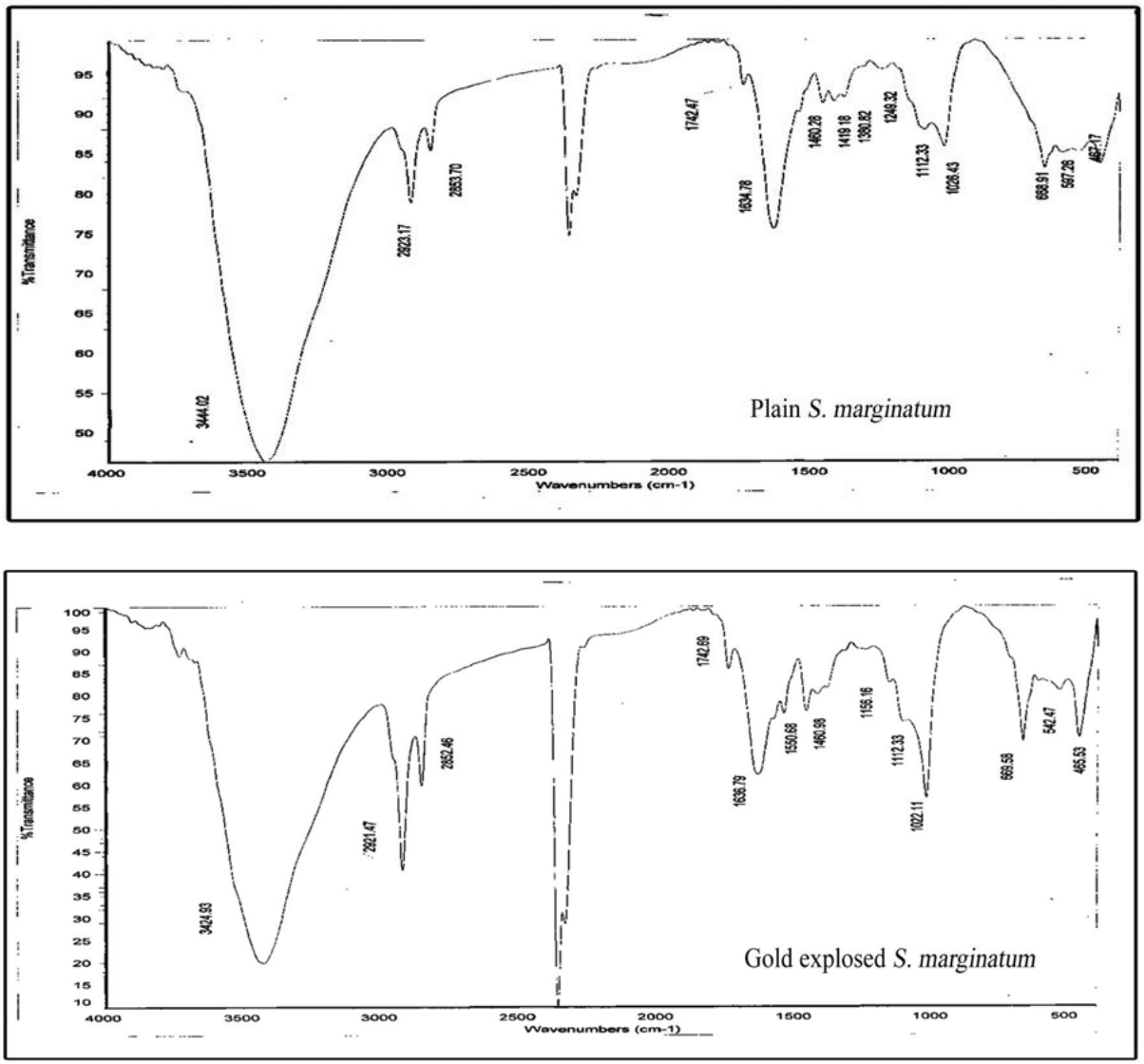


Plate 8: The FT-IR Spectra of dried powder of Plain *S. myriocystum* and gold exposed *S. myriocystum*

Scanning Electron Microscopy (SEM):

The size and shape of the synthesized nanoparticles were determined by the SEM analysis. The SEM analysis revealed the size of the gold nanoparticles synthesized by the reduction of gold chloride by seaweed *S. myriocystum*. The size (diameter) of the synthesized gold nanoparticles lie within 40-85 nm and the average size of the nanoparticles is ~ 60 nm (Plate: 9).

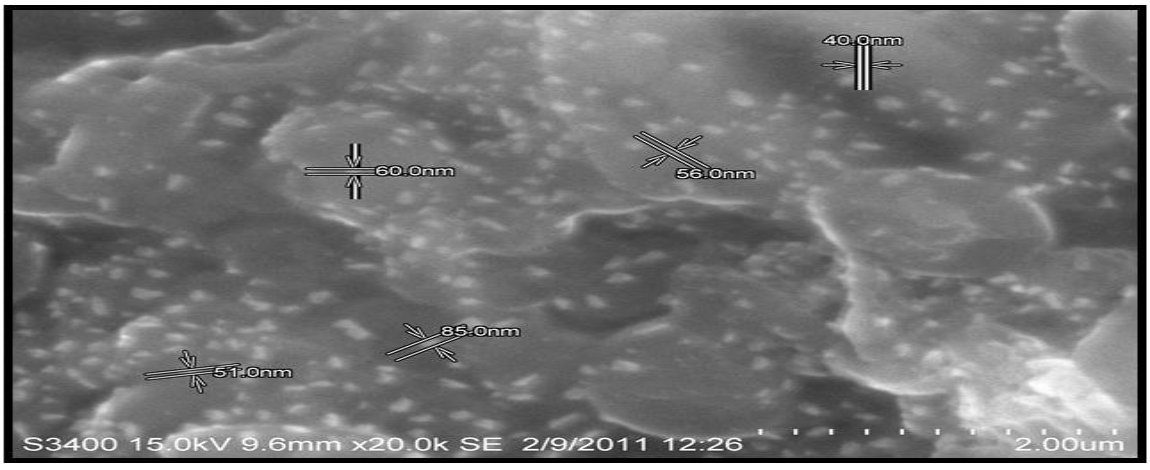
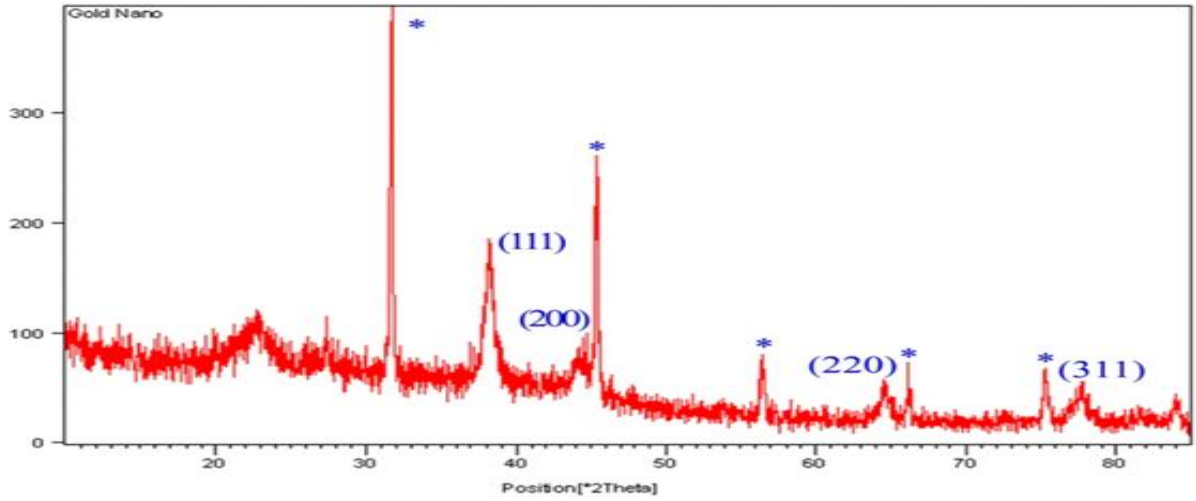


Plate 9: The SEM micrograph of gold exposed *S. myriocystum* biomass

X-Ray Diffraction (XRD) analysis

Further X-ray diffraction studies was carried out to confirm the crystalline nature of Au-Exposed *Sargassum myriocystum* the particle and the XRD pattern obtained was presented in (Plate: 10). Bragg's reflections representative of the Face-Centered-Cubic (FCC) lattice structure of gold. A few unassigned peaks were also noticed in the vicinity of the characteristic peaks. The slight shift in the peak positions may be due to the presence of some strain in the crystal structure which is a characteristic of nanocrystallites synthesized through bio-route. The bottom area of the peak is broad. Generally the broadening of peaks in XRD patterns of solids is attributed to particle size effects. Average crystal size of AuNPs was calculated by applying Debye–Scherer's equation by determining the width of the (1 1 1) Bragg's reflection. The XRD patterns thus clearly showed that the gold nanoparticles formed by the bio reduction of $AuCl_4^-$ ions using *Sargassum myriocystum*.



*** Unassigned peaks**

Plate 10: The XRD patterns of synthesized gold nanoparticles by treating with chloroauric acid aqueous solution

Particle size analysis

Particle size distribution of gold nanoparticles was analysed based on measuring the time dependent fluctuation of scattering of light by nanoparticles undergoing Brownian movement. The increased particle size could be due to bio-organics present in the *Sargassum myriocystum.* The particle size of gold nanoparticles is lesser than 100 nm (Plate: 11).

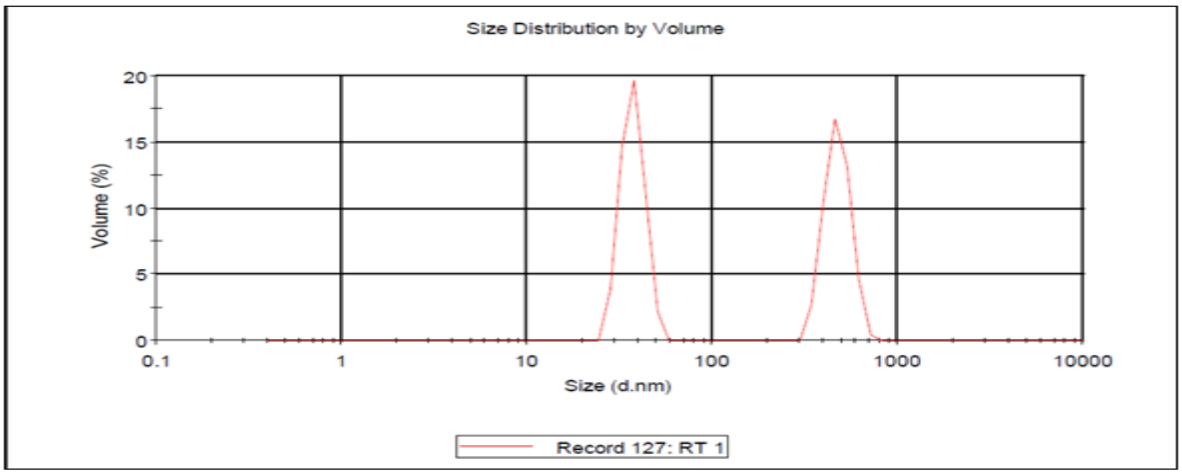


Plate 11: The Particle size distribution of gold nanoparticles synthesized by *S. myriocystum*

Antibacterial activity of gold nanoparticle

The gold nanoparticles were tested against various organisms and the observed result was tabulated (Table: 1). From the table, it was confirmed that gold nanoparticle has most of its activity against gram negative organism.

Table 1: Antibacterial activity of gold nanoparticles

Bacterial Pathogens	Sample	Positive control	Negative control
Pesudomonas aeroginesa	8	13	0
Klebisella oxyctoca	7	14	0
Enterobacter fecalis	11	9	0
K.pneumonia	6	12	0
V.cholerae	8	15	0
E.coli	0	12	0
S.typi	6	13	0
S.paratypi	8	13	0
V.para	9	17	0
P.vulgaris	8	14	0

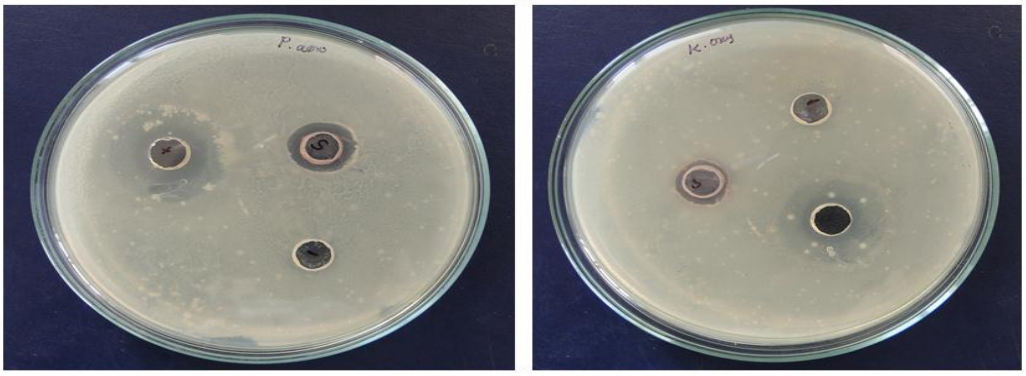


Plate12. *Pesudomonas aeroginosa* **Plate 13.** *Klebisella oxytoca*

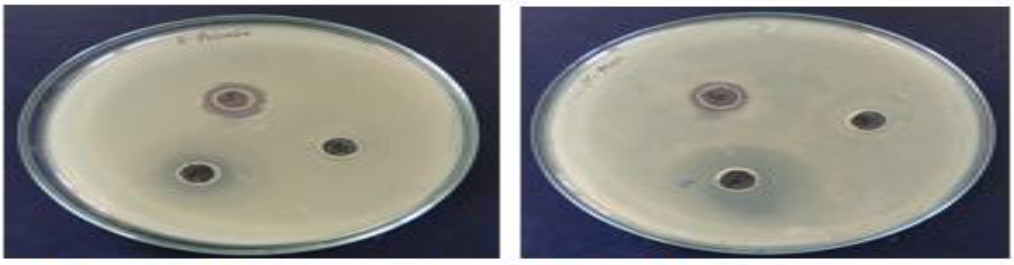


Plate 14. *Enterobacter fecalis* **Plate 15.** *Klebsiella pneumonia*

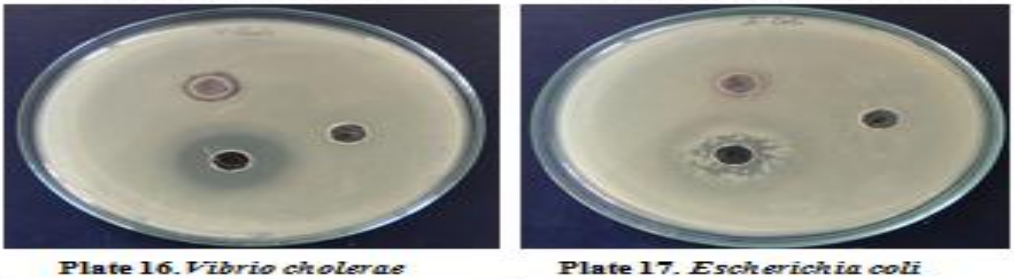


Plate 16. *Vibrio cholerae* **Plate 17.** *Escherichia coli*

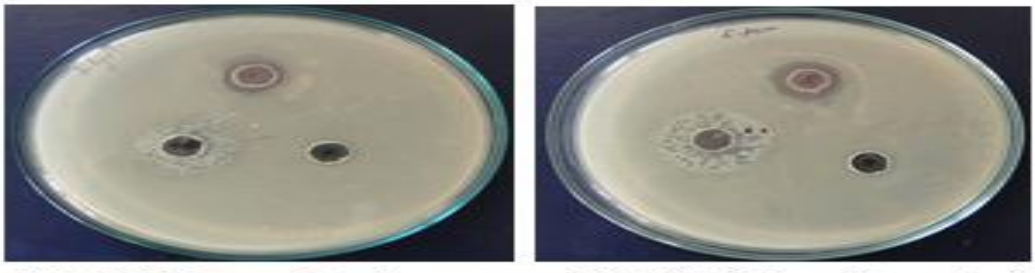


Plate 18. *Salmonella typi* **Plate 19.** *Salmonella paratypi*

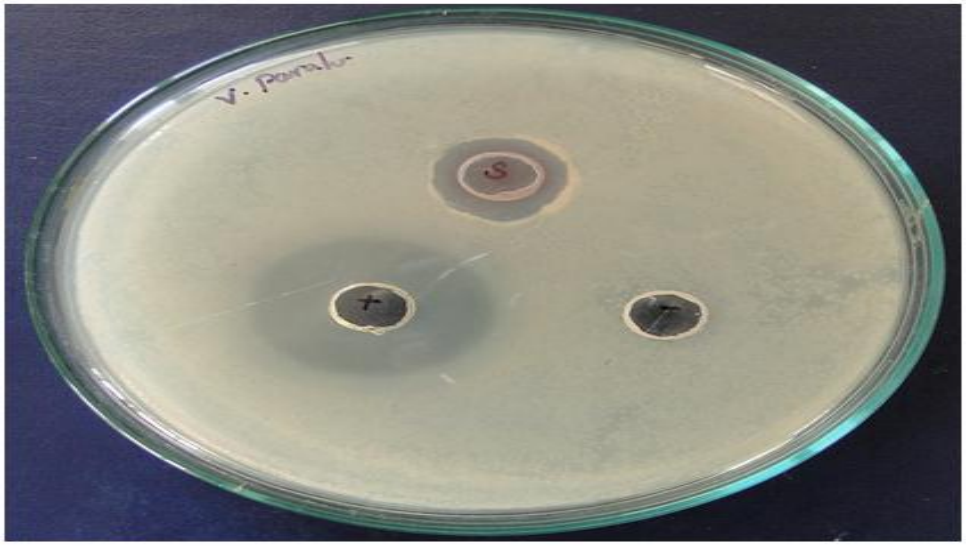


Plate 20. ***P. vulgaris***

SUMMARY AND CONCLUSION

Gold in its colloidal form has been remarkably useful since ancient times in fields ranging from medicine to staining glass and silver. Novel procedures for nanoparticles synthesis suitable for diverse applications are continuously being explored. The unique electronic, magnetic, and optical properties exhibited by nanometer-sized materials are enabling a broad spectrum of biomedical applications.

Seaweeds are one of the most beautiful groups of photosynthetic organisms which grow under the ocean's blue waters. In India, about 1153 species of marine algae belonging to 271 genera have been reported and the total standing stock is estimated to be one-lakh tons, exploited mainly for the industrial production of phycocolloids such as agar- agar, alginate and carrageenan and not for health related aspects. Seaweeds are well distributed in the southeast coast of Tamilnadu, India, especially in the Mandapam coastal region.

The experiments were conducted for the synthesis of stable gold nanoparticles by the reduction of aqueous $AuCl_4^-$ by the biomass of marine alga *Sargassum myriocystum*. The color change of the reaction mixture (Biomass extract and Gold Chloride solution) on formation of gold nanoparticles was quite evident from the conversion of turbid brown color reaction mixture to a ruby red colour. This color change from turbid brown color reaction mixture to a blood red was noticed within the first 10 mins of reaction time.

In the present investigation synthesized gold nanoparticles by the reduction of seaweed *S. myriocystum* consist of nanoparticles of various size in reaction with chloroauric acid concentration at 1mM and $HAuCl_4$ and seaweed Biomass ratio at 0.100: 10 mg/ml were maintained during experimentation. The synthesized particle sizes varied from very small (40.0 nm) to very large particles (85.0 nm). and these results were well conformed with SEM, FTIR, and XRD analysis.

In conclusion we have reported the antibacterial activity of Au NPs over bacteria (both Gram positive and Gram negative). The zone of inhibition using Hi antibiotic zone scale clearly shows that all the bacterial strains which were resistant to antibiotics are highly susceptible to gold nanoparticles. Thus it is proven from this study that the gold nanoparticles synthesized from biomass of *S. myriocystum* seem to be promising and effective antibacterial agent against different strains of bacteria.

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