Volume 65, Issue 3, 2021
Journal of Scientific Research
Institute of Science,
Banaras Hindu University, Varanasi, India.

Antimicrobial Activity of Biologically Synthesized Gold Nanoparticles from Wild Mushroom *Cantharellus Species*

Prachi Jha[*1], Ashish Saraf[1], and Jasmeet Kaur Sohal[2]

[*1]Faculty of Biological and Chemical Sciences, MATS University, Raipur, C.G.
prachi.jha0@gmail.com*, ashish.saraf22@gmail.com
[2]Dept. of Home Science, D.B. Girls P.G. Autonomous College, Raipur, C.G. jasmeetkaursohal@gmail.com

Abstract: **To combat the increasing cases of drug resistance is one of the biggest challenges for the scientists all over the world. Thus, development of novel antimicrobial agent has become the need of the hour. Nanotechnology is emerging as the new technology in the field of medical science to overcome such problems. In the present study, the Gold Nanoparticles were synthesized by mycelial extracts of a wild edible mushroom *Cantharellus sp*. These Nanoparticles were tested for their antibacterial and antifungal properties on a wide range of microorganisms. The nanoparticles showed significant anti bacterial and antifungal property in our study.**

Index Terms: **Antibacterial, Antifungal, *Cantharellus*, Gold Nanoparticles, Green Technology.**

I. INTRODUCTION

Nanotechnology has been one of the most explored research fields in modern materials science. Nanoparticles (NPs) are small particles having the size of 1–100 nm in at least one dimension. At nano scale, metal particles exhibit different and novel properties than that of bulk of the same materials (Esparza *et al*. 2005). Their biocompatibility, easy surface modification, and adsorption ability makes them very useful for research purpose (Khlebtsov and Dykman, 2011).These characteristics owing to their size, shape , structure and surface has contributed significantly for their applications in medical imaging, disease detection, and drug delivery etc. (Mukherjee *et al*. 2016).

Increasing cases of drug resistance in microorganisms has raised a concern for the society. The overuse and misuse of antimicrobials has led to the grave condition of drug resistance in microbes. Almost all the bacteria have developed resistance to one or another antibiotic. All major groups of antibiotics generally act by disrupting bacterial cell wall, hindering translational machinery, and interfering in DNA replication (Magiorakos *et al.,* 2012). Unfortunately, resistance may be developed against each one of these. Therefore, development of a novel antimicrobial agent has become a challenge for researchers from all over the world. During the last few decades, nanotechnology has proved its potential in medical science.

There is no exact mechanism of action of nanoparticles on microbes. However, some studies have shown that NPs act by directly adhering to bacterial cell wall, without penetrating the cells of bacteria (Thill *et al*., 2006). This raises the hope that nanoparticles would be less prone to promote resistant in bacteria.

Many studies have revealed the significance of green synthesis of nanoparticles over chemical and physical methods as they have many concerns including use of toxic chemicals, high energy requirements and release of harmful by products (Sahayaraj and rajesh, 2011). Therefore, green or Biological synthesis of nanoparticles using microorganisms like bacteria, fungi and plants are proved to be an efficient and excellent route for benign synthesis.

Cantharellus, also known as bamboo mushroom, is one of the most popular edible mushrooms in central India, generally found in the forest of Balaghat District of Madhya Pradesh. It grows naturally on the decayed stems of bamboo during the rainy season, thus locally known as "Bans Pihri". Several studies have confirmed that the nutritional value of this mushroom is very high and it is a rich source of amino acids, carbohydrates, fats and fibres (Caglarirmak *et al*., 2002; Colak *et al*., 2007).

* Corresponding Author

Figure 1: *Cantharellus species*

Taking into consideration the above aspects, this study was carried out to estimate the antimicrobial properties of biologically synthesized Gold Nanoparticles (AuNPs) mediated by wild edible macrofungi *Cantharellus species*.

II. EXPERIMENTAL

A. Collection and Identification of Mushroom

The mushroom, *Cantharellus sp*. was collected from Balaghat district of Madhya Pradesh during rainy season from July to September. The collected mushroom samples were identified by comparing the sample with descriptions given in previously available authentic literatures (Figure 1).

B. Preparation of Mushroom Extract

The sample was first cultured in Potato Dextrose Agar (PDA).Mycelial extract of *Cantharellus sp*. was prepared by inoculating the fungal mycelia in Potato Dextrose Broth and incubated for 7 days at 120 rpm, in a rotary shaker at 28°C. After 7 days, the filtrate obtained from the above culture was stored and used as extract.

C. Preparation of aqueous $HAuCl_4$ solution

1mM, 3mM and 5mM aqueous solution of $HAuCl_4$ was prepared for comparative study of the synthesis of AuNPs.

D. Synthesis of Gold Nanoparticles (AuNPs)

50 ml. mycelial extracts was mixed with 100 ml of 1 mM, 3mM and 5mM aqueous $HAuCl_4$ respectively in separate flasks ((Nachiyar *et al*.,2015), and incubated for 48 hrs at 28°C in a rotary shaker at 120 rpm. Change in colour from yellow to red indicated the synthesis of AuNPs (Figure 2).

Figure 2: Visual confirmation of synthesis of AuNPs

The formation of AuNPs was confirmed by UV visible spectrometer (Systronics, Double beam spectrophotometer, 2203) under the range of 200-600 nm. UV-Vis absorption spectrum gives characteristics peaks for the surface plasmon resonance of different nanoparticles (Abboud *et al*, 2013; Yin *et al*., 2005; Rodriguez *et al*.,2007; Swarnkar *et al*., 2011). AuNPs were purified by centrifugation at 10,000 rpm for 15 min. The solid residue obtained was washed twice with deionised water to get AuNPs free from any biological molecule.

E. Antibacterial Activity of AuNPs:

The antibacterial properties of AuNPs synthesized by mycelial extract of the *Cantharellus species* were studied by Kirby Bauer method (Bauer *et al*.,1966). 5 Gram positive (*Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis, Bacillus cereus and Listeria monocytogenes*) and 5 gram negative (*Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella enterica, Escherichia coli, Aeromonas hydrophila*) were procured from MTCC, Pune.

6 mm sterilized filter papers disks (Whatmann No. 1) were saturated with mycelial extract, 1mM, 3Mm and 5mM $HAuCl_4$ solution, AuNPs, distilled water, and ampicilin. These discs were dried and placed onto previously prepared Muller-Hinton Agar (MHA) plates and kept in incubator at 35°C for 24 hours. Antibacterial activity was determined in terms of zone of inhibition. All experiments were carried out in triplicates and mean value was considered.

F. Antifungal Activity

Poisoned food technique (Grover and Moore, 1962) was used to determine the antifungal activity of AuNPs by measuring the reduction in growth of the microorganism on the medium.

G. Characterization of AuNPs

AuNPs showing highest antimicrobial activity were characterized using different techniques including UV-Vis spectrophotometer, Fourier Transform Infrared Spectroscope (FTIR), Transmission Electron Microscope (TEM) and Atomic Absorption Spectroscope (AAS).

III. RESULT AND DISCUSSION

Present study describes the synthesis of AuNPs from *Cantharellus sp*. and its antimicrobial activity. When mycelial extract was mixed with 1, 3 and 5 mM $HAuCl_4$ aqueous solution, mycelial extract was reduced by 3mM $HAuCl_4$ aqueous solution forming Gold Nanoparticles (AuNPs), which was evident from change in color from yellow to red. It was further confirmed by UV-Vis spectroscopy.

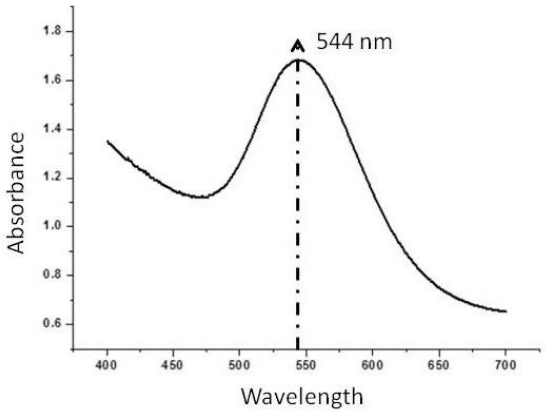

Figure 3. UV-Vis spectroscopic analysis of AuNPs

UV-Visible Spectra Analysis

For the confirmation of NP synthesis, Purified AuNPs were analyzed by Systronics Double beam spectrophotometer (2203), where they showed sharp absorbance at 544 nm, specific for AuNPs (Figure 3) (Daizy, P., 2010). When an incident light is passed through a sample, Surface plasmon resonance (SPR) induces a strong absorption of the light causing the change of the colour as reported in some previous studies (Das *et al*., 2012). The wavelength of the SPR band of NPs depends on the type, composition of mixture, dielectric constant shape and size of the metal (Huang and El-Sayed, 2010).

A. Antimicrobial susceptibility Test:-

NPs have been reported to have many biomedical applications. The higher surface to volume ratio enables them to interact with bacteria, resulting in antimicrobial properties. In this study antimicrobial potential of AuNPs was determined against various gram-positive and Gram- negative bacteria using Kirby-Bauer method (Disc Diffusion method).As it is clear from the table 1, when treated with AuNPs, among Gram positive bacteria *Bacillus subtilis* and among gram negative bacteria *Salmonella enterica* showed highest zone of inhibition i. e. 29±0.8 mm and 32±1.2 mm.

These results can be explained by the fact that the interaction of AuNPs with the negatively charged group of the cell wall leads to the structural change in the cell surface. It also suggests that this interaction results into formation of a complex which interrupts the transport of essential solutes inside the cell (Palza, 2015; Kon and Rai, 2013; Zhou *et al.*, 2012).

These results are in Accordance with the experiment conducted by Baek and An (2011), and Saranya S. (2015) where they found Gram-negative bacteria are more susceptible to AuNPs as compared to Gram-positive bacteria.

Table 1: Antimicrobial activities of AuNPs

S. No	Micro-organism	Zone of Inhibition (mm)				
		Mycelial extract	$HAuCl_4$	AuNPs	+VE control	-VE control
1	Staphylococcus aureus	13±0.5	12±0.4	27±0.8	35±1.3	0
2	*Bacillus subtilis*	14±0.6	12±0.4	29±0.8	32±1.1	0
3	*Bacillus cereus*	14±0.5	13±0.5	26±0.6	30±0.9	0
4	*Micrococcus luteus*	15±0.6	13±0.6	28±0.7	34±1.2	0
5	*Listcaeria monocytogenes*	13±0.4	13±0.5	28±0.7	30±1.1	0
6	*Salmonella enteric*	16±0.6	13±0.5	32±1.2	35±1.4	0
7	*Aeromonas hydrophila*	14±0.5	12±0.6	28±0.8	34±1.3	0
8	*Klebsiella pneumonia*	13±0.4	13±0.4	30±0.9	33±1.3	0
9	*E. coli*	13±0.5	12±0.5	28±0.8	35±1.5	0
10	*Pseudomonas aeruginosa*	14±0.5	12±0.5	29±0.9	34±1.5	0

*Values are expressed as mean±SD

* positive control – Ampicilin, Negative control – water

B. Antifungal activities of AuNPs by Poisoned food technique:-

Using this technique, *Fusarium moniliforme* showed highest % inhibition of mycelial growth i.e 75. Our result was supported by the findings of Eid *et al.*, (2011). They showed that 130 µm of AuNPs solution completely

inhibited the growth of Penicillium in the medium. It seems that the fungal cell absorbs Nanoparticles. Their accumulation inside the cell has detrimental effect on the cell membrane.

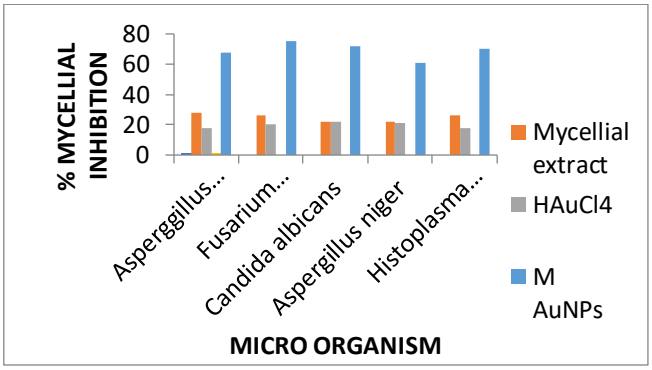

Figure 4: Antifungal activity of AuNPs

C. Characterization of the synthesized AuNPs

1) Fourier Transform Infrared Spectroscopic Analysis (FTIR)

FTIR is one of the most commonly used methods to detect the functional groups present in pure compounds and mixtures. FTIR characterization was conducted to investigate the interaction between functional groups of *Cantharellus* mycelial extract and AuNPs. FTIR spectra showed the wave number shift of mycelial extract functional groups before and after AuNPs formation.

The FT-IR result shows there are some common functional groups present in both the solution in similar positions or with small changes in the place. These are the groups that acts as capping agent and prevents clustering of NPs. The extract samples have four main peaks in the range of 1107.57, 1421.22, 1637.72, and 3446.58 cm^{-1} and two weak peaks 569.59 and 2934.08 cm^{-1}. The absorption peak at 1107.57 cm^{-1} can be associated with Ester linkages. The peak at 1421.22 cm^{-1} represents lignin and cellulose. The presence of amide I group is confirmed by the peak at 1637.72 cm^{-1}.Likewise, the presence of proteins, carbohydrates, flavonoids and tannins is evident from absorption peak at 3446.58 cm^{-1}. These peaks were also present with minor changes in the position in the spectrum of solution containing AuNPs which confirmed that the carbonyl group and peptides might have formed a layer on the AuNPs and might be responsible for reduction of the Au ions to atoms (Philip, 2009; Bhat *et al*., 2013).These groups seem to have prevented agglomeration of the NPs making them stable. The results are supported by the findings of Narasimha *et al.* (2011) and Eskandari *et al*. (2016). In their study, some functional groups present in A. *bisporus* extracts were found to be involved in the synthesis and stabilization of silver and gold NPs.

2) Transmission Electron Microscope (TEM)

TEM analysis was done using TECNAI G2 spirit equipped with Gatan digital camera to estimate the size and morphology of Synthesized AuNPs. AuNPs were found to have an average size of 60.6 nm and spherical in shape (Fig. 5).

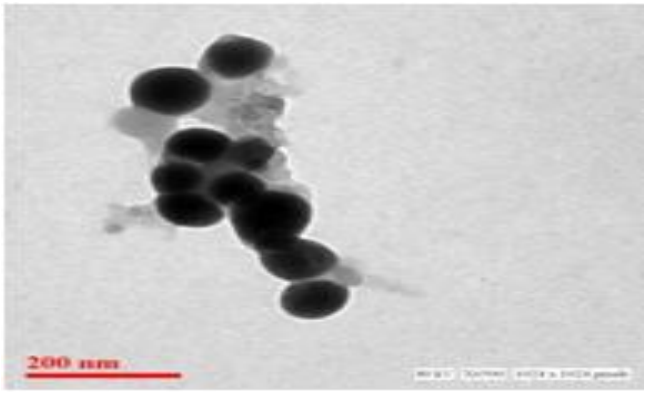

Figure 5: TEM image of AuNPs

3) Atomic Absorption spectroscopic analysis (AAS)

Gold ion concentrations were analyzed by AAS. A standard solution of 10 ppm of $HAuCl_4$ was prepared for analysis. The solution was analyzed at 0 min in AAS. The Au^{3+} ion concentration in the reaction mixture was monitored after adding mycelial extract to it, at different time interval. With time, a decrease in the ion concentration was observed indicating the conversion to Au^0 NPs after 40 min of reaction time at 75^0C.

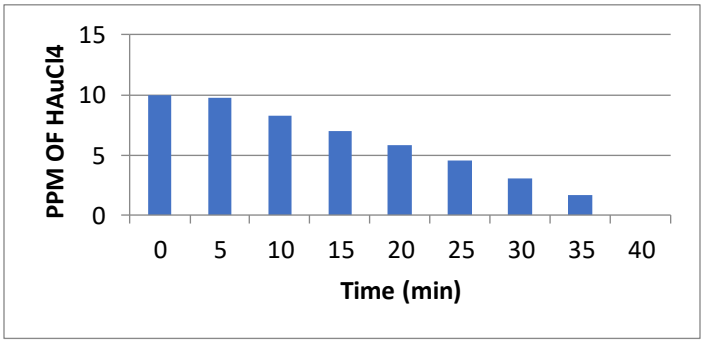

Figure 6: AAS analysis

CONCLUSION

The Present study reports the Green synthesis of AuNPs using mycelial extracts of wild edible mushroom *Cantharellus sp*. This method is environment friendly, cost effective, less toxic and proficient. It overcomes the problems associated with chemical and physical methods of nanoparticles production. This method can be used for large scale production of metal NP as it's very fast and efficient. AuNPs synthesized using this method showed significant antimicrobial activity against a range of microorganisms.

ACKNOWLEDGMENTS

The Authors express their gratitude to the Head, MATS School of Biological and Chemical Sciences, MATS University, for providing central laboratory facilities.

REFERENCES

Abboud, Y., Saffaj, T., Chagraoui, A., Bouari, E,. Brouzi, K., Tanane, O., and Ihssane, B. (2013). Biosynthesis, characterization and antimicrobial activity of copperoxide nanoparticles (CONPs) produced using brown alga extract (*Bifurcaria bifurcata*), *Appl Nanosci*, 4, 571–576

Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45, 493-496.

Bhat, R., Sharanabasava, V., Deshpande, R., Shetti, U., Sanjeev, G., Venkataraman, A. (2013).Photo-bio-synthesis of irregular shaped functionalized gold nanoparticles using edible mushroom Pleurotus florida and its anticancer evaluation. J. Photochem. Photobiol. B , 125, 63–69.

Caglarirmak, N., Unal, K., Otles, S. (2002). Nutritional value of edible wild mushrooms collected from the Black Sea region of Turkey. Micologia Aplicada International 14(1),1–5.

Colak, A., Kolcuoğlu, Y., Sesli, E., Dalman, Ö. (2007). Biochemical composition of some Turkish fungi. Asian Journal of Chemistry 19(3) ,2193–2199.

Daizy, P. (2010).Green synthesis of gold and silver nanoparticles using Hibiscus rosa sinensis. Physica E: Low-dimensional Systems and Nanostructures, 42(5),1417–1424.

Das, R.K., Babu, P.J., Gogoi, N., Sharma, P., Bora, U. (2012). Microwave-mediated rapid synthesis of gold nanoparticles using Calotropis procera latex and study of optical properties. ISRN Nanomater. 1–6

Eid, K.A., Salem, H.F., Zikry, A.A., El-Sayed, A.F., Sharaf, M.A. (2011). Antifungal Effects of Colloidally Stabilized Gold Nanoparticles: Screening by Microplate Assay. Nature and Science.9(2). 29-33.

Eskandari-Nojehdehi, M., Jafarizadeh-Malmiri, H., and Rahbar-Shahrouzi, J., (2016). Optimization of processing parameters in green synthesis of gold nanoparticles using microwave and edible mushroom (Agaricus bisporus) extract and evaluation of their antibacterial activity. Nanotechnol Rev, 5(6), 537–548.

Esparza, R., Ascencio, J.A., Rosas, G., Sánchez Ramírez, J.F., Pal, U. and Perez, R. (2005). Structure, stability and catalytic activity of chemically synthesized Pt, Au, and Au-Pt nanoparticles. *J Nanosci Nanotechnol* **5**, 641– 647.

Grover, R.K. and Moore, J.D. (1962) Toximetric Studies of Fungicides against the Brown Rot Organisms, Sclerotinia fructicola and S. laxa. Phytopathology, 52, 876-879.

Huang, X., El-Sayed, MA. (2010). Gold nanoparticles: optical properties and implementations in cancer diagnosis and photothermal therapy. J. Adv. Res., 1, 13–28.

Khlebtsov, N. and Dykman, L. (2011). Biodistribution and toxicity of engineered gold nanoparticles: a review of in vitro and in vivo studies. *Chem Soc Rev* **40**, 1647– 1671.

Kon, K., Rai, M.(2013). Metallic nanoparticles: mechanism of antibacterial action and influencing factors. J. Comp. Clin. Pathol. Res. 2, 160–174.

Magiorakos, A.-P., Srinivasan, A., Carey, R. B. et al., (2012)."Multidrugresistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance," *Clinical Microbiology and Infection*, 18(3), 268–281.

Mukherjee, S., Sau, S., Madhuri, D., Bollu, V.S., Madhusudana, K., Sreedhar, B., Banerjee, R. and Patra, C.R. (2016). Green synthesis and characterization of monodispersed gold nanoparticles: toxicity study, delivery of doxorubicin and its bio-distribution in mouse model. *J Biomed Nanotechnol* **12**, 165– 181.

Nachiyar,V., Sunkar, S., Prakash, P.S and Bavanilatha. (2015). Biological synthesis of gold nanoparticles using endophytic fungi. Der Pharma Chemica, 7(2),31-38

Palza H. (2015). Antimicrobial polymers with metal nanoparticles. Int. J. Mol. Sci., 16, 2099–2116.

Philip, D. (2009). Biosynthesis of Au, Ag and Au-Ag nanoparticles using edible mushroom extract. Spectrochim. Acta Mol. Biomol. Spectrosc., 73, 374–381.

Rodriguez, E., Parsons, J.G., Peralta-Videa, J.R., Cruz-Jiménez, G., Romero-González, J., Sánchez-Salcido, B.E., Saupe, G.B., Duarte-Gardea, M., Gardea-Torresdey, J.L. (2007). Potential of Chilopsis linearis for gold phyto-mining: Using XAS to determine gold reduction and nanoparticle formation within plant tissues, Int. J. Phytorem., 9-2, 133-147.

Sahayaraj, K. and Rajesh, S. (2011). *Bionanoparticles: synthesis and antimicrobial applications. Science against microbial pathogens: communicating current research and technological advances*. Formatex Research Center, Spain.

Saranya, S. (2015). Biosynthesis of gold nanoparticles (AuNPs) from C. orchioides and study their antimicrobial efficacy. Int. J. Phytopharm., 5, 58–64.

Swarnkar, R.K., Singh, S.C., and Gopal, R. (2011). Effect of aging on copper nanoparticles synthesized by pulsed laser ablation in water: structural and optical characterizations, *Bull. Mater. Sci.*, 34*(7), 1363–1369*.

Thill, A., Zeyons, O., Spalla, O., Chauvat, F., Rose, J., Auffan, M., and Flank, AM. (2006). Cytotoxicity of CeO2 nanoparticles for Escherichia coli. Physico-chemical insight

of the cytotoxicity mechanism. Environmental Science & Technology 40(19):6151-6.

Yin, M., Wu, CK., Lou, Y., Burda, C., Koberstein, JT., Zhu, Y., O'Brien, S. (2005). Copper oxide nanocrystals. J Am Chem Soc 127:9506–9511

Zhou Y, Kong Y, Kundu S, Cirillo JD, Liang H. (2012). Antibacterial activities of gold and silver nanoparticles against Escherichia coli and bacillus. Calmette-Guérin. J. Nanobiotechnol., 10, 19–28.

***