



## Green-chemical Fabrication of Silver Nanoparticles by Marine macro Algae and its Fungicidal Activity

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### Abstract

The green synthesis of eco-friendly nanoparticles is an evergreen area of bionanotechnology for biomedical advantages. Here, we report the synthesis of silver nanoparticles in aqueous medium using marine brown seaweed *Turbinaria conoides* extract as the reducing and stabilizing agent. On treating silver nitrate solutions with *T. conoides* algae extract rapid reduction of silver ion is observed leading to the formation of highly stable silver nanoparticles. The surface Plasmon resonance band formed at 440 nm indicates the formation of silver nanoparticles. TEM and SEM images of the silver nanoparticles indicated their spherical shape and size ranging from 14 to 26 nm with average size of 20 nm. Furthermore, silver nanoparticle synthesized by this green method is toxic to fungi tested by antimicrobial susceptibility test. This green method of synthesis of silver nanoparticles using algae extract is simple, rapid and an environmentally benign process. Thus algae assisted synthesized silver nanoparticles shows significantly higher inhibition activity against human pathogenic fungi.

**Key words:** Green synthesis, Nanoparticles, Spectrophotometer, Absorbance.

### INTRODUCTION

Nanomaterials are widely used in many fields due to their great unique optical, physical, chemical and electronic properties than the bulk materials [1]. These properties are based on the size, shape and morphology of the nanomaterials [2]. Silver is a prehistoric metal that has been used in medicine for curing several diseases. Silver nanoparticles are more attractive because they are non toxic to human at low concentration and it has high inhibitory activity against pathogenic microorganisms due to their large surface volume ratio [3]. Many physical, chemical and biological methods are adopted

for nanoparticles synthesis. Nanoparticles synthesized by green methods are environmentally acceptable, non toxic of low cost and constitute a single step synthesis process [4-8]. Algae extract mediated synthesis of nanoparticles exhibits numerous advantages such as eliminating need for cell culture maintenance, use of capping agent and stabilizing agent and is environmentally acceptable [9].

Many bacteria and fungi cause high mortality in human population and other organisms. Drugs and chemicals can tackle these problems. Some microorganisms are resistant to commercially available drugs and antibacterial agents: often increasing serious

health problems [10]. There is an increasing demand of selecting therapeutic drugs from natural products such as seaweed etc, but in natural drugs sometime some other chemicals added to enhance the biological activity leads to adverse side effects [11]. Therefore, it is very urgent and important to explore the other approaches to effectively suppress the variety of pathogenic bacteria and ocular fungal pathogens. Silver nanoparticles have the great efficiency at low concentration to suppress the fungal pathogens without side effects. Silver nanoparticles synthesized here with a green method have high antimicrobial activity. The action of the commercially available antibiotics against targeted organisms was enhanced by impregnating the silver nanoparticles with them. It is of great interest to develop nanoparticles based antibiotics that decrease the toxicity and side effects of free drugs and ensure the targeted drug delivery.

In the marine environment, macro algae play an important role in energy transfer and as bioindicator of pollution [12, 13]. Marine algae are classified according to their photosynthetic pigments as brown, red and green algae [14]. Marine algae are used as food, feed and fertilizer in most countries in the world and they are the renewable living resources. Marine algae contain more than 60 trace elements with higher concentrations than the terrestrial plants and also contain proteins, iodine, vitamins, polysaccharides [15] and they produce a great variety of secondary metabolites [16]. *Turbinaria conoides* is a brown algae belonging to the order Fucales, family Sargassaceae, Phaeophycophyta. This seaweed is having a broad range of biological activities such as antibacterial, antifungal, anti-inflammatory, anticancer and antioxidant [17,18]. Naturally, seaweeds have the ability of synthesizing industrially and biotechnologically valuable several organic compounds like primary and secondary metabolites. Due to these properties, we carried out seaweed mediated synthesis of silver nanoparticles using the brown algae *T. conoides* by green route. This route is a non toxic, eco-friendly and takes less time because it eliminates culture maintaining and toxicity of chemicals over bacteria and chemical mediated methods. Marine macro algae are easily available natural renewable resource. Silver nanoparticles were evaluated for use of antifungal activity against pathogenic fungi by well diffusion method.

## MATERIALS AND METHODS

### Chemicals

Analytical grade silver nitrate, Potato Dextrose Agar, Rose Bengal agar, sodium hydroxide and hydrochloric acid were purchased from Himedia laboratories private limited, Mumbai.

### Green synthesis of silver nanoparticles using marine algae

The brown algae *T. conoides* were collected from Gulf of Mannar coastal area in South Tamilnadu, India. The seaweeds were washed thoroughly and kept at room temperature for drying. After that, the dried algal materials were crushed and 1 g powder was dissolved in 100 ml of double distilled water and boiled for 10 min at 60 °C. Further, the boiled extract was collected and used for the nanoparticles synthesis. About 10 mL of pure algal extract solution was added into 90 ml of 1 mM AgNO<sub>3</sub> for synthesis of silver nanoparticles. After that, the solution was kept on magnetic stirrer for constant stirring at room temperature and pH 7.2. The colour change of the solution indicates that silver is reduced to Ag nanoparticles. The reaction of silver nitrate solution with algal extract was optically measured using UV-vis spectrophotometer.

The roles of pH and temperature on the nanoparticles synthesis were determined. For this optimization pH of the algal extract was changed to 6.2, 7.2, 8.2, and 9.2 by using 0.1 N sodium hydroxide and 0.1 N hydrochloric acid. To study the effect of temperature in the nanoparticles synthesis, the experiments were carried out at different temperature of 30 °C, 40 °C and 50 °C. The nanoparticles exhibited different colours based on the pH and temperature as seen by UV-Vis spectrophotometer.

### Characterization of algae assisted synthesized silver nanoparticles

The reduction of silver ions to nanoparticles was primarily identified by double beam UV-Visible Spectrophotometer (Perkin Elmer, Singapore). The shape and size of the silver nanoparticles were characterized by using Scanning Electron Microscope (Hitachi, Model: S-3400N) and Transmission Electron Microscope (PHILIPS, CM200).

### Antifungal activity of silver nanoparticles

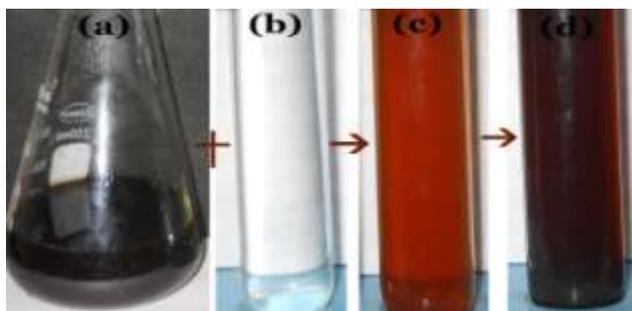
The opportunistic infections causing fungi were *Aspergillus niger*, *Aspergillus fumigatus*, *Candida spp*, *Fusarium sp* and *Aspergillus flavus* which were grown in Potato Dextrose agar at 28 °C for 3 days. Inoculum suspensions were prepared by scraping the surface of the colonies using sterile needle and the fungal spores were mixed with 10 ml sterile distilled water. Each fungal suspension was swabbed uniformly using sterile cotton swabs onto the individual plates containing sterile Rose Bengal agar. About 2 wells of 5 mm diameter were prepared with the help of a sterilized forceps. The 50 µL concentration of silver nanoparticles and algae extract solution were loaded in each well on all plates. Similarly, the disc diffusion method was performed to find the inhibition activity of silver nanoparticles against pathogenic fungi. About 5 mm in diameter sterile paper discs were dipped into the 50 µL concentration of silver

nanoparticles solution and algae extract and these were air dried. After that the fungal spores were spread on the Rose Bengal agar plates and the discs were placed on it. The commercial antibiotic discs streptomycin was used as control. Then the plates were incubated at 37 °C for 48-78 h. A clear zone of inhibitions around the wells was examined and the diameter of zone of inhibition was measured for each organism. The diameter of zone of inhibition was expressed in millimeter. Triplicate of experiments was carried out to find the statistical analysis.

## RESULTS AND DISCUSSION

### Visual identification

The present study shows the synthesis of silver nanoparticles by using the brown seaweeds *Turbinaria conoides*. Herein, the silver nitrate was reduced into silver nanoparticles identified by colour change. Figure 1 shows the images of (a) algal extract (c) after the addition of silver nitrate with algal extract and (d) after 24 hr incubation of silver nitrate with algal extract. The colour of the solution was changed to brown colour immediately after addition of pure extract of *T. conoides* to the aqueous silver nitrate solution. The intensity of brown colour was raised while increasing the time of incubation. The colour was changed into dark brown after the 24 hr incubation (Figure 1c&d) reaction mixture [19,20]. After 24 hr, there was no colour change which indicates that the Ag nanoparticles synthesis process was completed.

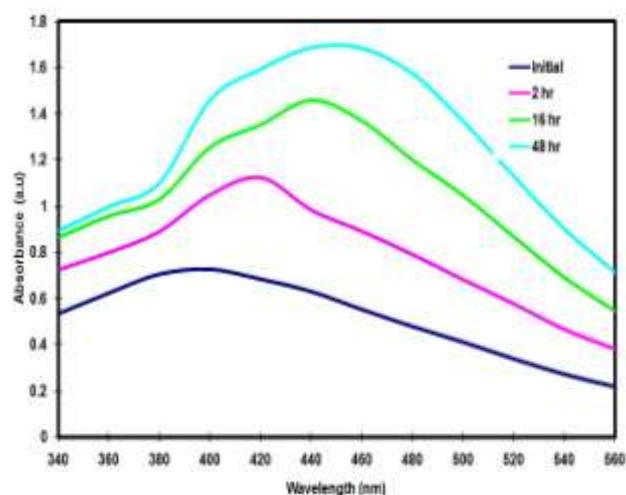


**Figure 1: Colour change of reaction mixture of 10 ml algae extract with 90 ml of 1 mM AgNO<sub>3</sub> indicates the nanoparticles synthesis (a) pure algae extract (b) silver nitrate solution (c) after the addition of algae extract with silver nitrate (d) after 24 hr incubation**

### UV-vis spectrophotometer analysis

In silver NPs synthesis, the absorbance due to AgNPs was observed at 2 hr and it gradually increased up to 48 hrs (Figure 2). The UV-vis spectra show the peaks at around 400-440 nm which correspond to the characteristic surface plasmon resonance due to excitation of electrons in the synthesized silver nanoparticles. The broadening of the absorption band started at the beginning of the reaction due to the formation of large sized

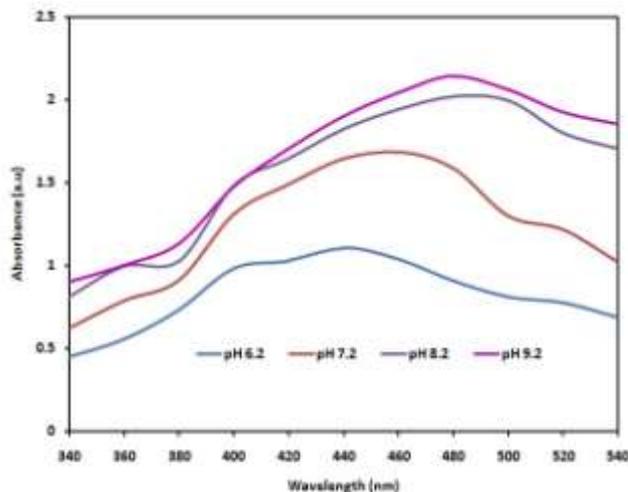
nanoparticles with poly-dispersed nature [21]. With an increasing incubation time, there is an enhancement in the nanoparticles synthesis which was noticed by increasing in the intensity of absorbance from 420 to 440 nm. From initial time to 8 hrs, the SPR band of silver nanoparticles was observed in the range of 400 to 420 nm. Then, SPR band was changed to 440 nm during 16 hrs to 48 hrs indicating an increase in the sizes of the particles [22]. At the beginning of the nanoparticle synthesis reaction (Initial to 2 hr), the small sized spherical nanoparticles are synthesized due to the presence of enough amount of reducing agent. Afterwards (16 to 48 hr), less amount of reducing agent resulted in the production of large sized spherical nanoparticles.



**Figure 2: UV-vis spectrum of synthesized silver nanoparticles recorded as different functional time indicates respective SPR band at 440 nm.**

### Effect of pH

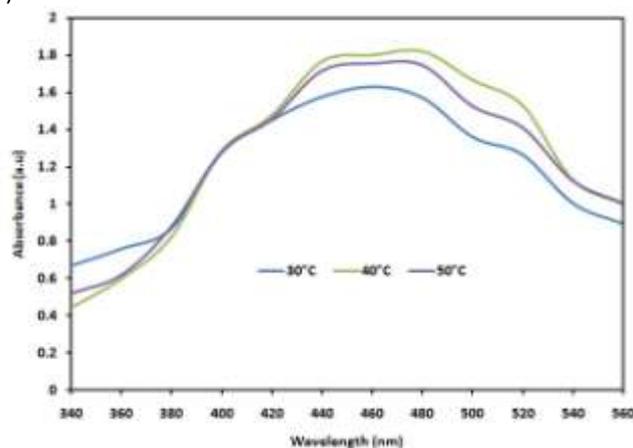
The behavior of pH in the nanoparticles synthesis was determined by changing the pH of the algae extract from 6.2 to 9.2. Overall, the SPR band of silver nanoparticles was observed in the range of 400-500 nm with broadened peak (Figure 3). The absorbance of silver nanoparticles was increased with increasing pH which indicates the synthesis of large sized silver nanoparticles. The alkaline pH favors (8.2 and 9.2) the reaction rate, but due to the presence of enough amount of reducing agent large sized nanoparticles was obtained. The absorption peak character of silver nanoparticles was different at the various pH [23]. Several studies revealed that stable silver nanoparticles were synthesized at high pH range [24]. From this study we concluded that at low pH the polydispersed nanoparticles with the decreased sizes were synthesized.



**Figure 3: Effect of pH in the silver nanoparticles synthesis**

#### Effect of temperature

Temperature is one of the important factors for all chemical reactions. In this work we studied the role of reaction temperature in the formation and growth of silver nanoparticles using *T. conoides* extracts. With increase in the temperature from 30 to 40°C, the absorbance of silver nanoparticles also increased (Figure 4).

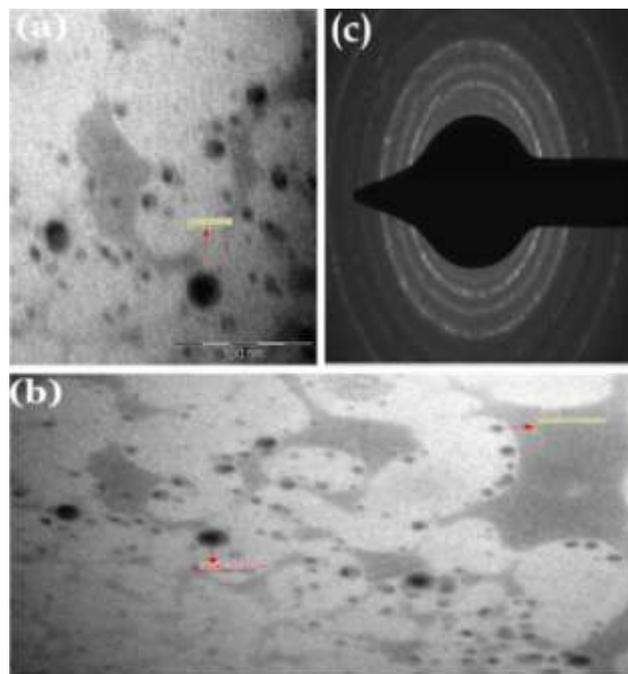


**Figure 4: Effect of temperature in the synthesis of silver nanoparticles**

At 30°C, the SPR band was at 460 nm and it changed into 480 nm at 40°C. The absorbances at 50°C and at 40°C reveal the completion of silver nanoparticle synthesis process at 40°C. The red shift of SPR bands from 460 nm to 480 nm was due to the formation of large sized nanoparticles. More than two bands are formed at 40 °C which indicates presence of anisotropic nanoparticles in the reaction mixture. Huang et al [25] reported that maximum reduction of metal ions to nanoparticles occurred at high temperature.

#### TEM and SAED analysis

The size, shape and distributions of nanoparticles were clearly observed by using TEM analysis [26]. Figure 5(a) exhibits the TEM images of silver nanoparticles synthesized by using *T. conoides*. The shape of the nanoparticles was clearly observed and most of the particles are spherical and some of the particles are rounded rectangle like structures. The average size of *T. conoides* synthesized nanoparticles was 14 nm and 26 nm, respectively. Figure 5(b) corresponds to high resolution lattice image of some particles. The nanoparticles synthesized from plant extracts such as *Desmodium triflorum* and *Diopyros kaki* were predominantly spherical in shape and they appeared to be monodispersed [27, 28]. The selected area electron diffraction (SAED) pattern shown in Figure 5(c) suggested the synthesized silver nanoparticle to be crystalline in nature. The rings bound in the distance which corresponds to (1 1 1), (2 0 0) and (2 2 0) confirm the crystalline silver nanoparticles.



**Figure 5: TEM and SAED pattern of algae mediated synthesized silver nanoparticles**

#### SEM and EDS analysis

A similar tendency is also observed in the SEM images obtained by the reaction of brown seaweed *T. conoides* broth and 1 mM silver nitrate solution (Figure. 6a). It is shows spherical structures with sizes up to 50 nm. The EDS spectra recorded from the silver nanoparticles are shown in figure. 6b. The EDS profile shows a strong silver signal at 3 keV along with weak oxygen and carbon peaks, the weak peaks may have originated from the biomolecules bound to the surface of the silver nanoparticles [29]. It has been reported that nanoparticles synthesized using algal extracts are

surrounded by a skinny layer of some capping biological material from the algae extracts and are, thus, stable in solution up to 90 days after synthesis. This is another advantage of nanoparticles synthesized using green materials over those synthesized using toxic chemical methods.

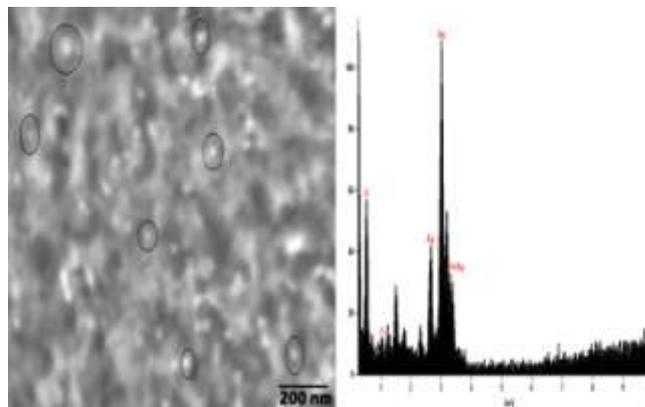


Figure 6: SEM and EDS spectrum of silver nanoparticles

#### Antifungal activity

The antifungal activity of *T. conoides* synthesized silver nanoparticles was investigated against pathogenic fungi *A. flavus*, *A. niger*, *A. fumigatus*, *C. albicans*, and *Fusarium sp.* by disc diffusion method. These selected fungi are human pathogenic and cause the skin and hair infections. Synthesized silver nanoparticles showed high inhibition zone against all the pathogenic fungus at 50  $\mu$ L concentration of silver nanoparticles (Figure 7&8). Maximum zone of inhibition was found to be with *Candida albicans*, *Fusarium spp*, *Aspergillus fumigatus*, *Aspergillus niger*, and minimum inhibition zone was found to be with *Aspergillus flavus*. *A. flavus* is more pathogenic and greater virulence, it produces mycotoxins. Toxin segregation was controlled and decreased while treating with silver nanoparticles. *C. albicans* causes skin diseases and *Aspergillus* species causes aspergillosis. In our study we found that algae mediated synthesized silver nanoparticles acts as good antifungal agent to control these opportunistic fungi. Similarly, Govindaraju et al [29] reported the inhibitory action of silver nanoparticles against *A. niger* and *A. flavus* are 14.8 mm and 15.2 mm, respectively at 100  $\mu$ L concentration. Prasad and Elumalai [21] studied the antimicrobial activity of silver nanoparticles against *C. albicans* reported 10 mm of inhibition. In this study, synthesized silver nanoparticles from algae are toxic to multi drug resistant microorganisms and when compared to previous report we got best results against the pathogenic fungal strains. It shows that they have great potential in biomedical applications. High inhibitory activity of silver nanoparticles against fungi may be due to the interaction with proteins leads to the inactivation of fungi growth and direct interaction with DNA resulted

in inhibition of replication [31, 32]. Mutation in fungal DNA can be caused by displacing of hydrogen bonds in the base pairs [33, 34]. Silver nanoparticles form the pits in the cell wall and damage the cell permeability [35] and induce the proton leakage caused by ROS in the membrane [36] resulting cell death. Woo et al [37] demonstrated that silver nanoparticles inhibits the conidial germination on fungi.

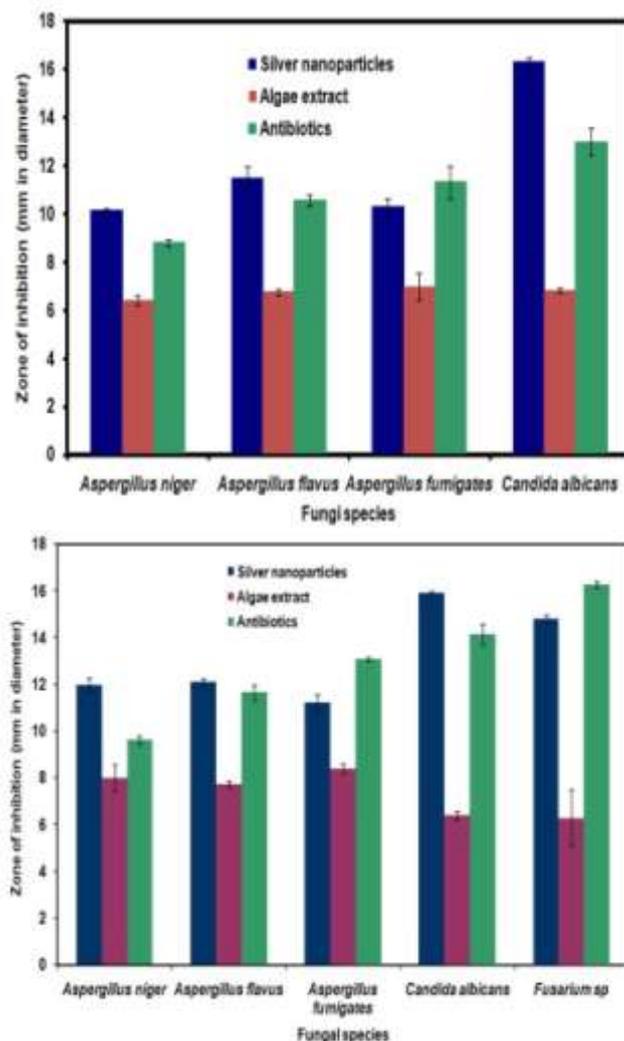


Figure 7: Antifungal activity of *T. conoides* assisted silver nanoparticles shows zone of inhibition around the well and disc.

Specially, the cell wall of seaweeds contain many functional molecules like amine, carboxyl, sulphate, phosphate and imidazoles associated with polysacchride, alginic acid and proteins which enable them to bind with heavy metal ions i.e. silver to cause reduction to silver nanoparticles. These functional molecules associated with synthesized silver nanoparticles resulted in more antifungal activity. Because these combinations inhibit the spore germination and control their spreading in the environment. Finally we concluded that marine macro

algae mediated synthesized the silver nanoparticles have a great potential to controlling the spore producing fungi.

## CONCLUSIONS

This study demonstrated that algae mediated rapid and stabilized synthesis of silver nanoparticles. The Surface plasmon resonance band was formed at 440 nm. The most effective parameters pH and temperature were studied. TEM and SEM were used to characterize the size and shape of nanoparticles. Crystalline structure was confirmed by SAED and presence of elemental silver at 3keV was analyzed by EDS spectrum. Algae mediated synthesized silver nanoparticles shows high antifungal potential against human pathogenic fungi *Candida sp*, *Fusarium sp*, *A. flavus*, *A. niger* and *A. fumigatus*. It shows higher zone of inhibition against clinical pathogenic fungi. It can be concluded that the formulation of silver nanoparticles could be used as effective antibacterial agent. This study also revealed the formulation of new types of fungicidal effects against antibiotic resistant fungi and eco-friendly synthesis of silver nanoparticles for pharmaceutical applications.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

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