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Int. J. Nano Dimens., 9 (3): 273-285, Summer 2018

ORIGINAL ARTICLE

Biochemical profiling of microbes inhibiting Silver nanoparticles using symbiotic organisms

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Received 8 January 2018; revised 27 March 2018; accepted 25 April 2018; available online 26 April 2018

Abstract

Silver nanoparticle therapeutics using symbiotic organisms can offer solutions to the current obstacles in antimicrobial therapies, because of cost-effective and eco-friendly properties over chemical and physical methods. In this study, we aim to synthesize silver nanoparticles using lichen (*Parmotrema tinctorum*) extract and evaluation of its antibacterial properties. Synthesized silver nanoparticle were characterized on the basis of morphology, size, shape and nature by UV-visible spectroscopy, Transmission electron microscopy (TEM), Particle size analyzer, Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) analysis. TEM analysis showed that synthesized silver nanoparticles were spherical in shape with maximum particles in size range within 15 ± 5.1 nm. Prolonged stability of synthesized silver nanoparticles was due to the presence of capping and stabilizing agent in form of biomolecules, which were confirmed by FTIR analysis. Furthermore, the bio-potentiality of synthesized silver nanoparticles was done against five pathogenic bacteria *viz*., *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Klebsiella pneumoniae* using the agar well diffusion method. On the basis of zone of inhibition we can say that silver nanoparticles had antibacterial properties. Our results suggested that, prepared silver nanoparticle might be used for production of antibiotics and applied as potential microbial cell inhibitors.

Keywords: *Antibacterial Activity; Biosynthesis; Silver Nanoparticles; Biochemical Profiling; Lichen Extract.*

How to cite this article

Khandel P, Kumar Shahi S, Kanwar L, Kumar Yadaw R, Kumar Soni D. Biochemical profiling of microbes inhibiting Silver nanoparticles using symbiotic organisms. Int. J. Nano Dimens. 2018; 9 (3): 273-285.

INTRODUCTION

Nanotechnology involves the application of scientific knowledge from a variety of disciplines in science and engineering to understand, manipulate and control the properties of matter at nanoscale (1–100 nm) size dimensions [1]. Nanoparticles such as gold, silver and platinum are widely used in medical applications. Nanoparticles can be synthesized by using various methods that includes physical, chemical and biological [2]. However, the chemical method of nanoparticle synthesis requires less time for synthesis of a large quantity of nanoparticles, but that chemicals used for the reduction and stabilization are lead to toxic in nature and produces non-ecofriendly by-products [3]. So, there is a need to develop an eco-friendly approach for the biosynthesis of nanoparticles that does not utilize toxic chemicals.

Nowadays various eco-friendly approaches using several biological systems like yeast, fungi, bacteria, algae and plant extracts are used for the biosynthesis of metal nanoparticles. Silver nanoparticles biosynthesized by using biological materials have special characteristics features such as a large surface area, high dispersion and smaller in size [4]. It has been found morphology of silver nanoparticles is very useful in preventing infections in burns and wounds [5]. It has been also reported that silver nanoparticles have great antifungal [6], antiviral [7], antibacterial [8] and antiplatelet activity [9, 10]. On other hand, mechanisms of silver nanoparticles formation by using biological materials are still conflicting.

Lichens are composite algae living with

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fungi in a symbiotic manner. Aqueous extract of lichen contain a variety of active ingredients and metabolites such as dibenzofurane, depside and depsidon (Phenolic compounds) and some polysaccharides like homo-D-glucan that possibly act as a reducing agents for silver ions [11]. Some species of *Parmotrema* lichen also consist aliphatic acids *i.e.* praesorediosic acid and protopraesorediosic acid which may also involved in bioreduction of silver nitrate into silver nanoparticles. It has been reported that *Parmotrema tinctorum* of lichen consist some secondary metabolites. In upper cortex atranorin and chloroatranorin, and in medulla lecanoric acid (major) with orsellinic acid (trace) are present which can play an important role in the bio-genesis of silver nanoparticles [4, 11]. It has been also reported that they have significant antioxidant activity and inhibitory potential against carbohydrate digestive enzymes such as α-glucosidase and α-amylase [12]. Although the mechanism and mode of action is still not well known [13]. At high concentration silver is also toxic for human beings; however in low concentration it is nontoxic.

Keeping these points in our mind, the present study aims the biosynthesis and characterization of silver nanoparticles using lichen extract (*P. tinctorum)* and evaluation of its antibacterial properties against some pathogenic bacterial strains.

EXPERIMENTAL

Chemicals and Collection of lichen sample

Silver nitrate and all analytical grade chemicals were purchased from Hi-media Chemicals. Double distilled water was used for the experiment. Lichen *(Parmotrema tinctorum),* (Fig. 1) samples were collected from Kabirchabutara, Achnakmar Amarkantak Biosphere Reserve, Bilaspur, Chhattisgarh, India. Bacterial strains *Pseudomonas aeruginosa* (MTCC-424), *Escherichia coli* (MTCC-44), *Bacillus subtilis* (MTCC-441), *Staphylococcus aureus* (MTCC-96) and *Klebsiella pneumoniae* (MTCC-39) were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India.

Biosynthesis of silver nanoparticles

Lichen samples were collected, shade dried and washed thoroughly with double distilled water and then incised into small pieces. About 3 gm of finely cut lichen samples were weighted and transferred into 250 ml beaker containing 40 ml double distilled water, mixed well and boiled for 30 minutes at 70 °C. The extract obtained was then filtered through Whatman filter paper no. 1 and the filtrate was collected in 250 ml Erlenmeyer flasks and stored at 4 °C for further

Fig. 1: Photograph of lichen *Parmotrema tinctorum* used in this study.

use. Aqueous solution (1mM) of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. Silver nanoparticles were synthesized by treating the aqueous extract of lichen in different mixing ratios with silver nitrate solution (1:1, 1:2, 1:3 and 1:4) and allowed to react in dark condition at room temperature. After 24 hours of incubation, observing a reddish brown color solution indicating the formation of silver nanoparticles.

Characterization techniques

To examine the optical characteristics of synthesized silver nanoparticles, sample was introduced for UV-Visible spectroscopic studies (Systronics, Double Beam Spectrophotometer, 2203) at room temperature regulated at a resolution of 1 nm between 300-700 nm ranges. Size distribution pattern of synthesized nanoparticles was measured using (Zetasizer Nano, Malvern UK). Further characterization was done using FTIR spectrophotometer. The reaction mixture was centrifuged at 12,000 rpm for 20 minutes, to remove the biological biomass residues. The silver nanoparticles pellet obtained after centrifugation were redispersed in distilled water. Centrifugation and redispersion process repeated for two to three times. Finally the samples were dried and grinded with KBr pellets and then analyzed by using FTIR instrument (Thermo-Nicolet, Avatar 370). TEM was used for the characterization of shape and size of the synthesized nanoparticles. For this the samples was firstly sonicated for 20 minutes. Then one drop of this sonicated solution was loaded on grid (Carbon Coated Copper grid), and then it was allowed to evaporated under infrared light for 30 minutes, resulting an image is formed from the introduction of the electron transmitted through the specimen. The X-ray diffraction pattern of biosynthesized silver nanoparticles was performed on a diffractometer [14]. Synthesized silver nanoparticle solution was cast onto glass slides, and then operated at a voltage of 20mA with Cu, radiation of 1.5406 nm wavelengths. Scanning was done in the range of 2θ from 10 °C to 90 °C. Finally the size of biosynthesized nanoparticles was calculated by using Scherer's equation [14].

Antibacterial assay

Synthesized silver nanoparticles were tested for antibacterial activity against five different groups of bacteria *viz., Pseudomonas aeruginosa, Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Klebsiella pneumoniae*. Nutrient agar media were used as medium to culture bacteria. The pure cultures of bacteria were subculture on petridishes containing nutrient agar medium. The bacterial strains were stored in refrigerator. The bacterial suspensions were prepared in 0.86% saline [15]. The antibacterial activity of silver nanoparticles was determined by following standard Nathan's agar well diffusion technique [16]. The bacterial suspension was spread on nutrient agar media in petriplates to create confluent lawn of bacterial growth. The wells of 6 mm were prepared by cork borer. Various volumes (10, 30 and 50 µL) of silver nanoparticles of concentration (1:3) were poured to the centre of the each well. Antimicrobial effect of silver nitrate (1mM) solution as well as lichen extract was also assessed on the basis of zone of inhibition. For control, distilled water was used. Inoculated plates were incubated to 24 hours at 35°C. The susceptibility of test organisms was determined after 24 hours by measuring zone of inhibition around each well.

RESULTS AND DISCUSSIONS

Biosynthesis of silver nanoparticles

Biosynthesis of silver nanoparticles through lichen extract (*P. tinctorum*) was carried out. It has been found that the chemical synthesis approaches leads to the use of toxic chemicals that may have adverse effect in the biomedical applications [17]. So there is a need of a rapid, eco-friendly and cost effective approach. Biological synthesis methods provides an additional benefits over physical and chemical methods of synthesis because it is environmental friendly, cost effective, less time consuming, easily scaled for large scale production and in this approaches there is no additional need to use increase pressure, energy, temperature and toxic chemicals. When we mix the aqueous extract of lichen (Fig. 2b) with silver nitrate solution (1mM) (Fig. 2a) a change in color from pale yellow to reddish brown was observed (Fig. 2c). The change in color from pale yellow to reddish brown confirms that there was reduction of Ag^+ occurs, that indicates the formation of silver nanoparticles [18]. The change in color indicates that *P. tinctorum* extract could be used as reducing and stabilizing agent for silver nanoparticle synthesis and also play as a key of evidence for biosynthesis of silver nanoparticles. Similar results

were reported by Kharat and Mendhulkar [19], when they mix the leaf extract of *Elephantopus scaber* with the aqueous solution of 1mM silver nitrate solution, after few minutes change in color was observed by them, which shows the synthesis of silver nanoparticles. The change in color indicated the presence of silver nanoparticles that could be due to the excitation of surface plasmon resonance arising due to collective oscillation of free electrons produced by an electromagnetic field, typical of the silver nanoparticles [20].

Characterization of silver nanoparticles

Formation of silver nanoparticles was confirmed by using UV-visible spectral analysis. It is generally known that UV-Visible spectroscopy could be used for the examination of size and shape of the nanoparticles in aqueous suspension [21]. Fig. 3 shows the UV-visible spectra of the nanoparticles obtained by the various ratios (1:1, 1:2, 1:3 and 1:4) of reaction mixture. In our results the nanoparticles synthesized at (1:3) extract to $AgNO_3$ (1mM) ratio, shows maximum (strong plasmon resonance band) absorbance value. Peak specific for silver nanoparticles at (1:3) ratio was observed at 410 nm by using UV-Visible spectroscope in the form of sharp peak (Fig. 3), which was specialized for the biosynthesis of silver nanoparticles. The reaction mixtures showed a single SPR band, which confirms the spherical shape of silver nanoparticles, which was further confirmed by TEM micrographs. No change in absorbance was recorded after 96 hours of incubation, confirming the complete reduction of Ag^+ ions to silver nanoparticles. The stability of the synthesized silver nanoparticles was studied by measuring its intensity by UV visible spectrophotometer over a period of 3 months in same reaction conditions. No significant change in the absorbance was observed, which proved its stability over a longer period (data not shown). Further experiments were carried out with (1:3) extract to $AgNO_3$ (1mM) ratio combination.

In this study formation of silver nanoparticles was initially confirmed by using SPR phenomenon. For the silver nanoparticles λ_{max} value were remains in the visible range of 400-500 nm [22]. It is well known that metal nanoparticles like silver and gold have free electrons, which give rise to surface plasmon resonance (SPR) band [23]. Our findings corroborate with the findings of Jacob *et al.* [24] who reported the same results in which they studied that when *Piper longum* leaf extract was treated with 1mM aqueous silver nitrate solution the resulting reaction mixture containing silver nanoparticles showed the absorption peak at about 410 nm due to the excitation of longitudinal plasmon resonance vibration. The localized SPRs are due to the collective oscillation of the conduction of electrons confining to metal nanoparticles. Due to the excitation of the localized surface plasmon, an electric field causes a strong light scattering, where resonance occurs at a particular wavelength, this occurrence results in the development of strong SPR bands [22]. The exact mechanism of biosynthesis of silver nanoparticles was not clearly known, but later it was hypothesized that the silver ions requires NADH-dependent nitrate reductase enzyme for their reduction [25], this enzyme was secreted by the fungal biomass extracellularly. Therefore it is clear that in case of lichens the fungal part is actively involved in the biosynthesis of silver nanoparticles. Pal *et al.* [26] also studied the mechanism of biosynthesis of silver nanoparticles and confirmed that the NADH-dependent nitrate reductase enzyme in extracellular cell filtrate of the fungi is used the synthesis of silver nanoparticles. The band at 410 nm confirmed that the particles were well dispersed without aggregation. Similar results were also reported by Mie *et al.* [4] and Samsudin *et al.* [11]. An intense reddish brown color of the reaction mixture further supports the reduction of silver ions and the formation of silver nanoparticles [18]. In this study for the determination of optimum process parameters of the biological process “one factor at a time” method was employed for the factorial experimental design [22]. According to that experiment one experiment factor was varied at a time and other factor keep constant.

Results obtained from DLS pattern reveal that the biosynthesized silver nanoparticles have an average diameter of 57.08 nm with polydispersity index (PDI) of 0.295 (Fig. 4). With the help of this we can analyze particle size range of the nanoparticles along with its polydispersity. This analysis tool is also helpful for determining the mean size of particles inside the sample. Particle size was determined based on measuring the time dependent fluctuation of scattering of laser light by the nanoparticles undergoing Brownian motion. Silver nanoparticles biosynthesis by using some plant extracts are reportedly having good

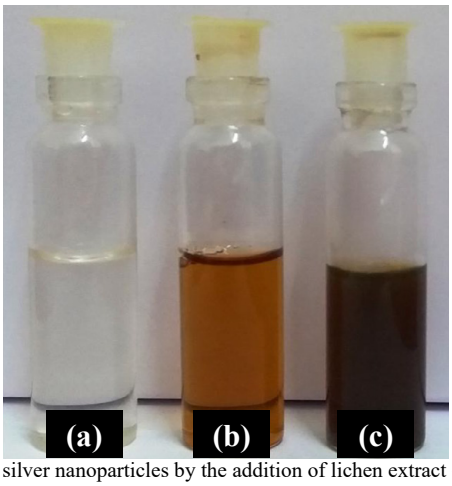

Fig. 2: Color change of silver nitrate to silver nanoparticles by the addition of lichen extract (A) 1mM $AgNO_3$ solution (B) Aqueous extract of *P. tinctorum* (C) Reduction of Ag+ into silver nanoparticles after 24 hrs of reaction.

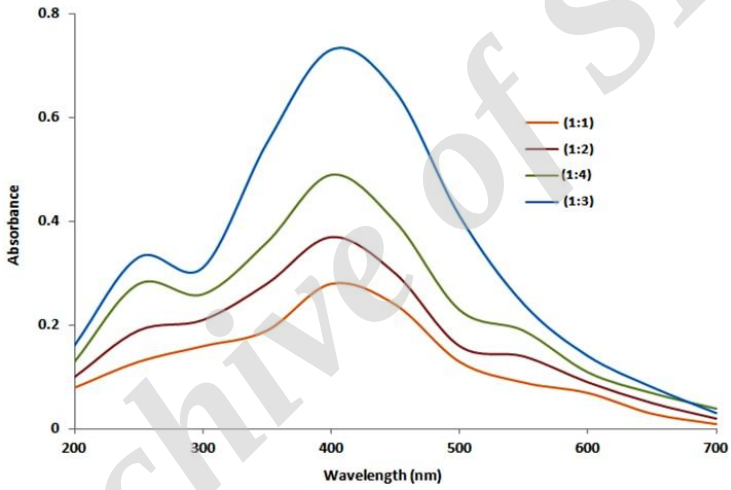

Fig. 3: UV–visible spectra of biosynthesized silver nanoparticles obtained on varying the mixing ratios at a range of 300-700 nm.

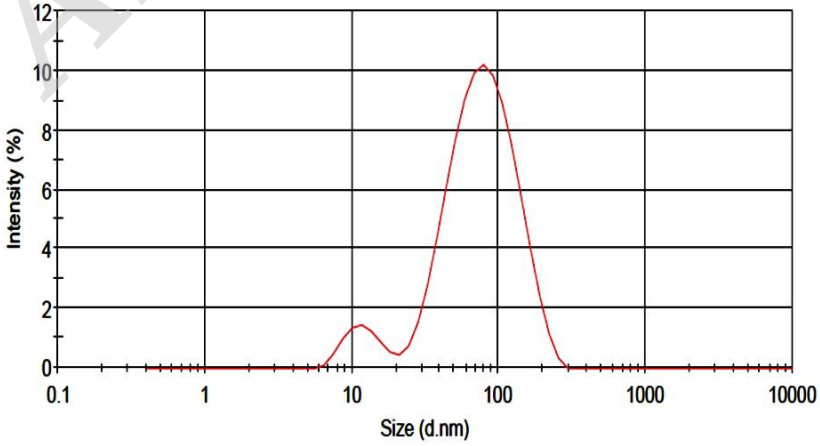

Fig. 4: Graph showing particle size distribution for silver nanoparticles.

monodispersity as well as stability [18]. Particle size distribution has been made by using dynamic light scattering pattern. The differences in size as observed in TEM images is may be due to the presence of impurities of bio-active molecules of lichen on silver nanoparticle surface. The dynamic light scattering measured size is bigger than the particle size analyzed by the TEM micrographs, this is because of the DLS mainly measures the hydro-dynamic radius of the nanoparticles [27]. The DLS pattern analysis was done from Rajiv Gandhi Proudyogiki Viskwavidyalaya, Bhopal, India.

FTIR analysis were carried out to characterize the possible biomolecules responsible for the reduction of Ag^+ ions and the stabilization of the bioreduced silver nanoparticles to prevent agglomeration of the nanoparticles and their capping in the aqueous medium [28]. FTIR analysis showed the peak range in the 500-4000 cm^{-1} which all was correspondents to different functional groups and reported the presence of protein stabilizing molecules. Results of FTIR analysis shows sharp absorption peak at 548.59, 1638, 2074 and 3452 cm^{-1} corresponding to several functional groups (Fig. 5). The prominent peaks of FTIR are corresponding to the amide I and II and the -OH stretch of alcohols and phenolic compounds present commonly in lichen extract. Absorption peak at 1638 cm^{-1} may be designated to the amide I bond of proteins, similarly the band at 3452 cm^{-1} is close to that reported for -OH stretching in alcohols and phenolic compounds [29]. It is reported that absorption band at 1638 cm^{-1} is found to be close to the native proteins which were reported for the interacting with biosynthesized silver nanoparticles and it was also confirmed that this protein does not loses its secondary structure when it binds or reacts with silver nanoparticles [30]. This FTIR analysis reports confirms that carbonyl group of amino acid residue have strong binding affinity with the silver nanoparticles and that may be act as a reducing agent and stabilizing agent to avoid agglomeration and that leads to the providing stability to the silver nanoparticles in the medium [31]. Absorbance peak near at 1630 cm^{-1} is associated with stretch vibration of -C=C- [35] which is assumed for the amide I bonds of proteins [30]. The presence of the peak in the amide I and II regions were assigned for the proteins and enzymes which are responsible for the reduction of metal ions for synthesis and stabilization process. These bands indicate the presence of bio-organic compounds of terpenoid group present in the aqueous lichen extract. Similar observations is noticed by the Nabikhan *et al.* [32] they carried out a study in which they synthesizes silver nanoparticles by the callus and leaf extract of *Sesuvium portulacastrum*. It is well known that the proteins can bind to silver nanoparticles through either cysteine residue or free amines group in the proteins and this surface bound protein stabilizes during synthesis. Other reports on enzyme NADH dependent reductase and polysaccharides which majorly involved in the stabilization and biosynthesis process [33, 34]. Sivaraman *et al.* [35] reported that the tannic acid which is a polyphenol and it's a plant derived compound can be effectively reduces the silver nanoparticles. Kaviya *et al.* [8] reveals the FTIR analysis in which they found the absorption bands at 1383, 1601, 1226 and 2993 cm^{-1} which were associated with germinal methyl group, C=C aromatic and C-N stretch aliphatic amines, C-O stretch acidic and C-H stretching alkanes respectively. Obtained results also supports the finding of Gole *et al.* [36] they found that proteins can bind to nanoparticles through free amines groups or through electrostatic attraction of negatively charged carboxylate groups in enzymes that is present in the cell wall of fungi and this way proteins stabilizes the nanoparticles. Our results show resemblance to that reported by Jagtap and Bapat [37], who reported the Green synthesis of silver nanoparticles using *Artocarpus heterophyllus Lam.* seed extract and their FTIR analysis they found that absorption peaks at 3398–3359 (bonds due to N-H stretching, amides), 2924–2922 (bonds due to C-H stretching, alkanes), 1632–1638 (characteristic of amino acids containing NH_2 groups, amide I band), 1415–1401 (due to C-H deformation, ketones and esters), 1154–1147 (germinal skeletal vibrations of dimethyl) and 1021–1019 (bonds due to P-O stretching). The application of nanoparticles is also depends in terms of chemical stability without undergoing the degradation like partial oxidation. With the FTIR results we can conclude that some of the biological compounds from lichen extract formed a strong capping agent on the nanoparticles for their stabilization.

TEM analysis was employed to visualize the size and shape of silver nanoparticles formed. The typical TEM images of the biosynthesized silver nanoparticles are shown in Fig. 6 (i &

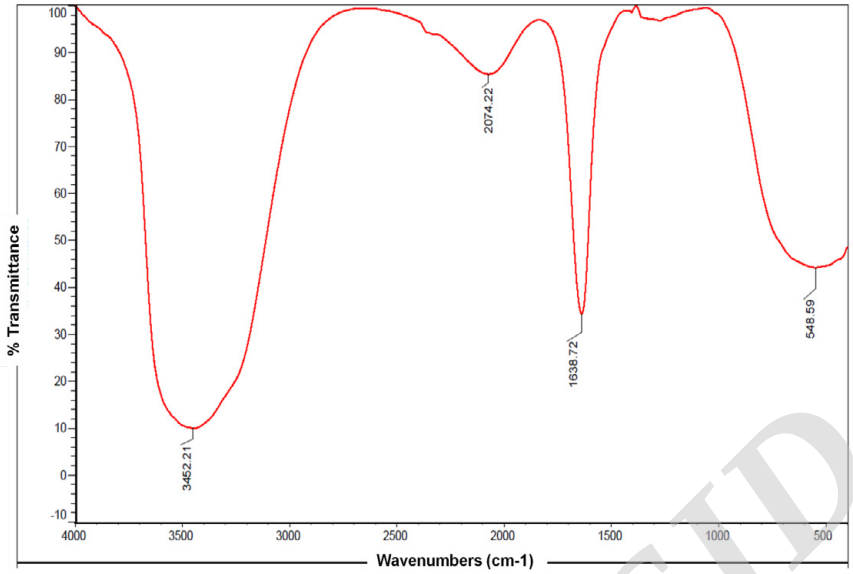

Fig. 5: FTIR spectrum of biosynthesized silver nanoparticles.

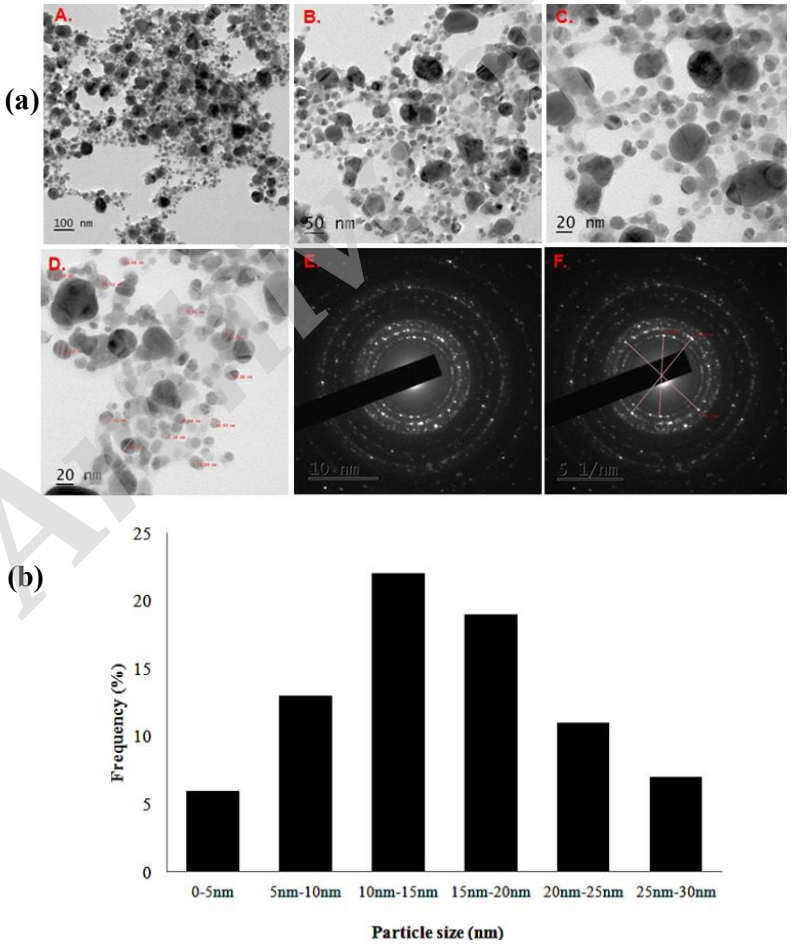

Fig. 6: (i) TEM image of biosynthesized silver nanoparticles at (A) 100 nm, (B) 50 nm and (C), (D) 20 nm scales (E), (F) SAED pattern, (ii) Particle size histogram of biosynthesized silver nanoparticles.

ii). TEM analysis confirms the formation of silver nanostructures and gives monographs of synthesized silver nanoparticles at 100 nm, 50 nm and 20 nm scales (Fig. 6 A, B, C & D). TEM images reveal that synthesized silver nanoparticles are polydisperse, mostly in spherical and near spherical shapes with non specific distribution. The average diameter of synthesized silver nanoparticles was found to be range of 1-25 nm with average diameter of 15.14 nm. A significant proportion of largely spherical silver nanoparticles within the range of 10-15 nm were observed in TEM monographs. We also observed that there are few traces of clustered silver nanoparticles which may contribute for the variation in particle size. In TEM micrographs it was also observed that the edges of the nanoparticles were lighter than the centers, suggesting presence of biomolecules in lichen metabolites, such as proteins have capped the silver NPs and were attached to their surfaces [37]. Selected area electron diffraction (SAED) pattern of silver nanoparticles was shown in Fig. 6 (E and F). The diffraction rings also suggested the nanoparticles were crystalline in nature. Fig. 6 (ii) shows the histogram pattern of synthesized silver nanoparticles, which shows the average particle size of these spherical shaped nanoparticles. Measurement of the maximum size and their diagonal lengths of the individual particles were also taken into account. In monographs it is clear that the synthesized nanoparticles were not in direct contact even within the aggregates, which indicates the stabilization of the nanoparticles by a capping agent. With these images we can conclude that synthesize silver nanoparticles were polydispersed and round in shape, with uniform distribution in the prepared aqueous solution. The presence findings corroborate the observation of Bar *et al.* [38] who studies the synthesis of nano-crystalline silver particles by using *Jatropha curcas.* They found that the synthesized silver nanoparticles were mostly spherical particles with diameter ranging from 15 to 50 nm. While Mie *et al.* [4] synthesize silver nanoparticles with the extract of lichen with an average size of 42 nm. Similarly Samsudin *et al.* [11] synthesize silver nanoparticles using aqueous extract of *Parmatrema praesorediosum.* The synthesized silver nanoparticles were spherical in shape, polydispersed and ranged in size from 5 nm to 40 nm with average diameter of 19 nm. Obtained results represent a significant consensus

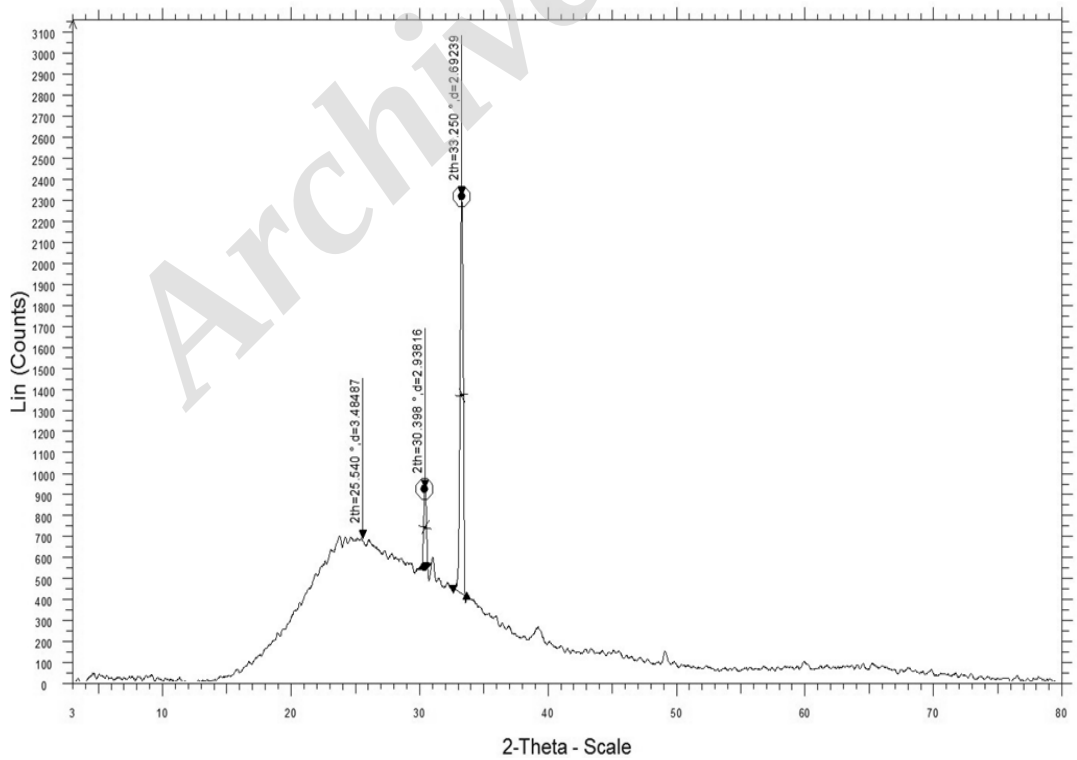

Fig. 7: XRD pattern of biosynthesized silver nanoparticles.

with earlier findings reporting synthesis of silver nanoparticles using plant extracts and lichen extracts [39].

The XRD spectrum is important to analyze the exact nature of the synthesized silver nanoparticles. It is mainly used to analyze the crystallization of the biogenic phase that appears on the surface of the metal nanoparticles [40]. X-ray penetrates into the nanomaterials and shows different diffraction patterns which are further compared with standards to get structural information's. XRD diffraction pattern of synthesized silver nanoparticles of *P. tinctorum* was shown in Fig. 7. In our results the Bragg's reflection peaks corresponding to crystal structure of silver nanoparticles were observed at 25.540°, 30.398° and 33.250°. The XRD pattern obtained was consisted with earlier reports. Full width at half maximum (FWHM) data was used with Debye Scherrer's formula to determine the average particle size. The mean size of silver nanoparticles was calculated using Debye-Scherrer's equation by determining the width of the Braggs reflection [19]. A typical XRD pattern of the silver nanoparticles synthesized was found to exhibit an fcc structure [14]. It is well known that the results data obtained from XRD of particles having very small size, is difficult to analyze. XRD patterns of nanoparticles display several different size dependent features corresponding to anomalous peak position, height and width. The appearance of this so called twinned particles combined with fcc structure makes the XRD data interpretation more complicated for metal nanoparticles [22]. XRD pattern analyzed that synthesized nanoparticle was in the form of nano crystals.

Antibacterial assay of synthesized silver nanoparticles

Antibacterial activity of silver nanoparticles synthesized from *P. tinctorum* was also successfully analyzed by agar well diffusion method against five different pathogenic strains of bacteria *viz., P. aeruginosa, S. aureus, E. coli, B. subtilis* and *K. pneumoniae* (Fig. 8a & b). We found that the silver nanoparticles inhibited the growth of gram positive bacteria as well as gram negative bacteria. At the concentration of 50µL the highest zone of inhibition was observed against *P. aeruginosa* (17±0.50 mm), *K. pneumoniae* (14±0.10 mm) and *E. coli* (11±0.10 mm), lowest zone of inhibition against the *B. subtilis* (8±0.30 mm) and *S. aureus* (7±0.30 mm). This result is might be due to the variation in the structure of the cell wall of the gram +ve and gram -ve bacteria. It was also observed that as the concentration of synthesized silver nanoparticles was increased, growth of the microorganisms decreases in all cases. Concentration of silver nanoparticles varied as 10, 30 and 50µL. It should be noted that a significant variation in the size of the inhibition zones was observed. The results of antibacterial activity were compared with control experiment. In control, no zone of inhibition was observed (Fig. 8b); indicating that the activity is due to bio-inspired silver nanoparticles.

In recent years, the nanoparticles have been found as an interesting alternative substitute of antibiotics and appear to have a high potential in solving bacterial multi-drug resistance in human pathogenic bacteria. It is reported that silver nanoparticles has been utilized to control a wide variety of diseases caused by both Gram -ve and Gram +ve bacteria. The antibacterial activity of silver nanoparticles is due to the strong interaction of silver with the thiol groups of respiratory enzymes of bacteria [41]. It is also reported that silver nanoparticles not only interact with the cell wall of bacteria, but it may also penetrate inside the bacteria [42]. Smaller particles with a large surface area have greater antibacterial effects as compared to larger particles. Among the other nano sized metal antimicrobial agents, silver has found to be most efficient and effective due to its broad spectrum activity against a wide range of pathogenic microorganisms including bacteria, fungi, viruses and eukaryotic microorganisms [43]. Silver nanoparticles are widely used in antimicrobial coating in medical instruments and textiles.

Table 1 shows a zone of inhibition in diameter of various volumes of silver nanoparticles against different pathogenic bacterial strains. When concentration is increased the activity of silver nanoparticles was also getting improved. The antibacterial activity of biosynthesized nanoparticle is possibly due to certain changes in the membrane structure of bacterial cell wall. The silver nanoparticles increase the membrane permeability of bacteria and consequently leading to their death. It is well known that the cell wall of gram +ve bacteria is made up of thick layer of peptidoglycan, with a polysaccharide chain cross linked by small peptides forming a more rigid structure, which is difficult to penetrate

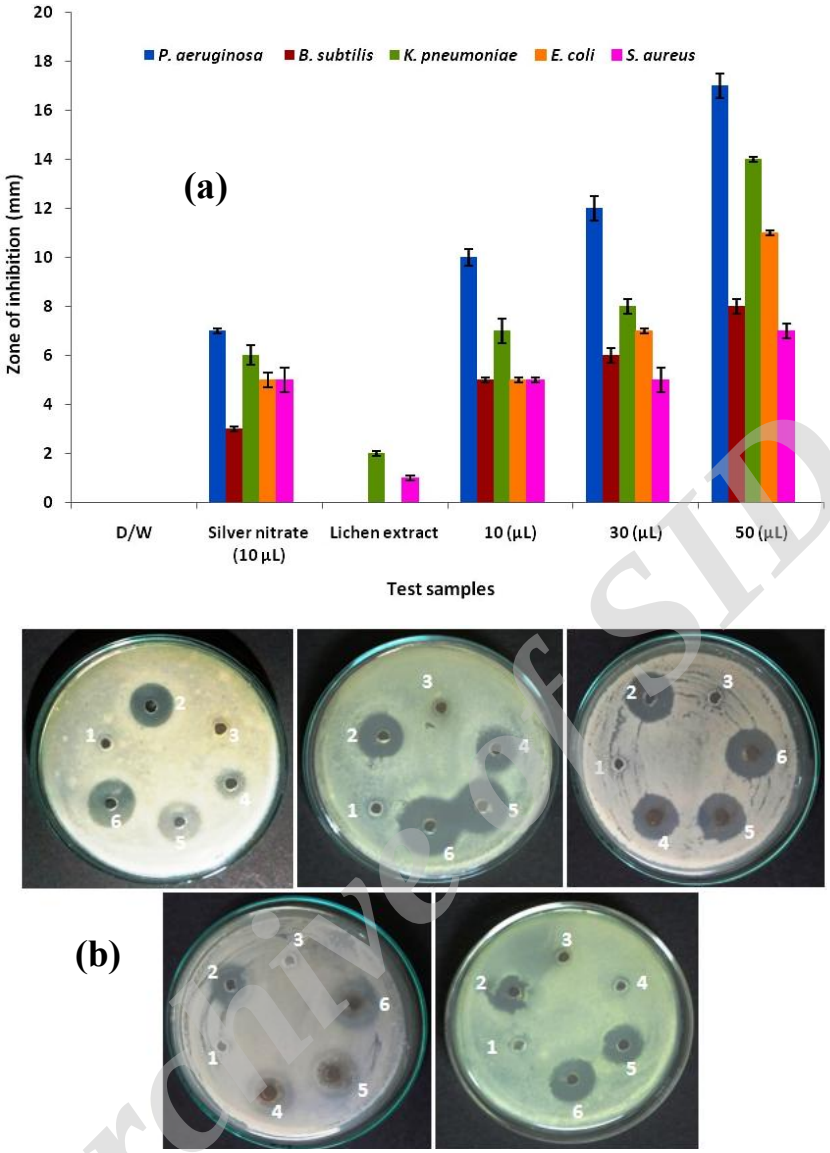

Fig. 8: (A) Antibacterial activity of the synthesized silver nanoparticles against disease causing pathogenic strains of bacteria. (B) Zone of inhibition produced by biosynthesized silver nanoparticles against various bacterial strains [D/W (Distilled water) (*1*), Silver nitrate (*2*), Lichen extract (*3*), AgNPs 10 µL (*4*), 30 µL (*5*), 50 µL (6)].

Table 1: The results of antibacterial activity with zone of inhibition (mm) of P. tinctorum synthesized silver nanoparticles against various microorganisms.

Microorganisms	Zone of inhibition (mean ± SD* in mm)					
	D/W	$AgNO_3$ (1mM)	Lichen Extract	Synthesized silver nanoparticles (µL)		
				10	30	50
Pseudomonas aeruginosa	--*	7 ± 0.10	--	10 ± 0.33	12 ± 0.50	17 ± 0.50
E. coli	--	5 ± 0.30	--	5 ± 0.10	7 ± 0.10	11 ± 0.10
Bacillus subtilis	--	3 ± 0.10	--	5 ± 0.10	6 ± 0.30	8 ± 0.30
Staphylococcus aureus	--	5 ± 0.50	1 ± 0.10	5 ± 0.10	5 ± 0.50	7 ± 0.30
Klibsiella pneumoniae	--	6 ± 0.40	2 ± 0.10	7 ± 0.50	8 ± 0.30	14 ± 0.10

*(--) - No inhibition zone, (SD) - Standard deviation

by the silver nanoparticles as compared to gram -ve bacteria which consist thinner layer of peptidoglycan [44].

Silver ions form complex with the electron donor groups on thiols and phosphates of amino acid and nucleic acid. Also the silver nanoparticles attach to the cell surface of the bacteria and inhibit its normal function, penetrate the bacterial cell and releases silver. The wells filled with lichen extract (concentration which is used for synthesis of silver nanoparticles) did not show any zone of inhibition suggesting that at these concentrations lichen alone is not antibacterial. Similar reports have been also reported by Mahita *et al.* [45] in which they have studied the antibacterial activity of silver nanoparticles against gram +ve (*B. subtilis*, *S. aureus*) and gram -ve (*E. coli, K. pneumoniae*). Mubarakali *et al.* [46] reported antibacterial activity of silver nanoparticles against clinically isolated pathogens and obtained results suggested them for the formulation of new bactericidal agents. Huang *et al.* [27] reported that the silver nanoparticles ranging 10-25 nm are highly effective against pathogenic microbes. Shrivastava *et al.* [21] carried out antimicrobial study of silver nanoparticles against *E. coli* in which they reported that the at initial stage of interaction of silver nanoparticles attach to the surface of bacterial cell wall and subsequently by penetrating the bacteria leads to the death of microbes by degrading the cell membrane. Nazeruddin *et al.* [47] reported the extracellular biosynthesis of silver nanoparticles using leaf extract of *Azadirachta indica* and they studied its antibacterial activity towards the test pathogenic gram +ve bacterial strain *Bacillus subtilis* NCIM 2635 and gram -ve bacterial strain *Salmonella typhinorium* using standard well diffusion method. Another study was carried out by Anandalakshmi *et al.* [48], they reported the antibacterial effect of silver nanoparticles synthesized by using *Pedalium murex* leaf extract and their bactericidal effects on different types of bacteria such as *E. coli, K. pneumoniae, P. aeruginosa* (Gram negative) *M. flavus, B. subtilis, B. pumilus and S. aureus* (Gram positive). There are only few reports concerned with silver nanoparticles synthesis by lichen. On the basis of obtained results we concluded that the present study showed an extracellular and one step process for the biosynthesis of stable antimicrobial silver nanoparticles.

CONCLUSION

Here we report a green, bio-inspired and single step method for silver nanoparticles synthesis using lichen metabolites. The process represents an example of nontoxic, clean and eco-friendly approach for biosynthesis of silver nanoparticles. The present study shows that extract of *Parmotrema tinctorum* lichen can be effectively used for the reduction of $AgNO_3$ into silver nanoparticles of average diameter of range of 15.14 nm. Synthesized silver nanoparticles are quite stable, no UV-Visible changes are recorded even observed after 3 months, which is might be due to presence of proteins, enzymes and sugars that may be act as a capping agent; still further research is needed in this field to understand the possible mechanism for bioreduction process. Characterization from UV-visible, FTIR, DLS, TEM and XRD analysis results also supports the stability of nanoparticles. Synthesized silver nanoparticles also exhibit good antibacterial activity against five different pathogenic strains of bacteria. So this can be potentially applicable in food industries, medicines and cosmetic industries. In present study it confirms that biosynthesized nanoparticles having capacity of interpreting great antibacterial activity and therefore has huge possibilities in the preparation of antibiotics and drugs against various pathogenic strains of bacteria.

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies with human and animal subjects.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Head, Department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur, C.G. for providing facilities and UGC and GGV for financial assistance for the fulfillment of this study. The authors also gratefully acknowledge STIC SAIF Cochin, India for FTIR, TEM and XRD analysis and School of Pharmaceutical Sciences, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal, M.P., India for particle size analysis.

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