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Biosynthesis and Characterization of Zinc Oxide Nanoparticles Using *Pleurotus ostreatus* and Evaluation of Their Antioxidant Activity

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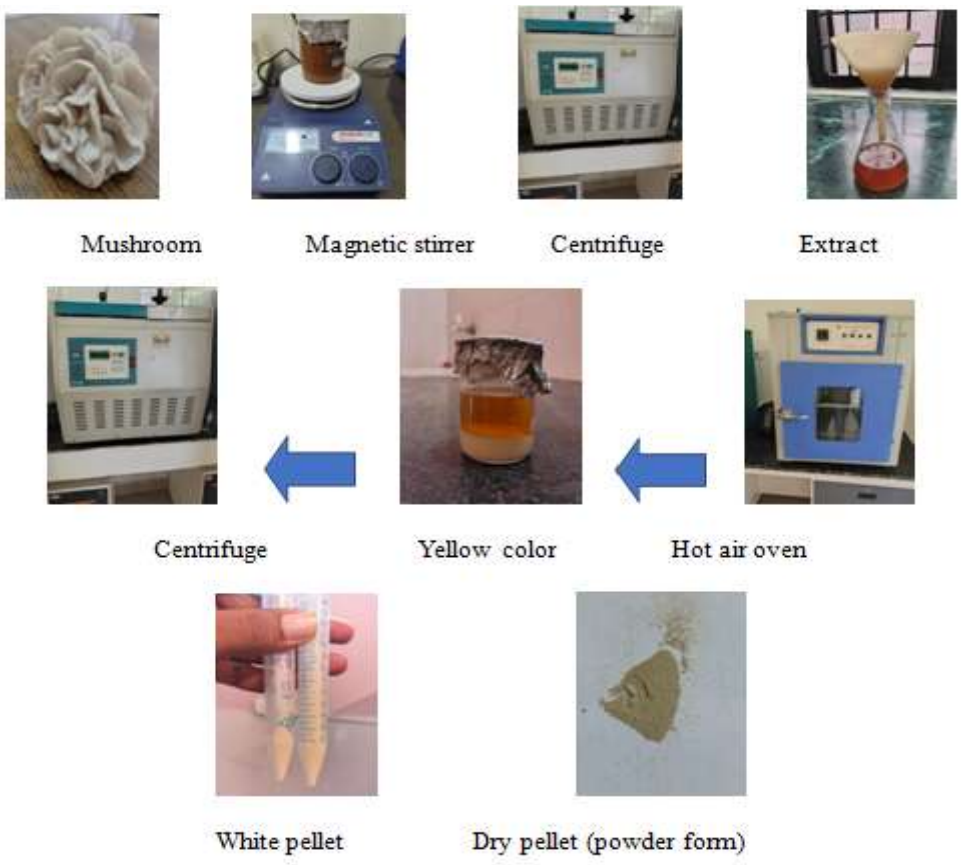
ABSTRACT

Nanotechnology has emerged as a promising field for the development of eco-friendly nanomaterials using biological resources. In the present study, the ZnO NPs were biologically synthesized using oyster mushroom (Pleurotus ostreatus) which acted as a both reducing and capping agents. The synthesized ZnO NPs were characterized by UV-Visible spectroscopy, Scanning Electron Microscopy (SEM), HR Transmission Electron Microscopy (TEM), EDS and Mapping. UV–Visible spectroscopy confirmed the successful synthesis of ZnO nanoparticles by exhibiting a characteristic absorption peak between 370 and 380 nm.SEM analysis revealed irregular and agglomerated particles, where as a TEM graph demonstrated the flower like nanostructure which composed of interconnected petal-like nanostructures. EDS analysis confirmed the presence of zinc and oxygen as major constituents while the elemental mapping indicated the homogenous distribution of zinc confirming the purity of ZnO NPs. The antioxidant potential of ZnO NPs demonstrating their potential ability to neutralize the free radicals which suggests the role in strengthening the antioxidant defense system.

Keywords: Green synthesis, Zinc oxide nanoparticles (ZnO NPs), Pleurotus ostreatus, Oyster mushroom extract, Biosynthesis, Nanotechnology, UV–Visible spectroscopy, SEM, TEM, Energy-dispersive X-ray spectroscopy (EDS), Antioxidant activity, Biogenic nanoparticles

GRAPHICAL REPRESENTATION



CHARACTERIZATION AND ANTIOXIDANT ACTIVITY

INTRODUCTION

Nanotechnology has become one of the fastest growing fields of research due to its ability to develop novel materials with its unique biological and physiochemical properties The simplest definition of nanotechnology is "technology on the nanoscale. Nanomaterials generally possess dimensions ranging the size of 1nm to 100nm possess a high surface to volume ratio (1). The antibacterial, antifungal, antiviral, antidiabetic, larvicidal, and anticancer properties of biogenically produced nanoparticles make them special (2, 3).

A material's characteristics may drastically change when its dimensions are lowered below 100 nm. Any technology that operates at the nanoscale and has practical applications, such as using individual atoms and molecules to create functional structures, is referred to as nanotechnology (4). The development and use of chemical, physical, and biological systems with structural characteristics ranging from single atoms or molecules to submicron dimensions, as well as the integration of the resulting nanostructures into larger systems, are all included in the field of nanotechnology (5). Research on the synthesis of biocompatible, eco-friendly, quick, safe, effective, and economical nanomaterials has been spurred by their uses (6). These days, a lot of research has been done on the creation and application of different nanoparticles in a variety of ways (7). Among the biological resources, mushroom have attracted increasing attention due to its extract contains a variety of bioactive metabolites contains phenolic compounds such as flavonoids, proteins, amino acids, enzymes that reduce metal ions and stabilized synthesised nanoparticles. These fungi are currently being grown as edible mushrooms due to their straightforward, better biological efficiency and simple, inexpensive production methods (8).

These are well acknowledged as abundant stores of several phytocompounds that underpin their function as effective biological nano-factories. Flavonoids like quercetin and catechin, which function as strong reducing and antioxidant agents, and phenolic acids like gallic, caffeic, and protocatechuic acids are abundant in edible and medicinal species like Pleurotus ostreatus, Lentinula edodes, Agaricus bisporus, and Morchella esculenta (9). The creation of nanoparticles with varying sizes, shapes, chemical compositions, and controlled dispersity, as well as their possible application for human benefit, are the main concerns of nanotechnology. ZnO NPs is used fungal extracts to create environmentally friendly nanoparticles (10).

Because of the tiny size and increased surface chemical reactivity, ZnO NPs are a significant class of metal oxide nanoparticles with intriguing biological and photocatalytic capabilities (11). Since phenolic compounds in plant extracts are essential for the formation and development of nanoparticles, choosing the right plant source to prepare the extracts is a critical step in obtaining ZnO NPs with the suitable physicochemical properties. Therefore, the goal of this study was to find a straightforward and efficient way to create strong, environmentally friendly ZnO NPs using high-purity fruit extract from oyster mushroom as a stabilising and reducing agent (12). Numerous techniques have focused to characterizes the biogenically synthesized ZnO NPs to confirm nps formation. In the present study ZnO NPs were characterized using UV vis. SEM and elemental mapping, HRTEM. After physiochemical characterization the synthesized ZnO NPs was by evaluating their antioxidant activity.

MATERIALS AND METHODS

2.1. Source and cultivation of oyster mushroom(Pleurotus ostreatus)

Healthy spawn of oyster mushroom was obtained from the department of plant pathology,G.B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand India. The oyster mushroom was cultivated under controlled environmental conditions until mature fruiting bodies were produced for experimental purpose.

2.2. Cultivation of oyster mushroom

For preparing a substrate 50 L of water were taken in a clean container. Calcium carbonate ($CaCO_3$) was added to adjust the substrate pH, while carbendazim was used to minimize fungal contamination. Now add about 10kg of wheat straw in a treated water tightly sealed and left overnight. To remove excess moisture the soaked straw was exposed to a sunlight and now packed into a cultivation bags. After these bags were maintained under dark room with regular watering until mature mushroom was harvested.

2.3. Preparation of a mushroom extract

Fresh harvested mushroom was dried and make a fine powder with a grinder machine. Approximately 10g of mushroom powder was mixed in a 100ml of distilled water and stirred continuously for 2 to 3 hours by using a magnetic stirrer. The mixture was centrifuged at 8000 to 9000 rpm for 15 to 20 min and the supernatant (extract) was collected and stored in fridge overnight.

2.4. BIO synthesis of ZnO NPs

20ml of extract was taken in a small beaker and 20gm of zinc acetate was mixed in this. Now NAOH solution was used to bring the pH about 9 to 10. After this mixture was kept in a hot air oven. The appearance of a yellowish-white precipitate indicated the formation of ZnO nanoparticle precursor.

2.5. Drying of ZnO NPs

Now centrifuge this mixture about 7000rpm to 8000rpm for 20 min, supernatant was disposed and remaining pellet was washed 2 to 3 times with a distilled water to remove impurities followed by a high speed until the pellet was completely dispersed in a distilled water. This purified pellet transferred to a petri dish and dried in a hot air oven until a fine powder was obtained.

2.6. Characterization of ZnO NPs

There are several characterization techniques which confirms the synthesis ZnO NPs. The synthesized ZnO NPs were characterized using UV-Vis which confirm the formation of ZnO NPS and to evaluate their optical properties by recording the characteristic absorption peak around 370 to 380nm. SEM was used to examine the morphology of surface ,particle distribution and approximate size of synthesized ZnO NPs. TEM was used to determine the size, shape, crystallinity and morphology of the synthesized also provide the information of the aggregation and distribution pattern of the synthesized ZnO NPs.

2.7. Antioxidant activity of ZnO NPs

The antioxidant activity was evaluated by using DPPH free radical scavenging assay which determines the ability of ZnO NPS to donate electrons of hydrogen atoms to neutralize stable free radicals, thereby reducing oxidative stress. The percentage of DPPH radical scavenging activity was calculated by measuring the decrease in absorbance at 517nm. A higher percentage of radical scavenging activity and a lower IC50 value indicate stronger antioxidant potential of the synthesized nanoparticles.

RESULTS AND DISCUSSION

3.1 UV-VIS Spectroscopy

An important method for examining the optical characteristics of semiconductor nanoparticles is Uv-Visible spectroscopy (13). The Uv-Visible spectroscopy of the synthesized nanoparticles exhibited a strong absorption in a range from 370nm to 380nm which is a distinguishable feature of the synthesized ZnO NPs (Fig1). In the spectrum, the absorbance remained high in the uv region which shows a sharp decrease near 375nm which indicating the confirm formation of ZnO NPs. This absorption edge corresponds to the electronic transition from the valence band to the conduction band of ZnO due to its wide gap band of ZnO. Beyond 400nm the absorbance gradually decreased towards visible region suggesting minimal absorbance in the visible range and indicating low absorption in the visible region, which is characteristic of ZnO nanoparticles. The observed absorption behavior is consistent with previously reported studies on green synthesized ZnO NPs.

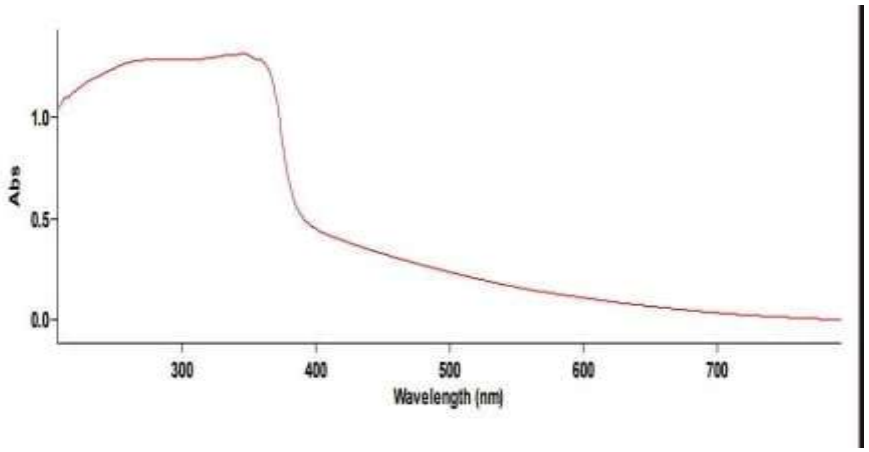


Fig 1. UV-Visible absorption spectrum of biosynthesized ZnO NPs exhibiting the characteristic absorption band around 370-380 nm.

3.2 SEM, MAPPING AND EDS

The image of Zn elemental mapping clearly shows a uniform distribution pattern of Zn throughout the NPs sample in which red color signals represent the presence of Zn indicating that Zn was homogenously dispersed over the analyzed group (Fig2.a). This uniform elemental distribution further confirms the successful synthesized NPs of zinc whereas EDS (energy dispersive x ray spectroscopy) spectrum confirmed the composition of elements which shows the characteristics peaks corresponding to zinc and oxygen which indicating the successful formation of zinc oxide NPs. The intense peaks of Zn around 1.0 keV and O near 0.5 keV confirmed the presence of ZnO NPs and no further impurity peaks were detected (Fig2.b) which suggested the high purity of ZnO NPs. This result agrees the percentage composition of zinc was 66.16% and that of oxygen was 33.84% for ZnO NPs synthesized by oyster mushroom extract (14). One more characterization of SEM revealed that synthesized NPs exhibit a highly and irregular agglomerated

morphology due to high surface energy and presence of biomolecules from oyster mushroom extract which act as a reducing and capping agents in which particles appeared as cluster composed of small crystallites with rough surfaces (Fig2.3). The average NPs were observed in the micrometer(µm) range while the crystallites were much smaller. Additionally, because green-synthesized NPs often have a high surface area, which enables NPs to adhere together, the green-synthesis approach is known to typically produce agglomeration in NPs (15).

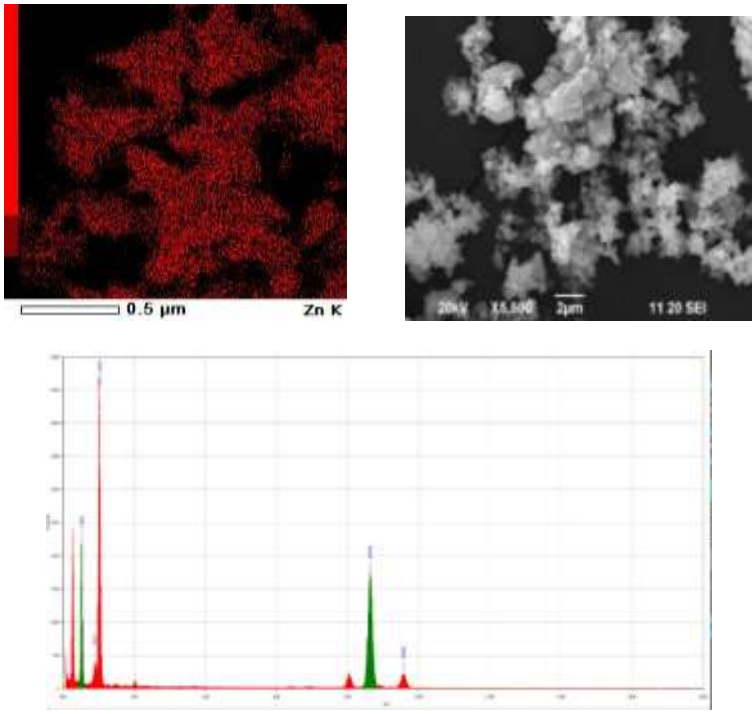


Fig2. (a)Zn elemental mapping of bio synthesized ZnO NPs, (b)SEM image showing aggregated particles with irregular morphology, (c)EDS spectrum confirming the elemental composition of Zn and O.

3.3 HR-TEM

The internal structures and morphology of the synthesized ZnO NPs were investigated by HR-TEM (Fig 3) which revealed the formation of flower like nanostructures composed of several interconnected nanosheets radiating from a common center which overlapped with each other producing 3D flower like assembles. High temperature calcination is frequently used in the manufacture of ZnO nanoparticles, which raises surface reactivity and encourages agglomeration(16).The ZnO NPs shows a high degree of aggregation due to their high surface energy and presence of biochemical from oyster mushroom. The images further shows that the primary crystallites were within the nanometer range whereas flower-like structures show the agglomerates of ZnO NPs. Given that ZnO nanoparticles are also prone to forming hard or soft agglomerations—hard agglomeration is caused by the chemical reaction of the surface groups, while soft agglomeration is caused by other physical effects—this suggests that the synthesized ZnO NPs were most likely agglomerated (17). Based on the 100 nm scale bar the nanoflowers appeared to have an overall size of 150 to 300 nm. The well define flower like architecture suggests good crystallinity and successful formation of ZnO NPs. Overall, the TEM analysis the confirmed the successful synthesized of ZnO NPs with a distinctive flower like morphology which provides a large specific area and also contributes to enhanced antioxidant activity.

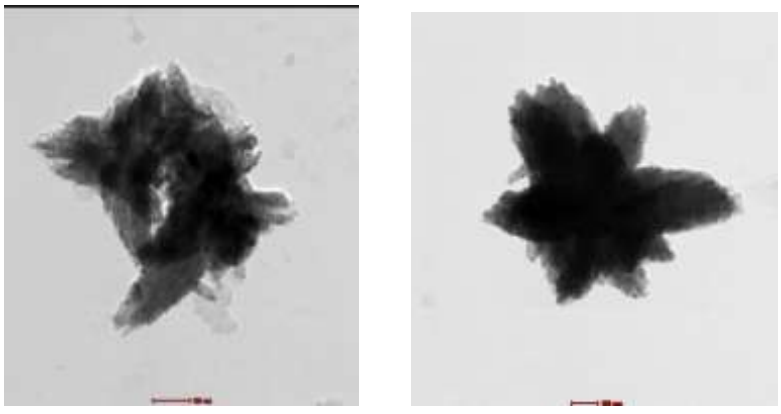


Fig3. (a) and (b) showing the TEM images of bio synthesized ZnO NPs

4) ANTIOXIDANT ACTIVITY

Antioxidants are compounds that inhibits or delay the oxidation of another molecules by scavenging the free radicals. Excessive reactive free radicals are produced in the human body by a number of factors, including poor nutrition, mental stress, smoking, and other illnesses (18). In biological systems ROS(reactive oxygen species) can cause oxidative stress which leads to cellular damage. To counteract this, we evaluate the radical scavenging potential of various compounds such as zinc oxide nanoparticles. The free radical scavenging potential of the ZnONPs extract was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay method. For this study, ZnONPs samples (1 mg/mL) were prepared in 99.9% methanol at variable concentrations ranging from 62.5 to 1000 \mug/mL, while ascorbic acid was employed as the standard at concentrations of 6.25–100 \μ g/mL. The violet colour of DPPH disappears when it is combined with hydrogen-donating nanoparticles (19) Each sample was treated with 1 mL of buffer, followed by the addition of 1 mL of 0.135 mM DPPH solution (20). The resulting mixture was shaken well and incubated at 20°C in the dark for 30 minutes. Post-incubation, the absorbance was measured at 517 nm using a spectrophotometer, with the absorbance of a freshly prepared DPPH solution serving as the control. The percentage of antioxidant activity or Radical Scavenging Activity (RSA) was calculated using the formula: % RSA = [(Ac - As) / Ac] × 100, where Ac represents the control absorbance and As represents the testing specimen absorbance. Finally, the % RSA values were plotted against the different concentrations of extract to derive a trendline equation, which was used to calculate the specific IC_{50} concentrations for each sample(11).

In (Fig4) the percentage RSA values were plotted against the respective concentrations to generate a linear trendline. The IC_{50} value, which indicates the concentration required to inhibit 50% of the free radicals, was calculated using the linear regression equations derived from the graphs. The standard ascorbic acid showed a high correlation coefficient (R^2 = 0.9919) and an IC_{50} of 18.9 \pm 0.51 \ug/mL. The ZnO NPs sample also exhibited excellent linearity (R^2 = 0.9958) with an IC_{50} value of 131.60 \pm 3.25 \ug/mL. In this study Ascorbic acid proved to be a more efficient scavenger at lower concentrations as compare to ZnO NPS. The ZnO NPs also demonstrated a clear concentration-dependent antioxidant effect. The IC50 value of ZnO NPs at 131.60 ug/ml shows that these NPs also neutralizes the free radicals and making them candidates for biomedical and environment applications.

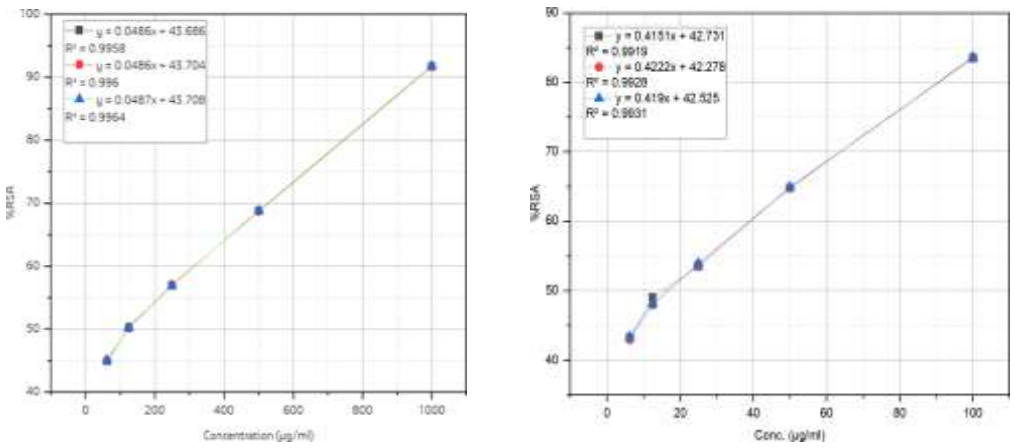


Fig4. DPPH free radical scavenging activity of bio synthesized ZNO NPs and ascorbic acid.

Research Gap

Although green synthesis of zinc oxide nanoparticles (ZnO NPs) using plant extracts has been extensively investigated, comparatively fewer studies have explored the use of edible mushrooms such as Pleurotus ostreatus as sustainable bio-reducing and stabilizing agents. Existing reports primarily focus on nanoparticle synthesis, while comprehensive characterization combined with biological evaluation remains limited. Furthermore, the influence of mushroom-derived bioactive compounds on the physicochemical properties and antioxidant potential of ZnO nanoparticles has not been adequately investigated. Therefore, there is a need for systematic studies integrating biosynthesis, detailed characterization (UV–Visible spectroscopy, SEM, TEM, and EDS), and antioxidant activity to establish P. ostreatus-mediated ZnO nanoparticles as eco-friendly nanomaterials with potential biomedical and pharmaceutical applications.

CONCLUSION

The present study successfully demonstrated the eco-friendly biosynthesis of zinc oxide nanoparticles using the aqueous extract of Pleurotus ostreatus. The biomolecules present in the mushroom extract acted as natural reducing and stabilizing agents, eliminating the need for hazardous chemicals during nanoparticle synthesis. UV–Visible spectroscopy confirmed the formation of ZnO nanoparticles, while SEM, TEM, and EDS analyses revealed their morphology, nanoscale dimensions, elemental composition, and purity. The synthesized nanoparticles also exhibited significant antioxidant activity, indicating their ability to scavenge free radicals effectively.

Overall, the findings suggest that Pleurotus ostreatus is a promising biological resource for the green synthesis of ZnO nanoparticles with desirable physicochemical and biological properties. The developed method is simple, cost-effective, environmentally benign, and suitable for large-scale production. These biosynthesized ZnO nanoparticles hold considerable potential for applications in biomedicine, pharmaceuticals, food preservation, cosmetics, and antioxidant formulations. Future studies should focus on evaluating their antimicrobial, anticancer, cytotoxicity, and in vivo therapeutic properties to further expand their practical applications.

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