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Mycosynthesis and Characterization of Zinc Oxide Nanoparticles from Shiitake Mushrooms (*Lentinula edodes*): Potential Antidiabetic, Antimicrobial and Antioxidant Efficacy

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Zinc oxide nanoparticles (ZnO NPs) are widely used because they are affordable, reliable and easy to produce. They have special characteristics which render them useful in different medicines. Due to their health benefits, affordability and minimal impact on the environment, mushrooms are a highly favourable option for biomedical applications. In this study, we used a water-soluble component of a methanolic extract of *Lentinula edodes* to produce ZnO NPs. The biosynthetic approach utilized flavonoids, polyphenols and other constituents from *L. edodes*, which possess reduction and encapsulation capabilities, to transform metal ions into ZnO NPs. Various analytical methods, including UV-Vis, FTIR and SEM, have been used to characterize the biogenic ZnO NPs. The FTIR analysis identified the presence of the hydroxyl (OH) group based on the functional group observed at 3425.08 cm^{-1}, moreover, the peaks observed at 1631.32 cm^{-1} indicated the presence of C=O carboxylic stretch bonds. Scanning electron microscopy (SEM) demonstrated the presence of a porous shape with diameters of 35-200 nm. The biologically produced ZnO NPs showed promising antibacterial, antioxidant and antidiabetic properties. At specific concentrations, it showed strong antioxidant (70.51 ± 0.35%) and antidiabetic (82.36 ± 0.41%) effects, indicating that it could be used therapeutically for the treatment of diabetic conditions and could be suitable for incorporation into food and nutritional supplements.

Keywords: Zinc nanoparticles, *Lentinula edodes*, Antioxidant activity, Antidiabetic activity, Mushroom.

INTRODUCTION

The exceptional properties of nanoparticles have driven technological advancements to the forefront of numerous fields, including environmental, agriculture and medicine fields. Nanoparticles possess many forms, but zinc oxide nanoparticles (ZnO NPs) have recently emerged in prominence because of their numerous beneficial application and excellent physical and chemical characteristics [1-3]. Pharmaceutical delivery mechanisms, neuroimaging agents and medical therapies for chronic illnesses are only a few biomedical potentials for these nanoparticles due to their robustness, biological compatibility and large area of surface [4,5].

Mycosynthesis, the microbial biosynthesis of ZnO NPs, has recently developed as a viable and eco-friendly substitute for existing chemical processes. In this technique, plants, algae and fungi are used to convert metal ions into nanoparticles. Despite the fact that fungi release several chemicals and proteins that aid in metal ion reduction and stabilization, mushrooms have garnered a great deal of attention as a possible biosynthetic source for nanoparticles [6,7].

Despite the fact that fungi release several chemicals and proteins which assist in metal ion reduction and stabilization, thus, mushrooms have garnered a great deal of attention as a possible biosynthetic source for biogenic nanoparticles [8-10]. The bioactive substances produced by shiitake mushrooms, such as polysaccharides, polyphenols and flavonoids, are well-known for their ability to combat inflammation, diabetes and free radicals. Incorporating these bioactive compounds into the nanoparticle fabrication process contributes to decreasing metal ions and provides the final product with further therapeutic advantages [11,12]. The shiitake mushroom, scientifically

known as *Lentinus edodes*, a great demand among consumers across the world and consequently has a significant influence in the mushroom marketplace. There are several bioactive polysaccharides in its nutritional characteristics, including β-D-glucan, heteroglucan, xylomannan, lentinan and eritadenine. It also contains a variety of natural sugars, such as arabinose, arabitol, mannose, mannitol, trehalose and glycerol [13,14]. In addition to being a good source of dietary fiber and vital vitamins (B_2, B_{12} and D_2), it also has pharmacological effects that may help with a variety of medical ailments. Further strengthening its therapeutic value, studies ascribe antifungal, antibacterial and antioxidant activities to its biofunctional compounds. *L. edodes* is a fascinating opportunity for pharmacological and nutritional applications due to its potential in the treatment of diabetes, high blood pressure, high cholesterol, cardiovascular diseases, immunodeficiencies, hepatocellular illnesses and malignancies [15].

To effectively use nanoparticles in biomedical research, it is crucial to comprehend the biological production mechanisms and characterize their physico-chemical features, as recently highlighted by advances in nanotechnology. Nanoparticles produced by mycosynthesis were characterized using various techniques like SEM, DLS, XRD, UV-Vis and FTIR. In the context of chronic diseases, oxidative stress and inflammation play crucial roles in the development of illnesses like diabetes mellitus [16]. ZnO NPs produced from shiitake mushrooms have great therapeutic promise for treating diabetic problems caused by oxidative stress. According to research, these nanoparticles may neutralize free radicals, shield pancreatic β-cells from oxidative stress and improve insulin sensitivity. As a result, glucose metabolism is improved and diabetes mellitus may not advance as progression [17].

To produce well-defined ZnO NPs at a lower cost and with less effort than conventional synthetic methods, this study intends to use green synthesis approaches using mushroom extracts. In this research, we employ a water-based fraction of a methanolic extract from *Lentinus edodes* to produce ZnONPs in an environmentally friendly method. In addition, it delves into the potential antimicrobial, antioxidant and antidiabetic uses of these ZnO NPs.

EXPERIMENTAL

Shiitake mushrooms source and extraction of bioactive compounds: The Umai dried shiitake mushroom (*Lentinula edodes*) was acquired from an online seller from Flipkart, India. The specific product, Umai Dried Shiitake mushroom, originated from C K Industrial Co., Ltd, located at No. 98 Huanghai Road, Tianjin, China. Initially, about 1 g of *L. edodes* mushrooms was allowed to dry and clean at room temperature (25 ± 1ºC). Before being steeped in methanol at ambient temperature, the mushrooms that had been dried were ground into a fine consistency. After filtering the methanol extract, a partially solid extract was obtained by evaporating it utilizing a rotary evaporator. Additional fractionation of this product was carried out using *n*-hexane and distilled water [18].

Green synthesis of ZnO NPs: With few modifications, Selim *et al.* [19] procedure was adopted for the biological synthesis of ZnO NPs. About 50 mL of water-soluble portion of *L. edodes* methanolic extract was combined with 50 mL of 100 mM $Zn(NO_3)_2{\cdot}6H_2O$ and then stirred magnetically at 65-85 ºC. The pH dropped to 12 when it reached 60 ºC after the dropwise addition of 0.1% NaOH with swift shaking in a 1:1 molar ratio. A white precipitate was formed after 2 h of holding time in the solution and after drying in a hot air oven set at 70 ºC, the powder was transformed into a thicker cream mixture. The mixture was separated by washing it with an ethanol and distilled water solution multiple times. Finally, the mixture was calcined in a porcelain furnace container for 2 h at 400 ºC in an oven to obtain a white powder as ZnO NPs, which was stored for further studies [20].

Characterization: Utilizing a UV-visible spectrophotometer from Shimadzu, Kyoto, Japan, a 100-700 nm wavelength range double-beam V-630 spectrophotometer was used to monitor the UV absorption of ZnO NPs. A Perkin-Elmer spectrometer instrument was used to characterize the functional groups on the interfaces of ZnO NPs, with a wavelength resolution of 4 cm^{-1} and an emission spectrum ranging from 4000-450 cm^{-1}. A Zeiss EVO LS10 scanning electron microscope (SEM) operated at 12 keV was used to investigate the shape of ZnO NPs produced from the water-based portion of methanol extract. A small quantity was placed on a copper grid coated with carbon and allowed to dry for 5 min under a mercury lamp to prepare the samples. The SEM images were acquired at different magnifications. A carbon sheet was used to dropcoat the dried powdered ZnO NPs sample after it was analyzed utilizing an EDX detector linked to the SEM for the elemental analysis [21].

Biological applications

Antioxidant potential (FRAP assay): To determine the antioxidant capacity, a 96-microtiter plate was used to mix 100 µL of ZnO NPs at various concentrations (100, 200, 300, 400 and 500 µg) with 900 µL of freshly prepared FRAP reagent. The FRAP reagent consisted of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-*s*-triazine) in 40 mM of HCl and 20 mM of $FeCl_3$ in a 10:1:1 ratio. The mixture was then incubated in dark at 37 ºC for 30 min in order to attain the completion. An ELISA reader was used to detect absorbance at 593 nm using the iMark Microplate Absorbance Reader (Bio-Rad Laboratories India Pvt. Ltd.), which estimate the reduction of Fe^{3+}-TPTZ complex to the ferrous form (Fe^{2+}-TPTZ), which has a strong blue colour. Standard antioxidant, such as ascorbic acid, was diluted in 1 mL of distilled water at varying doses. An indicator of antioxidant activity, the FRAP value was determined by comparing the 593 nm absorbance change of test reaction mixtures with those containing ferrous ions at known quantities [22].

$$\text{Inhibition (\%)} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

Antibacterial activity: The antimicrobial efficacy of ZnO NPs was evaluated by disc diffusion method against several bacterial species *viz*. Gram-positive *Staphylococcus aureus*, Gram-negative *Escherichia coli* and *Klebsiella pneumoniae*. With the help of glass spatula, nutrient agar plates were unifor-

mly coated with bacterial cultures. The next step was to deliver different concentrations of ZnO NPs sample onto sterile discs, which were subsequently placed onto the agar plates. The plates were kept at 37 ºC for 24 h for incubation. Ampicillin discs and DMSO were applied as a positive and negative controls, respectively. After the incubation period, the zones of inhibition surrounding each disc were examined using a clear reader to determine the efficacy of the synthesized ZnO NPs against bacteria [23].

Antidiabetic activity: The starch-iodine technique was used to assess the α-amylase inhibition by ZnO NPs at different concentrations (100, 200, 300, 400 and 500 µg). At first, the standard solutions of various concentrations were incubated with 5 µL of α-amylase solution and 195 µL of nanoparticle sample in DMSO for 10 min at 37 ºC. The resulting mixture was incubated for another 60 min after the first incubation after which 50 µL of starch solution was added. Afterward, the iMark Microplate Absorbance Reader was used to determine the intensity at 630 nm after 100 µL of 1% iodine solution was added. The positive control consisted of metformin, whereas the negative control was PBS14. The following formula was applied to determine the inhibition percentage:

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Statistical analysis: Statistical significance were analyzed with the help of computer oriented program Two-way ANOVA using software Graph Pad Prism, version 2022 (GraphPad Sofware, San Diego, USA). The values were represented as mean ± SE.

RESULTS AND DISCUSSION

Harnessing the advantages of beneficial bioactive compounds present in shiitake aqueous fractions, the biological generation of nanoparticles is an innovative approach for detection and medical treatment. Nanoparticle production using this approach is a fascinating field of study since it is both simple and inexpensive [24]. The key bioactive components of the stabilizing agent-containing aqueous portion of *Lentinula edodes* methanolic extract, which includes flavonoids and phenolics, were used to generate ZnO nanoparticles in this investigation. The stability and enhanced biological properties of the biogenic ZnO nanoparticles were confirmed by the green synthesis method, which was both environmentally friendly and economically efficient.

Characterization of ZnO NPs: Several important properties pertinent to their potential uses in many domains have been explored *via* the characterization of ZnO NPs synthesized from *L. edodes* (shiitake mushroom). According to results, ZnO NPs obtained using this biogenic approach have many desired characteristics, such as uniform size dispersion and a large area of surface, both of which are important for their use in ecological and biomedical applications. Potentially beneficial as antibacterial compounds and pharmaceutical delivery mechanisms, these nanoparticles are usually round and have diameters between 35 and 200 nm. The appealing potential of these nanoparticles for long-term use is enhanced by the fact that their biological production from *L. edodes* is less toxic to the environment and more economical than traditional chemical processes.

UV-visible spectral studies: To validate their formation, the solution was subjected to UV-vis analysis after being suspended in aqueous methanolic extract in a 1:1 proportion of ZnO NPs. In order to verify the effective formation of ZnO nanoparticles, the colour of the product witnessed a transition from yellow to light yellow, and finally to milky white, as the chemical reaction progressed. *Lentinula edodes* synthesized ZnO NPs showed a broad UV absorption band, with a sharp maximum at 237 nm (Fig. 1). The excited valence electrons in the ZnO nanoparticles are responsible for this absorption band.

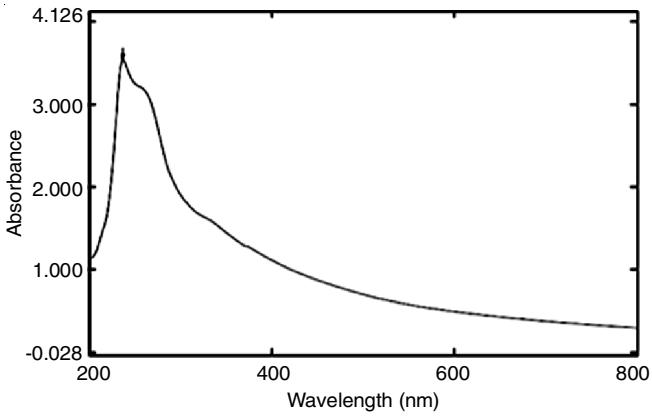

Fig. 1. UV-visible spectrum of ZnO NPs synthesized from *L. edodes* aqueous fraction

FT-IR spectral studies: In the process of synthesizing ZnO nanoparticles using *L. edodes*, the FTIR technique is often used to detect biologically active compounds and functional groups. There were bands in the spectra between 3550 and 3200 cm^{-1}, which correspond to hydroxyl (OH) groups and bands between 1720 and 1706 cm^{-1}, which indicate carboxyl (C=O) groups. Fig. 2 shows the results, which highlight the key functional groups in the synthesis [25].

Morphological studies: The SEM images showed the bunches and isolated ZnO NPs, which were mostly spherical but could aggregate into bigger particles with a variety of geometries (Fig. 3). These micrographs were analyzed using ImageJ® software and the results showed that the biogenic ZnO NPs had a very narrow size distribution, with an average diameter of 200 nm.

Antioxidant activity: Biologically synthesized ZnO NPs showed antioxidant activity similar to that of the water-based mushroom extract when evaluated using the FRAP assay. In FRAP assay, the nanoparticles' shift frequency at 593 nm is decreased due to the transfer of electron count from an oxygen atom to the odd electrons on a nitrogen atom, which is facilitated by the smaller ZnO NPs [22]. Fig. 4 shows the percent of inhibiting an enzyme target when different quantities of ascorbic acid and ZnO NPs (100, 200, 300, 400, 500 µg/mL) were used. At all concentrations, ascorbic acid surpasses biogenic ZnO NPs in terms of inhibitory efficiency. When the ZnO NPs were concentrated to 300 µg, the highest inhibitory effect was measured

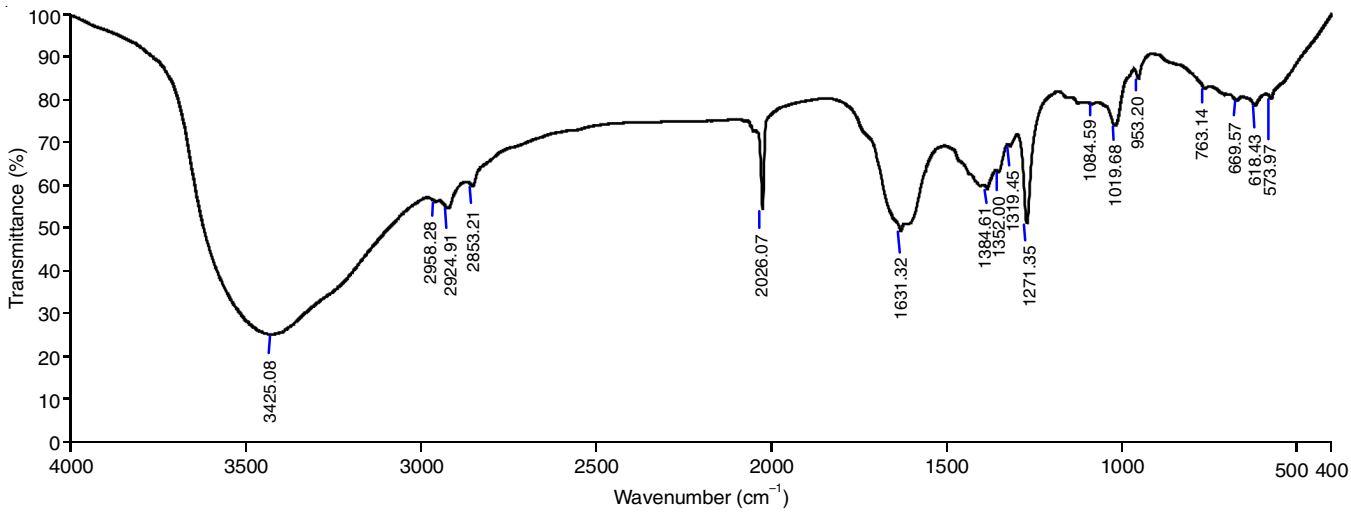

Fig. 2. FT-IR spectrum of ZnO NPs from aqueous fraction of *L. edodes*

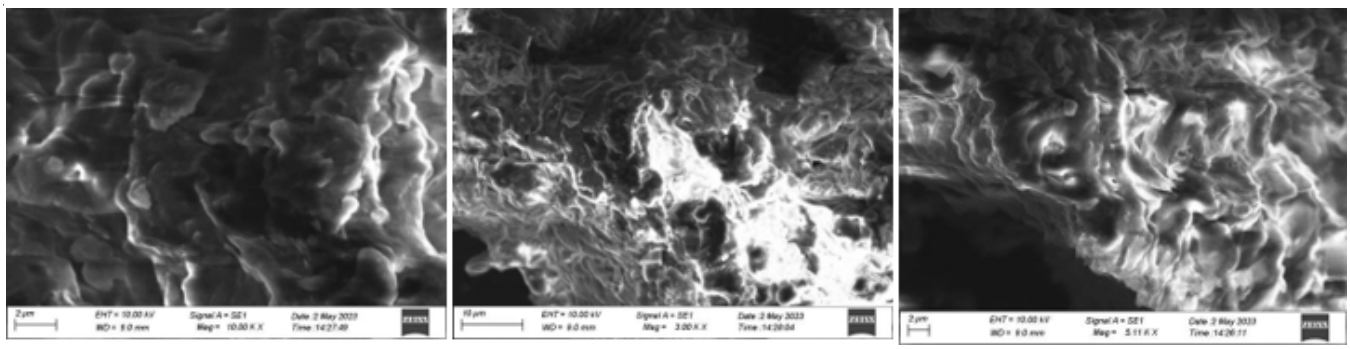

Fig. 3. SEM image of ZnO NPs from aqueous fraction of *L. edodes*

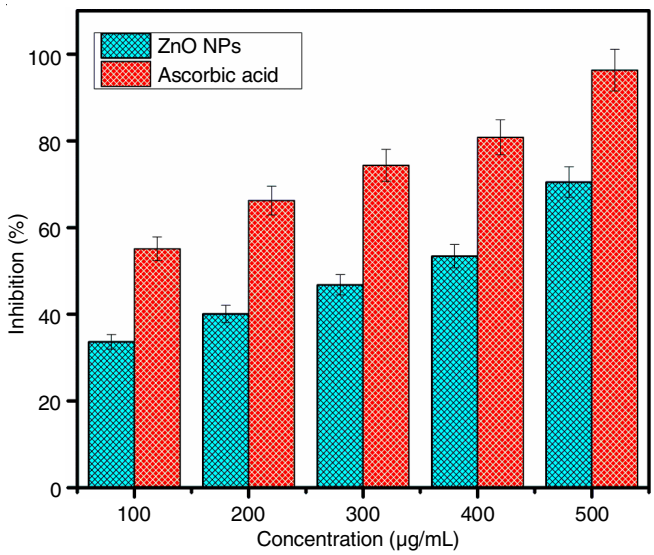

Fig. 4. Free radical scavenging activity of ZnO NPs of aqueous fraction of *L. edodes*. The values are presented as the Mean ± SD

at 65.7 ± 1.09%. It was established that the nanoparticles had antioxidant properties and showed intense blue colour. Around 55.13 ± 0.27% inhibition is achieved by ascorbic acid at 100 µg/mL, whereas biogenic ZnO NPs reach around 33.65 ± 0.16%. At increasing concentrations, the inhibitory effects of both treatments are dose-dependent; at 500 µg/mL, ascorbic acid reaches a peak of about 96.31 ± 0.48 % inhibition while ZnO NPs reach around 70.51 ± 0.35 %. By comparing their efficacy with that of ascorbic acid, a standard antioxidant, the findings from the biogenic ZnO nanoparticles are crucial for evaluating the potential biological applications of ZnO NPs. The reference standard for this comparison was ascorbic acid. With a high ratio of surface to volume the ZnO NPs that were generated by adsorbing bioactive compounds from the mushroom extract interacted with and reduced DPPH radicals more effectively [26].

Antibacterial efficacy: The effectiveness of ZnO NPs as an antibacterial agent is dose-dependent, as shown in Table-1, which displays the zone of inhibition for various pathogens at different doses. As the concentration increased from 20 µg to 80 µg, the inhibitory zones for *E. coli* increased from 8 ± 0.06 mm to 15 ± 0.09 mm, whereas the zones increased from 8 ± 0.07 mm to 14 ± 0.10 mm in *K. pneumoniae*, which showed comparable patterns. The degree of activity was much higher in *P. aeruginosa*, with inhibition zones increasing from 10 ± 0.10 mm to 17 ± 0.12 mm. A significant rise in inhibitory zones from 9 ± 0.06 mm to 16 ± 0.08 mm was also seen in *S. aureus*, while the inhibition of *C. albicans* was increased from 8 ± 0.02 mm to 13 ± 0.05 mm. These results highlight the potential of biogenic ZnO NPs as an antibacterial agent for treating diseases triggered by Gram-positive and Gram-negative bacteria, fungi and other microorganisms, as well as their strong and broad-spectrum antimicrobial activity [27,28].

TABLE-1
ANTIMICROBIAL ACTIVITY OF SYNTHESIZED ZnO NPs DERIVED FROM *Lentinula edodes* (SHIITAKE MUSHROOM) AGAINST SELECTED PATHOGENIC ORGANISMS

Organisms	Zone of inhibition (mm) (Agar well diffusion); Various concentrations of LE ZnO NPs extract			
	20 µg	40 µg	60 µg	80 µg
Escherichia coli	8 ± 0.06	11 ± 0.07	13 ± 0.08	15 ± 0.09
Klebsiella pneumoniae	8 ± 0.07	10 ± 0.08	12 ± 0.09	14 ± 0.10
Pseudomonas aeruginosa	10 ± 0.10	12 ± 0.11	15 ± 0.12	17 ± 0.12
Staphylococcus aureus	9 ± 0.06	11 ± 0.06	13 ± 0.07	16 ± 0.08
Candida albicans	8 ± 0.02	10 ± 0.04	11 ± 0.04	13 ± 0.05

Antidiabetic activity: Different concentrations of the ZnO NPs used in the investigations were conducted in order to ascertain the extent to which ZnO NPs are able to inhibit α-amylase. The findings showed that the percentage of inhibition decreased with decreasing levels, suggesting that the degree of inhibition was dependent on concentration. At 500 µg/mL, the highest indicated inhibition percentage was 82.36 ± 0.41 %, which was similar to the standard drug metformin, which showed inhibition of 85.63 ± 0.42 % (Fig. 5). Based on these results, biogenic ZnO NPs may provide a natural substitute as well as a supplement to conventional diabetes therapies, as they demonstrate a strong antidiabetic effect.

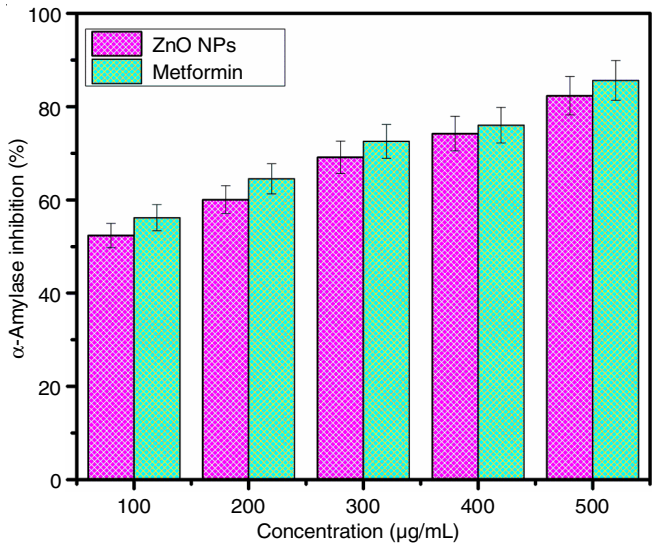

Fig. 5. α-Amylase inhibition (%) of ZnO NPs of aqueous fraction of *L. edodes* methanolic extract. The values are presented as mean ± SD

Conclusion

The present investigation used a green synthesis process to synthesize ZnO NPs, which is advantageous since it is inexpensive, safe for the ecosystem and produces fewer pollutants. To stabilize and reduce size nanoparticles, this method makes use of bioactive chemicals present in the Shiitake mushrooms (*Lentinula edodes*). The mycosynthesis of ZnO NPs using zinc nitrate was significantly facilitated by the presence of several compounds, such as polyphenols, flavonoids and carboxylic acid derivatives present in *entinula edodes*, especially those that include carboxylic and hydroxyl groups. The biogenic ZnO NPs showed as promising antibiotics, antidiabetic agents and sugar regulators.The findings support the concept that the ZnO NPs may have therapeutic applications and illustrate the potential synergy between mycology and nanotechnology in addressing diabetes treatments.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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