International Journal of Current Science Research
www.drbgrpublications.in

Volume: 2; Issue: 11; November-2016; pp 1001-1013. ISSN: 2454-5422

Biosynthesis of Silver Nanoparticles using Brown Seaweed *Sargassum tenerrimum* and It's Antibacterial Activity

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Abstract

In this present study fruit peel of three different plants *Citrus sinensis, Punica granatum, Mangifera indica* were taken to investigate in their potential for synthesizing silver nanoparticle and analyses antibacterial activity. There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, catalysis, chemistry, energy and medicine. It is gaining attention due to its cost effective, eco-friendly and large scale production possibilities. The formation of silver nanoparticles was observed by change of colour from transparent yellow to brown colour by addition the fruit peel extracts. The characterization studied was done by UV-Vis spectroscopy and Scanning Electron Microscopy (SEM). All the three fruits peel synthesized silver nanoparticles show good antibacterial activity against clinically important pathogen *Escherichia coli.*

Keywords: Nanoparticles, Silver, Seaweed

Introduction

Nanotechnology is an important field of modern research dealing with design, synthesis, and manipulation of particles ranging from approximately 1-100nm in size, i.e. one billionth (or 10^{-9}) of a meter. Nanoparticles are the fundamental building blocks of nanotechnology. Nanobiotechnology has emerged as the integration between biotechnology and nanotechnology for developing biosynthetic and environment friendly technology for synthesis of nanomaterials.

In recent years researchers in the field of nanotechnology are finding that metal nanoparticles have all kinds of previously unexpected benefits in both the conventional technology and experimental medical industries. Researchers have already developed a series of products

based on nanoparticles, some of which have been released for public consumption. For those with severe auto-immune disease like HIV and AIDS, nanoparticles could essentially serve as a synthetic immune system.

Nanoparticles of free metals such as Gold, silver and copper have been extensively researched and are used mostly, for synthesis of nanoparticles, because of their unique physical properties, chemical reactivity and potential applications in catalysis, biological labeling, biosensing, drug delivery, antibacterial activity, and detection of genetic disorders, gene therapy and DNA sequencing (*Thirumurugan et al*., 2010).

There are many ways depicted in various literatures to synthesize silver nanoparticles. These include **physical, chemical,** and **biological** methods. The physical and chemical methods are numerous in number, such as chemical reduction of silver ions in aqueous solutions with or without stabilizing agents (*Liz-Marzan and Lado-Tourino,* 1996), thermal decomposition in organic solvents (*Esumi et al.,* 1990), chemical reduction and photoreduction in reverse micelles (Pileni, 2000*; Sun et al.,* 2001), and radiation chemical reduction (Henglein, 1993). Most of these methods are extremely expensive and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks. Hence an alternate, feasible method was introduced to synthesize silver nanoparticles. This was achieved by employing biological methods of using microbes and plants.

In biologically synthesized nanoparticles are of considerable interest in the area of biology and medicine; due to their unique particle size and shape dependent various properties (*Sun et al.,* 2008). Utilization of plants for the synthesis of silver nanoparticles is advantageous over other biological methods. The rate of biosynthesis of silver nanoparticles from plants is cost effective and does not use toxic chemicals, temperature and high pressure (*Parashar et al.,* 2009). During the past three decades, seaweed research has been increased considerably for the search of new and effective medicines of natural origin. Several compounds include primary and secondary metabolites synthesized by seaweeds are promising source for both industrial and biotechnological applications (Renn, 1997).

Among the most important bioreductants are seaweeds. Seaweeds are macroscopic, benthic marine algae that live either in marine or brackish water environment. They are abundant along the coastline of India. They are commonly termed as **gulfweeds.** The elements abundant in seaweeds include potassium, sodium, calcium, magnesium, zinc copper, chlorine,

phosphorous, vanadium, cobalt, manganese, selenium, bromide, iodine, arsenic, iron and fluoride. Seaweeds are being harvested for the extraction of alginate, agar and carrageenan, gelatinous substances collectively called as hydrocolloids or phycocolloids that have attained commercial significance as food additives. Seaweeds are relatively easy to handle, readily available and low cost. It produces variety of primary and secondary metabolites that have been isolated and reported to have excellent biological activities such as antibacterial, anti-cancer, anti-diabetic, anti-tumor, anti-coagulant and anti-oxidant (*Lakshmanasenthil et al.,* 2014).

Hence the present study focuses on the toxic free, eco-friendly, biological synthesis of silver nanoparticles from the marine brown seaweed *Sargassum tenerrimum* and study its antibacterial activity against the bacterial pathogen *Escherichia coli*.

Materials and Methods

Chemicals used for synthesis of AgNPs:

Silver nitrate (pure) the precursor for silver was used without further purification. De-ionized water was used for the preparation of silver nitrate solution.

Equipments used for characterization:

UV-visible measurements were made on UV-visible-NIR spectrophotometer in a standard optical cell of 10mm path-length. The XRD pattern was done using XPERT-PRO X-ray diffractometer and SEM images were taken using Scanning Electron Microscope.

Preparation of Aqueous Seaweed Extract:

The seaweed sample of *Sargassum tenerrimum* was collected from the coast of Manapad, Tamilnadu, India. The seaweed was initially sterilized with tap water to remove extraneous substances and salt from the surface of the sample, followed by distilled water.

Fresh seaweed extract:

The fresh seaweed sample was taken and was cut into small pieces. The extract was prepared by adding 10gm of sample in 100ml sterile distilled water and the mixture was heated at 60^0c for 15 minutes. The solution was then filtered using whattman filter paper and was used as the aqueous seaweed extract.

Dry seaweed extract:

The seaweed was shade dried for one week and was powdered using mixer grinder. Later, the extract was prepared by dissolving 10gm of powdered seaweed in 100ml of sterile distilled water and the mixture was heated at 60^0c for 15 minutes. The solution was then filtered using whattman filter paper and was used as the aqueous seaweed extract.

Synthesis of Silver Nanoparticles (Ag-NPs):

In an Erlenmeyer's flask, 95 ml of 1mM Silver nitrate ($AgNO_3$) solution and 5ml of **fresh** aqueous seaweed extract was added. Similarly, In another Erlenmeyer's flask, 95 ml of 1mM Silver nitrate ($AgNO_3$) solution and 5ml of **dry** aqueous seaweed extract was added. These reaction mixtures were gradually heated to 90^0c for 15 minutes and the change in color from light to dark brown was noticed after 24hrs of incubation at 30^0c.

Characterization of Silver Nanoparticles (Ag-NPs):

The bioreduction of AgNPs was characterized by using UV-Visible spectroscopy, XRD (X-Ray Diffraction) and SEM (Scanning Electron Microscope) analysis, which confirms the presence of AgNPs.

The mean or average size of the silver nanoparticles were calculated using

Debye – Scherrer's Equation:

$$\mathbf{D = K\lambda / \beta \cos\theta}$$

Where,

$\beta = \pi/180 \times$ FWHM (FWHM – Full Width Half Maximum)

$K = 0.96$

$\lambda = 1.5406$ Å

$K\lambda = 0.96 \times 1.5406$

$= 1.4789$ Å

Preparation of nutrient agar

Weigh out 5.6 grams of nutrient agar powder and is added to 200ml of deionized water with gentle stirring. Sterilize the media by autoclaving at 121^0c for 15 minutes. Allow the mixture to cool to 50^0c. Then pour the Agar into the petriplate and keep this undisturbed to solidify.

Inoculation of the culture

A reusable metal spreader should be flame sterilized and is then allowed to cool. The spreader is placed in contact with the inoculum on the surface of the plate and positioned to allow the inoculums to run evenly along the length of the spreader. Evenly distribute the inoculums and to allow it to be absorbed into the agar. Avoid disturbing plates for 10 to 20 minutes after spreading. After that the spread plates have been permitted to absorb the inocula for 10 to 20 minutes they may be inverted and incubated as desired. After appropriate incubation, plates are inspected and the cultured colonies were again sub cultured for isolation.

Isolation of the culture

Sterilize the inoculating needle by flame until the metal turns red and cool it. Touch the inoculating needle to an individual colony growing on the plate. Carefully lift up the lid of the plate inoculating onto. Touch the inoculating needle to the very center of the surface of the nutrient agar. Place the lid on the plate, and flame the inoculating needle to kill any remaining bacteria. Seal the plate with a layer of paraffin around the edges. The isolated cultures were again sub cultured for the analysis of antimicrobial activities.

Antibacterial Activity

The antibacterial activities of the crude extracts were determined in accordance with the agar-well diffusion method. Wells were bored into the agar using a sterile well cutter. These wells were filled with the solution of the extract and is allowed to stand at room temperature for about 2 h and then incubated in the microbial incubator. The plates were observed for zones of inhibition after 24 h. The effects were compared with those of streptomycin and tetracycline (purchased from HIMEDIA, Mumbai) respectively.

Result

The formation of silver nanoparticles from the seaweed extract of *Sargassum tenerrimum* was examined. The reduction of $AgNO_3$ in aqueous seaweed extract by heating at 60^0c showed visible colour change. The colour of the solution gradually intensified on heating, which indicated the synthesis off silver nanoparticles. The change in colour is an attribute to the excitation of Surface Plasmon Vibration of silver nanoparticles. The colour change was noted for both the fresh and dry samples at 30minutes and after 24hours of incubation **(Fig. 1 and 2)**.

Silver nanoparticles were characterized by UV-Visible spectroscopy which is a convenient preliminary method for characterizing the silver nanoparticles based on optical properties called Surface Plasmon Resonance (SPR). **Fig. 3 and 4** depicts the UV-Visible spectra of silver nanoparticles synthesized from the fresh sample at 30 minutes and after 24 hours of incubation. Similarly, **Fig. 5 and 6** shows the UV-Visible spectra of AgNPs synthesized from the dry seaweed sample. The UV-Visible spectra showed the maximum absorption of 405.2nm at 30 minutes and 439.8nm after 24 hours of incubation, whereas the dry sample showed the maximum absorption of 398.6nm at 30 minutes and 444.6nm after 24 hours of incubation. The samples showed the peak areas between 380nm and 450nm, which indicates the presence of silver nanoparticles.

The dry powders of the silver nanoparticles were used for XRD analysis. The diffracted intensities were recorded from 10^0 to 80^0 at 2θ angles (**Fig. 7 and 8**). To study the nature of the nanoparticles the XRD analysis was undertaken. The XRD pattern shows that the nanoparticles are crystalline in nature. The size of the formed silver nanoparticles were estimated using Debye-Scherrer's Equation and the average size of the nanoparticles were calculated to be 29.57nm in fresh sample and that from the dry sample was around 27.38nm.
The biosynthesized nanoparticles were further characterized by the SEM analysis, which showed the structure of silver nanoparticles at 5000X, 7500X and 10000X Magnification (**Fig.9 and 10**) synthesized from both the fresh and dry seaweed extracts of *Sargassum tenerrimum*. The synthesized nanoparticles were Spherical in shape.

Further these nanoparticles were investigated for the antibacterial property against water borne pathogenic bacteria, *Escherichia coli.* The synthesized nanoparticles from both the fresh and dry seaweed extracts showed effective antibacterial activity against the *E.coli*. (**Fig. 11**).

Figure 1: Synthesis of silver nanoparticles at 30mts.

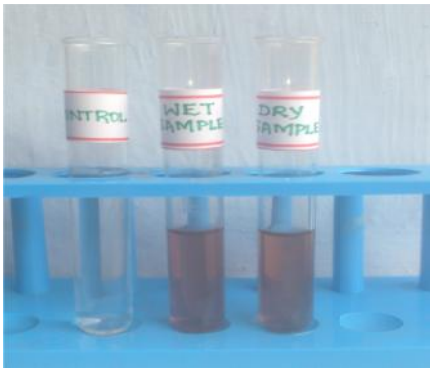


Figure 2: Synthesis of silver nanoparticles after 24hrs.

Figure 3: UV–Vis absorption spectrum of silver nanoparticles synthesized from FRESH SAMPLE of *Sargassum tenerrimum* at 30 minutes

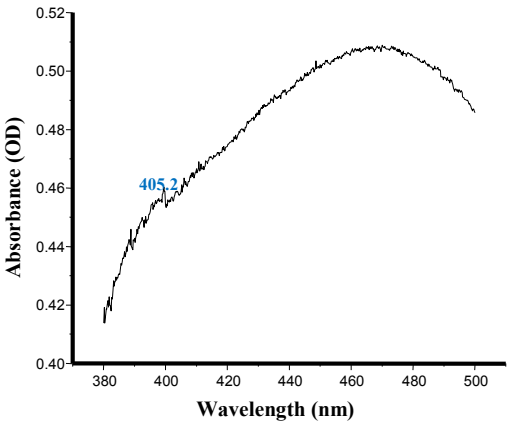


Figure 4: UV–Vis absorption spectrum of silver nanoparticles synthesized from FRESH SAMPLE of *Sargassum tenerrimum* at 24 hours

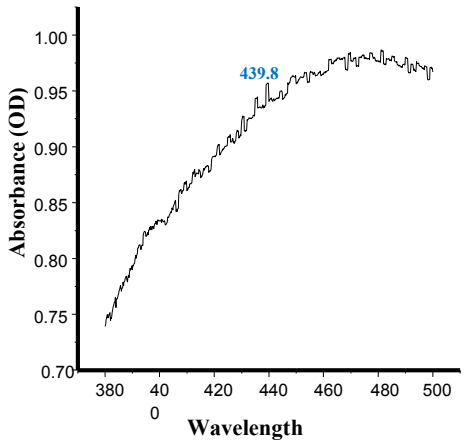


Figure 5: UV – Vis absorption spectrum of silver nanoparticles synthesized from DRY SAMPLE of *Sargassum tenerrimum*

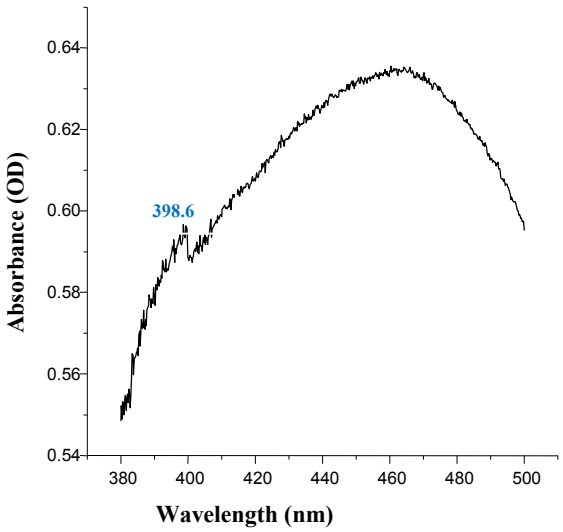


Figure 6: UV – Vis absorption spectrum of silver nanoparticles synthesized from DRY SAMPLE of *Sargassum tenerrimum* at 24 hours

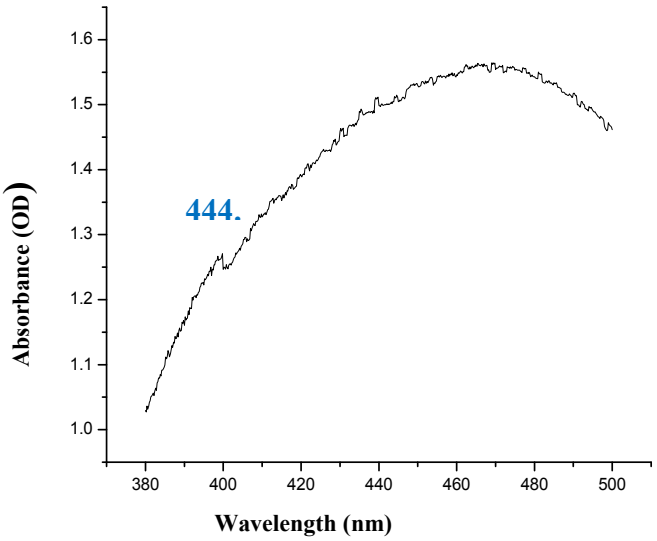


Figure 7: X-ray diffraction (XRD) pattern of silver nanoparticles (AgNPs) synthesized from fresh seaweed sample of *Sargassum tenerrimum*.

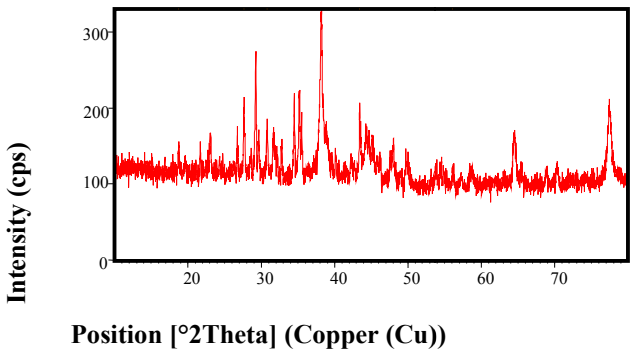


Figure 8: X-ray diffraction (XRD) pattern of silver nanoparticles (AgNPs) synthesized from dried seaweed sample of *Sargassum tenerrimum.*

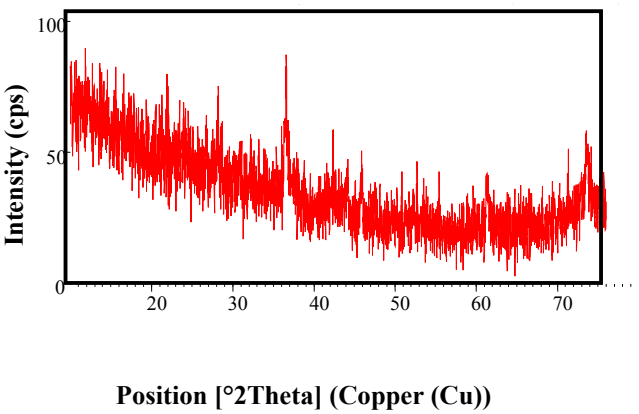


Figure 9: SEM image if silver nanoparticles synthesized from the FRESH SAMPLE of *Sargassum tenerrimum*.

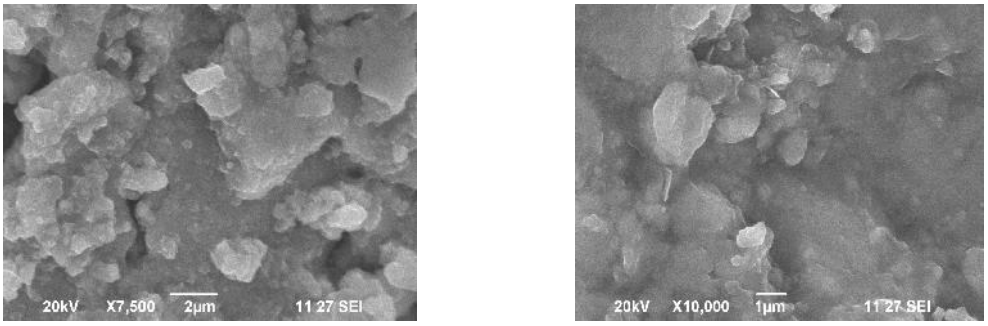


Figure 10: SEM image if silver nanoparticles synthesized from the DRY SAMPLE of *Sargassum tenerrimum*.

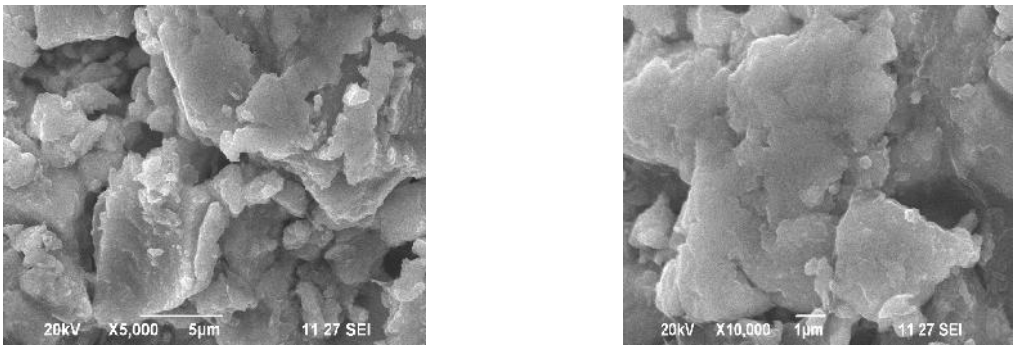
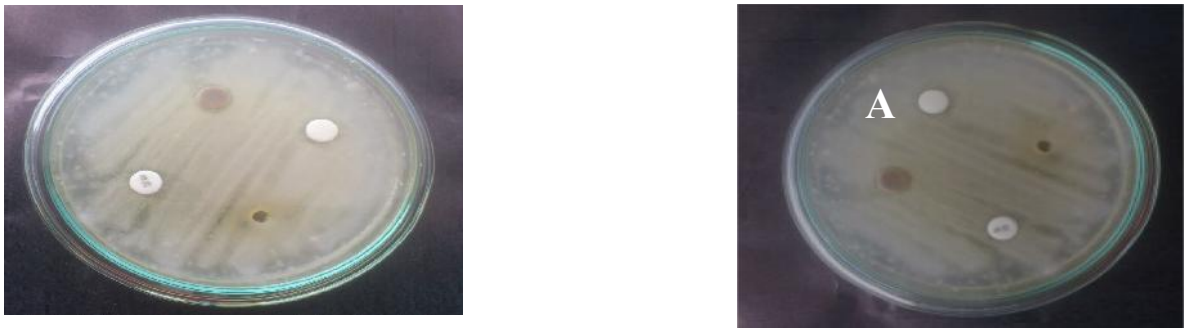


Figure 11: Antibacterial activity of silver nanoparticles against ***Escherichia coli***

A – Action of AgNPs prepared from FRESH seaweed sample

B - Action of AgNPs prepared from . FRESH seaweed sample



Discussion

Silver nanoparticles have gained considerable interest because of their unique properties and proven applicability in diverse areas such as medicine, catalysis, textile engineering, biotechnology, Nanobiotechnology, electronics, optics and water treatment. These nanoparticles have significant inhibitory effect against microbial pathogens and are widely used as antimicrobial agents in a diverse range of products.

Biological method of synthesizing nanoparticles provides a convenient way of synthesizing nanoparticles using natural reducing and stabilizing agents which are environmentally and economically friendly alternatives to chemical and physical approaches.

UV-Visible Spectrophotometer analysis

Silver nanoparticles are formed by the reduction of Ag^{+} to Ag^{0} with the addition of seaweed extract to the solution of 1mM $AgNO_3$. The colour of the solution gradually intensified on heating, which indicates the synthesis of silver nanoparticles. The solution was then incubated at 30^{0}C for 24 hours. Notably even after 24 hours, there was no remarkable deepening of the colour indicating the saturation of the reaction. This reflects that the particles may be well dispersed in the solution with mild agglomeration (*Nabikhan et al*., 2010). The silver nanoparticles were synthesized within 30 minutes. The formation of silver nanoparticles was monitored by UV-Visible absorption spectra within a range of 300 to

600nm, for both the fresh as well as dry sea weed extracts. The UV-Visible spectrum of the reaction mixture showed progressive increase in absorbance upon increasing time in both the samples i.e. between 380nm to 450nm, indicating the synthesis of nanoparticles (*Vinoth Kumar Thirumalairaj, et al*., 2014). In our present work, the dry and fresh sample extracts take 24 hours for change in colour, which shows that the silver ions have been converted into silver nanoparticles and the absorbance showed peak at 444.6nm and 439.8nm.

SEM Analysis

In the present work, the surface morphological appearance of silver nanoparticles was photographed by Scanning Electron Microscope. The silver nanoparticles were spherical in shape, which could be compared to the spherical structure of nanoparticles, synthesized from *Sargassum ilicifolium,* by P. Kumar et al., 2012. The nanoparticles synthesized were well distributed in aggregations. We have observed that, the particles tend to stick on the surface and form agglomerates (*Mani Arunkumar et al.,* 2014).

XRD Analysis

The crystalline pattern of synthesized AgNPs was confirmed by XRD spectrum. The typical XRD pattern revealed that the sample contains a mixed structure of silver nanoparticles. The average size of the AgNPs synthesized from the fresh sample 29.57nm, was slightly larger than the nanoparticles synthesized from the dry sample 27.38nm. A similar study by *Kumar et al*., 2013, with the brown seaweed *Gracilaria corticata* resulted in spherical shaped nanoparticles, with the average size ranging between 18–46 nm. The 2θ value of the XRD values was ranging from 10^0 to 80^0. The observed peak broadening and noise were probably related to the effect of nanosized particles and the presence of various crystalline biological macromolecules in the plant extract (Mehrdad Forough *et al*., 2010).

Antibacterial Analysis

The adsorption on bacterial surface and intracellular enzyme activity is the main reason for antibacterial reaction (Rajesh kumar *et al*., 2012). Nowadays, due to the resistibility and tolerance, most of the microorganisms often change to pathogens and cause severe infection in human beings. Hence new antimicrobial agents from natural and inorganic substances are needed to eradicate the multi-drug resistant bacteria (*Kim et al*., 1998; cho, 2005). Our experimental result shows that the silver nanoparticles synthesized both the fresh and dry seaweed sample shows effective antibacterial property against *Escherichia coli*, confirmed by the formation of clear zone of inhibition around the wells.

Conclusion

In this study, *Sargassum tenerrimum* (fresh & dry) extracts have been used as a reducing agent for the synthesis of silver nanoparticles. This seaweed is easily found and available over the coastal areas, hence it has been selected for the present study. Synthesized silver nanoparticles were confirmed from the prominent yellowish brown color. This was the first report of synthesized silver nanoparticles from both the fresh and dry seaweed samples of *Sargassum tenerrimum.* Further these nanoparticles were characterized by UV -visible spectroscopy. The SEM analysis showed the spherical shape of the nanoparticles. The XRD pattern revealed the crystalline structure of silver nanoparticles. It was also concluded that the silver nanoparticles synthesized from both the fresh and dry seaweed extracts demonstrated a strong activity against bacteria, hence could be used in the treatment of many diseases including bacterial infection. The nanoparticles with different sizes obtained from the reaction medium can directly put forward to various biomedical applications because of the green technology procedure in their synthesis. This is the route in which there is no involvement of any toxic or hazardous reducing agents. Thus the eco-friendly synthesis of silver nanoparticles has been achieved in the present study using sea weed, *Sargassum tenerrimum.* It also can be scaled up for large scale synthesis of silver nanoparticles without using high pressure, energy, costly chemicals as reducing agents.

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