ABSTRACT

Currently, Green nanotechnology principle is highly applicable in synthesis of nanoparticles through simple techniques. In this study, we demonstrate the synthesis of silver nanoparticle by ethanol extract of algae fungi intermediate lichen Parmotrema perlatum. The synthesized silver nanoparticles were analyzed by UV visible spectrophotometer, XRD, FTIR, and SEM analysis. Lichen extract could be able to synthesize silver nanoparticles as proved by gradual change in the color of the reaction mixture consists of 10 ml extract in 1mM AgNO3 to dark brown color. Silver nanoparticles formation was confirmed by surface Plasmon resonance (SPR) peak obtained around 400 – 420 nm in UV Visible spectrophotometer. The morphology of the biogenic silver nanoparticles was identified by SEM analysis and is polydispersed in nature. The occurrence of silver in the sample and its crystalline nature was confirmed by X-ray diffraction technique. Silver nanoparticles showed 2Θ values within the range of Bragg’s reflection (38.06 and 77.43) consequent to the existence of silver nanocrystal as obvious by X-ray diffraction spectrum. FTIR results were used to identify the biomolecules which is responsible for the silver ion reduction. The FTIR results of lichen extract synthesized silver nanoparticle displayed the prominent peaks in (3210.17, 2849.93, 2917.38, 1652.85, 1609.87, 1452.63, 1378.45, 1145.25, 823.36, 585.50, 527.27) in different ranges. These biogenic silver nanoparticles exhibited substantial
antibacterial efficacy against both Gram positive and Gram negative organisms such as Enterococcus, streptococcus, Escherichia coli, pneumonaiæa, klebsiella, serratia, planomicrobium. The Invitro antibacterial test 100μg disk⁻¹ concentration displayed the results of maximum inhibition zone with the Enterococcus (30 mm) and minimum inhibition zone with klebsiella sp (7mm). The antibacterial activity of silver nanoparticles against these bacterial cultures was evaluated to obtain their promising use in silver containing antibacterial product. Lichen extract has the ability to treat various skin diseases and applying in wound healing treatments. Thus the silver nanoparticles synthesized by lichen extract may have the potential biomedical applications.

KEYWORDS: Parmotrema perlatum, silver nanoparticle, Characterization, antibacterial activity.

INTRODUCTION

Nanotechnology field is one of most active research ground in current materials science. Applications of nanoparticles and nanomaterials are rising fastly due to its improved properties such as size, distribution and morphology (Silvestre et al., 2011). The production of nanoparticles above the last 25 years leads to the industrialists and consumers have profited from the technological revolution. Through their antimicrobial properties, silver nanoparticles have been used in textiles, food contact surfaces, building materials, vehicle interiors, cosmetics, paints, air conditioners and medical applications (Han et al., 2005; Blaser et al., 2008; Panacek et al., 2009; Marambio-Jones and Hoek, 2010). Manufacture of silver nanoparticles expected to be over 500 tones yearly worldwide (Mueller and Nowack, 2008). The rapid production time, environmental friendly nature, low cost of development and the capability to up fabrication quantity construct plants an utilizable platform for synthesizing nanoparticle (Njagi et al., 2011).

Silver nanoparticles accumulated at 50 nm to a high level in Brassica juncea (mustard greens) and Medicago sativa (alfalfa) while grown on silver nitrate as substrate (Harris and Bali 2008). Inspite of the actuality that green synthesis of nanoparticles via plant material is of significant interest, it is value learning the uniformity of the nanoparticles synthesized through physical and chemical methods to their potential applications and creation scalability. It is well recognized that appliances of conventional physicochemically synthesized metal nanoparticles in drug delivery, molecular imaging, waste water treatment, fuel elements,
catalysis, coatings, cosmetics, biosensor, and as antiseptics. Green synthesized nanoparticles have been examined in small number of practical applications. Nanoparticles manufactured in plant extracts previously have a functionalized surface that can includes protein, polysaccharides, organic ligands, polyatomic alcohols which are lack in physicochemically synthesized nanoparticles. Biological components presence upholds the stability of the nanoparticle and if essential, may also make possible consequent attachment of functional molecules such as antibodies or DNA to nanoparticles (Sintubin et al., 2012). The intention of this study was to synthesize silver nanoparticles using the ethanolic extract of the lichen *Parmotrema perlatum*.

Lichens are a composite organism comprises of a mutual relationship between a fungus and green algae (Jain et al., 2009). Lichen belongs to the family parmeliaceae. *Parmotrema perlatum* is collected in huge amount as a food ingredient in India. It is utilized in treating wounds, infections, inflammation, skin diseases, diarrhea, dysentery, cough, fever and renal calculi (Tay et al., 2004). The synthesis, characterization and antibacterial activity were evaluated in this study. To the best of our knowledge no data about the nanoparticle synthesis of the lichen *Parmotrema perlatum* is available for the past decades. Furthermore the antibacterial activity of biosynthesized nanoparticles was investigated by disc diffusion method.

**MATERIALS AND METHODS**

*Parmotrema perlatum* was purchased from the local market in tenkasi. Silver nitrate, Mueller-Hinton broth, and nutrient broth were purchased from Hi media laboratories, mumbai.

**BIOSYNTHESIS OF SILVER NANOPARTICLES**

An extract of *Parmotrema perlatum* was prepared by mixing 10 g of powdered lichen with 100 ml of ethanol. After that the solution was filtered through Whatman No 1 filter paper to remove plant debris. About 10 ml of ethanolic extract of lichen was mixed with 90 ml of an 1 mM AgNo3 solution and allowed to react at room temperature for 24 hours. The color change from light brown color into brown color indicating the formation of AgNps.

**CHARACTERIZATION OF SILVER NANOPARTICLES**

The reduction of silver ions to AgNPs solution was examined by UV-spectra at the wavelength from 340 to 740 nm (Perkin Elmer Lambda double beam UV-
Spectrophotometer). The obtained silver nanoparticles were centrifuged for purification process at 15,000 rpm for 2-3 times. After that the solution was filtered and pellet was dried in the hot air oven. Then the dried powder was further examined by XRD (Philips PW 1830). The functional group present in the nanoparticle was monitored on a SHIMADZU instrument with the sample as KBR pellet in the wavenumber region of 500-4,000 cm⁻¹. The physical appearances of the synthesized nanoparticles were analyzed by Scanning Electron microscopy (Philip model CM 200).

**GERMICIDAL EFFECT OF SILVER NANOPARTICLES**

The germicidal effects of silver nanoparticles were estimated by using well diffusion method. Seven bacterial cultures such as *Escherichia coli, Enterococcus, Streptococcus, Pneumoniae, planomicrobium, serratia* and *klebsiella planticola* were purchased from MTCC. Sterile nutrient agar plates were prepared and latter test organism swabbed along with 24 h growing cultures were grown in Mueller hinton agar petridishes with different concentrations of silver nanoparticle of well such as 25μl, 50μl and 75 μl and 100 μl respectively. The antibacterial activity was measured by the zone formation in the petridishes after incubation for 24 hours.

**RESULTS AND DISCUSSION**

![Figure 1: A - 1mM Agno3 solution B- Lichen extract C- Initial color change of reaction mixture  D – After 24 hours of reaction](image)
Figure 2: UV Visible spectrum of biogenic synthesized silver nanoparticles by using Parmotrema perlatum ethanol extract

In this work biosynthesis of silver nanoparticles by utilizing Parmotrema perlatum was performed and the detailed study is reported. Silver nanoparticle formation by reduction of aqueous metal ions during the exposure of Parmotrema perlatum extract may be followed by UV-visible spectroscopy. UV-visible spectrophotometer analysis screened the development and stability of the reduced silver nanoparticle in aqueous solution. It was observed that the solution color changed light brown and next dark brown following 1 hr and 24 hr of reaction which confirmed the nanoparticle synthesis in Fig 1 C & D. The surface Plasmon absorption band was observed at 420 nm with different reaction times in Fig 2. This examination revealed that the reduction of Ag$^+$ ions obtained extracellularly by Parmotrema perlatum extract. Sequentially to confirm UV-visible spectral analysis results, the samples were further examined by XRD analysis. Metallic nanoparticles exhibited color because of the consistent excitation of all free electrons within the conduction band directing to an in-phase oscillation which is recognized as surface Plasmon Resonance- SPR (Akanna et al., 2010). Based upon the size and shape of the metal nanoparticles, dielectric constant and surrounding medium the frequency and width of the surface Plasmon absorption takes place in the solution (Mukherjee et al., 2002). Literature survey exposed that the nanoparticle synthesis using algal fungi intermediate resource has been unfamiliar and unexploited. Currently few reported that symbiotic partner being utilized for metallic nanoparticle synthesis. The fabrication of silver, gold and silver-gold nanoparticle by using mushroom extract was achieved (Daizy Philip,
and utilization of marine alga for synthesis of silver nanoparticle (Govindraju et al., 2009). Marine fungus *Pencillium fellutanum* responsible for formation of extracellular synthesis of silver nanoparticle has been depicted by kathiresan and coworkers (Kathiresan et al., 2009). The commonly obtainable fungus *cladosporium cladosporiodes* originated in marsland regions was used to silver nanoparticle biosynthesis (Balaji et al., 2009).

XRD pattern obviously examined the crystalline nature of silver nanoparticles. The diffraction peak of silver nanoparticles obtained at (Fig 3) which can be assigned to sets of lattice planes. XRD examination proved that the silver nanoparticles synthesized in the experiments were in the nanostructures form as confirmed by the peaks at 2ϴ values of 38.06°, 44.23°, 64.40°, 77.43° were indexed with the planes (111), (200), (220) and (311) for the face centered cubic silver as per the JCPS file no 84-0713 and 04-0783. In literature, similar results were found for silver nanoparticles (Jagtap and Bapa 2013; Das et al 2013; Raman et al., 2012; Guidelli et al 2011). The unassigned peak was present due to crystallization of bioorganic molecule which may arise on the nanoparticle surface and also the XRD peaks were broadening all around their bases showed that the silver nanoparticles were in nanorange (Ahmad and Sharma 2012).

![XRD spectrum of biosynthesized silver nanoparticles using Parmotrema perlatum](image)

**Figure 3:** XRD spectrum of biosynthesized silver nanoparticles using *Parmotrema perlatum*

FTIR analysis revealed the size distribution and characterization of green synthesized nanoparticle by using *Parmotrema perlatum*. The presence of biomolecules in the
*Parmotrema perlatum* extract is responsible for the production of nanoparticles was recognized using FTIR analysis. The FTIR spectra of silver nanoparticle were characterized in the Fig 4 and the spectra exhibits numerous absorption bands signify the potential functional groups present in the lichen synthesized nanoparticles. The spectra exhibited the strong and broad peak at 3210 cm\(^{-1}\) assigned to O-H stretch and H bonded from alcohol and Phenolic compounds. The absorption was observed at 2917 cm\(^{-1}\) was identified as O-H stretching vibrations from carboxylic acids.

![FTIR spectrum of biosynthesized nanoparticles using *Parmotrema perlatum*](image)

**Figure 4:** FTIR spectrum of biosynthesized nanoparticles using *Parmotrema perlatum*.

The medium intense band was observed at 2849 cm\(^{-1}\) was assigned to C-H stretching of alkanes denotes the presence of alkanes in the lichen extract. The medium band observed at 1652 cm\(^{-1}\) was assigned due to the presence of \(-C=\text{C}-\) stretching vibrations of the functional group alkenes. The peak was observed in the region at 1609 cm\(^{-1}\) indicates the presence of primary amine in the lichen extract. The peak obtained at 1452 cm\(^{-1}\) corresponds to N-O asymmetric stretching vibrations from nitro compounds. The peak was observed in the region from 823 cm\(^{-1}\), 585 cm\(^{-1}\), 527 cm\(^{-1}\) indicates the presence of C-Cl stretching of vibrations from alkyl halides. FT-spectrum proved the presence of alcohols, Phenolic compounds, carboxylic acids, alkanes; alkenes, nitro compounds and alkyl halide may be responsible for the silver nanoparticle synthesis. These chemical bands are confirmed to have promising reducing agents in silver nanoparticles synthesis previously (Cho et al. 2005). The presence of vibrational peaks at 1654 cm\(^{-1}\) in extract indicates the chance of an aromatic compound (Bahgat and Ragheb 2007).
The surface morphology and size of the silver nanoparticles was examined by scanning electron microscope. SEM image had revealed the individual nanoparticles and polydispersed in nature. It shows the nanoparticles are non uniform in shape and larger particles with no well defined morphology in Fig 5. In the current research small silver nanoparticles are attached to large silver nanoparticles. The obtained results were agreed with ashok Kumar who synthesized silver nanoparticle using the leaf extracts of Parthenium hysteroporus.

![SEM images of biosynthesized silver nanoparticles using Parmotrema perlatum](image_url)

**Figure 5:** SEM images of biosynthesized silver nanoparticles using *Parmotrema perlatum*

The formation of silver nanoparticles was confirmed by EDAX analysis. EDAX (Fig 6) evidenced from silver nanoparticles illustrated signal of silver from 2.9 kev. Weak signals was arises due to the x-ray emissions from other compounds present within the *Parmotrema perlatum*. All over the scanning range of binding energies, other elemental (O, C, Na) signals are traced due to the presence of proteins or enzymes within the *Parmotrema perlatum* extract. The absorption peak nearly at 3 kev shows the metallic silver nanoparticle due to surface Plasmon resonance (Magudapatty et al., 2001).
Figure 6: EDX analysis of biosynthesized silver nanoparticles prepared by Parmotrema perlatum

ANTIBACTERIAL ACTIVITY

The mechanism of antibacterial activity of the biosynthesized silver nanoparticle against bacteria was depicted in the Fig 7. The antibacterial effect of biogenic nanoparticles against *Streptococcus, Enterococcus, klebsiella, Escherichia coli, pneumoniae, serratia* and *planomicrobium* were investigated by using well diffusion method. The various concentration of silver nanoparticle was used are 25 µl, 50µl, 75µl, 100µl. Zone inhibition was increased while the concentration of silver nanoparticle was also increased.
Biogenic silver nanoparticles exhibited maximum antibacterial activity against pathogenic bacteria *Streptococcus* (30 mm) and minimum activity observed against *Klebsiella planticola* (7 mm). Through this current study we exposed that the silver nanoparticle synthesized using *Parmotrema perlatum* displayed potent activity against both gram positive and gram negative organism which is shown in the Fig 8.

![Image of Fig 8]

**Figure 8 displaying the rate of inhibition of silver nanoparticles against both gram positive and gram negative organisms.**


The zone formation was apparently around the well consisting the silver nanoparticle was observed in Fig 9 A-D. The antibacterial activity of silver nanoparticles within bacterial cells will develop a strong connection with bacterial cellular components and once inside the cell, interference of nanoparticles with the bacterial growth signaling pathway through altering tyrosine phosphorylation of putative peptide substrates critical for viability of the cell and division.

Nanoparticle is able to interact with DNA, therefore losing its replication capability that may lead to cell death. Nanoparticle and bacterial cell wall interaction of bacteria would be assisted by the negative charge abundance on the gram negative bacteria. The gram negative bacterial growth was more intensely affected by the AgNps than that of gram positive organisms (Fayaz et al 2010; Mulvaney 1996). Correspondingly removal of hydrogen atoms on cell wall takes place by the oxygen links with silver as well as reacts with the sulfhydryl groups initiating the atoms of sulphur to form an R-S-S-R bond, prevented the respiration and make happening toxic effect of bacterial cells (Siva Kumar et al 2004). Naturally silver
nanoparticles act together with the bacterial membrane and thus interrupt the integrity of the membrane; silver ions combine to sulfur, oxygen and nitrogen of fundamental biological molecules as well as block the bacterial growth (Juan et al 2010).

Fig 9: Antimicrobial activity of biosynthesized silver nanoparticles against A- Pneumoniae, B- Serratia, C-Streptococcus and D- Escherichia coli.

CONCLUSION
In this current study biological synthesis of silver nanoparticle by utilizing lichen extract has been successfully demonstrated. The Phytochemical present in the Lichen extract can act as reducing as well as stabilizing agents. The characterization techniques from UV-Visible spectrophotometer, Fourier Transform infrared spectroscopy, Scanning electron microscope and X- ray diffraction confirmed the development and stability of biologically synthesized silver nanoparticle. In the current study we have identified that Parmotrema perlatum could be an excellent resource for silver nanoparticle synthesis. It is indispensable to discover novel sources of antimicrobials due to constant increasing of bacterial resistance against recognized antibiotics in global public health. Currently there has been awareness concentrated on creating medicinal products from natural source. Biologically synthesized silver nanoparticle using algal fungi intermediate extracts showed potent antibacterial activity against both Gram positive and Gram negative bacteria. Hence the biosynthesized silver nanoparticle antimicrobial activity based on our results could lead to effective inventions in several areas including medical devices as well as in pharmaceutical and biomedical industries.

REFERENCES


