

Green Synthesis and Characterization of Silver NPs Using Oyster Mushroom Extract For Antibacterial Efficacy

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ABSTRACT

Background: Silver NPs have attracted to scientists due to their broad spectrum antimicrobial and antifungal properties. The biogenic extracts including mushrooms fruitings have potential compounds including amino acid, many sugars etc. to reduce metal ions into the zerovalent ions.

Purpose: The purpose of present study was to synthesize silver nanoparticles (AgNPs) using the extract of oyster mushroom *Pleurotus sajor-caju*, characterization of synthesized NPs and evaluation of their antibacterial activity.

Methods: The aliquot of mushroom extract was prepared using fruitings of oyster mushroom powder that was added into 20 mM silver nitrate solution followed by stirring on hot plate for 2 hours. The prepared AgNPs was then used for its antibacterial testing against *Escherichia coli* through broth test. The prepared nano-material was characterized by UV-Visible spectrophotometer, and transmission electron microscopy (TEM).

Result: The initial confirmation of silver NPs synthesis was observed with the alteration of the colour of the solution from colourless to wine red. The TEM revealed spherical structure of synthesized AgNPs and the particle size between the ranges of 11-44 nm. The bactericidal efficacy of silver NPs tested against *Escherichia coli* confirmed the lowest 50 µg/L concentration of silver NPs effectively bactericidal.

Conclusion: Based the observations of the study; silver NPs at the level of its 50 µg/L can be used for the purposes of potential water disinfection, killing of bacteria, disinfection of medical equipments, wound washings, preservation of food stuffs and in hand sanitization. The approach of green synthesis of silver NPs is fast, simple, environmentally friendly and economically viable.



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1. Introduction

Since historic time silver is well known for antimicrobial noble and coinage metal and is being deliberately used to store potable water of daily use to improve silver content. In recent decade silver NPs shown its significance of antimicrobial and antifungal nature compared to its bulk counterpart because of increased surface to volume ratio (Davies & Etris, 1997; Bhardwaj et al., 2019b). Silver NPs got popularity in the incorporation of a diverse range of commercial products, including plastics, soaps, pastes, food and textiles, increasing their market value due to broad spectrum bactericidal and fungicidal activity (Ahamed et al., 2010; Bhardwaj et al., 2019a). The rapidly emerging tendency of drug resistant and infectious microbes through fast evolution by mutation has created thrust need to develop advance formulation for the modification or inactivation of such microbes (Mandal et al.,

2014). In recent few years several attempts of green synthesis of nanoparticles using the microorganisms such as bacteria, fungi and algae as well as various higher plants part have been made (Maurya et al., 2016; Sanjivkumar et al., 2019; Tomer et al., 2019). The biogenic extract have diversity of phytochemical compounds (alkaloids, amino acids flavonoids, inositol, phenolic compounds, resins, saponins, tannins, terpenes, and vitamins), particularly, secondary metabolites available in plant and animal tissues with unique potential of NPs synthesis (Abbasi et al., 2018). Such natural components have ability to act in reducing and stabilizing of metal ions into zerovalent ions. These green approaches are always nontoxic, biocompatible, simple process and environmentally benign.

Mushrooms extract have various unique metabolites and enzymes able to reduce metal ions under extracellular or intracellular environment. Vigneshwaran et al. (2007) reported effectiveness of *P. sajor-caju* mushroom based

silver NPs against pathogenic bacteria *K. pneumonia* and *S. aureus*. Aygun et al. (2020) utilized reishi mushroom for the preparation of silver NPs and proved antimicrobial activity against various gram-positive and gram-negative bacteria; *S. aureus*, *E. hirae*, *B. cereus*, *E. coli*, *P. aeruginosa*, *L. pneumophila* sub sp. *Pneumophila*, *C. albicans*. Moreover, Maurya et al. (2016) demonstrated synthesis of silver NPs using oyster mushroom extract with the successful employment in bacterial deactivation particularly against *S. aureus* and *E. coli*.

In the present study, silver NPs were prepared using mushroom extract of oyster mushroom *Pleurotus sajor-caju* through a very fast, simple, economical and environmental friendly process. The aliquots of synthesized silver NPs were further used for the antibacterial activity against *Escherichia coli*.

2. Materials and Methods

2.1. Procurement of Chemicals and Culture Media

All chemicals used in the present work were of analytical grade. The silver nitrate (AgNO_3 , 99.98%), and Milli-Q grade water were purchased from Merck Pvt. Ltd. However, the nutrient agar culture media (NA) was procured from Himedia Pvt. Ltd.

2.2. Preparation of Mushroom Extract

The fresh and healthy fruiting bodies of oyster, *Pleurotus sajor-caju* (Figure 1) were achieved from the mushroom cultivation facility of Mushroom Training Research Laboratory (MTRC), Veer Bahadur Singh Purvanchal University, Jaunpur, India. The fruiting bodies were washed properly using double distilled water and were oven dried at 40 °C for 2 hours and powdered for the exercise of NPs synthesis. For this 6g of powder was added into 50 mL of hot and boiled (100 °C) distilled water and kept for cooling at room temperature, which was then filtered using Whatman-4 filter paper, thus obtained filtrate was used as mushroom extract.



Figure 1: *Pleurotus sajor-caju* fruiting body grown over the wheat straw substrate.

2.3. Green Synthesis of Silver Nanoparticles (AgNPs)

The synthesis of silver NPs was done according to the standard process (Bhardwaj et al., 2018). The 25 mL aliquot of prepared extract was added into 25 mL (20 mM) silver nitrate solution and maintained final volume up to 50 mL. Thereafter, solution was kept on hot plate with rotating magnetic stirrer for 2 hrs at 50°C. In consequence, colourless solution was turned into wine red colour that indicated initial confirmation of silver NPs synthesis.

2.4. Characterization of Silver NPs

Qualitative imaging of synthesized Ag NPs samples was done by standard photography which shows the change in colour colourless to wine red of Ag NPs. The concentration and surface Plasmon resonance (SPR) of silver NPs was measured through UV-Visible absorption double beam spectrophotometer (PerkinElmer Lambda 35). Furthermore, transmission electron microscopy (TEM) was used to determine size and shape of silver NPs.

2.5. Antibacterial Assay

The antibacterial susceptibility assay of silver NPs was carried out by bacterial broth test. In this assay, *E. coli* was selected as the model bacteria, which was cultured in nutrient broth for overnight at 37°C. The initial cell concentration of bacterial inoculants was 10^7 cfu/mL. The silver NPs with 50 ppb concentration was used as bactericidal, where as the control set contained only silver nitrate and mushroom extract. Thereafter, NPs treated broth was poured on nutrient agar plates to check the viability of *E. coli* cells.

3. Results and Discussion

Mushroom assisted green synthesis is a one of the most promising method with rapid simple and environmentally benign scope (Maurya et al., 2016; Owaid et al., 2019). The green synthesis of silver NPs cannot be possible without the presence of functional materials in the mushroom extract and other biologicals. The presence of such functional group play a significant role in the reduction of silver ions (Ag^+) into zerovalent silver (Ag^0), after that the nucleation process starts and finally reaction completes within 2 hrs to finish the synthesis of nanomaterials. *Pleurotus* mushroom is recognized to serve an excellent amount of riboflavin (Imran et al., 2011) in their fruiting bodies. The riboflavin acts as a catalyst for various reduction-oxidation reactions and functions in the bound coenzyme forms; flavin mononucleotide (FMN) and flavinadenine dinucleotide (FAD) (Bhat et

al., 2011; Khatun et al., 2015; Bhardwaj et al., 2018). According to several observations flavins are known for their strong light sensitivity and excellent oxidizing properties (Eichler et al., 2005). In accordance to these facts it appears that flavoproteins with appreciable redox potential, play a significant role in the production of NPs. Hence, flavins (flavoproteins) existing in the aliquots of mushroom extract accomplish reduction of Ag^+ ion into Ag^0 NPs once exposed to sunlight (Bhardwaj et al., 2018). In our study the colour of transparent solution was turned into wine red which was the indication of NPs synthesis and this whole reaction was completed within a period of two hours. Change in colour of mixture and the green synthesis of silver NPs represented in Figure 2. Similar observations were also made by other authors also (Owaid et al., 2019)



Mushroom Extract
& AgNO_3

Silver NPs

Figure 2: Reduction of silver nitrate into silver NPs in the combination of mushroom extract.

3.1. UV-Visible Spectroscopy of the Synthesized Silver NPs

The wine red colour of synthesized silver NPs was the initial confirmation of the presence of silver particles of nano-range in the prepared colloidal solution. The UV-VIS spectra of mushroom extract and colloidal solution of synthesized silver NPs has been illustrated in Figure 3. The highest peak of the absorbance spectra of the nanoparticle sample was obtained in between the range of 430-450 nm are due to the characteristic peaks of surface plasmon resonance phenomenon; the collective oscillation of the conducting electrons on the surface of the nanosized particle absorbs visible electromagnetic waves which confirms silver particles are nano-sized (Tan et al., 2013; Bhardwaj et al., 2017a; Bhardwaj et al., 2018).

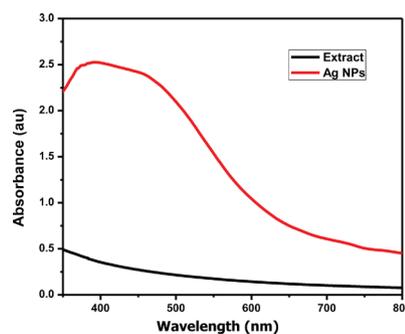


Figure 3: Comparative UV-VIS spectra of mushroom extract and mushroom assisted synthesized silver NPs.

3.2. Transmission Electron Microscopy (TEM) of NPs

The colloidal aqueous silver NPs synthesized using mushroom was characterized by transmission electron microscopy (TEM) for the shape and size measurements. According to the observations it was found that synthesized spherical, small, and mono-dispersed silver NPs particles are uniformly present (Figure 4). The average particle size of NPs was recorded as 18 nm with a standard deviation of 8.13 nm. The TEM image recorded found fully supported with the data obtained through UV-VIS spectrophotometer which have shown the broad peak due to range of particle distribution. Our results are also in accordance to the findings of other studies (Parmar et al., 2020; Oyanedel et al., 2008).

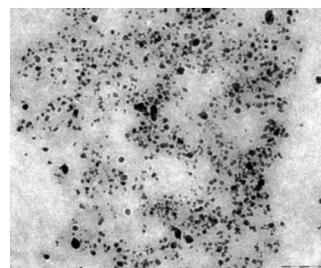


Figure 4: TEM image of silver NPs synthesized with assistance of mushroom extract (inset, scale bar, 200 nm).

3.3. Antibacterial Efficacy of Silver NPs

It has been found that the prepared silver NPs demonstrated high bactericidal effect against *E. coli*. Control was tested using mixture of silver nitrate, bacteria, and mushroom extract but does not contained. The growth of tested bacteria was completely inhibited at the lesser concentration 50 ppb of NPs shown in Figure 5. Study clearly shows that the smaller concentration is much effective for complete inhibition of the growth of bacteria using mushroom assisted silver NPs. The significant inhibition observed might be due to the smaller size of Ag NPs and their surface volume

ratio would be break integrity of cell wall (Srinivasan et al., 2013). Bhardwaj et al., (2018) reported that the mushroom assisted silver NPs synthesis and inhibitory nature against *E. coli*. Moreover, Nadafan et al., (2016) also reported effective antibacterial property of silver nanoparticles.

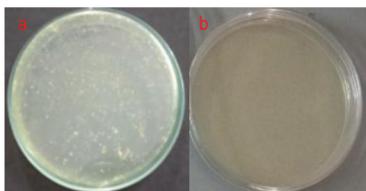


Figure 5. Complete inhibition of *E. coli* growth after the treatment of prepared AgNPs (50 µg/L); (a) untreated (b) treated with AgNPs.

Conclusion

Based on the results of the present study AgNPs synthesized through green synthesis method exhibited strong bactericidal properties. The green method of synthesis found rapid, simple, environmental friendly and highly cost effective. The silver NPs observed by the TEM were well dispersed and with small size and round shape. Silver NPs employed against *E. coli* successfully deactivated the cells at the level of 100% using smaller concentration of 50 µg/L. The results of the present study suggests that the prepared NPs can be used in the field of water disinfection and safe disinfectants, safe medical equipment, wound healing, and also for the purpose of preservations, safe mask and the hand sanitization.

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