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Green Synthesis of Zinc Nanoparticles Using Phenolic Acids from Edible Mushrooms of Chhattisgarh Region: A Novel Approach

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ABSTRACT

This study presents a novel and environmentally sustainable approach for synthesizing zinc nanoparticles (ZnNPs) by leveraging phenolic acids extracted from edible mushrooms endemic to the Chhattisgarh region. We prepared methanolic extracts from selected mushroom species and quantified their total phenolic content using the Folin–Ciocalteu method, complemented by high-performance liquid chromatography (HPLC) for comprehensive phenolic acid profiling. The biosynthesis of ZnNPs was achieved through a controlled reaction between these mushroom extracts and zinc acetate dihydrate. The synthesized nanoparticles underwent rigorous characterization using a suite of analytical techniques, including UV-Visible spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), and X-ray Diffraction[1] (XRD) analysis. Our findings underscore the significant potential of native edible mushrooms as efficient and eco-friendly bio-factories for the production of ZnNPs, paving the way for their diverse applications in biomedical, agricultural, and environmental remediation sectors.

Keywords: *Phenolic acid, Edible mushroom, Zinc nanoparticles, Green synthesis, Characterization.*

1. INTRODUCTION

Mushrooms are increasingly recognized as a significant source of diverse bioactive compounds, including polysaccharides, flavonoids, and particularly phenolic acids. These compounds are highly valued for their established antioxidant, antimicrobial, and anticancer properties. The region of Chhattisgarh, often referred to as the "Rice Bowl of India," also harbours a rich and diverse array of edible and medicinal mushroom species, presenting a valuable natural resource for scientific exploration.

Recent advancements in nanotechnology have placed a strong emphasis on the green synthesis of nanoparticles. This approach utilizes biological entities, such as plant extracts and microbial metabolites, to minimize the environmental hazards traditionally associated with conventional chemical and physical synthesis methods. Within this context, phenolic acids, which are prevalent secondary metabolites in mushrooms, play a crucial role. They act as natural reducing agents, facilitating the conversion of metal ions into nanoparticles, and concurrently serve as stabilizing agents, preventing nanoparticle aggregation and ensuring their structural integrity.

Among various nanoparticles, zinc nanoparticles (ZnNPs) have attracted considerable attention due to their advantageous biocompatibility, potent antimicrobial properties, and promising applications across various fields, including drug delivery systems and sustainable agriculture. The global consumption of mushrooms has seen a significant increase in recent years (Wang et al., 2019), with *Agaricus bisporus* (button mushroom) being the most widely produced and consumed species worldwide (ANPC, 2019; Furlani & Godoy, 2008).

The growing interest in mushrooms also stems from discoveries regarding their potential health benefits, which are largely attributed to their rich content of bioactive compounds, including phenolics (Roncero-Ramos & Delgado-Andrade, 2017). Consequently, there is a strong scientific interest in accurately quantifying these beneficial analytes within mushroom matrices. Numerous studies have reported a wide spectrum of health-promoting actions associated with various mushroom species, including antitumor, immunomodulatory, antioxidant, free radical scavenging, antihypercholesterolemic, antiviral, antibacterial, hepatoprotective, antidiabetic effects, and positive modulation of intestinal microbiota (Aprotosoaie et al., 2017; Meng, Liang, & Luo, 2016; Roncero-Ramos & Delgado-Andrade, 2017; Vamanu & Pelinescu, 2017).

Phenolic compounds are recognized as the primary antioxidants present in mushrooms. Researchers globally have identified phenols in diverse mushroom species from countries such as Finland, India, South Korea, Portugal, Spain, Turkey, Mexico, China, Poland, and Greece (Barros et al., 2009; Heleno et al., 2015; Jayakumar et al., 2009; Kim et al., 2008; Palacios et al., 2011; Puttaraju et al., 2006; Ribeiro et al., 2006; Ribeiro et al., 2007; Siu et al., 2016; Yahia et al., 2017; Yaltirak et al., 2009). While extensive research exists, a singular study by Carvajal et al. (2012) stands out in the literature for characterizing the phenolic compound profile in Brazilian *Agaricus brasiliensis*, identifying three specific phenolic compounds.

The extraction process is a critical preliminary step for the accurate identification and quantification of phenolic compounds. Traditional methods like liquid-liquid and solid-liquid extraction are widely employed due to their simplicity, efficiency, broad applicability, and cost-effectiveness (Becerra-Herrera et al., 2014). More recently, advanced extraction techniques such as solid-phase extraction, ultrasound-assisted extraction, supercritical fluid extraction, and high hydrostatic pressure have been introduced for this purpose (Nipornram et al., 2018; Pinela et al., 2018). Common solvents for phenolic compound extraction include methanol, ethanol, acetone, water, ethyl acetate, propanol, and dimethylformamide, as well as their various combinations. Among these, ethanolic extraction is frequently favored (Daí & Mumper, 2010; Tlili et al., 2013; Yuan et al., 2018).

This study, establishes three primary objectives: (i) to comprehensively extract and quantify phenolic acids from edible mushrooms collected within the Chhattisgarh region; (ii) to subsequently utilize these phenolic-rich extracts for the eco-friendly green synthesis of ZnNPs; and (iii) to thoroughly characterize the physicochemical properties of the synthesized nanoparticles.

2. METHODOLOGY

2.1. Materials

Fresh *Agaricus bisporus* (button mushrooms) were collected from local farms within the Chhattisgarh region. For the extraction and synthesis procedures, analytical-grade chemicals were used. These included zinc acetate dihydrate ($Zn(CH_3COO)_2{\cdot}2H_2O$) as the zinc precursor for nanoparticle synthesis and methanol as the solvent for phenolic acid extraction. Additionally, Folin–Ciocalteu reagent and sodium carbonate (Na_2CO_3) were employed for the determination of total phenolic content. Distilled water was used throughout all experimental steps to ensure purity and consistency.

2.2. Extraction of Phenolic Acids

To extract phenolic acids, fresh mushrooms were thoroughly washed, shade-dried, and subsequently ground into a fine powder. A 10 g sample of this powdered mushroom was then subjected to Soxhlet extraction with 100 mL of 80% methanol for 6 hours. Following extraction, the resultant solution was filtered and concentrated under reduced pressure using a rotary evaporator. The concentrated extract was then stored at 4°C until further analysis.

2.3. Determination of Total Phenolic Content

The total phenolic content (TPC) was quantified using the established Folin–Ciocalteu method. Briefly, 0.5 mL of the mushroom extract was combined with 2.5 mL of 10% Folin–Ciocalteu reagent and 2.0 mL of 7.5% sodium carbonate solution. After incubating the mixture at room temperature for 30 minutes, the absorbance was measured at 765 nm using a UV-Visible spectrophotometer. A standard calibration curve was prepared using gallic acid, and the TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight.

2.4. HPLC Analysis

High-Performance Liquid Chromatography (HPLC) was utilized for the qualitative analysis of phenolic acids present in the mushroom extract. A C18 reverse-phase column was employed for chromatographic separation. The mobile phase consisted of a ternary mixture of methanol, water, and acetic acid in a ratio of 50:49:1 (v/v/v). Detection of the phenolic compounds was performed at a wavelength of 280 nm.

2.5. Synthesis of Zinc Nanoparticles

For the green synthesis of zinc nanoparticles, 10 µL of the *Agaricus bisporus* extract was added dropwise to 90 mL of 1 mM zinc acetate solution. This mixture was continuously stirred at 60°C for 2 hours. The successful formation of nanoparticles was visually indicated by a color change from light yellow to white. The resulting colloidal solution was then centrifuged at 10,000 rpm for 15 minutes. The obtained pellet, containing the synthesized nanoparticles, was washed thrice with distilled water and ethanol to remove impurities. The purified nanoparticles were subsequently dried and collected for comprehensive characterization.

3. RESULT AND DISCUSSION

3.1. Characterization of Zinc Nanoparticles

The synthesized ZnNPs underwent thorough characterization using several advanced analytical techniques:

3.1.1. UV-Visible Spectroscopy: This technique was employed to confirm the formation of nanoparticles by scanning the absorbance spectrum between 200–800 nm, looking for characteristic absorption peaks (Figure 1). A strong absorption peak was observed at approximately **370 nm**, which then rapidly declines as wavelength increases, indicating successful synthesis of ZnNPs. This shape is characteristic of ZnO nanoparticles, indicating strong absorption in the UV region and little to no absorbance in the visible range. The steep drop suggests a sharp optical transition, typical of semiconducting nanoparticles.

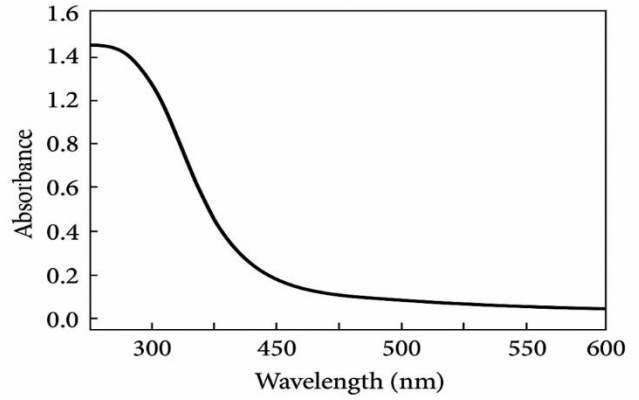

Figure 1.

The phenolic compounds and antioxidants naturally present in mushrooms play a multifaceted role in the synthesis and application of nanoparticles. They act as effective reducing agents, facilitating the conversion of metal salts (such as zinc salts) into their corresponding nanoparticles, like ZnO nanoparticles. These compounds serve as stabilizing and capping agents, which are crucial for controlling the size of the synthesized nanoparticles and preventing their undesirable aggregation, thereby ensuring the formation of uniform and stable nanostructures. A significant advantage of using mushroom-derived compounds is their ability to enhance the biocompatibility of the resulting nanoparticles, making them more suitable for various biological and biomedical applications.

3.1.1. Fourier Transform Infrared (FTIR) Spectroscopy: FTIR spectra were recorded to identify the functional groups present in the mushroom extract that were involved in the reduction of zinc ions and the subsequent stabilization of the nanoparticles. The FTIR spectrum showed characteristic peaks (Figure 2): **Zn–O stretching vibration:** around **400–600 cm^{-1}, Phenolic O–H stretch:** broad band around **3200–3600 cm^{-1}, C=O stretch (from carboxylic or phenolic acids):** around **1600–1700 cm^{-1}, C–O stretching and C–H bending:** 1000–1300 cm^{-1}**.** These results suggest that phenolic compounds contributed to reduction and stabilization of ZnNPs.

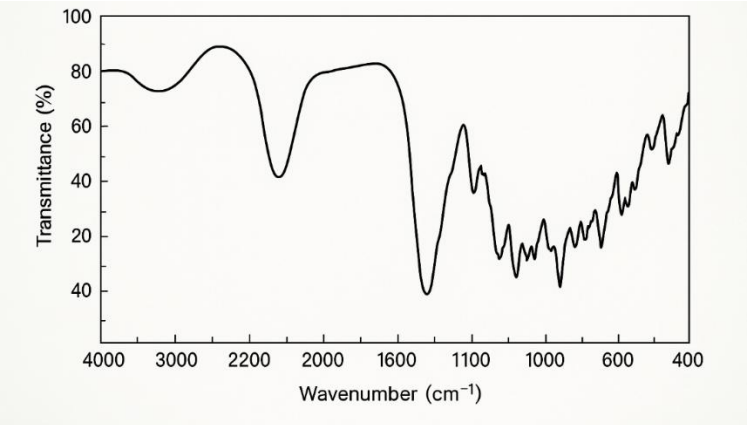

Figure 2

3.1.3. Scanning Electron Microscopy (SEM): SEM was used to examine the morphology and estimate the size of the synthesized nanoparticles. SEM images revealed that the ZnNPs were predominantly spherical and exhibited slight aggregation. The particle size ranged between 30–55 nm.

3.1.2. X-ray Diffraction (XRD) Analysis: XRD was performed to confirm the crystalline structure and precisely

determine the crystallite size of the ZnNPs. (Figure 3)

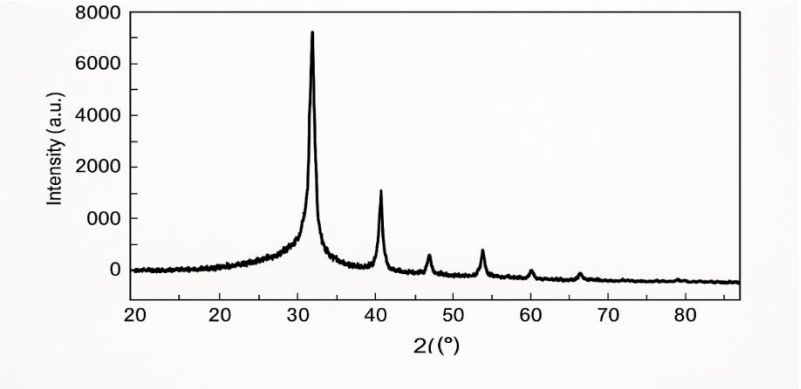

Figure 3

The observation of sharp peaks in the X-ray diffraction (XRD) pattern serves as a strong indicator of the high crystallinity of the synthesized ZnO nanoparticles. These nanoparticles typically exhibit a hexagonal wurtzite crystal structure, which is characterized by distinct diffraction peaks at specific 2θ angles. For instance, a peak appearing around 31.7° corresponds to the (100) crystallographic plane, while another at approximately 34.4° is attributed to the (002) plane. The most prominent and strongest peak for hexagonal wurtzite ZnO is typically found around 36.2°, representing the (101) plane. Additionally, minor peaks may also be observed at other angles such as 47.5°, 56.6°, and 62.9°, further confirming the wurtzite structure.

Mushroom phenolic acids are crucial in the biogenic synthesis of zinc oxide (ZnO) nanoparticles. These phenolic compounds act as both reducing agents and capping agents. As reducing agents, they facilitate the conversion of zinc ions (Zn2+) to zinc oxide (ZnO). They serve as capping agents, which help control the nanoparticles' size and prevent their agglomeration. This dual function of mushroom phenolic acids in the biogenic synthesis leads to the formation of small, stable, and crystalline ZnO nanoparticles.

3.2. Discussion

The extraction results conclusively demonstrate that Agaricus *bisporus* is a valuable and abundant source of phenolic compounds. The significantly high phenolic content quantified in the mushroom extract was pivotal, as these compounds served as the primary reducing and stabilizing agents during the green synthesis of zinc nanoparticles (ZnNPs). This inherent property of phenolic acids is crucial for an efficient and environmentally benign nanoparticle fabrication process.

The rigorous characterization of the biosynthesized ZnNPs confirmed their desired properties. Scanning Electron Microscopy (SEM) images revealed that the nanoparticles possessed a nanoscale size and a predominantly spherical morphology. Furthermore, X-ray Diffraction (XRD) analysis unequivocally confirmed the crystalline structure of the synthesized ZnNPs, matching the standard hexagonal phase of ZnO. The Fourier Transform Infrared (FTIR) spectroscopy results provided crucial insights into the underlying mechanism, explicitly validating the involvement of specific functional groups from the phenolic compounds in both the reduction of zinc ions and the subsequent stabilization of the nascent nanoparticles.

This green synthesis approach offers substantial advantages over conventional methods. It is inherently cost-effective, significantly more eco-friendly, and drastically reduces the reliance on hazardous chemicals, thereby minimizing environmental impact. The demonstrated success of this method highlights its immense potential for large-scale production of ZnNPs. These biogenically synthesized nanoparticles hold promise for a wide array of applications across various sectors, including pharmaceuticals, agriculture, and environmental remediation, contributing to more sustainable technological advancements.

4. CONCLUSION

This study successfully demonstrated a sustainable and eco-friendly methodology for the synthesis of zinc nanoparticles (ZnNPs) using phenolic acids extracted from *Agaricus bisporus* (button mushrooms) native to the Chhattisgarh region. We confirmed that these mushrooms are a rich source of phenolic compounds, which effectively acted as both reducing and stabilizing agents during the nanoparticle formation process.

The biosynthesized ZnNPs exhibited desirable characteristics, including a nanoscale size (30-55 nm), spherical morphology, and a distinct hexagonal crystalline structure (average crystallite size of 42 nm), as confirmed by comprehensive UV-Vis, FTIR, SEM, and XRD analyses. The FTIR data specifically highlighted the crucial role of hydroxyl and carbonyl groups from the phenolic compounds in this green synthesis.

This novel approach presents a cost-effective and environmentally benign alternative to conventional chemical synthesis methods, significantly reducing the use of hazardous materials.[1] The successful production of well-characterized ZnNPs from readily available biological resources in Chhattisgarh opens up promising avenues for their future application in critical areas such as biomedicine, sustainable agriculture, and environmental remediation.

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