KAVAKA 58 (2) : 53-56 (2022) DOI: 10.36460/Kavaka/58/2/2022/53-56

Synthesis and characterization of ZnO nanoparticles using *Pleurotus florida* extract

Kumar Manimaran[1,2] and Subban Murugesan[2*]

[1]*Department of Chemical Engineering, Sri Sivasubramaniya Nadar College of Engineering, Kalavakkam, Chennai- 603110, Tamil Nadu, India.*

[2]*Department of Botany, School of Life Sciences, Periyar University, Periyar Palkalai Nagar, Salem-636 011, Tamil Nadu, India*

[*]*Corresponding author Email: drsmbtpu@gmail.com*

(Submitted on March 03, 2022; Accepted on May 29, 2022)

ABSTRACT

In recent years, eco-friendly, simple, cost-effective synthesis of nanoparticles through green method is developing interest in the modern research. In this present work, the ZnO nanoparticle (ZnO NPs) was synthesized by the facile green process using *Pleurotus florida* extract. The structure, morphology, size, elemental properties of the synthesized ZnO NPs were characterized by UV visible spectroscopy, X-ray diffraction, Fourier transform infrared (FT-IR) spectra, scanning electron microscope (SEM), transmission electron microscope (TEM) analyses. The XRD pattern show pure crystalline nature of the ZnO NPs and spherical shape morphology of NPs was noted by SEM image. The broad spectrum of antibacterial activity was observed in both gram-positive and gram-negative bacteria and maximum growth inhibitory zone was recorded in *Klebsiella pneumoniae* (14.36±0.41 mm) and *Bacillus cereus* (14.12±0.41 mm) followed by other tested organisms. Based on the findings of present study the biosynthesized ZnO NPs from *P. florida* can be served as an alternative, eco-friendly nano medicine in the near future.

Keywords: *Pleurotus florida*, Biosynthesis, ZnO NPs, Antibacterial activity

INTRODUCTION

Nanobiotechnology is a modern and empowering technology by involving the various fields of science. In recent years, biologically mediated nanoparticles have played a significant consideration due to their non-toxic chemicals, antibacterial, antiviral, diagnostics, anticancer, targeted drug delivery, environmental as minable solvents and renewable materials (Muthuvel *et al.,* 2020). Over a period of few decades, the medicinal mushroom was utilized as an antidiabetic nutrition (Agrawal *et al.,* 2006). Among them, *Pleurotus sajor-caju* contains a potential immunological enhancer and non-cellulosic β-glucans in the fruit body was reported by Alanazi *et al.* (2010).

Currently, many researchers have started to explore the use of edible and medicinal mushroom for synthesis of metallic nanoparticles. Owaid *et al.* (2019) analyzed the phyto-constituents like amino acids, proteins, and polysaccharides in the mushrooms which have been utilized for the synthesis of both extracellular and intracellular metal nanoparticles. The phyto-constituents found in the edible and medicinal mushroom lead to form the nanoparticles with high stability and good dispersion characteristics. Mattila *et al.* (2020) investigated the use of macrofungi (mushrooms) for the synthesis of an eco-friendly, non-toxic, and stable nanoparticles. The pioneer research for mushroom mediated nanoparticles was started in 2004 the year (Mukherjee *et al.,* 2001). The oyster mushroom *P. ostreatus* have been used for synthesis of AgNPs was reported by Owaid *et al.* (2019). Similarly, the edible mushroom used for successful synthesize of nanoparticles was carried out by Shang *et al.* (2013).

Molds, yeasts, and edible mushrooms are used in the biological synthesis of nanoparticles in nanotechnology. The highest amount of metallic nanoparticles was obtained from the farm and laboratory mushroom, particularly from edible mushroom, mycelia, and enzymes. (Owaid *et al.,* 2017). To avoid the toxicity from chemical reducing agents, an environmentally friendly approach was applied in the current study (Virkutyte *et al.,* 2011). Metal oxides including zinc oxide (ZnO), titanium dioxide (TiO_2), and magnesium oxide (MgO) are not only stable, but also considered to be healthy for humans. Considering the above cited information, the present study was focused on the synthesis, characterization and antibacterial activity of mushroom-mediated ZnO NPs.

MATERIALS AND METHODS

Preparation of *Pleurotus florida* extracts

The *Pleurotus florida* was collected from edible and medicinal mushroom research laboratory, Department of Botany, Periyar University, Tamil Nadu, India. The collected mushroom fruit body was thoroughly washed with deionized water to remove dirt and other adherent material, and then manually cut into small pieces. About 15g of fruit body was transferred to 500 mL beaker containing 100 mL of distilled water, and boiled for 15 minutes. The obtained solution was then filtered using Whatman no. 1 filter paper and the filtrate solution was directly used for preparation of ZnO Nps.

Biosynthesis of ZnO NPs using *P. florida* extract

The NPs were obtained through the reduction of zinc nitrate hexahydrate. In a typical experiment, 40 mL of *P. florida* extract was mixed with 20 mL solution of zinc nitrate hexahydrate (0.5 M), and then, the volume of the mixture was adjusted to 100 mL by adding distilled water and magnetically stirred for 20 min. Afterwards, the above resulting mixture was transferred into a 100 mL Teflon-lined stainless steel autoclave and hydrothermally treated at 150°C for 10 hrs under autogenous pressure. The suspension was cooled to room temperature and filtered to obtain ZnO NPs precipitates. They were washed thrice with distilled water and absolute ethanol. The final obtained product was then dried at 60°C for 24 hrs in a hot air oven and used for characterization and further tests.

Characterization

The structural and chemical properties of the synthesized ZnO NPs were characterized by a variety of different physicochemical techniques. X-ray diffraction (XRD) patterns of sample were performed using a Bruker D8

Advance diffractometer with Cu Kα radiation (λ = 1.54 Å). Fourier-transform infrared (FT-IR) spectra of sample were measured on a Bruker Vertex 70 spectrometer using pressed KBr pellets. The surface morphology of the synthesized ZnO NPs was observed through the field-emission scanning electron microscope (SEM). The chemical composition of the ZnO NPs was studied using energy-dispersive spectroscopy (EDS), which is attached to the SEM instrument. High-resolution Transmission electron microscopy (HR-TEM) images TEM images of sample were taken with a JEOL JEM-2100 microscope to view their internal structure. The UV-vis diffuse reflectance spectrum was recorded on by a Shimadzu UV-2700 UV-vis spectrophotometer.

Antibacterial activity

Antibacterial activity of biosynthesized ZnO NPs was tested against *S. aureus, E. coli, K. pneumonia* and *B. cereus* by agar well diffusion method. The tested bacteria were obtained from the Department of Botany, Periyar University, Salem, Tamil Nadu, India. Briefly, the pure culture of tested organisms was sub cultured in nutrient broth at 37°C in an incubator for 24 hrs. For antimicrobial experiments, approximately 100 mL of each culture of fresh bacteria (106 (CFU) mL^{-1}) was spread uniformly to the nutrient agar plate using a sterile glass-rod spreader. The various concentrations (50, 75, 100, and 125 µg/mL) of ZnO NPs were placed on a nutrient well in agar plate well and these plates were then incubated at 37°C for 24 hrs and the standard antibiotic chloramphenicol (10 µl) was used as control. The anti bacterial activity was evaluated and the diameter of the growth inhibition zones around the well was measured in millimeter (mm).

RESULTS AND DISCUSSION

UV-visible spectrum analysis

The UV-vis spectrum of ZnO NPs obtained using *P. florida* fruit body extract is displayed in **fig. 1**. The strong absorption peak in the spectral range of 250-400 nm was observed at 258 nm, which confirmed the existence of ZnO NPs. The outcome of the UV-Vis spectrum was in good agreement with the previous findings (Kadam *et al.,* 2019).

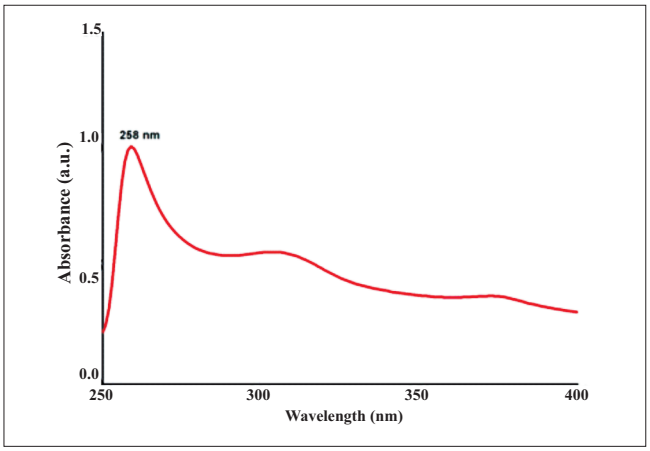

Fig. 1: UV-Visible spectroscopy analysis of synthesized ZnO NPs using *P. florida*

FTIR analysis

The synthesized ZnO NPs were observed through FTIR to find out the interfaces (Functional groups) between the ZnO and bioactive compounds of mushroom extract (**Fig. 2**). FTIR spectrum of *Pleurotus florida* medicated ZnO NPs showed intense peaks of 2920.67, 2854.39, 1631.53, 1584.58, 1119.38, 984.56, 867.08 and 416.35 cm-1 were directly related to free C-H, vinyl ethers, -C=C- and beta-lactones, respectively. The sturdy aromatic ring and carboxylic acid occurrence in the FTIR band was responsible for the ZnO NPs synthesis. The present FTIR frequency peaks confirmed the synthesis of metal oxide nanoparticles (Li *et al.,* 2019).

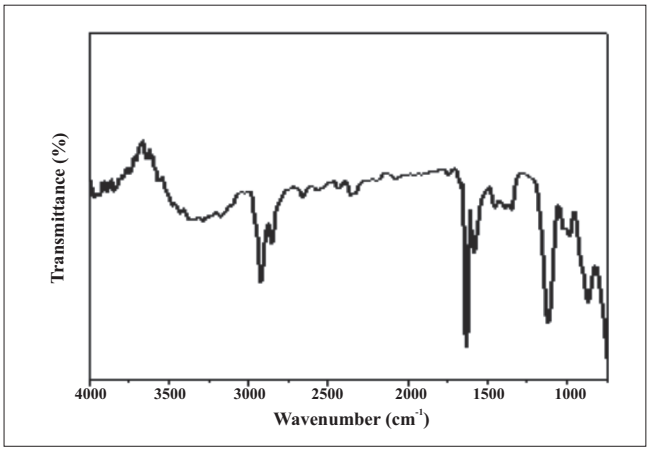

Fig. 2: FTIR spectrum analysis of synthesized ZnO NPs using *P. florida*

XRD analysis

The XRD pattern (**Fig. 3**) of ZnO NPs exhibited 2θ peaks at 31.79°, 34.46°, 36.28, 47.56°, 56.62°, 62.87°, 66.38° and 67.95° attributed to (100), (002), (101), (102), (110), (103), (200) and (202) planes of hexagonal ZnO NPs, respectively. Similarly, the XRD patterns from the fungal biomass mediated ZnO NPs were reported by Gandhi *et al.* (2017). Therefore, XRD result shows that the hexagonal phase and crystallization of the biogenic phase occurs on the surface of the ZnO NPs.

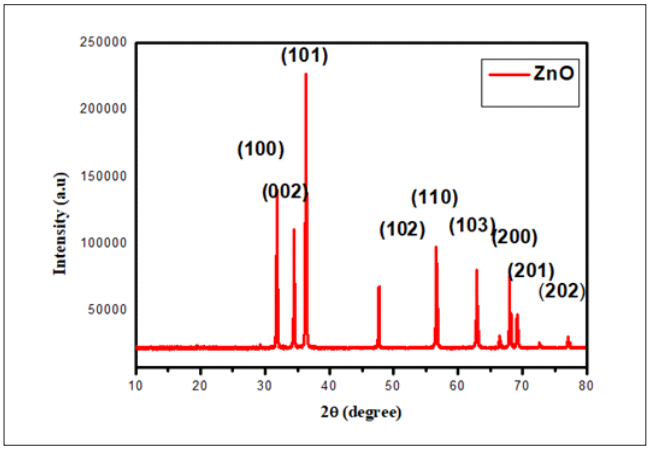

Fig. 3: X-ray diffraction (XRD) pattern analysis of synthesized ZnO NPs from *P. florida*

Scanning electron microscopy (SEM) analysis

The SEM image of green synthesized ZnO NPs were prepared by a hydrothermal method. It can be noticed that the obtained product are composed of spherical like nano structures and a smooth surface without aggregation. The diameters of these ZnO are 25 nm. SEM images confirmed that the fungus mediated ZnO nanoparticles were spherical in structure (**Fig. 4**) and the nanoparticles thickness of ZnO was about 25 to 50 nm.

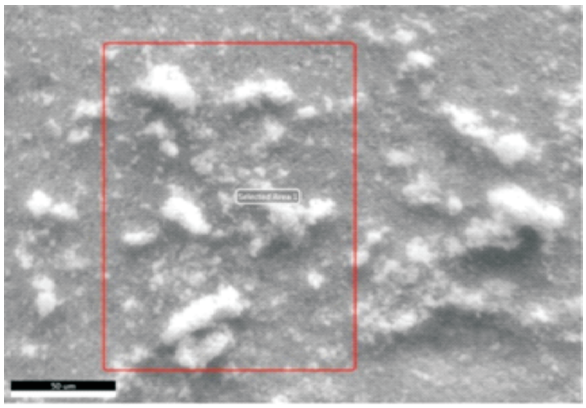

Fig. 4: SEM analysis of ZnO NPs from *P. florida*

High resolution transmission electron microscopy analysis

The morphology and size of ZnO NPs in the colloidal solutions were analyzed by HR-TEM. The ZnO NPs showed irregular shape and few particles were spherical in shape with an average size of 40-60 nm (**Fig. 5**). Similarly, ZnO NPs biosynthesized using *P. djamor* mushroom extract exhibited particle size varying between 20 and 70 nm as given by Manimaran *et al.* (2021).

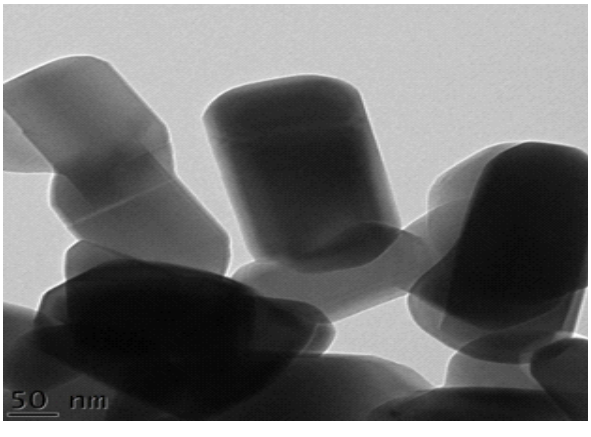

Fig. 5: HR-TM analysis of ZnO NPs from *P. florida*

Antibacterial activity of synthesized ZnO NPs

The antibacterial activity of synthesized ZnO NPs using *P. florida* extract (50 and 75 µl of NPs solution) and the commercial antibiotics (by disc diffusion method) is depicted in **table 1** and **fig. 6**. The biosynthesized ZnO NPs show a broad spectrum of antibacterial activity against most of the tested bacterial pathogens (**Table 1**). The better growth inhibitory zone was recorded in *S. aureus* (14.36 ± 0.41), *B. cereus* (14.12 ± 0.41), *E. coli* (15± 0.2 mm) followed by K. pneumoniae (8.75 ± 0.31) and at 75 µl dose of ZnO NPs, no zone formation was observed in the *P. florida* extract (negative control) (**Table 1**). The standard antibiotic chloramphenicol was reported the antibacterial activity with the average inhibitory effect between the range of 16 mm to 20 mm. The results clearly show that the gram positive bacteria have higher zone of inhibition than gram negative bacteria. The present results were in agreement with the findings of previous study on antibacterial activity of various mushroom and fungal extracts from *Tricholoma matsutake* and *Phytophthora infestans* medicated silver nanoparticles (Anthony *et al.,* 2014; Kannan *et al.,* 2020).

Table 1: Antibacterial activity of synthesized ZnO NPs against selected human pathogens

Bacterial strain	Zone of inhibition (in mm) of synthesized ZnO NPs.			
	50 (µL)	75 (µL)	ME	Chloramphenicol 25 (µL)
Staphylococcus aureus	8.21 ± 0.45	8.75 ± 0.31	-	18.81 ± 0.12
Klebsiella pneumoniae	12.13 ± 0.22	14.36 ± 0.41	-	23.36 ± 0.17
Escherichia coli	8.21 ± 0.45	8.75 ± 0.31	-	18.81 ± 0.12
Bacillus cereus	12.13 ± 0.22	14.12 ± 0.41	-	13.36 ± 0.40

Data (mean ± SE) represent the zone of growth inhibition (mm). ME- *P. florida* aqueous extract; - Nil activity

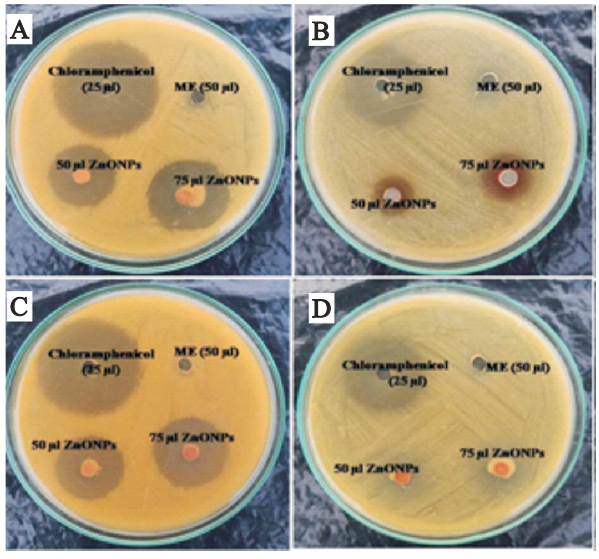

Fig. 6: Antibacterial activity of ZnO NPs against human pathogens, a) *Staphylococcus aureus*, b) *Klebsiella pneumoniae,* c) *Escherichia coli* and d) *Bacillus cereus*.

CONCLUSION

In the present investigation, the ZnO NPs was successfully synthesized from aqueous extract of *P. florida*. The UV-Vis spectral analysis of sample confirms the ZnO NPs peak at 258

nm. The antibacterial activity of synthesized ZnO NPs was also assessed on clinically isolated human pathogens such as *K. pneumoniae* (14.36 ± 0.41 mm), *B. cereus* (14.4 ± 0.41 mm), *S. aureus* (8.75 ± 0.31 mm), and *E. coli* (8.75 ± 0.31 mm). Overall, the present study suggests that *P. florida* fruit body extract mediated ZnO NPs may emerge as an effective alternatives for the existing antibacterial agents. Further in-depth research on *in-vivo* experiments to understand the mechanism of ZnO NPs toxicity is needed, and would be useful for biomedical applications.

REFERENCES

Agrawal, A. and Avill, R. 2006. Mesh migration following repair of inguinal hernia: a case report and review of literature. *Hernia* **10:** 79-82.

Alanazi, F.K., Radwan, A.A. and Alsarra, I.A. 2010. Biopharmaceutical applications of nanogold. *Saudi Pharm. J.* **18:** 179-193.

Anthony, K.J.P., Murugan, M., Jeyaraj, M., Rathinam, N.K. and Sangiliyandi, G. 2014. Synthesis of silver nanoparticles using pine mushroom extract: A potential antimicrobial agent against *E. coli* and *B. subtilis. J. Ind. Eng. Chem.* **20:** 2325-2331.

Gandhi, P.R., Jayaseelan, C., Kamaraj, C., Rajasree, S.R. and Mary, R.R. 2018. *In vitro* antimalarial activity of synthesized TiO_2 nanoparticles using *Momordica charantia* leaf extract against *Plasmodium falciparum*. *J. Appl. Biomed.* **16:** 378-386.

Gandhi, P.R., Jayaseelan, C., Mary, R.R., Mathivanan, D. and Suseem, S.R. 2017. Acaricidal, pediculicidal and larvicidal activity of synthesized ZnO nanoparticles using *Momordica charantia* leaf extract against blood feeding parasites. *Exp. Parasitol.* **181:** 47-56.

Jiang, P., Gu, S., Pan, D., Fu, J., Sahu, A., Hu, X. and Liu, X. S. 2018. Signatures of T cell dysfunction and exclusion predict cancer immunotherapy response. *Nat. Med.* **24:** 1550-1558.

Kadam, V.J., Jadhav, S.M. and Vijayakumar, K. 2019. Breast cancer diagnosis using feature ensemble learning based on stacked sparse autoencoders and softmax regression. *J. Med. Syst.* **43:** 1-11.

Kalaimurugan, D., Sivasankar, P., Lavanya, K., Shivakumar, M.S. and Venkatesan, S. 2019. Antibacterial and larvicidal activity of *Fusarium proliferatum* (YNS2) whole cell biomass mediated copper nanoparticles. *J. Clust. Sci.* **30:** 1071-1080.

Kannan, K., Radhika, D., Vijayalakshmi, S., Sadasivuni, K.K., Ojiaku, A. and Verma, U. 2022. Facile fabrication of CuO nanoparticles via microwave-assisted method: photocatalytic, antimicrobial and anticancer enhancing performance. *Int. J. Environ. Anal. Chem.* **102:** 1095-1108.

Li, H., Chang, J., Qin, Y., Wu, Y., Yuan, M. and Zhang, Y. 2014. Poly (lactide-co-trimethylene carbonate) and polylactide/polytrimethylene carbonate blown films. *Int. J. Mol. Sci.* **15:** 2608-2621.

Mattila, P., Suonpaa, K. and Piironen, V. 2000. Functional properties of edible mushrooms. *Nutrition* **16:** 694-696.

Mukherjee, P., Ahmad, A., Mandal, D., Senapati, S., Sainkar, S.R., Khan, M.I. and Sastry, M. 2001. Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis. *Nanoletters* **1:** 515-519.

Muthuvel, A., Jothibas, M. and Manoharan, C. 2020. Effect of chemically synthesis compared to biosynthesized ZnO NPs using *Solanum nigrum* leaf extract and their photocatalytic, antibacterial and *in-vitro* antioxidant activity. *J. Environ. Chem. Eng.* **8:** 103-705.

Owaid, M.N. and Ibraheem, I.J. 2017. Mycosynthesis of nanoparticles using edible and medicinal mushrooms. *Europ. J. Nanom.* **9:** 5-23.

Owaid, M.N., Rabeea, M.A., Aziz, A.A., Jameel, M.S. and Dheyab, M.A. 2019. Mushroom-assisted synthesis of triangle gold nanoparticles using the aqueous extract of fresh *Lentinula edodes* (shiitake), *Omphalotaceae*. *Environ. Nanotechnol. Monit. Manag.* **12:** 100-270.

Rai, M., Yadav, A. and Gade, A. 2008. CRC 675- current trends in phytosynthesis of metal nanoparticles. *Crit. Rev. Biotechnol.* **28:** 277-284.

Shang, Y., Min, C., Hu, J., Wang, T., Liu, H. and Hu, Y. 2013. Synthesis of gold nanoparticles by reduction of $HAuCl_4$ under UV irradiation. *Solid State Sci.* **15:** 17-23.

Virkutyte, J. and Varma, R.S. 2011. Green synthesis of metal nanoparticles: biodegradable polymers and enzymes in stabilization and surface functionalization. *Chem. Sci.* **2:** 837-846.