Mycosynthesis, Characterization and Antibacterial activity of Silver Nanoparticles (Ag-NPs) from fungus *Ganoderma lucidum*

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ABSTRACT

Lingzhi or Reishi therapeutic fungus, *Ganoderma lucidum* has been used over the ages as highly medicinal herb in the Orient. We report here a new facet of this “elixir of life” as a mycosource for synthesis of metal nanoparticles. Treating the extracellular suspension extracts of *G. lucidum* with silver nitrate reduces the metal ions to nanoparticles. Optical detection followed by confirmation through spectroscopic analysis suggests that this fungus can be used for the purpose of safe and sure synthesis of silver nanoparticles, demand for which is growing day by day in all fields of human life. In this study the Mycosynthesis of silver nanoparticles using *G. lucidum* extract has been reported. Characterizations of nanoparticles were done using different methods, which include; ultraviolet-visible spectroscopy (UV-Vis), powder X-ray diffraction (XRD), energy dispersive X-ray (EDX), Fourier transform infrared (FT-IR) spectroscopy and scanning electron microscopy (SEM). Surface Plasmon resonance confirmed the formation of silver nanoparticles in UV-Visible spectra at 430 nm. The XRD study showed that the particles are crystalline in nature, with a Face Centered Cubic (FCC) structure. The Fourier transform infrared spectroscopy (FTIR) analysis was carried out to identify and study the functional groups responsible for the bio-reduction of silver ion. The synthesized Ag-NPs were polydispersed spherical particles ranging in size from 5 to 30 nm and stabilized in the solution. These nanoparticles have shown strong bactericidal activity against pathogens *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus mutants*, *Klebsilla pneumoniae* and *Pseudomonas aeruginosa* and also exhibited their efficiency in enhancing the activity of the antibiotics. Of these, silver nanoparticles are playing a major role in the field of nanotechnology and nanomedicine.

Keywords: *Ganoderma lucidum*, Mycosynthesis, Silver Nanoparticles, Antibacterial activity.

1. INTRODUCTION

Metal nanoparticles have significant roles due to their properties such as Plasmon absorption and surface accessibility for functionalization. Synthesis of metal nanoparticles using biological systems has been explored well in the past few decades. Living organisms such as plants, algae, bacteria, and fungi
are used as vectors or a system to fabricate submicron-sized particles for varied applications [1]. This tremendous wave of interest in the biosynthesis of metal nanoparticles is due to their ever-increasing applications in all fields of human life, including medicine, surgery, cosmetics, biophysics, chemistry, space, electronics, agriculture, sports, and more [2-4]. The magnetic and optoelectronic properties possessed by nanometer depend upon their size, shape, source of origin, physicochemical and biological parameters, and method of synthesis [5, 6]. Biosynthetic methods are preferred over physical and chemical methods because the nanoparticles synthesized by biosynthetic processes are environment friendly and economically effective [7].

Recent publications have highlighted the potential of microorganisms, particularly bacteria (including thermopiles) and fungi, to synthesize or sequester metallic and/or oxide nanoparticles [8-12]. Extracellular synthesis of silver and gold nanoparticles by the fungus Colle-totrichum sp [13] or Aspergillus fumigatus have been reported [14]. Similarly, extracellular synthesis of silver nanoparticles in the fungus Fusarium semitectum was also reported while possible medicinal applications of these silver nanoparticles have also been envisaged [15].

In recent years nanoparticles of silver have been found to exhibit interesting antibacterial activities [16]. Presently, the investigation of this phenomenon has regained importance due to the increase of bacterial resistance to antibiotics, caused by their overuse. Antibacterial activity of the silver-containing materials can be used, for example, in medicine to reduce infections as well as to prevent bacteria colonization on prostheses [17], catheters [18, 19], dental materials [20], stainless steel materials [21] and human skin [22]. The use of silver nanoparticles as antibacterial agent is relatively new. Because of their high reactivity due to the large surface to volume ratio, nanoparticles play a vital role in inhibiting bacterial growth in aqueous and solid media. Silver containing materials can be employed to eliminate microorganisms on textile fabrics [23] or they can be used for water treatment [24]. Silver nanoparticles also exhibit a potent cytoprotective activity toward HIV infected cells [25]. Contrary to bactericide effects of ionic silver, the antimicrobial activity of colloid silver particles are influenced by the dimensions of the particles the smaller the particles, the greater antimicrobial effect [16]. Our aim of the present contribution was to mycosynthesis, characterization and antimicrobial activity of silver nanoparticles from therapeutic fungus Ganoderma lucidum.

2. MATERIALS AND METHODS

2.1. Chemicals

All chemicals were of analytical grade and procured from Sigma Aldrich (India) and Merck (India). The culture media were purchased from Hi-Media (Mumbai, India).

2.2. Preparation of fungal extract

20 g of fruiting bodies of fungus Ganoderma lucidum (GL) were rinsed thrice in distilled water, dried on a tissue paper and cut in fine pieces until they were made into a paste, and finally boiled in 100 ml of sterile distilled water up to 5 minutes. It was filtered using whatman No: 2 filter paper and stored at 4°C.

2.3. Synthesis of silver nanoparticles

1 mM aqueous solution of silver nitrate (AgNO₃) was prepared and used for the synthesis of silver nanoparticles. 10 ml of mushroom extract was added into 90 ml of aqueous solution of 1 mM silver nitrate for reduction into Ag⁺ ions and incubated overnight at room temperature in dark.

2.4. UV-Vis Spectral analyses of Silver Nanoparticles

The reduction of pure Ag⁺ ions was monitored by measuring the UV-vis spectrum of the reaction medium after overnight incubation, after diluting a small aliquot of the sample into distilled water. Silver nanoparticles (Ag-NPs) are soluble in distilled water and the color changes were observed visually. The reduction of silver ions was monitored by measuring the UV-VIS Spectrum the reaction medium at 24h and their absorbance was recorded 300 to 800 nm using spectrophotometer.

2.5. X-ray diffraction (XRD) analysis

The synthesized silver nanoparticles were centrifuged at 10,000 rpm for 15 min. and collect the pellet. The pellet was washed with distilled water to remove impurities and dried to get the powder. The X-Ray diffraction assay was performed for the detection of crystalline nature of the metal nanoparticles was done by X-Ray diffractometer (Phillips, Holland model: X" Pert), operating at 40 kV and current of 30 mA with Cu Kα radiation (λ = 1.5404 Å) and the 2θ scanning range was 0-90° at 2° min-1. The crystallite domain size was calculated from the width of the
XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer formula,

$$D = \frac{0.94 \lambda}{\beta \cos \theta}$$  \hspace{1cm} (1)

where D is the average crystallite domain size perpendicular to the reflecting planes, \(\lambda\) is the X-ray wavelength, \(\beta\) is the full width at half maximum (FWHM), and \(\theta\) is the diffraction angle. To eliminate additional instrumental broadening the FWHM was corrected, using the FWHM from a large grained Si sample.

$$\beta \text{corrected} = \frac{( \text{FWHM}_{2\text{sample}} - \text{FWHM}_{2\text{si}} )}{2}$$  \hspace{1cm} (2)

2.6. FTIR spectroscopy analysis

The synthesized silver nanoparticles were lyophilized and mixed with KBr pellets, and then subjected to a wide range of FTIR spectral analyses (Spectrum RX1, Perkin Elmer). Different peaks were obtained for the test samples.

2.7. Energy dispersive X-ray (EDAX) spectroscopy

The presence of elemental silver was carried out by using Scanning Electron Microscope (make Philips, Netherlands) equipped with Energy Dispersive X-ray system EDAX XL-30 operating at 15-25 kV.

2.8. Preparation of Silver Nanoparticles Sample for SEM Studies

The morphological features of synthesized nanoparticles were examined by scanning electron microscopy (JEOL 6380A, Japan).

2.9. Antibacterial Activity Analysis of Ag-NPs

The antibacterial activity of Ag-NPs synthesized from \(G.\) lucidum against \(E._{\text{cherichia coli}},\) \(P.s.aeruginosa,\) \(S.aureus,\) \(K.pneumoniae,\) \(S.mutants\) (IMTECH, Chandigarh, India) was investigated using a disk diffusion assay. Each strain was swabbed uniformly onto individual plates, and a concentrated solution of Ag-NPs was poured into each cup (20 mg per cup) on all the plates. After incubation at 37°C or 28°C for 24 hr, the diameter of inhibition zone was measured using caliper. Antibiotics were used individually as the negative control. The assays were performed in triplicate.

3. RESULTS AND DISCUSSION

The Mycosynthesis of silver nanoparticles using the medicinal fungus \(G.\) lucidum was studied with a view to explore the unstudied characteristic of this popular and highly desirable mushroom. When the \(G.\) lucidum extract was suspended in water overnight and the filtrate was treated with AgNO₃, the extracellular enzymes responsible for reduction of AgNO₃ were supposed to convert the silver ions to nanoparticles. The following detection and characterization studies confirm the formation of silver nanoparticles and suggest their exact size and concentration in the reaction mixture.

3.1 UV-Vis Spectral Analyses

Mycosynthesis of silver nanoparticles using 0.02mmol/mL AgNO₃ is shown in Figure 1. The fresh suspension of \(G.\) lucidum was yellowish in color. However, after addition of AgNO₃ and exposing to bright sunlight for 1h, the suspension turned reddish brown. This change in color is due to the excitation of the surface Plasmon vibrations in the metal nanoparticles, as suggested by [26]. This is an efficient method for detection of synthesized nanoparticles, also supported by many studies [27, 28, 29]. An earlier study reported that upon addition of silver ions into the filtered cell–free filtrate in the dark, samples changed color from almost colorless to brown, with the intensity increasing during the period of incubation. [30] reported the conversion of 3 mM silver nitrate solution to nanosilver by \(F.\) oxysporum in an aqueous medium based on the change in color of the reaction mixture from pale yellow to dark brown (Figure 2). Formation of silver nanoparticles was confirmed using UV-Vis spectral analysis and showed silver surface Plasmon resonance band at 430 nm, broadening of peak indicated that the particles are polydispersed (Figure 3). Observation of the strong but broad surface Plasmon peak has been well known in the case of various metal nanoparticles over a wide size range of 2–100 nm [30]. In the present study the peak value was observed at 430 nm. [31] Another study report similar observations in the edible fungus \(P.\) sajorcaju.

Figure 1. Schematic illustration of the mycosynthesis of silver nanoparticles (Ag-NPs) using aqueous extract of the fungus \(G.\) lucidum. Abbreviations: Aq soln, aqueous solution; t, time; T, temperature.
**Figure 2.** Photograph showing color changing (a) 0.02mmol/mL AgNO₃ without *G. lucidum* extract (b) Aqueous extract of *G. lucidum* extract (c) Color changed from yellowish to reddish brown after adding 0.02mmol/mL AgNO₃ after 1hr.

**Figure 3.** UV–Vis absorption spectra of reduction of Silver ions to silver nanoparticles after 1h of reaction.

3.2 *X-ray diffraction (XRD) analysis*

The X-ray diffraction pattern of the mycosynthesized silver nanoparticles produced by the extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image (Figure 4). The XRD pattern showed (38.05°, 77.32° and 44.07°) in the whole spectrum of 2θ value ranging from 0 to 85 and indicated that the structure of Ag-NPs is Face-centered cubic (FCC). These are (111) and (200) planes for silver, respectively. The lattice Constant calculated from this pattern was $a = 4.086\, \text{Å}$ and the data obtained was matched with the database of joint Committee on Powder Diffraction Standards (JCPDS) file No.04-0783. The size of the silver nanoparticles was calculated by Debye-Scherrer’s equation using FWHMs obtained from the diffraction peaks as shown in Table 1.

Debye-Scherrer’s equation,

$$D = \frac{K \lambda}{\beta \cos \theta}$$

Where $\beta = \pi / 180 \times \text{FWHM}$ (FWHM= Full Width Half Maximum), $K=0.94, \lambda=1.54059\, \text{Å}$, $K \lambda=0.94 \times 1.54059\, \text{Å} = 1.4482$

For example, in our result we obtain three major peaks. We take 3rd peak for calculation by Debye-Scherer equation,

$$D = \frac{K \lambda}{\beta \cos \theta}$$

$K \lambda= 0.94 \times 1.54059\, \text{Å} = 1.4482$

$\beta = \pi / 180 \times \text{FWHM}= 3.14 / 180 \times 1.0178 = 0.01775$

$2\theta = 44.475, \text{So, } \theta = 22.2375$

and $\cos \theta = 0.9256$

now, $D = \frac{K \lambda}{\beta \cos \theta} = \frac{1.4482}{0.01775} = 81.58\, \text{nm}$.

Average$ = 81.58\, \text{nm} + 73.51\, \text{nm} + 57.51\, \text{nm} / 3 = 70.86\, \text{nm}$. The presence of structural peaks in XRD patterns and average crystalline size around 70.86 nm clearly illustrate that Ag-NPs were crystalline in nature.

**Table 1.** Measurement of the size of Ag-NPs of *G. lucidum* extract by using Debye-Scherrer’s equation

<table>
<thead>
<tr>
<th>S.No</th>
<th>$2\theta$</th>
<th>FWHM</th>
<th>$\beta = \pi / 180 \times \text{FWHM}$</th>
<th>$\cos \theta$</th>
<th>$D = \frac{K \lambda}{\beta \cos \theta}$</th>
</tr>
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<tr>
<td>1</td>
<td>38.0589</td>
<td>1.01780</td>
<td>0.01777</td>
<td>0.9256</td>
<td>81.58 nm</td>
</tr>
<tr>
<td>2</td>
<td>77.3247</td>
<td>1.12950</td>
<td>0.0197</td>
<td>0.9256</td>
<td>73.51 nm</td>
</tr>
<tr>
<td>3</td>
<td>44.0708</td>
<td>1.44170</td>
<td>0.0251</td>
<td>0.9256</td>
<td>57.69 nm</td>
</tr>
</tbody>
</table>

**Figure 4.** XRD pattern of silver nanoparticles synthesized using *G. lucidum*. 
3.3 FTIR spectroscopy analysis

FTIR spectroscopy is typically used for the qualitative measurement of organic functional groups, especially O-H, N-H, and C-O. Various absorption bands within the 4000-400 cm range were recorded in the FTIR spectra of Ag-NPs, prepared from Ganoderma lucidum as shown in Figure 5. The possible biomolecules were identified that are responsible for capping and stabilization of the Ganoderma-synthesized silver nanoparticles. The infrared spectrum shows six independent peaks. The peaks at 3398.89, 2924.88, 2853.70, 1647.18, 1554.25, 1456.89, 1383.82, 1316.17, 1243.79, 1154.42, and 1043.46 cm⁻¹ indicate the shift in bandwidth after synthesis of silver nanoparticles. Three absorption peaks located around 888, 788, and 1049 cm⁻¹ can be assigned as the absorption peaks of –C–N stretching vibrations of the amine, –C–O–C or –C–O groups, respectively [32]. The bonds or functional groups such as –C–O–C, –C–O and –C=C– derived from heterocyclic compounds, e.g., alkaloid, or flavones, and the amide I bond derived from the proteins which are present in the stem bark extract are the capping ligands of the nanoparticles [33]. This spectrum clarifies the presence of N-H, N-O, C-N, C-C, C-H, C-O, amide linkages, and linkages for nitro compounds that may be present between the nanoparticles synthesized here as stabilizing caps, along with proteins and amino acid residues.

Figure 5. FT-IR spectrum of silver nanoparticles synthesized using G. lucidum.

3.4 Energy dispersive X-ray (EDAX) spectroscopy

The EDAX pattern thus clearly shows that the silver nanoparticles are crystalline in nature by the reduction of silver ions made in this study. The EDAX analysis obtained in the present study also confirmed the presence of silver nanoparticles synthesized from Glucidum extract (Fig. 6). Metallic silver nanocrystals generally show typical optical absorption peak 3 keV due to surface Plasmon resonance. The extract of G. lucidum synthesized silver nanoparticles also produces a strong signal at 3 keV which reveals the presence of silver nanoparticles. The presence of the strong signal from silver (70.02%) atoms in the nanoparticles and weaker signals from oxygen (26.52%) and chlorine (03.45%) atoms is confirmed (Figure 5). The strong signals of silver correspond to the peaks in the graph confirming presence of Ag-NPs. The weaker signals are recorded possibly due to elements (Cl, Ca, and O) from organic moieties like enzymes or proteins present within the extract of the pomegranate fruit [34]. In an earlier study, individual spherical-shaped silver nanoparticles were obtained in the range 2.5–4 keV by using Memecylon edule leaf extract [35].

3.5 Scanning electron microscopy (SEM) analysis

Scanning electron microscopy (SEM) analysis of silver nanoparticles was done using Hitachi S-4500 Scanning Electron Microscopy. The surface morphology (ie. shape and size) of the silver nanoparticles was shown in Figure 7. The uniform spherical shape nanoparticles were obtained with the sized ranging from ~5-30 nm. Similarly, the spherical shaped silver nanoparticles with a diameter ranging from 30-40 nm were synthesized using Boswellia ovalifoliolata [36]; 30-50 nm using Merremia tridendata [37], Plant extracts of Elaeagnus latifolia [38].

Figure 6. EDAX spectrum of synthesized Ag-NPs using G. lucidum

3.6 Antibacterial Activity of Silver Nanoparticles (Ag-NPs)

The Mycosynthesis of silver nanoparticles showed a higher level of restriction zone against human bacterial pathogens strains when compared to three antibiotics namely Gentamicin, Co-Trimoxazole and Roxithromycin. Ag-NPs showed a higher inhibition...
zone against human bacterial pathogens than three antibiotics. The zone of inhibition observed in *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsilla pneumoniae*, *Staphylococcus mutants* are 28.12±0.76 mm, 30.28±0.78 mm, 29.48±0.80 mm, 22.26±0.17 mm and 30.65±0.43 mm respectively (Figure 8).

Silver in a nanometric scale (less than 100nm) has different catalytically properties compared with those attributed to the bulk form of the noble metal, like surface Plasmon resonance, large effective scattering cross section of individual silver nanoparticles, and strong toxicity to a wide range of microorganisms [39]. Earlier study demonstrated the antibacterial activity of silver nanoparticles in four types of Gram negative bacteria: *Escherichia coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, and *Salmonella typhi* and suggested that silver nanoparticles attach to the surface of the cell membrane and disturb its function, penetrate bacteria, and release silver ions [40]. Other groups determined a similar antibacterial activity in Gram positive bacteria, such as *Bacillus subtilis* [41], *Staphylococcus aureus* [42] and *Enterococcus faecalis* [43]. Silver nanoparticles have also been found to exert antibacterial activity against some drug resistant bacteria [44].

The silver nanoparticles showed efficient antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles get attached to the cell membrane and also penetrated inside the bacteria. The bacterial membrane contains sulfur containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. When silver nanoparticles enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity [45, 40].

**4. CONCLUSION**

Synthesis of silver nanoparticles using fungal extracts was developed in a very simple and eco-friendly method. The Ag-NPs with an average size of 24.40 ± 3.27 nm and spherical in shapes were synthesized using extract of *G. lucidum*. The Ag-NPs were characterized by UV-visible, XRD, SEM, EDAX and FT-IR spectrum. Mycosynthesis of Ag-NPs using fungal resources like *G. lucidum* is a better alternative to chemical synthesis, since this fungal-mediate synthesis is pollutant free and eco-friendly. Generally, mushrooms containing proteins have played a major role in acting as a reductant as well as a capping material in order to synthesize a novel Ag-NPS and functioned more effectively as an antimicrobial agent against human pathogenic bacterial strains.

Antibiotics inhibit growth of only prokaryotic micro-organisms, while silver nanoparticles inhibits growth of fungi also which indicates that the silver nanoparticles inhibit growth of both prokaryotes and eukaryotes. From the results obtained in this effort, one can confirm that *G. lucidum* extracts can play an important role in the bioreduction and stabilization of silver ions to Ag-NPs. The biologically synthesized silver nanoparticles could be of enormous use in medical field for their efficient antimicrobial function.
Conflict of Interest

The authors declare that they have no conflicts of interest.

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References


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