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Biosynthesis of silver nano particles using marine brown alga *Lobophora variegata* and assessment of its bactericidal activity

Thennarasan. S and Murugesan, S[#]

[#]PG and Research Dept. of Botany, Unit of Algal Biotechnology and Bionano Technology, Pachaiyappa's College, Chennai 600 030, India.
**Corresponding Author: E Mail: smurugesan5@gmail.com;*
Mob. No.: +919840276446;
Ph.: +914426412844; Fax: +914426426900

Abstract

The biosynthesis of silver nano particles are an eco-friendly method in the field of nanotechnology. In the present study, the brown marine alga *Lobophora variegata* aqueous extract was used as a reducing agent for the synthesis silver particles (Ag-NPs), which has economic benefits over chemical and physical processes of synthesis. The synthesized nano particles have been characterized systematically by using UV–Vis spectroscopy, FTIR, TEM and XRD. The formation of Ag-NPs was confirmed through the presence of an intense absorption peak at 420 nm using a UV–visible spectrophotometer. A TEM image showed that the particles are spherical in shape with size ranging from 20 to 50 nm. The nano particles were crystalline in nature. This was confirmed by the XRD pattern. From the FT-IR results, it can be seen that the reduction has mostly been carried out by sulphated polysaccharides present in *Lobophora variegata*. Further, these biosynthesized silver nano particles were found to be highly toxic against gram positive bacteria *Bacillus cereus*, gram negative bacteria *Salmonella typhii* was analyzed by a zone of inhibition method.

***Keywords*:** Silver nano particles, *Lobophora variegata,* bactericidal activity.

Introduction

Nano particles do exhibit many interesting properties of materials in the form of nano-sized particles. Currently, a large number of physical, chemical, biological, and hybrid

methods are available to synthesize different types of nano particles (Mahdavi *et al*., 2013). Though physical and chemical methods are more popular for nano particle synthesis, the use of toxic compounds limits their applications. To overcome the problem of toxicity in synthesis, safe, eco-friendly green methods have a major role for producing nano particles (Arockiya Aarthi Rajathi *et al*., 2013). Several methods have been used for the green synthesis of nano particles using various biological materials as reducing agents such as microorganisms, marine organisms, micro-fluids, and plant extracts (Govindaraju *et al*., (2009).

Marine algae are well-known as a functional food for their richness in lipids, minerals and certain vitamins, and also several bioactive substances like polysaccharides, proteins and polyphonies, with potential medicinal uses against cancer, oxidative stress, inflammation, allergy, thrombosis, lipidemia, hypertensive and other degenerative diseases (Namvar *et al*., 2012; Mohamed *et al*., 2012). Thus, *L. variegata* phytochemicals include hydroxyl, carboxyl, and amino functional groups, which can serve both as effective metal-reducing agents and as capping agents to provide a robust coating on the metal nano particles in a single step (Mahdavi *et al*., 2013). Recently there are a few, reports that algae is being used as a biofactory for synthesis of metal nano particles (Rajasulochana *et al*., (2010); Vivek *et al*., (2011); (Bhuvaneswari *et al*., (2011); Murugesan *et al*., (2011) and Swaminathan *et al*., (2011).

Historically, silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. It has been reported that silver nano particles (SNPs) are non-toxic to humans and most efficient against bacteria, virus and other eukaryotic microorganisms at low concentrations without any side effects (Jonge *et al*., 2005). The most important application of silver and SNPs is in the medical industry such as tropical ointments to prevent infection against burn and open wounds (Ip *et al*., 2006). It is a need of today to develop reliable, non-toxic, clean and eco-friendly experimental protocols for the synthesis of NPs, which is likely through ambient biological resources.

The present study describes a single step, green, and rapid synthesis of silver nano particles (Ag-NPs) prepared by biological (green) techniques using *Lobophora variegata.* Bactericidal activities of the medicinally valid algal mediated nano particles were also examined against gram positive and gram negative pathogenic bacteria such as *B. cereus* and *S. typhii.*

Materials and Methods

(1) Collection of seaweed and chemicals

The marine brown seaweed *Lobophora variegata* was collected from 2.5 meters depth in the rapid island Gulf of Mannar, a Mandapam Coastal area in South India.

(2) Preparation of Algal extract

Collected brown seaweed was washed with sea water to remove the epiphytes and sand particles. After dries, 1 gram of fresh materials was cut into small pieces; grind with 50 ml of distilled water with mortar and pestle and these extracts were boiled for

5 min. The boiled extract was filtered through Whatman No 1 filter paper and the supernatant was used and stored at 4°C for further process.

(3) Biosynthesis of silver nano particles

In the typically synthesis process of silver nano particles, add 10 ml of aqueous algal extract into the 90 ml of 1mM of silver nitrate solution in 250 ml conical flask. The reaction mixture was kept at room temperature under mechanically stirring.

(4) Characterization of bionano material

Biogenic synthesis of nano silver was monitored using UV-visible spectrophotometer (UV-1601 Shimadzu spectrophotometer). After the complete reduction of Ag^+ ions by the *L. variegata* extract. It was analyzed by FTIR. XRD pattern of dry nano silver powder was acquired by Cu Ká radiation (1.5406 Å; 45 kV, 30 mA). It was also analyzed to determine peak intensity, position and width. The size and shape of the biosynthesized nano particles were observed by Transmission Electron Microscope (TEM) (Hitachi, Model: S-3400N).

(5) Bactericidal activity

Bactericidal activity of *L. variegata* assisted, silver nano particles were carried out by disc diffusion method against pathogenic bacteria. Bacterial cultures were purchased from MTCC, India. These bacterial cultures were freshly cultivated for 24 hrs in nutrient broth. Each bacterial culture was spread on the Muller Hinton agar plates. Sterile paper discs containing three different concentrations of silver nano particles were placed and incubated. After the 24 hrs of incubation the zone formation was recorded. The experiments were repeated for three times.

Results and Discussion

The formation of silver nano particles was confirmed through visual assessment. The reaction mixture turned to dark brown colour from brownish-yellow colour within 20 min indicated the synthesis of silver nano particles (Fig.1). Colour changes appear after the completion of the reaction, it is well known that silver nano particles exhibit yellowish brown based on their size. (Li *et al*. 2009). The appearance of dark brown colour may be due to the excitation of surface plasmon resonance (SPR) effect and reduction of $AgNO_3$ (Mulvaney, 1996). Fig 2 shows the UV absorption spectra of the synthesized silver nano particles using the extract of brown seaweed *Lobophora variegata* recorded as the function of reaction time. Absorption spectrum shows that the peak positioned at 420 nm indicated the formation of silver nano particles. In the present study, a brown algae extract mediated synthesized silver nano particles was rapid process and stable for several months due to the presence of stabilizing agent.

Fig.1. The aqueous extract of *L. variegata* (A) before; and (B) after synthesis of Ag NPs. particles synthesized from the extract of *L.variegata*

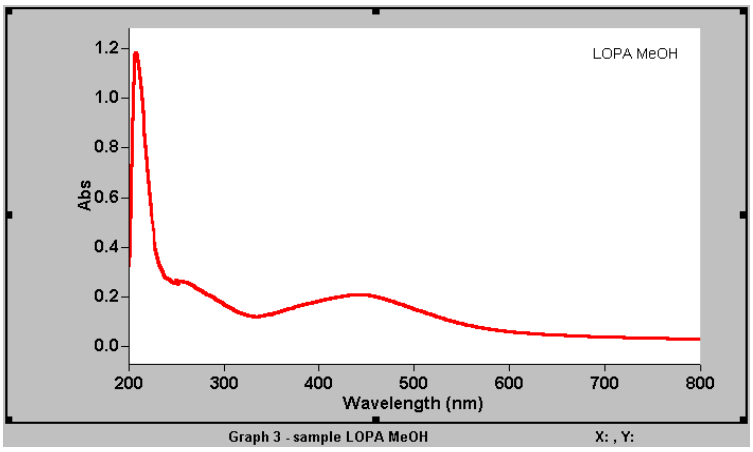

Fig.2 UV Spectroscopic analyses of silver nano

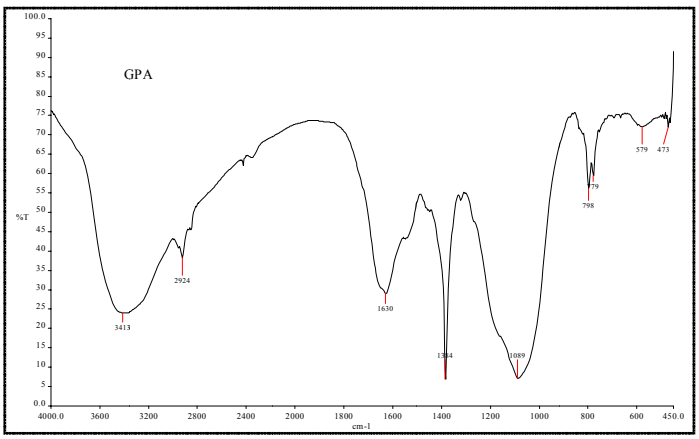

Fig.3 FT-IR spectrum of *L.variegata* mediated synthesized silver nano particles

Fourier Transform Infrared spectroscopy (FT-IR) measurements are carried out to identify the possible biomolecules responsible for the reduction of the Ag^+ ions and capping of the bio-reduced SNP's synthesized by *L. variegata*. The FT-IR spectrum of *L. variegata* biosynthesized nano silver is depicted in Fig.3. The representative spectra of nano particles obtained manifests absorption peaks located at about 3413 cm^{-1} (O–H stretch, H–bonded alcohols, phenols), 2924 cm^{-1} C-CH_3 stretch alkanes), 1630 cm^{-1} (-Amides (Non-conjugated), 1089 cm^{-1} (C–N stretch aliphatic amines), 798 cm^{-1} (C–Cl stretch alkyl halides) and 579 cm^{-1} (C–Br stretch alkyl halides). The result revealed that the capping ligand of the Ag-NPs may be an aromatic compound or alkanes or amines (Inbakandan *et al*., 2010).

Table. FT-IR Spectral qualities of silver nano particles in *L.variegata*

Assignments	**Wave number (cm^{-1})**
O–H stretch, H–bonded alcohols, phenols	3413
C-CH_3 stretch alkanes	2924
Amides (Non-conjugated)	1630
CH_3	1384
C–N stretch aliphatic amines	1089
C–Cl stretch alkyl halides	798
C–Br stretch alkyl halides	579

The XRD pattern (Fig.4) shows that the particles are crystalline in nature and some of the unassigned peaks were observed, it may be due to the fewer biomolecules of stabilizing agents are enzymes or proteins in the algal extract. The observed peak broadening and noise were probably related to the effect of nano sized particles and the presence of various crystalline biological macromolecules in the algal extracts. The obtained results illustrate that silver ions had indeed been reduced to Ag0 by the extracts under reaction conditions. The lattice planes {1 0 0}, {1 1 0}, {1 1 1}, {2 0 0}, {2 1 0}, {2 1 1} and {2 2 0} were identified with the corresponding Bragg's angles of 21.66°, 33.87°, 39.97°, 46.12°, 51.28°, 52.35°, 55.58° and 64.23° respectively, which confirm the face-centered cubic structure of the formed Ag-NPs. From the XRD spectra of our experiment indicate, the formation of silver nano particles is crystalline in nature and aggregation was formed due to the fewer action of stabilizing agents in the algal extract.

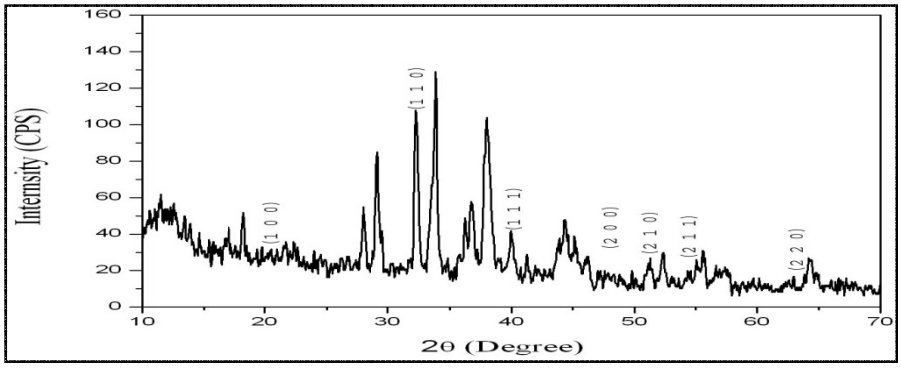

Fig.4. X-ray diffraction pattern of *silver nano particles using L.variegata*

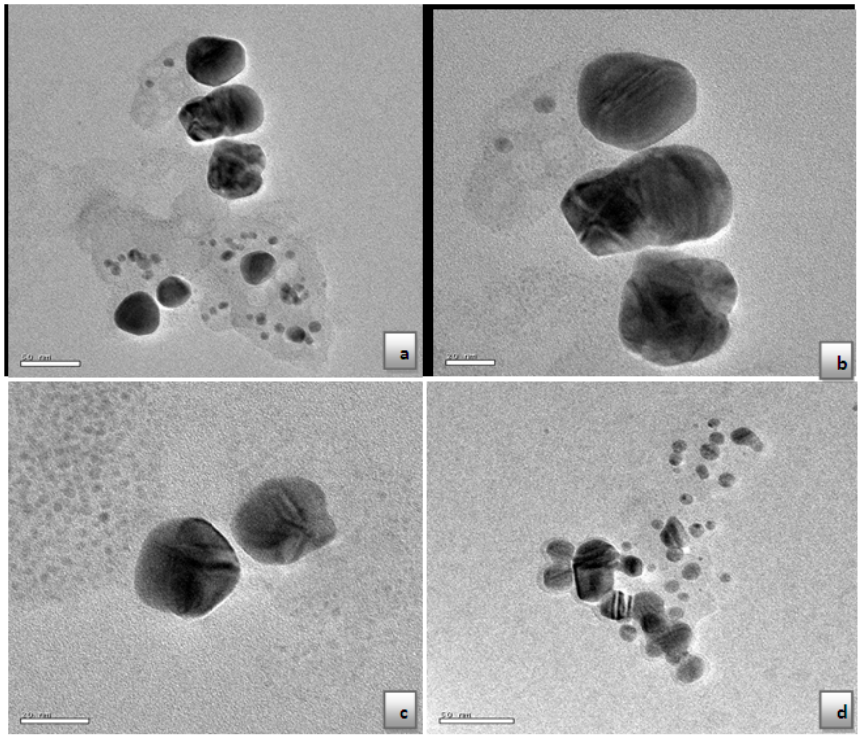

Fig.5 TEM images of silver nano particles by *L.variegata* extract ranging from 20 nm and 50nm.

TEM was utilized to characterize the particles and their size and distribution by taking micrograph from drop coated films of the silver nano particles synthesized by the treatment of silver complex solution *L.variegata* extract. Nano particles observed from the micrograph majority are spherical with a small percentage of elongated particles ranged in size of 20 nm and 50 nm. The average mean size of silver nano particles was 40 nm. (Fig.5a-d).

Bactericidal Activity

The bactericidal activity of *Lobophora variegata* mediated silver nano particles was performed against gram negative pathogenic bacteria such as *Salmonella typhii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and gram positive pathogenic bacteria's such as *Staphylococcus aureus, Bacillus cereus* and *Bacillus subtillus* using disc diffusion method. Table.1 shows the zone of inhibition (mm) around the well with *L. variegata* mediated synthesized silver nano particles. In gram positive bacteria the action of silver nano particles was very less zone of inhibition in the range of 8 ± 0.001 to 14 ± 0.002 mm in 100 µg/µl concentrations. The bactericidal activity of synthesized silver nano particles was high against gram negative bacteria *Salmonella typhii* in the inhibition range was 10 ± 0.001 to 16 ± 0.004 mm at the concentration of 100 µl. The inhibition variation was occurring due to the differences in cell wall composition in gram positive and gram negative bacteria. Gram positive bacteria were made up of thick cell wall contain peptidoglycon so that nano particles did not affect easily. But the nano particles were easily penetrated into gram negative bacteria due to the structure of cell wall contain thin lipid layers, so nano particles easily enter into the cell and disturb it (Jing *et al*., 2012). Still the exact mechanism of inhibitory action of nano particles against bacteria was not well known. The formation of free radicals from the surface of the silver nano particles were responsible for the antibacterial function (Shanmugam Rajeshkumar *et al*., 2012).

Silver nano particles were attaching to the surface of the bacteria and act against the cell wall protein and control the power of bacteria, apart from this small particle attach to the larger surface area was clearly explained (Ales Panacek *et al*., 2006). The numeral of bacterial colonies developed on agar plates as a role of the different concentration of brown seaweed assisted, silver nano particles when steadily declined when the nano particle concentration increased. Silver nano particles were playing a role in various medical applications. The results of the present study undoubtedly reveal that synthesized silver nano particles were assuring bactericidal agent against the pathogens employed.

Table.1 Bactericidal activity of silver nano particles of *L. variegate*

S. No	Bacteria zone of nhibition (mm in diameter)	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	Standard drug
1	*Staphylococcus aureus*	8 ± 0.001	10 ± 0.001	12 ± 0.002	14 ± 0.002	18 ± 0.005
2	*Bacillus cereus*	9 ± 0.001	12 ± 0.003	13 ± 0.003	14 ± 0.002	18 ± 0.005
3	*Bacillus subtillus*	10 ± 0.001	11 ± 0.001	12 ± 0.003	13 ± 0.002	17 ± 0.006
4	*Salmonella typhii*	11 ± 0.002	14 ± 0.003	15 ± 0.003	16 ± 0.004	18 ± 0.005
5	*Pseudomonas aeruginosa*	10 ± 0.001	11 ± 0.001	12 ± 0.002	14 ± 0.002	16 ± 0.004
6	*Klebsiella pneumoniae*	10 ± 0.001	12 ± 0.003	13 ± 0.003	14 ± 0.002	20 ± 0.007

Values are expressed as Mean ± SEM, n=3

Conclusion

In this study, silver nano particles were synthesized by using the *L. variegata* extract

and characterized by UV-Vis, FT-IR, XRD and TEM. The bactericidal activity was tested against human pathogens. The formed metal nano particle was found to have wider bactericidal activity against *Salmonella typhii* than *Bacillus cereus*. The present study is simple, safe, less expensive, non-toxic and eco-friendly as compare to the toxic chemical process. Marine algae could thus be used as an efficient choice of the cost intensive conventional methods. This study may be used in the development of value-added products from the seaweed for biomedical and nanotechnology based industries.

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