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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING MARINE BROWN ALGAE *TURBINARIA CONOIDES* AND ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

Improvement of eco-friendly procedures for the nanoparticles synthesis is the growing in to the field of nanotechnology. In this report we used the extract of marine brown seaweed *Turbinaria conoides* for the silver nanoparticles synthesis is the new one, cost effective and environmentally favorable. The surface plasmon resonance band positioned at 420 nm for silver nanoparticles was characterized by UV-Visible Spectrophotometer. X-Ray diffraction (XRD) data illustrated the crystalline nature of silver nanoparticles. Scanning electron microscope (SEM) shows the spherical shaped with the average size of 96 nm. The possible biomolecules are amines and poly phenols may responsible for reduction of silver ions was identified through Fourier transmittance infrared spectroscopy (FT-IR). Further these biosynthesized silver nanoparticles were found to be highly toxic against gram positive bacteria *Bacillus subtilis* (MTCC3053) and gram negative bacteria *Klebsiella planticola* (MTCC2277) was analyzed by zone of inhibition.

KEYWORDS: ***Turbinaria conoides*, Silver nanoparticles, Scanning electron microscope, Antimicrobial activity**

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INTRODUCTION

Nanotechnology is the enhancing the properties of materials in the form of nano-sized particles. Nanoparticles are the basic essential elements in the wall of nanotechnology and it exhibits fabulous advanced characteristic features based on their properties such as size, morphology and other size dependent properties[1]. In this current century, application of nanoparticles is hasty impetus due to their chemical, optical and mechanical properties[2]. Nanoparticles are one of the nearly everyone sought materials for the future significant in many of the fields. Noble metals are palladium, silver, platinum and gold they exhibit a particularly wide range of material behavior along the atomic to bulk transition[3]. Among these noble metals silver have wide applications in jewellery, dental alloy and health additive in traditional Chinese and Indian Ayurvedic medicine[4]. Silver nanoparticles have received considerable attention because of their unique chemical and physical properties, which differ greatly from those of bulk materials, as well as their potential for technological applications[5]. Silver nanoparticles exhibit tremendous applications in drug delivery[6], wound healing[7], sensor applications[8, 9, 10], cosmetics[11], textile industry and also used antimicrobial agent in paint[12]. Silver nanoparticles were actively involved in the medical sciences due to their antimicrobial actions in food pathogens *Staphylococcus aureus* and *Escherichia coli*[13] , *Bacillus subtilis* and *Klebsiella mobilis*[14], *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*[15], meningitis causative microbe *Cryptococcus neoformans*[16], methicillin-resistant *Staphylococcus aureus*, methicillin-resistant *Staphylococcus epidermidis*, *Streptococcus pyogenes*, and *Salmonella typhi* [17] and having good antifungal activities against *Candida albicans, Aspergillus niger, Penicillium citrium,* and *Aureobasidium pullulans* were isolated from waste suspension of kitchen drainage[11]

Synthesis of silver nanoparticles by biological method using bacteria, fungi, algae, enzymes and plant extracts has more advantages due to their environment benign process and ability of large scale production over physical and chemical methods[18]. Green synthesis of silver nanoparticles by algae extract shows more advantageous over other biological processes are bacteria and fungi, because it eliminates the cell culture maintaining process, and also it more suitable for large scale production of silver nanoparticles[19]. Marine brown seaweed *Turbinaria conoides* is the one of the medicinally valued macro algae and having numerous medicinal properties. These brown algae were coming under the order of Fucales in the family of Sargassaceae. The components found in that is major amount sterols some are Fucosterol, and different molecules containing vinyl and ethyl cholesterol types[20], cyclohexane[21], and some sulfated polysaccharides fucoidan, neutral glucan and guluronic and mannuronic acid residues containing alginic acid are found and providing a medicinal value for the brown algae. The healing properties of the Turbinaria conoides are antioxidants[22], antiproliferative, antiinvasive, antiangiogenic[23], antihistaminic[21] and antiviral (human embryonic lung, human epithelial (HeLa) cells and Vero cells). Apart from this it had the good antimicrobial effect against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger,* and *Candida albicans* [24]. In this study we used very low cost and medicinally significant green material marine brown seaweed *Turbinaria conoides* for the biosynthesis of silver nanoparticles. The synthesized silver nanoparticles were characterized using UV-visible spectrophotometer, SEM, XRD and FT-IR. Antibacterial activities of the medicinally valid algal mediated nanoparticles were also examined against *B. subtilis* and *K. planticola*.

MATERIALS AND METHODS

(i) ***Collection of seaweed and Chemicals*** The marine brown seaweed *Turbinaria conoides* was collected from Mandapam Coastal area in South India. Silver nitrate, Nutrient broth and Muller Hinton agar were purchased form Himedia Laboratories Private Ltd., Mumbai.

(ii) Preparation of pure algal extract (PAE): Collected brown seaweed *Turbinaria conoides* leaves were washed with distilled water and dried in shadow place for two to three days at room temperature. The leaves were cut into small pieces and grind into algal powder. The pure algal extract (PAE) was prepared by adding 1 g of algal powder into 100 ml of distilled water and 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.

(iii)Biosynthesis of silver nanoparticles: In the typically synthesis process of silver nanoparticles, add 10ml of pure algal extract into the 90ml of 1mM of silver nitrate solution in 250 ml conical flask. The reaction mixture was kept at room temperature under mechanically stirring. The colour change was noted and nanoparticles formation was monitored using UV-vis Spectrophotometer periodically.

(iv)Characterization of prepared nanoparticles: The reaction of silver nitrate solution with algal extract was optically measured using double beam UV-Visible Spectrophotometer (Perkin Elmer, Singapore) in the different wavelength range of 340 nm to 700 nm. The synthesized silver nanoparticles were centrifuged at 10,000 rpm for 20 min, and collect the pellet. The pellet was washed with distilled water for several times to remove impurities and dried to get powder. The X-Ray diffraction assay was performed for the detection of crystalline nature of the metal nanoparticles was done by powder X-Ray Diffractometer (Bruker, Jermany, model: D8 Advance). Shape and size of the silver nanoparticles were characterized by using Scanning Electron Microscope (Hitachi, Model: S-3400N). The FT-IR analysis was carried out in a MAKE – BRUKER Optik GmbH MODEL No - TENSOR 27. The dried silver nanoparticles were grinded with KBr pellets and measured at the wavelength range from 4000 to 400cm-1.

(v) Antimicrobial activity: Antibacterial activity of *Turbinaria conoides* assisted silver nanoparticles was carried out by disc diffusion method against Gram Positive and Gram Negative Bacteria. Bacterial cultures *Bacillus subtilis* (MTCC3053) and *Klebsiella planticola* (MTCC2277) were purchased from MTCC, India. These bacterial cultures were freshly cultivated for 24 h in Nutrient broth. Each bacterial culture was spread on the Muller Hinton agar plates. Sterile paper discs containing three different concentrations of silver nanoparticles were placed and incubated. After the 24 hours of incubation the zone formation was recorded. Experiments were repeated for three times.

RESULTS AND DISCUSSION

Pure algal extract (PAE) was added into silver nitrate solution. With in few minutes the appearance of brown colour was observed and it indicates the formation of silver nanoparticles (Fig 1a). The colour was changed into dark brown after the 1 h incubation (Fig 1b) due to the excitation of free electrons in the reaction mixture[25]. The reduction of silver ions into silver nanoparticles by using *Turbinaria conoides* seaweed was analyzed by UV-vis Spectrophotometer. Fig 2 shows the UV absorption spectra of the synthesized silver nanoparticles using the extract of brown seaweed *Turbinaria conoides* recorded as the function of reaction time. Absorption spectrum shows that the peak positioned at 420 nm indicated the formation of silver nanoparticles. During initial reaction time the band was broad and the peak positioned at 440 nm due to the formation of large size of nanoparticles in the initial time. Broadening of the peak indicates particles are polydispersed[26]. After 1 h of incubation the band shift into 420 nm and the reaction was took place very hasty. As increasing the reaction time, the reaction rate was gradually increased. Thus synthesized silver nanoparticles solution by using brown seaweed was very stable for three months which was detected by a surface plasmon resonance in the range of 420nm. In this study, algae extract mediated synthesized silver nanoparticles was rapid process and stable for several months due to the presence of stabilizing agent in the algal extract.

Formation of nanoparticles identified by colour change

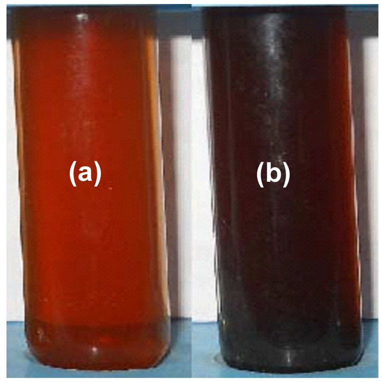

Figure 1
The formation of silver nanoparticles was identified by colour change (a) initial reduction of Silver ions (b) after 1 h of reaction between silver ions and pure algal extract.

UV-Vis Spectrophotometer analysis

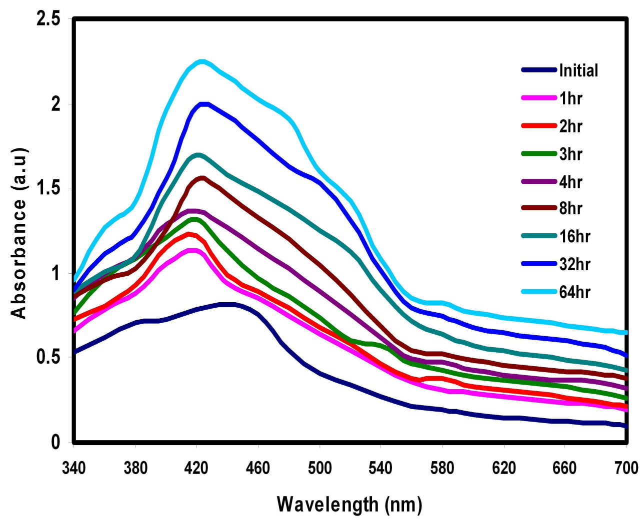

Figure 2
UV Spectroscopic analyses of silver nanoparticles synthesized from extract of Turbinaria conoides recorded as function of time.

The crystalline structure of silver nanoparticles was confirmed by X-ray diffraction pattern. The 2θ values of the XRD pattern was ranging from 30 to 80° and three strong peaks were observed at 38.1º, 46.3º and 64.3º were corresponds to the planes (111), (200) and (220) respectively (Fig 3), which are indexed to the face centered cubic structures of silver nanoparticles (JCPDS file no. 84-0713 and 04-0783). The XRD pattern of these peaks indicates the silver nanoparticles is 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 mushroom extract[27].

X-Ray Diffraction Analysis

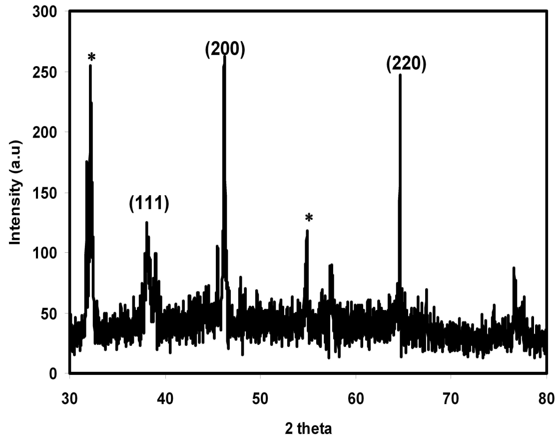

Figure 3
XRD patterns of silver nanoparticles shows crystalline nature of nanoparticles

The morphology of silver nanoparticles was characterized using Scanning Electron Microscope. SEM image shows the morphology of the nanoparticles. The Fig 4a shows silver nanoparticles were deposited on the surface of the algal extract (indicated by arrows). Similarly early reports illustrated that gold nanoparticles were deposited on the surface of the mycelium of *Phanerochaete Chrysosprium*[28]. The Figure 5b shows the magnified view of algal assisted silver nanoparticles with the spherical shape and average size of the nanoparticle was 96 nm. The more stable spherical shape and isotropic nanoparticles was formed by the action of large number of biomolecules ranged in the solution.

SEM image of silver nanoparticles.

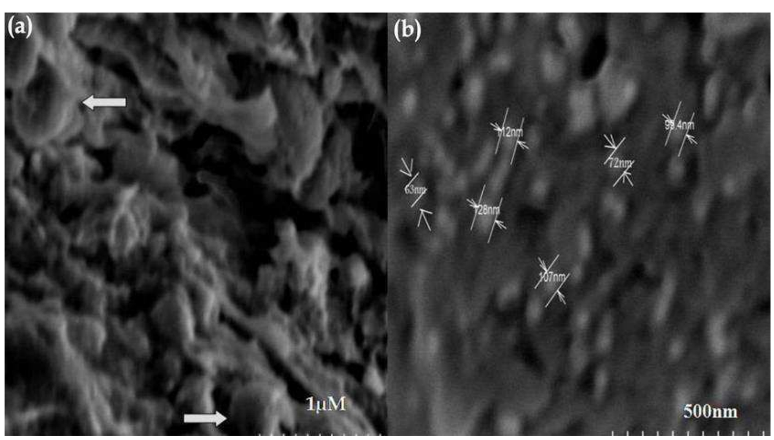

Figure 4
Scanning electron micrographs of the algal mediated synthesis of silver nanopartilces 1μM (a): inset arrows shows spherical like structure of nanopartilces in the surface of algae (b).

The possible potential biomolecules responsible for the reduction of silver ions to silver nanoparticles was identified using FT─IR analysis. Figure 5 shows the FT─IR spectrum of algal assisted silver nanoparticles. The band at 3421.3 cm^{-1} represents N─H stretching groups of amides. The band at 1627.3 cm^{-1} corresponds to N─H groups of primary amines. The peak at 1382.7 cm^{-1} corresponding to amide II and amide III of aromatic rings either may by poly phenols associated with synthesized silver nanoparticles which is segregated by algal extract. The band at 1,026.4 cm^{-1} shows that C─O stretching vibrations of alcohols, carboxylic and C─N stretching of aliphatic amines. The intense peak of 821.8, 637, 540, and 434.4 cm^{-1} shows that C─Cl, C─Br and C─I group of alkyl halides. The groups of monosaccharide and polysaccharides were found in the *T. conoides*[22] may be possible to involve in the synthesis process of silver nanoparticle. The biological molecules such as secondary metabolites could possibly play major role in the synthesis and stabilization of the metal nanoparticles was proved[29].

FT-IR Spectrum of synthesized silver nanoparticles

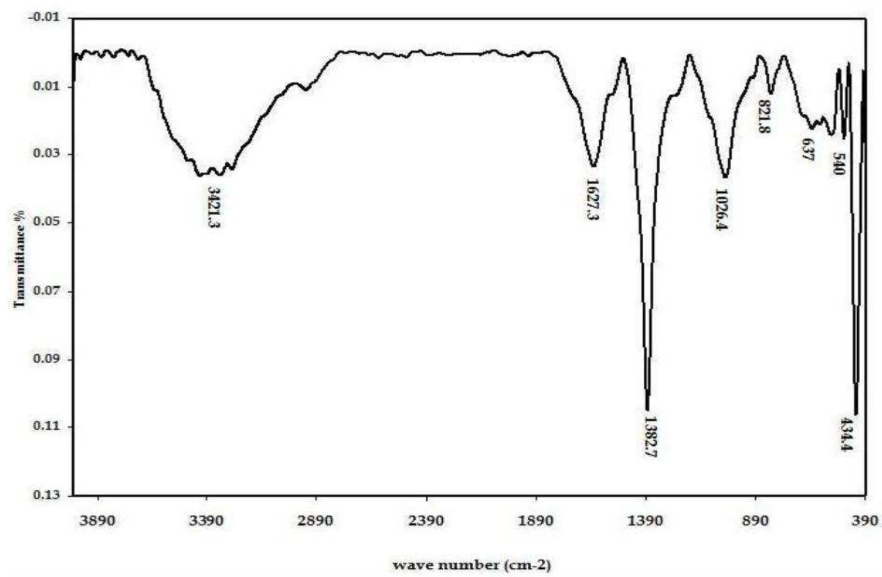

Figure 7
FT-IR Spectrum of Turbinaria conoides mediated synthesized silver nanoparticles

Antibacterial Activity

The antimicrobial activity of *Turbinaria conoides* mediated silver nanoparticles was performed against pathogenic bacteria *Bacillus subtilis* (MTCC3053) and *Klebsiella planticola* (MTCC2277) using disc diffusion method. Table 1 shows the three replicates experiments of zone of inhibition (mm) around the well with *T. conoides* mediated synthesized silver nanoparticles. In gram positive bacteria the action of silver nanoparticles was very less zone of inhibition in the range of 10.33±0.883 in 300µl concentration. The antibacterial activity of synthesized silver nanoparticles was high against gram negative bacteria *Klesiella planticola* in the inhibition range was 20.67±0.668 at the concentration of 300µl. The inhibition variation was occurs due to the differences of cell wall composition in gram positive and gram negative bacteria. Gram positive bacteria was made up of thick cell wall contain peptidoglycon so that nanoparticles did not affect easily. But the nanoparticles was easily penetrated into gram negative bacteria due to structure of cell wall contain thin lipid layer, so nanoparticles easily enter into the cell and disturb it. Still the exact mechanism of inhibitory action of nanoparticles against bacteria was not well known. The formations of free radicals from the surface of the silver nanoparticles were responsible for the antibacterial function[30]. Silver nanoparticles were attaching with the surface of the bacteria and act against the cell wall protein and control the power of bacteria, apart from this small particles attach with the larger surface area was clearly explained[31]. The numeral of bacterial colonies developed on agar plates as a role of the different concentration of brown seaweed assisted silver nanoparticles when steadily declined when the nanoparticles concentration increased. Silver nanoparticles were play a role in various medical applications are silver coated medical devices as nanogels, nanolotions, dental materials, sun screen lotions, water treatment and silver based dressings was proved[2]. These studied results undoubtedly reveal that newly synthesized nanoparticles were assuring antibacterial agent against the pathogens employed.

Table 1
Zone of inhibition of brown seaweed Turbinaria conoides mediated silver nanoparticles

Concentration of silver nanoparticles	Zone of inhibition (mm in diameter)	
	Bacillus subtilis	*Klebsiella planticola*
100 µl	6.67±0.334	10.33±0.883
200 µl	8..00±0.578	11.33±0.334
300 µl	10.33±0.883	20.67±0.668

± Standard deviation

CONCLUSION

In this present study, we have proposed a green approach for synthesizing silver nanoparticles using low cost marine brown seaweed *Turbinaria conoides* as a reducing mediator. We investigated the nanoparticles formation using UV-Vis spectrophotometer, the crystalline nature of formed nanoparticles was confirmed by using X-Ray Diffraction patterns, SEM micrograph demonstrated that the average size of the nanoparticles was about 96 nm and the structure was spherical. The possible biomolecules amide and polyphenol groups may responsible for the reduction of silver nitrate to silver nanoparticles are identified by FT-IR. The formed metal nanoparticle was found to have wider antibacterial activity in *Klebsiella planticola* than *Bacillus subtilis*. We trust that the silver nanoparticles have great promising for application in biological based nanomedicine, biosensors and food industries.

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