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Mushrooms (*Agaricus bisporus*) mediated biosynthesis of silver nanoparticles, characterization and their antimicrobial activity

ABSTRACT

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In this paper we report an eco-friendly route for the synthesis of silver nanoparticles using *Agaricus bisporus* (white button mushroom) extract. The synthesized silver nanoparticles were confirmed and characterized by UV-Visible spectrum of the aqueous solution containing silver ions showed a peak at 420 nm corresponding to the surface plasmon absorbance of silver nanoparticles. Transmission Electron Microscopy (TEM) micrographs showed the size of silver nanoparticles and were measured in the range of 8-50nm, Fourier Transform Infrared Spectroscopy (FTIR) spectrum detection of analysis showed peaks between 500-4000 cm⁻¹ confirmed the presence of proteins, carbonyl groups, esters and carboxylic acids for the synthesis and stabilization of silver nanoparticles. X-ray diffraction (XRD) spectrum of the silver nanoparticles exhibited 2θ values corresponding to the silver nanocrystal. Further, the antibacterial activity of synthesized silver nanoparticles showed effective inhibitory activity against pathogens and non-pathogenic bacterial strains *vis*, *Escherichia coli*, *Staphylococcus sps*, *pseudomonas sps*, and *Bacillus sps*.

Keywords: *Agaricus bisporus* (white button mushroom); silver nanoparticles synthesis (SNPs); Characterization; Antimicrobial activity

INTRODUCTION

Nanotechnology is mainly concerned with synthesis of nanoparticles of variable size, shapes, chemical composition and controlled dispersity and their potential use for human benefits. The most predominantly studied about nanoparticles today are those made from noble metals, in particular Ag, Pt, Au and Pd. Among the four, silver nanoparticles play a significant role in the field of biology and medicine.

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Nanotechnology is mainly concerned with the synthesis of nanoparticles of variable size, shape, chemical composition and controlled dispersity and their potential use for human benefits. Silver nanoparticles play a significant role in the field of biology and medicine. There is a growing need to develop clean, nontoxic and environmentally friendly (green chemistry) procedures for synthesis and assembly of nanoparticles, biosynthesis of silver nanoparticles using plants [1,2,3], bacteria [4,5], fungi [6,7] and yeast [8,9] are known to reduce silver ions into silver nanoparticles by both extra and intracellularly [10,11,12]. The application of nanoscale materials and structures usually ranging from 1 to 100 nanometers (nm) is an emerging area of nanoscience and nanotechnology. The biosynthesis of silver nanoparticles have numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for synthesis protocols. Chemical synthesis methods lead to the presence of toxic chemical absorbed on the surface that may have adverse effect in the medical applications. The strong toxicity of silver against wide range of micro organisms is well known and silver nanoparticles showed to be promising antimicrobial materials [13, 14, 15]. It can be expected that the high specific area and high fraction of surface atoms of silver nanoparticles (Ag-NPs) will lead to high bactericidal activity as compared with bulk silver metal. The role of reductase enzyme and reducing equivalents has been discovered; in one such an example nitrate reductase, from fungus (*Fusarium oxisporium*) has been documented to catalyze the reduction of silver nanoparticles utilizing NADPH as reducing agent [16]. In addition to these extracellular enzymes several naphthaquinones and anthroquinones with excellent redox properties have been reported in *F.oxisporum* that could act as an electron shuttle in metal reductions. These studies have demonstrated the synthesis of metal nanoparticles. The applications of silver nanoparticles greatly depend on their size. Nanoparticles range in size from 1 to 10nm readily interact with HIV-1 virus via preferential binding to gp120glycoprotein, this is one of applicative nature of silver nanoparticle to control HIV-1 infection [17].

The present study includes time dependent biologically synthesis of silver nanoparticles and

characterized using by UV-Vis spectrophotometer, size and morphology characterized by Transmission Electron Microscopy (TEM), structure from X-ray diffraction (XRD) technique and understanding of protein-silver nanoparticles interactions from Fourier transform infrared (FT-IR) spectroscopy. An antibacterial activity test was conducted to *E.coli*, *Staphylococcus sps*, *Pseudomonas sps*, and *Bacillus sps* to observe differences in antibacterial activity among the silver nanoparticles obtained. [18, 19, 20].

EXPERIMENTAL

Biological synthesis of Silver nanoparticles

A 20 gm of fresh *Agaricus bisporus* (white button mushrooms) was washed thoroughly with double distilled water and transferred to 100ml of sterile distilled water and boiled for 10 min and then filtered through whatman No. 1 filter paper. The extract was stored at 4°C for further experiments. The filtrate was used as reducing and stabilizing agent for 1mm of AgNO₃ (99.99%, Sigma-Aldrich). In a typical synthesis of silver (Ag) nanoparticles the mushroom extract was added to 50 ml of 10⁻³ AgNO₃ aqueous solution (prepared in deionized water) and incubated in shaker at 150 rpm at 37°C. Simultaneously, a positive control was maintained with mushroom extract and deionized water used as negative control, containing only silver nitrate solution.

UV-Vis spectroscopy analysis

UV-visible spectroscopy analysis was carried out on a JASCO V-530, UV-Visible absorption spectrophotometer with a resolution of 2.0 nm between 200 to 600 nm possessing a scanning speed of 300 nm/min. The process of reaction between metal ions and mushroom extract were monitored by UV-Visible spectra of silver nanoparticles in aqueous solution.

X-ray diffraction (XRD) analysis

The X-ray diffraction technique is used to analyze the metallic nature of particles. After bioreduction, silver nanoparticles solution thus obtained was purified by repeated centrifugation at 5000 rpm for 20 min followed by redispersion of the pellet of silver nanoparticles into 10ml of sterile deionized water. After freeze drying of the purified

silver particles, the structure and composition were analyzed by X-ray diffraction (XRD). The dried mixture of silver nanoparticles was collected for the determination of the formation Ag nanoparticles by INEL X-ray diffractometer.

Transmission electron microscopy (TEM) Measurements

The silver nanoparticle synthesized by *Agaricus bisporus* (mushroom) extract and a drop of aqueous solution containing the silver nanomaterials was placed on the carbon coated copper grids and dried under infrared lamp. Micrographs were obtained using a Phillips; TECHNAI FE 12 instrument operated at an accelerating voltage at 200Kv.

Fourier transmission infrared (FTIR) spectroscopy measurements

The residual solution after reaction was centrifuged at 10,000 rpm for 15min and the resulting suspension was repeated for three times, after that the purified suspension was washed with deionized water to get pure form i.e., free of proteins/enzymes which are not able to capping the silver nanoparticles. The sample was completely dried at 60°C. Finally the dried nanoparticles were analysed by FTIR (Thermo Nicolet nexus 670 spectrometer of resolution 4 cm⁻¹).

Antimicrobial activity

To determine the antibacterial activity of silver nanoparticles, against bacterial strains such as *E.coli*, *Staphylococcus sps*, *Pseudomonas sps*, and *Bacillus sps*. The cultures were grown overnight in nutrient broth on a rotary shaker (200 rpm) at 37°C and then they were seeded into nutrient agar plates. Different concentrations (10µl, 50µl, 100µl) of Ag-NPs are loaded to the wells. The plates were incubated at 37°C for 24hours, after incubation, zone of inhibition around the wells were measured.

RESULTS AND DISCUSSION

Several approaches have been employed to obtain a better synthesis of silver nanoparticles such as chemical and biological methods. Similarly in the present study silver nanoparticles were synthesized rapidly with in 1 h of incubation period using *Agaricus bisporus* extract. While the

mushroom extract incubated with deionized water (positive control) retained its original colour, the silver nitrate treated mushroom extract turned to brown colour after 1h due to deposition of silver nanoparticles. As it has been shown (Figure 1), the change in colour of the solution was noted by visual observation. The colour arises due to excitation of surface plasmon resonance (SPR) in the metal nanoparticles. The colour of the solution changed to intense brown after 24h of incubation, and in case of negative control (silver nitrate solution alone), no change in colour was observed even after 2 months.

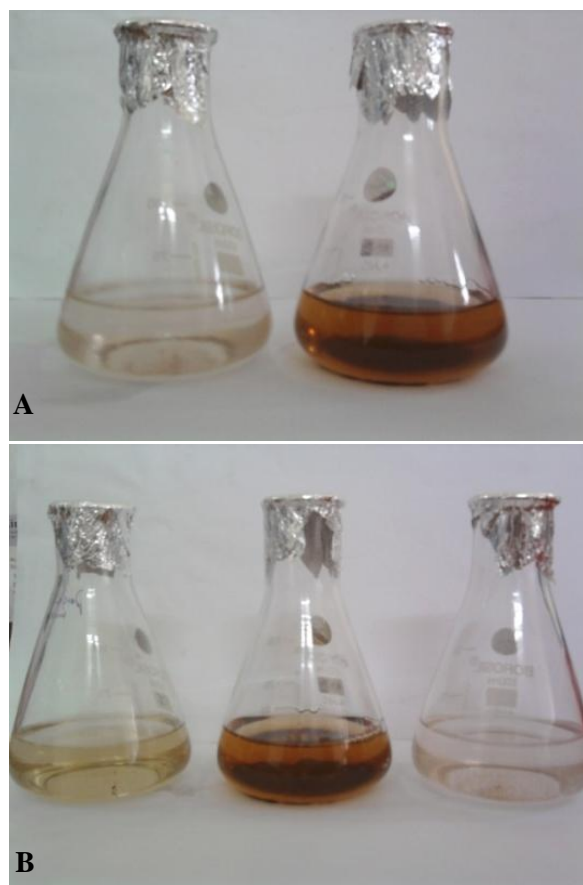


Fig.1. (A). The picture shows the colour changes before and after the process of reduction of Ag⁺ to Ag nanoparticles (negative control). (B) Picture shows the (positive) control without silver nitrate (AgNO₃)

The generation of dark brown colour is due to the surface plasmon resonance (SPR) exhibited by the nanoparticles. Figure 2 shows 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.

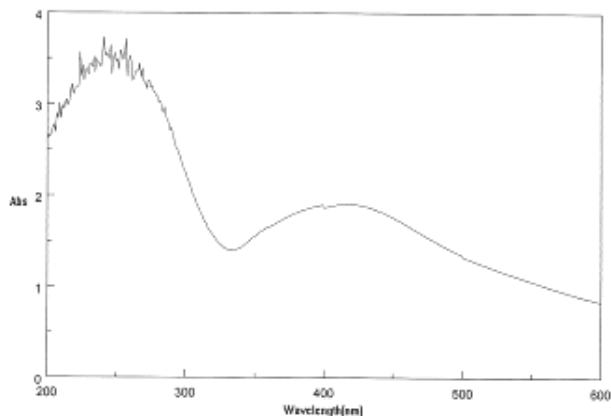


Fig.2. UV-Vis spectrum of Ag nanoparticles synthesized by reduction of Ag ion solution with the *Agaricus biporus* extract. The inset shows a digital image of the as-prepared Ag colloidal solution.

The XRD pattern of the silver nitrate-treated sample corresponds to that of silver nanoparticles as shown in the (Figure 3). The XRD pattern shows peak in the whole spectrum of 2θ values ranging from 10 to 120. It is important to know the exact nature of the silver particles formed and this can be deduced from the XRD spectrum of the sample. XRD pattern spectra clearly shows the pure crystalline silver structures. The data obtained was matched with the database of Joint Committee on Powder Diffraction Standards (JCPDS file No.04-0783). A comparison of our XRD spectrum with the standard and it was confirmed that the silver particles formed in our experiments were in the form of nanocrystals, as evidenced by the peaks at 2θ values of 38.250 corresponding to (111) plane for silver, respectively. The full width at half maximum (FWHM) values measured for 111 plane of reflection were used with the Debye-Scherrer's equation $d = 0.9 \lambda / \beta \cos\theta$ the average size of the nanoparticles was estimated as 8.5nm.

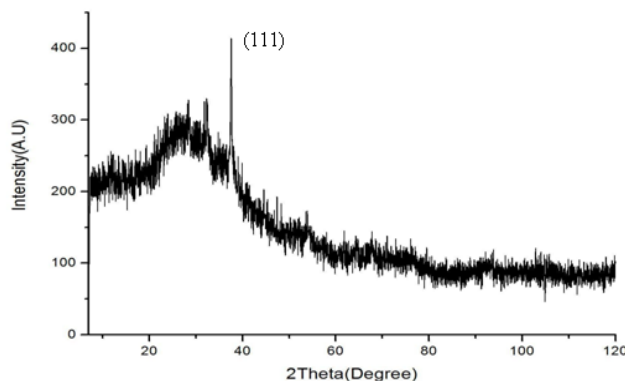


Fig.3. X- ray diffraction (XRD) pattern of as synthesized silver nanoparticles

TEM technique was employed to visualize the size and morphology of nanoparticles. Figure 4 shows most of the silver nanoparticles were spherical in shape. A few agglomerated silver nanoparticles were also observed in some places.

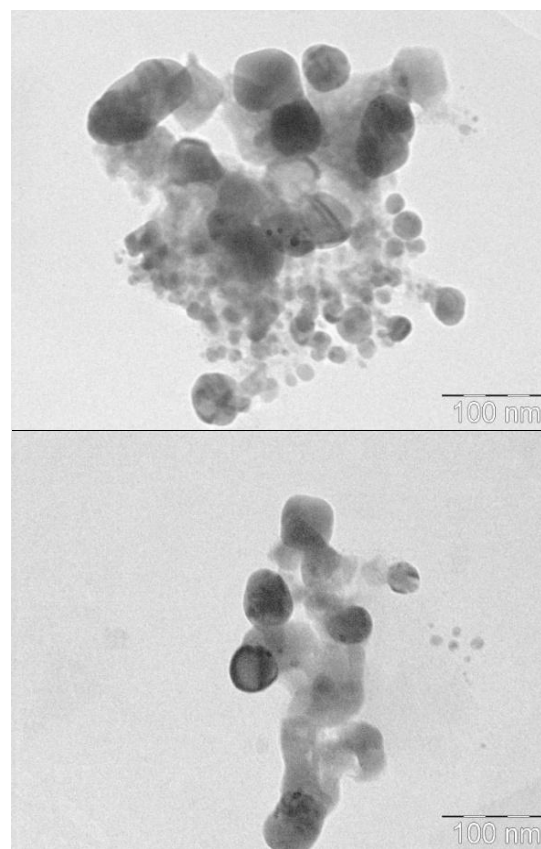


Fig.4. Transmission Electron Microscopy (TEM) images of synthesized silver nanoparticles

Figure 5 shows the histogram taken from a number of micrographs. It is evident that there is a variation in particle sizes and average size estimated was 8nm and the particles sizes ranged from 6nm to 50nm.

FTIR measurement were carried out to identify the potential biomolecule in *Agaricus biporus* extract responsible for the reduction of the silver ions and also capping reagent responsible for the stability of bioreduced silver nanoparticles. Figure 6 shows the FTIR spectra of

silver nanoparticles. The absorption bands centered at 1080, 1385, 1629, 3389 cm^{-1} and 1080 cm^{-1} is associated with vibration of C-O stretch and is assigned to the Ester linkages. 1385 cm^{-1} was notably enhanced in that NO_3^- existed in the residual solution. The peak at 1629 cm^{-1} corresponding to amide I, arising due to carbonyl stretch vibrations in the linkage of the protein, the peak at 3389 cm^{-1} refers to the stretching vibration of primary amines.

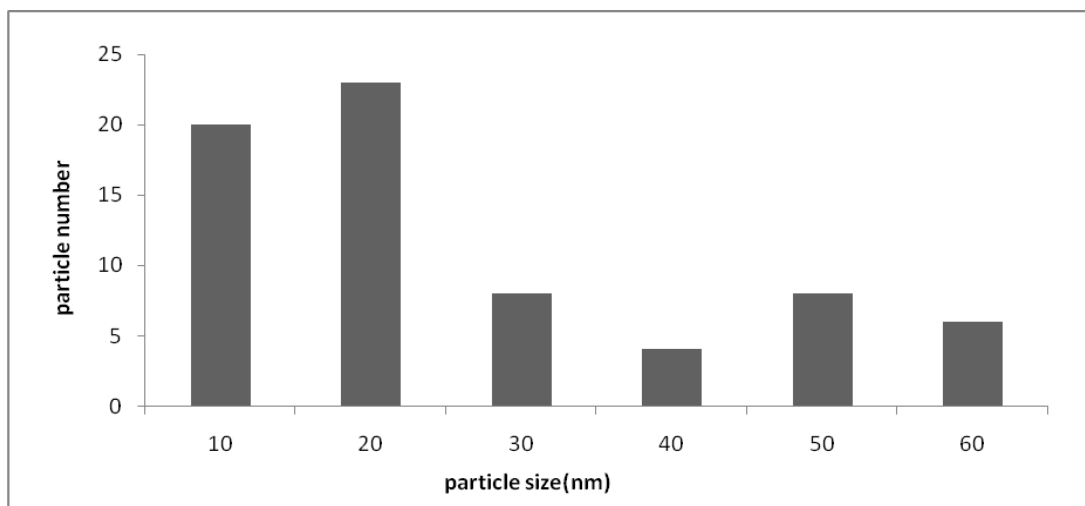


Fig.5. A particle size distribution histogram of as synthesized silver nanoparticles determined from Transmission Electron Microscopy (TEM) images.

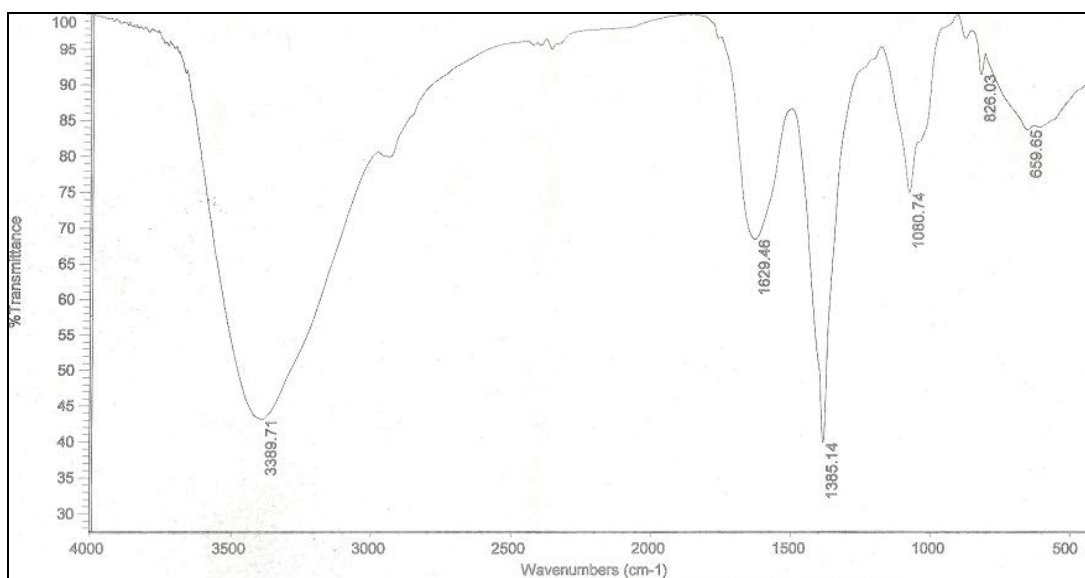


Fig.6. Fourier Transform Infrared Spectroscopy (FTIR) spectrum of Ag nanoparticles synthesized by reduction of Ag^+ ions by *Agaricus biporus*.

The size of metal nanoparticles ensures that a significantly large surface area of the particles is in contact with the bacterial species. Considering a hypothetical case with spherical particles size from 10nm to 20nm will increase the contact surface area. However, smallness in itself is not the goal. Synthesis and characterizations of nanoscaled materials in terms of novel physicochemical properties is of great interest in the formation of bactericidal materials. The synthesized silver nanoparticle shows an effective antibacterial activity against pathogens of gram positive and gram negative bacteria. The result suggests that silver nanoparticles undergo a interaction with bacterial cell and displayed the strong action against *E.coli*, *Staphylococcus sps*, *Pseudomonas sps*, and *Bacillus sps*. Klabs and co-worke demonstrated that reactive metaloxide

nanoparticles show an excellent bactericidal effect. It has been known for a long time that silver ions and silver compounds are highly toxic to most bacterial strains. Recently it was shown that the highly concentrated and nonhazardous nanosized silver nanoparticles can easily be prepared in a cost effective manner and tested as a new type of bactericidal and fungicidal nanomaterial agents. In this study different concentrations of silver nanoparticles ie 10µl/ml, 50µl/ml, and 100µl/ml was tested on bacterial strains *E.coli*, *Staphylococcus sps*, *Pseudomonas sps*, and *Bacillus sps* and shown in Figure 7. The formation of clear zone (restricted bacterial growth) around the cavity is an indication of antibacterial activity. The zone of inhibition of diameters was determined at concentrations, respectively (Table 1).

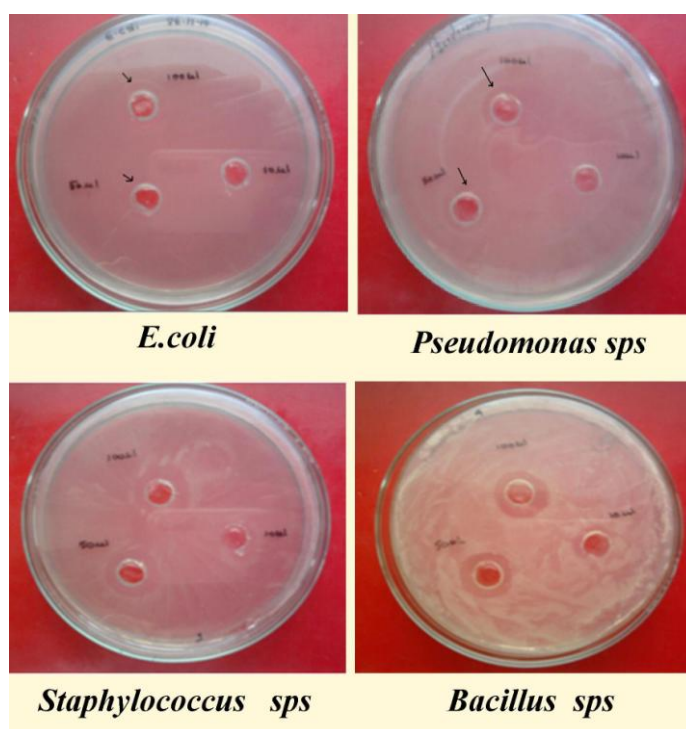


Fig.7. Appearance of inhibitory zones on agar plates at different concentrations of silver nanoparticles. In each figure the concentrations of silver nanoparticles are as 10ul, 50ul, 100ul. (*E.coli*, *staphylococcus sps*, *pseudomonas sps*, and *bacillus sps*)

Table 1. Inhibitory activity of silver nanoparticles on bacterial strains.

S.No	Organism	Zone of Inhibition(cm)at differen concentrations(μ l)		
		10 μ l	50 μ l	100 μ l
1	<i>E.coli</i>	1	1.6	1.8
2	<i>Staphylococcus sps</i>	1	1.4	1.9
3	<i>Pseudomonas sps</i>	0.8	1.3	1.6
4	<i>Bacillus sps</i>	1.4	1.6	1.8

*All values represented the in the table are average of conducted experiment.

CONCLUSION

The silver nanoparticles were synthesized from fungal strain *Agaricus biporus* (white synthesis). The synthesized silver nanoparticles were characterized by sophisticated analysis the silver nanoparticles showed potential anti bacterial activity against both pathogenic and nonpathogenic, gram positive and gram negative bacterial strains.

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