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GREEN SYNTHESIS OF SILVER NANO PARTICLES USING MARINE BROWN ALGA *LOBOPHORA VARIEGATA* AND ITS EFFICACY IN ANTIFUNGAL ACTIVITY

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ABSTRACT

The green synthesis of silver nano particles is an eco-friendly method. In the present study, the aqueous extract of brown marine alga *Lobophora variegata* was used as a reducing agent for the synthesis silver nano particles (AgNPs), which has economic benefits over chemical and physical processes of synthesis. The synthesized nano particles have been characterized by using UV–Vis spectroscopy, FTIR, TEM and XRD. The formation of AgNPs was confirmed through 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. From the FT-IR results, it can be seen that the reduction has mostly been carried out by sulphated polysaccharides present in *Lobophora variegata*. The green synthesized silver nano particles were found to be highly toxic against pathogenic fungi *Candida albicans* and *Trichophton mentagrophytes* was analyzed by a zone of inhibition method. Thus, the present study indicates AgNPs may have considerable antifungal activity, deserving further investigated for clinical applications.

***KEYWORDS*:** Silver nano particles, *Lobophora variegata*, XRD, HR-TEM, FT-IR, Antifungal activity.

INTRODUCTION

Nano particles 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.[1] 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.[2] 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.[3] 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 use against cancer, oxidative stress, inflammation, allergy, thrombosis, lipidemia, hypertensive and other degenerative diseases.[4,5] Thus, their 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.[1] It has been reported that silver nano particles (SNPs) are non-toxic to humans and most efficient against bacteria, fungi, virus and other eukaryotic microorganisms at low concentrations without any side effects.[6]

The present study describes a single step, green synthesis of silver nano particles (Ag-NPs) prepared by biological (green) techniques using *Lobophora variegata*. The anti-fungal activities of the medicinally valid algal mediated nano particles were also examined against pathogenic fungi such as *Trichophyton mentagrophytes*, *Aspergillus flavus*, *Candida tropicalis* and *Candida albicans*.

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, 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 dried, 1 gm 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 1 mM 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 characterized by FTIR. XRD pattern of dry nano silver powder was acquired by Cu Ká radiation (1.5406 Å; 45 kV, 30 mA). The size and shape of the biosynthesized nano particles were observed by Transmission Electron Microscope (TEM) (Hitachi, Model: S-3400N).

5. **Antifungal activity:** Antifungal activity of *L. variegata* assisted, silver nano particles were carried out by disc diffusion method against pathogenic fungi. Fungal cultures were purchased from MTCC, India. These fungal were cultured in a potato dextrose agar (PDA) at 35°C, respectively. The MIC for *Candida albicans* and *T. mentagrophytes* were determined by a broth micro dilution method based on the National Committee for Clinical Laboratory Standards (NCCLS); now renamed as Clinical and Laboratory Standards Institute, (CLSI, 2000) method outlined in documents M-27A [7] and M-38P, respectively. Sterile discs containing four different concentrations (25 µL, 50 µL, 75 µL, 100 µL) 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 to dark brown.[8] The appearance of dark brown colour may be due to the excitation of surface plasmon resonance (SPR) effect and reduction of $AgNO_3$ [6]. Fig.2 shows the UV

A B

Fig.1 The aqueous extract of *L. variegate* (A) before and (B) after synthesis of AgNPs.

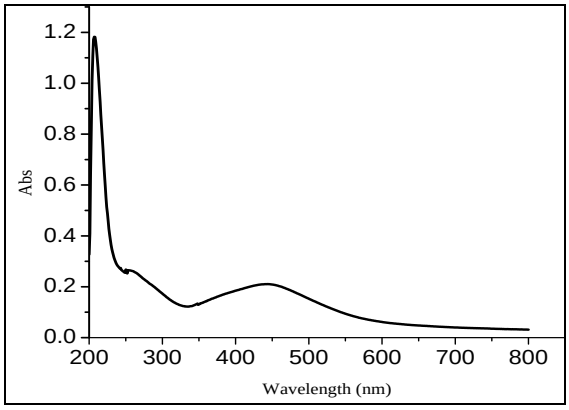

Fig.2 UV Spectroscopic analyses of silver nano particles synthesized from the extract of *L.variegata*

absorption spectra of the synthesized silver nano particles using the aqueous extract of brown seaweed *L.variegata*. 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.

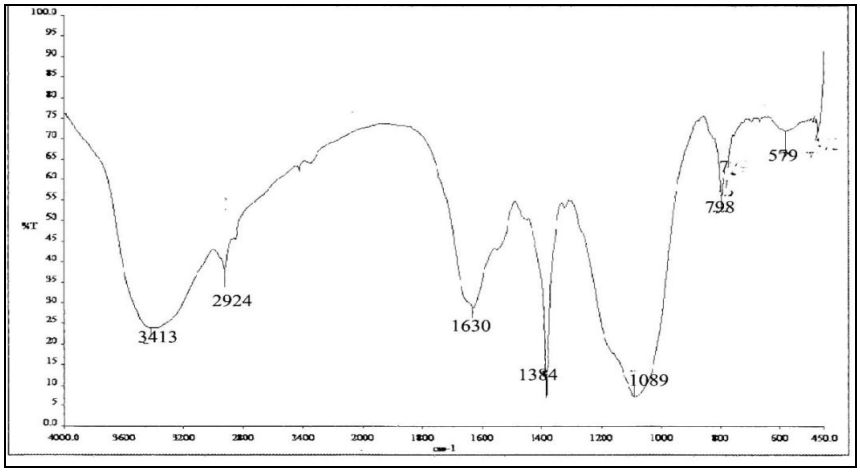

Fig.3 FT-IR spectrum of *L.variegata* mediated synthesized silver nano particles

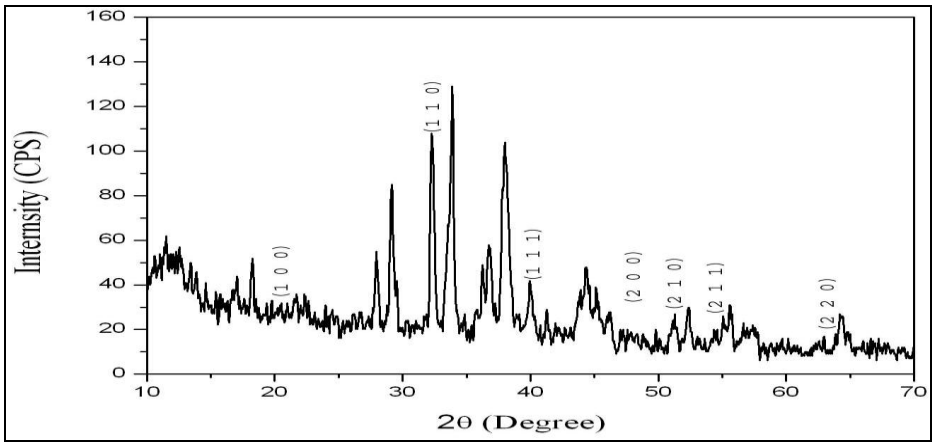

Fig.4. X-ray diffraction pattern of *silver nano particles using L.variegata*

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 SNPs 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.[9]

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 Ag by the extracts under reaction conditions. The lattice planes {1 0 0}, {1 1 0}, {1 1 1}, {2 0 0}, {210}, {2 1 1}, and {220} were identified with the corresponding Bragg's angles of 21.66°, 33.30°, 40.01°, 46.12°, 51.28°, 55.58°, and 64.23° respectively, which confirm the face-centered cubic structure of the formed Ag-NPs. The XRD spectra 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.

TEM was utilized to characterize the nano particles and their size 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.5).

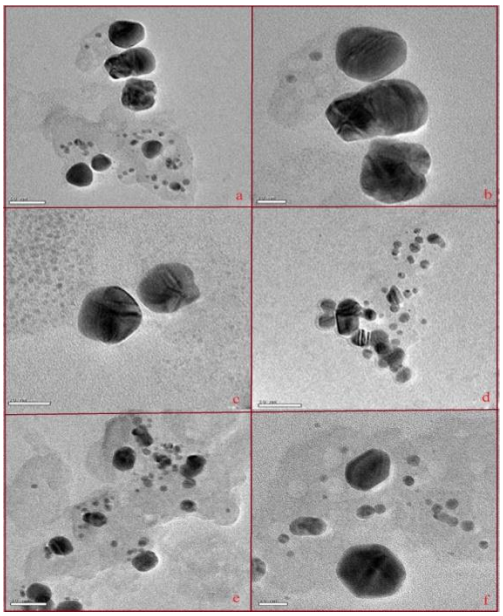

Fig.5 TEM images of silver nano particles by *L.variegata* extract ranging from 20 to 50nm.

Antifungal Activity

Many studies have shown the antimicrobial effects of nano-silver, [6; 10; 11] but very little work on the effects of nano silver against fungal pathogens including clinical isolates of *T. mentagrophytes* and *Candida* species. AgNP possesses some antifungal and antiviral activities.[12] The primary significance of this study is the observation that Nano-silver can inhibit the growth of dermatophytes, which cause superficial fungal infections. Secondly, the

fact that the preparation method of nano-silver described here is cost-effective is also importance. Recently, due to the emergence of antibiotic-resistant bacteria and limitations of the use of antibiotics clinicians have returned to using silver wound dressings, containing varying levels of silver.[13] For these reasons, the antifungal activity and its mechanism of silver, Nano Ag specifically, were investigated.[14, 15, 16]

Table.1 Antifungal activity of silver nano particles in various concentrations of *L. variegata*

S.No	Name of the Microorganism	Zone of Inhibition (mm)				
		25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL	Flucanozole (100 µg/mL)
1	*Candida albicans*	10.72 ± 0.001	13.16 ± 0.001	15.11 ± 0.001	16.54 ± 0.002	33.22 ± 0.002
2	*Candida tropicalis*	10.32 ± 0.001	12.16 ± 0.001	13.76 ± 0.002	14.78 ± 0.002	20.54 ± 0.002
3	*Trichophyton Mentagrophytes*	8.54 ± 0.001	12.83 ± 0.001	15.34 ± 0.002	16.25 ± 0.002	25.56 ± 0.002
4	*Aspergillus flavus*	-	7.52 ± 0.001	9.82 ± 0.001	12.71 ± 0.002	15.54 ± 0.002

The results of the antifungal studies with regard to percentage of radial growth inhibition in PDA plate are shown in Table.1. All the extracts exhibited different degrees of antifungal activity against *C. albicans, C.tropicalis*, *T.mentagrophytes* and *A. flavus*. Further, the nano particle synthesis by the green route by using *L. variegata* extract was found highly active against tested fungal species at a concentration of 100 µg/µl of synthesized silver nano particles. The results showed higher antifungal activity against *C. albiacans* (16.54 ± 0.002 µg/µL), whereas moderate activity was revealed against *T. mentagrophytes* (8.54 ± 0.001µg/µL), when compared with standard antifungal agent flucanozole (33.22 ± 0.002 µg/µL and 25.56 ± 0.002 µg/µL) respectively. The zone of inhibition clearly showed that the fungal strains tested were susceptible to silver nano particles. Thus the present study proved that the silver nano particles synthesized from *L. variegata* seems to be promising and effective antifungal agent against the pathogenic fungal strains.

CONCLUSION

In the present study, silver nano particles were synthesized by using the aqueous extract of *L. variegata* to characterized by UV-Vis, FT-IR, XRD and HR-TEM. Primarily, the fact that the preparation method of nano-Ag described here is cost-effective is also of importance. The results from this study exposed the antifungal property of the silver nano particles of *L.*

variegata contain certain constituents be a better alternative to the hazardous pathogens, deserving further investigated for clinical applications. Our results prompt further studies to isolate and identify the active compounds that evaluate a possible synergism between components with regard to their antifungal activity. The present study provides insight into the molecular basis of the therapeutic properties of *L. variegata* in pharmaceutical industry. Toxicity studies of silver nano particles on human pathogenic opens a door for a new range of antimicrobial agents. The overall results of the study revealed that the silver nano particles synthesized from marine brown seaweed *L. variegata* can act as a potential for the studies on the isolation and characterization of the silver nano particles necessary to realize new biological antibiotics.

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