

Research Article



Synthesis of Silver Nanoparticles from *Lentinula edodes* and Antibacterial Activity

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ABSTRACT

Lentinula edodes is a world second largest cultivated edible and medicinal mushroom. It is good source of carbohydrates, proteins and essential amino acids. In the present investigation an eco- friendly route for the synthesis of silver nanoparticles using *Lentinula edodes* fungus and its antimicrobial activity has been reported. The synthesized silver nanoparticles were confirmed by UV - VIS spectrum of the aqueous solution containing silver ions showed peak at 381 nm corresponding to the surface Plasmon absorbance of silver nanoparticles. Silver nanoparticle showed the antimicrobial activity against the gram positive and gram negative bacteria.

Keywords: *Lentinula edodes*, Silver nanoparticles, Antimicrobial activity, UV - VIS Spectrum, Mushroom.

INTRODUCTION

The black oak mushroom commonly known as shiitake, is the second most important edible mushroom in the world¹. This mushroom has been cultivated in Japan and China for about 2000 years as well in Thailand, Korea and Brazil^{2,3}. Among the countries, Japan is the major world producer of this mushroom, reaching production of 7.5 million tones.

The great interest in shiitake commercialization is due to its unique flavour / taste, nutritive value and medicinal properties^{4,5}. Its fungal mycelia has high content of proteins, fibers, vitamins, minerals and low content of lipid specifically cholesterol⁶.

Mushroom with their delicate flavor and texture are recognized as a nutritious food and an important source of biologically active compounds with medicinal values. Generally, mushroom are low in energy and high in dietary fibre⁷ and an external source for antioxidant as they accumulate a variety of secondary metabolites including phenolic compounds^{8,9}. Antioxidants are substances needed in minute quantity which are capable of counter acting with free radical to prevent oxidative damage. There are about 1200 species of mushroom used in 85 different countries for their gastronomic value and or medicinal properties¹⁰.

Nanotechnology has dynamically developed as an important field of modern research with potential effects in electronic and medicine¹¹⁻¹³. Nanotechnology can be defined as a research for the design, synthesis, and manipulation of structure of particles with dimension smaller than 100nm.

The medical properties of silver have been known for over 2,000 years. Since the nineteenth century, silver-based compounds have been used in many antimicrobial applications. Nanoparticles have been known to be used for numerous physical, biological, and pharmaceutical applications. Silver nanoparticles are being used as

antimicrobial agents in many public places such as railway stations and elevators in China, and they are said to show good antimicrobial action.

MATERIALS AND METHODS

Culture Maintenance

The strain *Lentinula edodes* were collected from Microbial Type Culture Collection Centre at Chandigarh. The sample was maintained at 25°C on malt agar medium and malt broth. The fungal filtrate used for biosynthetic experiments.

Formation of Silver Nanoparticle¹⁴

The cell free filtrate was obtained by filtration of the *Lentinula edodes* using Whatmann. No.1 filter paper. For the synthesis of silver nanoparticles 20ml of the cell free filtrate was brought in contact with 1mM final concentration in 150 ml Erlen Meyer flask and agitated at 25°C in dark conditions under normal pH. Simultaneously control without silver ions was also run along with the experimental flasks.

UV-VIS Spectrum Analysis¹⁴

The reduction of silver ions was monitored by measuring the UV-VIS spectrum of the reaction medium at 24 hr time interval by drawing 1cm³ of the sample and the absorbance was recorded at a resolution of 1 nm, between 300 and 500 nm, using 10 -mm -optical- path-length quartz cuvettes (ELICO SL 159 UV - VIS nanodrop spectrophotometer).

Antibacterial activity¹⁵

The antibacterial activity of the synthesis of silver nanoparticle, cell free filtrate tested against the selected bacterial strains. The 20ml of sterilized agar medium was poured into each sterile petriplates and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using a sterile cotton swab. Then a well of 0.5cm was made in the medium by



using a sterile cork borer, 20 μ l, 10 μ l of each as *Lentinula edodes* (synthesis of silver nanoparticle, cell free filtrate) were transferred into separate wells. These plates were incubated at 37°C for 24 hours. After incubation period, the results were observed and measured the diameter of inhibition zone around the each well.

RESULTS AND DISCUSSION

Color change of the cell free extract when challenged with 1mM AgNO₃ changed color from pale yellow to light brown in 48 hrs and attained maximum intensity after 72hrs with intensity increasing during the period of incubation indicative of the formation of silver nanoparticle. Control without silver ions showed no change in colour of the cell filtrates when incubated under same conditions¹⁴. Addition of silver ions into the filtered cell free filtrate in the dark samples changes its color from almost colorless to brown with intensity increasing during the period of incubation.¹⁶

The conversion of 3mM silver nitrate solution to nanosilver by *Fusarium oxysporum* in an aqueous medium due to the change in color of the reaction mixture from pale yellow to dark brown.¹⁷. In this study the cell free filtrate was changed from pale yellow to light brown. The brown colour of the medium could be due to the excitation of surface Plasmon vibration which is characteristics of silver nanoparticles. It is speculated that metal ions are reduced by enzymes and proteins in the cells leading to the nanoparticles formation.

The UV-Vis spectrum obtained from biologically synthesized silver nanosolution. It is observed from the spectra that the silver surface plasmon band occurs at 420 nm, it is well known that the size and shape of silver nanoparticles reflect the absorption peak apart from these two absorption peaks were observed in the UV region corresponding to 220 and 280 nm. While the peak at 220nm may be due to the amide band, the other peak at 280nm may be due to the tryptophan and tyrosine residues present in the protein that might have stabilized the nanoparticles.¹⁸

Silver nitrate solution when incubated with spent mushroom substrate synthesis of silver nanoparticles purified solution yielded the maximum absorbance at 436nm.¹⁹

Formation of AgNPs using 1mM solution of AgNO₃ was confirmed using UV- visible spectral analysis. Metal nanoparticles such as silver have free electrons, which gives rise to surface Plasmon resonance (SPR) absorption band. The characteristic surface Plasmon resonance band of biogenic AgNPs occurs at 300nm.¹⁵

Biosynthesis of nanosilver was also monitored using the UV – VIS nanodrop spectrophotometer the UV-VIS spectroscopy. An UV-Vis spectrum is one of the important and easy techniques to verify the formation of metal nanoparticles provided surface plasmon resonance exists for the metal. Plasmon vibrations and the peak at 343nm

corresponded. Maximum intensity was achieved after 24 hours of the reaction, which indicates the complete reduction of the Ag⁺ ions. An intense brown color of the reaction mixture further supports the complete reduction of Ag⁺ ions and the formation of AgNPs (Fig -1).

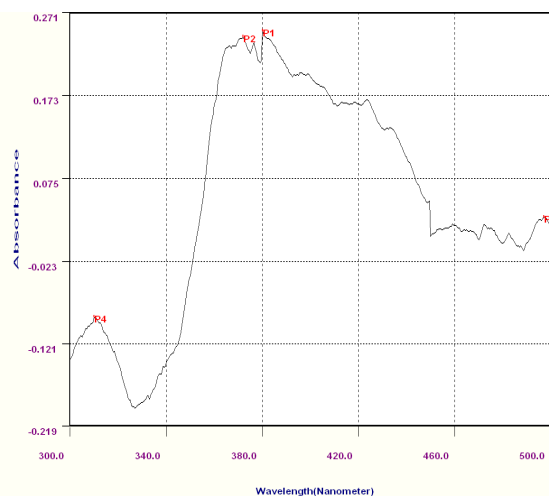


Figure 1: UV-VIS studies

The silver nanoparticles exhibited good antibacterial activity against both gram negative and gram positive bacteria. But it showed higher antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* (Gram negative) than *Staphylococcus aureus* (Gram positive). Silver nanoparticle undergo an interaction with bacterial cells.²⁰

The zone of inhibition of silver nanoparticles against Gram negative and positive organisms. The present study clearly indicates that *Pleurotus sajorcaju* has good antibacterial action against Gram negative organisms *Pseudomonas aeruginosa* and *Escherichia coli* when compared with the Gram positive organism *Staphylococcus aureus*¹⁴. The present investigation was clearly indicates that cell free filtrate of *Lentinula edodes* has good antibacterial activity against gram negative organisms *Escherichia coli*, *Klebsiella pneumoniae* and gram positive organism *Staphylococcus aureus*, *Bacillus subtilis* (Table 1).

CONCLUSION

The silver nanoparticles were synthesized and characterized from fungal strain *Lentinula edodes*. The synthesized silver nanoparticles has good antibacterial activity against gram positive and gram negative bacterial strains.

The ecofriendly process for the synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology.

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Table 1: Antibacterial activity of synthesized and non synthesized strains

Strains	<i>L.edodes</i> synthesized strain	<i>L.edodes</i> Non synthesized strain	1 mM AgNO ₃	Methicillin
<i>Escherichia coli</i>	9	-	3	-
<i>Klebsiella pneumoniae</i>	9	2	4	-
<i>Staphylococcus aureus</i>	8	-	5	-
<i>Bacillus subtilis</i>	4	2	3	-

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