BIOSYNTHESIS OF SILVER NANOPARTICLES BY GANODERMA APPLANATUM, EVALUATION OF THEIR ANTIBACTERIAL AND ANTIBIOTIC ACTIVITY ENHANCING POTENTIAL

*1Deepak K. Rahi and 2Madhurika Barwal

Department of Microbiology, Panjab University, Chandigarh- 160014, India.

ABSTRACT

The synthesis of various metal nanoparticles is now the most active area of research in nanotechnology. There are many chemical as well as physical methods, for the synthesis of metal nanoparticles however, green synthesis or biosynthesis involving different biological agents is the most emerging and preferred method of synthesis due to various reasons. In present communication we are reporting the synthesis of silver nanoparticles (AgNPs) using mushroom Ganoderma applanatum. The synthesized AgNPs have been characterized by UV/Vis spectroscopy, transmission electron microscopy (TEM), and Fourier Transform Infrared (FTIR) spectroscopy. The synthesized AgNPs were found to be in range of 4-22 nm as confirmed by TEM. FTIR analysis revealed the presence of proteins, amino acids, aldehydes, alcohol and carboxylic acids responsible for the reduction, stabilization and capping of AgNPs. The silver nanoparticles were found to possess remarkable antibacterial activity against standard test pathogenic strains, namely Methicillin- resistance Staphylococcus aureus (MRSA), Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli. Further, AgNPs were also evaluated for their efficacy to enhance the antibiotic activities of some important broad range commercial antibiotics (Methicillin, Penicillin, Amoxycillin and Ampicillin) and the result obtained revealed a manifold increase of antibacterial activity against the test pathogens.

KEYWORDS: Ganoderma, Amoxycillin, aureus, aeruginosa, AgNPs.
INTRODUCTION

Metallic nanoparticles have attracted scientist for over a century and are now widely utilized in biomedical sciences and engineering. They are the centre of interest due to their huge potential in the nanotechnology as they possess definite chemical, optical and mechanical properties. Metal nanoparticles can be modified with various chemical functional groups which allow them to conjugate with antibodies, ligands, and drugs of interest. Different types of metal nanomaterials like copper, zinc, titanium, gold and silver have been synthesized in the recent past.\(^{1, 2}\) Among the noble metals, silver has been used since the ancient time for burn wound treatment, dental work, catheters and bacterial infection control, in forms of metallic silver, silver nitrate, and silver sulfadiazine.\(^{3}\) Silver nanoparticles have also proved to be the most effective as it has good antimicrobial efficacy against bacteria, viruses and various other pathogenic microorganisms. Till date, the synthesis of AgNPs is extensively studied by using chemical and physical methods but these methods are expensive and involved toxic chemicals and by-products. Therefore, the development of some alternative technology to produce nanoparticles is an important aspect of nanotechnology. Biological synthesis is preferred as an important alternative to this as it provides a wide range of environmentally acceptable methodology with low cost of production.\(^{4}\) Many biological entities such as microorganisms (like bacteria, fungi, etc.), biochemical components (like enzyme, polysaccharide etc.) and plants are being used for the biological synthesis of nanoparticles\(^{5-8}\). However, the use of microorganisms especially the fungi is potentially exciting since they secrete large amount of enzymes and produce biomass which catalyses reduction reaction for biosynthesis of nanoparticles very efficiently and are also easy to handle. These properties of fungi have made them an ideal biological candidate for the synthesis of various metal nanoparticles. In the recent years several fungal species like *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium* spp. etc. have been reported for the biosynthesis of AgNPs.\(^{9-11}\) Comparatively, the higher fungi such as mushrooms in this regard have not received the much attention. Mushrooms are the fleshy fruiting bodies of the basidiomycetes fungi, typically found above ground on soil, rotten woods or trees. These fungi constitute a very favorable object of nanobiotechnological studies, as they have a high biomass yield and can accumulate large amounts of reduced nanoparticles in their mycelium.\(^{12}\) Therefore, in present study, the *Ganoderma applanatum*, an important medicinal mushroom has been explored for the synthesis of silver nanoparticles. The particles being synthesized were also characterized, evaluated for their antibacterial activities and assessed for their role as antibiotic activity enhancers.
MATERIALS AND METHODS

Materials

The culture of *Ganoderma applanatum* was obtained from Department of Microbiology, Panjab University, Chandigarh, India. All the chemicals used were of the reagent grade.

Cultivation of fungal biomass and biosynthesis of silver nanoparticles

The synthesis of silver nanoparticles by fungal biomass was done as per method of Hemath *et al.*[13] In this method the fungal biomass was grown in 100 ml Potato dextrose broth in 250 ml flask. Each culture flask was incubated in an incubator shaker at 28°C ± 1°C for 14 days. After incubation, the fungal biomass was harvested either by filtration or by centrifugation (at 10000 rpm for 10 minutes) and washed 3-4 times to remove any attached media components. 10 gm of this mycelial biomass was suspended in 100 ml sterilized water in 250 ml flasks separately and kept at 28± 1°C under shaking at 150 rpm for 3 days to obtain the cell free filtrate. Cell filtrate was obtained by filtration using Whatman filter paper No.1. The reaction for biosynthesis of silver nanoparticles was carried by mixing 0.5 ml of 0.1 M silver nitrate in 49.5 ml of cell free filtrate to get the concentration of silver nitrate to 1mM in the final solution. The reaction mixture was kept in dark at 28± 1°C under shaking at 150 rpm for 5 days. The cell free filtrate (without silver nitrate) was maintained as control under same conditions.

Characterization of silver nanoparticles

The silver nanoparticles produced, were characterized by visual detection, UV/Vis spectroscopy, transmission electron microscopy (TEM) and Fourier transform infrared spectroscopy (FTIR). The presence of silver nanoparticles was detected by the change in color from transparent/yellow to brown. Also, by UV/Vis spectra showing peak between 380-680 nm, average around 420 nm confirms the presence of silver nanoparticles. The spectroscopical readings were taken with Hitachi UV-2900 spectrophotometer after every 24 hours for 5 days. The characterization of the size and shape of silver nanoparticles was done by analyzing with transmission electron microscope (Hitachi 7500 TEM operating at 120 kV). The FTIR was carried out to identify the possible interactions between protein and silver nanoparticles on a Nicolet IR 200 (Thermo electron Corp) model.

Evaluation of antibacterial activity of silver nanoparticles

The antibacterial activity of biosynthesized silver nanoparticles was studied by agar diffusion method of Bauer *et al.*[14] The antibacterial activity was evaluated against standard bacterial
test pathogens, *Escherichia coli*, Methicillin resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The test pathogens were first inoculated on nutrient agar plate by spread plating method. Then wells on the plates were made by sterile borer and loaded with the 10µl of silver nanoparticles under aseptic conditions and incubated for 24 hours at 37°C. After 24 hours, the formation of zone of inhibition (if any) was observed.

**Evaluation of antibiotic enhancing activity of silver nanoparticles with commercial antibiotics**

Antibacterial activity of biosynthesized silver nanoparticles in combination with commercial broad spectrum antibiotics (Penicillin, Methicillin, Ampicillin and Amoxicillin) was evaluated as per disk diffusion method of Bauer.\(^{[14]}\) In this method, each of the antibiotic discs (10µg) were impregnated with biosynthesized silver nanoparticles (10µl) and placed on nutrient agar plates inoculated with bacterial test pathogens (*E. coli*, MRSA, *P. aeruginosa*, *S. aureus*). The plates were incubated at 37°C for 24 hrs and zones of inhibition (if formed) were observed after incubation, measured in millimeters and compared with control (i.e, disc impregnated with silver nitrate only and antibiotic discs with no silver nanoparticles). The increase in fold area of zone of inhibition was assessed as per Birla *et al.*\(^{[15]}\) by calculating the mean surface area of the inhibition zone of each antibiotic (A) and antibiotic + silver nanoparticles (B) by the equation \((B^2 - A^2)/A^2\), where ‘A’ and ‘B’ were zones of inhibition for antibiotic and antibiotic + silver nanoparticles respectively. In case of absence of inhibition zone, 6mm that represent the diameter of the disc was taken.

**RESULTS AND DISCUSSION**

**Screening for biosynthesis of silver nanoparticles**

The *Ganoderma applanatum* was used to screen its efficacy to synthesize silver nanoparticles. The biosynthesis was screen out by visual observation and UV/Vis spectroscopical studies.

**Visual detection**

The silver nanoparticles were visually detected by the change in the color from transparent to yellow or to dark brown of the reaction suspension, containing cell free filtrate and silver nitrate (Figure 1). The reduction of silver ions to silver nanoparticles (i.e. Ag\(^+\) to Ag\(^0\)) led to change of color from transparent or light yellow to brown, which indicated the formation of silver nanoparticles in the reaction mixture. The control did not show any change in its initial
color when incubated under the same conditions (Figure 1). This brown color was due to the excitation of the surface plasmon vibrations in the metal nanoparticles, as suggested by Ahmad et al. \cite{10}

![Image](image1.png)

**Figure 1:** The reaction mixture on 5th day of incubation, showing the biosynthesis of silver nanoparticles by *Ganoderma applanatum* indicated by change in colour from yellow to dark brown in ‘Test’ (T); No change in colour observed in Control (C).

**UV/Visible Spectrophotometry**

Along with colour change, UV/Vis spectrophotometry has also been used to detect the synthesis of silver nanoparticles. The samples containing the synthesized silver nanoparticles, showed a peak in range of 380-680 nm which is the defined range of the silver nanoparticles, taken after every 24 hours for 5 days. The highest peak was indicated on 5th day of AgNPs production (Figure 2). The width of the peak is indicative of polydispersed nature of nanoparticles ranging in sub-micron sizes.\cite{16}

![Image](image2.png)

**Figure 2:** Peak of silver nanoparticles synthesized by *Ganoderma applanatum* on 5th day of incubation as obtained by UV/Vis spectroscopy.
Characterization of silver nanoparticles

Transmission Electron Microscopy (TEM)

The characterization of size and shape of silver nanoparticles synthesized by *Ganoderma applanatum* with transmission electron microscopy (TEM) revealed the particles were mostly of spherical in shape, distributed randomly, polydispersed and ranged from 3 to 15 nm (Figure 3). Similar results were obtained by Karwa *et al.*[16] on silver nanoparticle synthesis by *Ganoderma lucidium*. They also reported the polydisperse nature of their nanoparticles, however their size ranged between 10 to 70 nm.

![TEM image of silver nanoparticles synthesized by *Ganoderma applanatum* showing shape and size distribution.](image)

**Figure 3:** TEM image of silver nanoparticles synthesized by *Ganoderma applanatum* showing shape and size distribution.

FTIR spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy is a useful technique to study the core shell morphology of the synthesized silver nanoparticles. It is stated that certain extracellular proteins are released by the fungal cells in the filtrate that could play role in synthesis and stability of silver nanoparticles. FTIR analysis of silver nanoparticles provide information about the chemical bonds and molecular structures that could play important role in
nanoparticle synthesis. The FTIR spectra (Figure 4) of silver nanoparticles synthesized by *Ganoderma applanatum* showed the band at 3400 cm\(^{-1}\) which has been identified as -NH group of amines, 2140 cm\(^{-1}\) as aromatic –CH stretching, 1645.26 cm\(^{-1}\) -NHCO of amide and 745.35 cm\(^{-1}\) (C-Cl). These spectrum clarifies the presence of N-H, C-N, C-C, C-H, C-O, amide linkages, and linkages for nitro compounds etc. that may be present between the synthesized nanoparticles as stabilizing caps, along with proteins and amino acid residues. Therefore, from the FTIR results it is clear that the synthesized nanoparticles were surrounded by proteins and amino acids which may be responsible for the stability of the silver nanoparticles and it can be assumed that the functional groups of alcohols, aldehydes and carboxylic acids present in the sample may be responsible for the reduction of silver nitrate to silver nanoparticle.

![FTIR spectra of silver nanoparticles biosynthesised by *Ganoderma applanatum*.](image)

**Figure 4**: FTIR spectra of silver nanoparticles biosynthesised by *Ganoderma applanatum*.

**Evaluation of antibacterial activity of silver nanoparticles**

The antibacterial activity of silver nanoparticles synthesized by the *Ganoderma applanatum* was measured as the diameter of the zone of inhibition in mm (Table 1 and Figure 5). The bigger the zone of inhibition, the higher the antibacterial activity. The results revealed that the silver nanoparticles synthesized by *Ganoderma applanatum* were quite effective in inhibiting the growth of all the test organisms. The zone of inhibition (Figure 5) clearly depicted the inhibitory effect of silver nanoparticles. The effect of silver nanoparticles on gram negative
bacteria was shown to be more as compared to gram positive bacteria. The reason behind this might be the cell wall composition of gram positive bacteria as described by Birla et al.\textsuperscript{[15]}. A similar case was also reviewed by Singh et al.\textsuperscript{[18]}. Different theories proposed to evaluate the mechanism of antibacterial activity of silver nanoparticles, reports that the silver ions interacts with the thiol groups of some of the major enzymes in bacteria which inactivates them.\textsuperscript{[19, 20]}

Table 1: Zone of inhibition (diameter in mm) obtained by silver nanoparticles synthesized by \textit{Ganoderma applanatum} against standard bacterial test pathogens.

<table>
<thead>
<tr>
<th>Bacterial Pathogens</th>
<th>MRSA</th>
<th>Staphylococcus aureus</th>
<th>Pseudomonas aeruginosa</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test (AgNps)</td>
<td>30mm</td>
<td>20mm</td>
<td>24mm</td>
<td>24mm</td>
</tr>
<tr>
<td>Control (AgNO\textsubscript{3})</td>
<td>20mm</td>
<td>8mm</td>
<td>12mm</td>
<td>8mm</td>
</tr>
</tbody>
</table>

T : Test (AgNps); C: Control (AgNO\textsubscript{3})

Figure: 4 Antibacterial activity of silver nanoparticles produced by \textit{Ganoderma applanatum} against (A) MRSA (B) \textit{Staphylococcus aureus} (C) \textit{Pseudomonas aeruginosa} (D) \textit{Escherichia coli}
Evaluation of antibiotic enhancing activity of silver nanoparticles with commercial antibiotics

After evaluating the antibacterial activity of biosynthesized silver nanoparticles, their ability of enhancing the antibacterial activity of some commercial antibiotics was also evaluated. The synergistic effect of antibiotics along with silver nanoparticles was seen against MRSA, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The commercial antibiotics used were Penicillin (Pnc), Ampicillin (Amp), Amoxycillin (Amp) and Methicillin (Met). Most of the bacteria have shown resistance against these commercial antibiotics, however the synergistic combination of these antibiotics with silver nanoparticles synthesized by *Ganoderma applanatum* remarkably enhanced the antibacterial activities of all the antibiotics as revealed by fold increase in the antibacterial activities of the antibiotics (Table 2; Figure 6). The highest fold increase (17.77) was seen against *E.coli* by the effect of silver nanoparticles in combination with amoxycillin (Table 2).

![Antibiotic enhancing activity of silver nanoparticles produced by Ganoderma applanatum against various bacteria](image)

C in the centre represent Control (AgNO₃)

Figure 7: Antibiotic enhancing activity of silver nanoparticles produced by *Ganoderma applanatum* against
1. *MRSA* a) Amc b) Amc with AgNps c) Pnc d) Pnc with AgNps e) Amp f) Amp with AgNps g) Met h) Met with AgNps.
2. *S. aureus* a) Amc with AgNps b) Pnc c) Pnc with AgNps d) Met e) Met with AgNps f) Amp g) Amp with AgNps h) Amc.
3. *P. Aeruginosa* a) Met b) Met with AgNps c) Amp d) Amp with AgNps e) Pnc f) Pnc with AgNps g) Amc h) Amc with AgNps.
4. *E. coli* a) Pnc b) Pnc with AgNps c) Amp d) Amp with AgNps e) Met f) Met with AgNps g) Amc h) Amc with AgNps.
Table: 2 Antibiotic enhancing activity of silver nanoparticles synthesized by *Ganoderma appalanatum* against the bacterial pathogens

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MRSA</th>
<th>S. aureus</th>
<th>P. aeruginosa</th>
<th>E.coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>- 11</td>
<td>2.36</td>
<td>- 23</td>
<td>13.6</td>
</tr>
<tr>
<td>Methicillin</td>
<td>7 10</td>
<td>1.41</td>
<td>- 24</td>
<td>15</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>- 7</td>
<td>13</td>
<td>- 22</td>
<td>12.4</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>- 7</td>
<td>13</td>
<td>- 25</td>
<td>16.36</td>
</tr>
</tbody>
</table>

(MRSA, S. aureus, P. aeruginosa & E.coli).  

Ab: Antibiotic ; Ab + AgNp: Antibiotic in combination with silver nanoparticle.  

Where, , [A] and [B] denotes Ab alone and Ab+ silver nanoparticle produced respectively and the increase in fold area of inhibition is shown by  

[B]²-[A]² / [A].
CONCLUSION
The medicinal mushroom *Ganoderma applanatum* has been explored for its enormous health benefits but existing literature reports no work on its capability to synthesize metal nanoparticles. The present work on silver nanoparticles synthesis using *Ganoderma applanatum* have shown a potential bactericidal activity against standard bacterial pathogens and also demonstrated a remarkable enhancement of antibacterial efficacy of important commercial antibiotics. Thus, if explored and characterized further, these nanoparticles may prove useful in various other medical as well as industrial applications.

ACKNOWLEDGEMENT
Authors are grateful to Chairperson, Department of Microbiology Chandigarh for providing the grants and necessary laboratory facilities to carry out the present work.

REFERENCES
7. Vigneshwaran N, Ashtaputre NM, Varadarajan PV, Nachane RP, Paralikar KM, Balasubramanya RH. “Biological synthesis of silver nanoparticles using the fungus
Aspergillus flavus,” Materials Letters., 2007; 61(6): 1413–1418. View at Publisher · View at Google Scholar · View at Scopus


