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Synthesis of Silver Nanoparticles from Edible Mushroom and Its Antimicrobial Activity against Human Pathogens

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Abstract: In the present study, we have reported the biological synthesis of silver nanoparticles (AgNPs) by using edible mushroom extract, Agaricus bisporus, as a bioreductant. The biosynthetic method developed in this study for producing silver nanoparticles has distinct advantages over chemical methods such as high biosafety and being ecofriendly and nontoxic to the environment. Furthermore, these functionalized silver nanoparticles showed a noticeable antimicrobial activity against different clinically important pathogenic microorganisms. Hence, such type of synthesis methods for the production of nanostructured materials at lower cost and with natural energy may encourage production of functionalized AgNPs on industrial scale. This is quite handy for using AgNPs on a wide range of applications in the field of Nano biotechnology.
Keywords: Silver nanoparticles, Agaricus bisporous, UV-Vis spectra, Antibacterial activity.

Introduction

Nanotechnology is the engineering of functional systems at the molecular scale. The field nanoscience and technology has gained great importance because of their potential applications in various areas such as chemicals, textile industries, materials industry, medical diagnostic (future nanobots), drug and gene delivery and electronics, diagnosis, artificial implants, tissues engineering[1], computing, biochemical sensors[2], medical imaging[3] and so on. It provides a platform to modify and develop the important properties of metal in the form of nanoparticles having promising applications in diagnostics, biomarkers, cell labeling, contrast agents for biological imaging, antimicrobial agents, drug delivery systems and nano-drugs for treatment of various disease [4,5]. One important use of silver nanoparticles is to give products a silver finish. Nanosilver's strong antimicrobial activity is a major reason for the development of nanosilver containing products. Of the more than 1000 consumer products that contain nanomaterials, roughly 25% are claimed to contain silver nanoparticles. Widely available consumer products that contain nanosilver include food contact materials (such as cups, bowls and cutting boards), odor-resistant textiles, electronics and household appliances, cosmetics and personal care products, medical devices, water disinfectants, room sprays, children's toys, infant products and 'health' supplements (6).The AgNPs were found to have reasonable antibacterial activity against a few pathogenic bacteria like *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. (7)

Materials and Methods

- **Preparation of Crude Extract of *Agaricus bisporus***

Fresh mushrooms *Agaricus bisporous* (white button mushrooms) were procured from commercial sources. About 10 gm. of the mushroom was weighed out and washed thoroughly with double distilled water. It is then crushed and transferred to a beaker containing 100ml of sterile distilled water. This mixture is stirred for about 2 hours and then filtered using Whattman No.1 filter paper. The resultant filtrate is the extract of mushroom used for the reduction of Ag+ to Ag0. The extract of mushroom can be preserved for further experiments by storing it at 40° C.

Standard Preparation of Silver Nanoparticle

- In a typical synthesis of silver nanoparticle, equal volume of mushroom extract is mixed to 1mM AgNO3 prepared in deionized water. The mixture is then incubated overnight in a shaker at 150 rpm at 37°C.
- Samples of different concentrations of mushroom extract and AgNO3 was prepared to derive the most efficient preparatory method for efficient and faster synthesis of silver nanoparticles.
- Sample 1 was prepared using 50ml of mushroom extract which was added to 50 ml of 1mM AgNO3 aqueous solution.
- Sample 2 was prepared using 10 ml of mushroom extract was added to 40 ml of distilled water into which 1mM of AgNO3 (approximately 8.5 mg) was added.
- Sample 3 was prepared using 450 ml of distilled water was taken in a conical flask into which 50 ml of mushroom extract was added. The above mixture is stirred well and into it about 1mM of AgNO3 (approximately 86.5 mg) was added.
- Sample 4 was prepared by simply adding 1mM of AgNO3 (approximately 8.5 mg) directly to the mushroom extract without preparing the aqueous solution of AgNO3.
- Control sample was prepared by mixing 40 ml of 1mM AgNO3 (approximately 8.5 mg) directly to 10 ml of sterilized soil extract.

UV -Visible Spectroscopy analysis

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

Scanning Electron Microscopy (SEM)

Scanning electron microscope analysis was used measure the size of silvernanoparticles. For SEM, the silver nanoparticle synthesized using mushroom extract was allowed to dry completely and grounded well to a powder. For SEM the specimen is normally required to be completely dry since the specimen is at high vacuum. The morphology of AgNPs is apparently spherical. From the SEM micrograph it is observed that the AgNPs formed are in a size range of 30 ± 15nm and poly-dispersed.

X- Ray diffraction Analysis (XRD)

After Bio reduction, 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 in to 10ml of sterile deionized water. After freeze drying of the purified silver particles, the structure and composition were analyzed by X-raydiffraction (XRD). The dried mixture of silver nanoparticles was collected for the determination of the

formation Ag nanoparticles by X-ray diffractometer. In order to verify the results of the UV spectral studies, the colloidal suspensions of AgNPs were examined by XRD to confirm its crystalline nature.

Anti-Bacterial Activity by Disc Diffusion Method

The antibacterial activities of the silver nanoparticles were determined by agar diffusion method according to NCCLS standards [8].This method is used to evaluate the antibacterial potential of Silvernanoparticles against human pathogenic bacteria like *Proteus vulgaris*, ESBL, *E.coli* and *Klebsiella species*. The Muller Hinton agar plates are prepared and the agar surface is inoculated with the test bacteria. A sterile disc is place on the surface of the agar and is impregnated with5µL, 10µL, 15µL solution of silver nanoparticles, control and soil solution. The plate is incubated at 27°C for 24 hours. After incubation, the plates were analyzed for the zones of inhibition and it was measured. The activity was evaluated by calculating the increase infold area.

Results and discussion

Biosynthesis of silver nanoparticles

Synthesis of silver nanoparticles using *Agaricus bisporous* extract was observed (Fig. 1). When mushroom extract of different concentrations was subjected to aqueous solution of 1mM silver nitrate, a gradual change of colour was observed after 12 hours and changed its colour to reddish brown. This change of colour could be due to the formation of silver nanoparticles of varying shape and size. The formation of reduced AgNPs reaction mixture was further characterized by UV-vis spectrophotometry.

UV Spectroscopy analysis

From the UV Spectroscopy analysis & absorbance peaks, it can be summarized that sample 2 shows absorbance peak in the visible region. The absorbance peak was obtained at 420 nm. Sample 2 as shown in figure 1 was selected and further characterization studies were performed. No absorbance peak was observed for sample E1 as shown in figure 1, indicating the absence of its role in reduction of silver nitrate to silver nanoparticles. Thus this indicates that mushroom enhances and plays a role in the reduction of silver nitrate to silvernanoparticles.

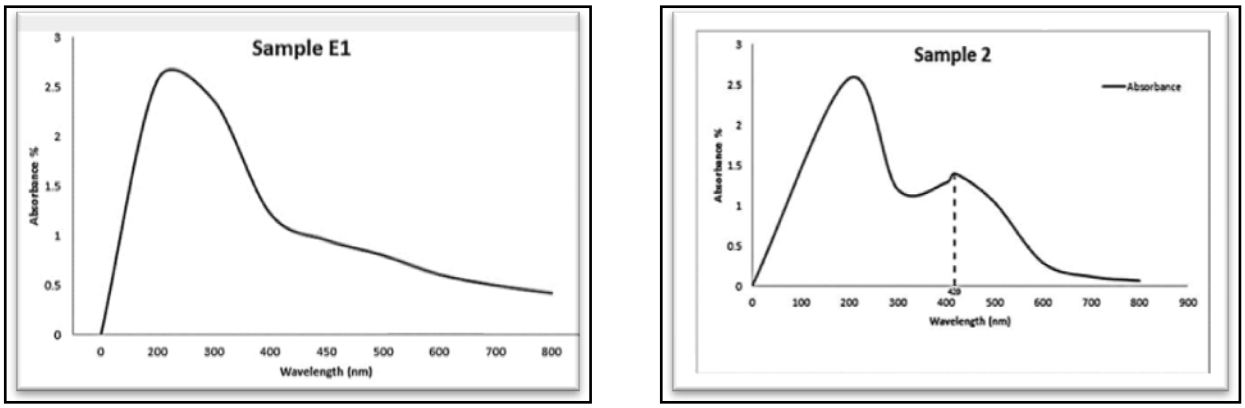

Fig. 1. Absorption peak of sample E1 and sample 2.

SEM Analysis of sample 2

SEM technique was employed to visualize the size and morphology of the particles.The particles obtained are spherical in shape, mono dispersed, uniform size and is in the size range 15-20 nm .

XRD Diffractions showing peaks

The XRD pattern of the silver nitrate- treated sample corresponds to that of silver nanoparticles. The XRD pattern shows peak in the whole spectrum of 2θ values ranging from 20 to 80. 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 fileNo.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 corresponding to (111) plane for silver as shown in figure 2.

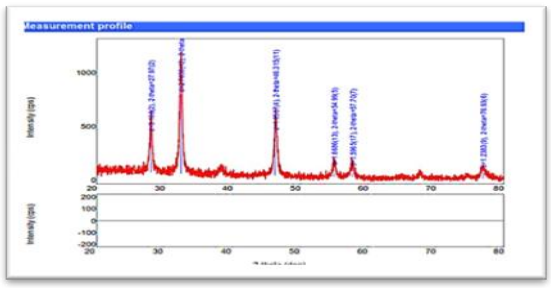

Fig. 2. XRD Diffractions showing peaks

Anti-bacterial activity of Silver Nanoparticles

Anti-bacterial potency of the obtained silver nanoparticles was checked against Escherchia coli, Proteus vulgaris, KlebsiellaSpp, ESBL. The zones of inhibition observed suggests the bactericidal property of the synthesized silver nanoparticles. Sample 2 was selected on the basis of characterization studies as shown in figure 3. It shows efficient antibacterial activity .Antibacterial activity of silver nitrate solution (control) was compared with that of sample E1. It was observed that both shows same level of activity, indicating that mushroom extract plays role in the reduction of silver nitrate to silver nanoparticles their by producing bactericidal properties.

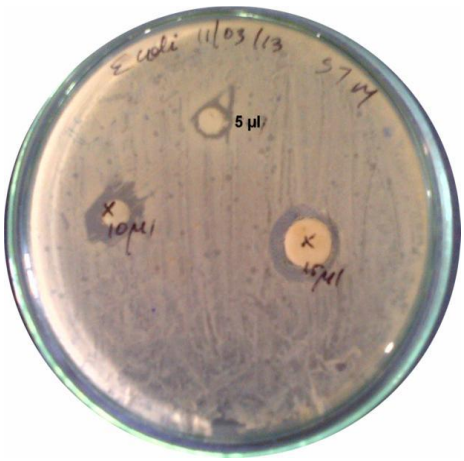

Fig. 3. Antimicrobial activity of Silver Nano Particles against pathogen

Discussion

Bio-synthetic method of synthesis of silver nanoparticles was followed to synthesize the silver nanoparticles. The silver nanoparticles were synthesized using the *Agaricus bisporous* extract. Silver nitrate solution was treated with the mushroom extract at different volumes to find out the optimal volume yielding better results. The mixture of extract and the silver nitrate solution was incubated at room temperature. The reduction of silver nitrate to silver nanoparticles was indicated by the color change from pale yellow reddish brown color. The color arises due to excitation of surface plasmon resonance (SPR) in the metal nanoparticles. The color change was observed within 24hrs of incubation. Silver nitrate solution was maintained as control, no color change was observed for it. The surface plasmon band occurs in the visible region of the light spectrum with absorbance peak was at 420 nm, this was correlating with the work of Narsimha et.al, 2011[9].

Further the silver nanoparticles were characterized by SEM and XRD analysis. SEM study revealed a uniform arrangement of particles having size in the range of 15-20nm and spherical in shape, Whereas Ravishankar Bhat et.al., 2011[10] synthesized silvernanoparticles using Pleurotusflorida and obtained particle size in the range 20 nm ± 5nm and of poly dispersed nature, Nithya et.al., 2009 synthesized siver nanoparticles using pleurotussajorcaju of size range 5-50 nm. But in our studies the particle obtained is in the size range 15- 20 nm. XRD pattern showed a small peak at 38°indicating the crystalline nature of the reduced silver nanoparticles, this was correlating with the work of Anuradhaet.al., 2010[11].Anti-bacterial potency of the silver nanoparticles was tested against various gram negative pathogens. The synthesized silver nanoparticle shows an effective antibacterial activity against all the pathogens tested, Whereas Nithyaet.al., 2009 synthesized siver nanoparticles using pleurotussajorcaju was tested against the pathogens, , *Escherichia coli, Pseudomonas aeruginosa Staphylococcus aureus* produced zone of inhibition of 12mm, 14mm & 11mm. But in our studies silvernanoparticles produced a zone of 19mm against Escherichia coli, thus suggesting better anti-bacterial efficacy. The result suggests that silver nanoparticles interact with bacterial cell and affinity towards cysteine residues and thiols, thereby resulting in bactericidal effect and the bactericidal effect is based on the size of the silvernanoparticles synthesized. Thus further studies can be carried out to reduce the particle size thereby increasing the bactericidal effect .

Conclusion

The present work is aimed at investigating the most efficient eco-friendly method for Biosynthesis of Silver nanoparticles using edible mushroom *Agaricus bisporus* and todo characterization of the synthesized Silver nanoparticle for shape and size analysis followed by antibacterial study for determining the Bio toxicity of the Silvernanoparticle.

Silver nanoparticles can be used effectively against multi-drug resistant bacteria due to their small size and relatively large surface area in comparison to their volume makes easy to interact with substances and increases their antibacterial efficacy. Silver may have an important advantage over conventional antibiotics in that it kills all pathogenic microorganisms, and no organism has ever been reported to readily develop resistance to it. Studies have also demonstrated that silver ions interact with sulfhydryl (-SH) groups of proteins as well as the bases of DNA leading either to the inhibition of respiratory processes or DNA unwinding leading to death of microorganism. Application of incorporation of Nano sized silver particles into or on the surface of products like cleaning sprays, skin creams, ATM buttons, and sports clothing etc. makes it attain antibacterial properties (12). The most important application of silver and silver nanoparticles is in topical ointments to prevent infection against burn and open wounds. The antifungal activity of Ag-NPs shows the usefulness of Nano silver in biostabilization of footwear materials. Studies are being carried out to study the anticanceractivity.

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