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(Review Article)

Medicinal and nutritional potential of mushrooms: A review on bioactive compounds, enzymes and isolation techniques

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Abstract

Mushrooms, the fruiting bodies of macro-fungi, have long been recognized for their culinary value and medicinal potential. Rich in bioactive compounds such as polysaccharides, terpenoids, phenolics, and proteins, mushrooms exhibit a wide range of therapeutic properties including antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, and antithrombotic effects. Due to their low-fat content and high nutritional profile, they are increasingly recommended in diets for patients with metabolic disorders such as diabetes, hypertension, and obesity. This review highlights the nutritional and therapeutic value of edible and medicinal mushrooms, with a focus on their bioactive molecules, including enzymes like fibrinolytic proteases, tyrosinase, and laccase. Various isolation and purification techniques used for obtaining these functional compounds are discussed, emphasizing their clinical and industrial relevance. The review also outlines the importance of mushrooms in modern biotechnology, particularly in enzyme-based diagnostics, pharmaceuticals, and food processing. As research advances, mushrooms emerge as a promising source of functional foods and therapeutic agents. Further clinical validation and molecular studies are recommended to harness their full medicinal potential.

Keywords: Medicinal Mushrooms; Bioactive Compounds; Mushroom-Derived Enzymes; Isolation Techniques; Immunomodulatory Effects; Antimicrobial Compounds

1. Introduction

Mushrooms are spore-bearing, fleshy fruiting bodies of macro-fungi that grow either above (epigeous) or below (hypogenous) the ground. Unlike green plants, mushrooms lack chlorophyll and are therefore unable to synthesize their own food through photosynthesis. Biologically, they are classified in the kingdom Fungi and are distinct from both plants and animals. The term "mushroom" generally refers to the visible fruiting body of macro-fungi, which may belong to the Basidiomycota or Ascomycota**,** and includes both edible and non-edible species [1].

The use of mushrooms dates back to prehistoric times, as evidenced by fossil records and archeological findings. Mushrooms have been valued not only as a food source but also for their medicinal properties and roles in folklore, rituals, and traditional medicine. Their attractive flavor, texture, and nutritional profile have made them a significant dietary component, while their bioactive compounds have contributed to their therapeutic applications [2].

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Mushrooms are considered both functional foods and sources of medicinal compounds. They are rich in proteins, vitamins, minerals, dietary fibers, and low in fats and calories, making them suitable for individuals with metabolic disorders such as diabetes, hypertension, obesity, and atherosclerosis. Beyond their nutritional role, mushrooms are a reservoir of bioactive molecules including polysaccharides, terpenes, phenolic compounds, and medicinal enzymes. These compounds contribute to antioxidant, antitumor, antimicrobial, immunomodulatory, anti-inflammatory, hepatoprotective, and cardiovascular protective effects [3, 4].

Aim and Objective of the Review

The purpose of this review is to explore the medicinal properties of mushrooms, with special emphasis on bioactive compounds and enzymatic components. Additionally, the review highlights different isolation and purification techniques for medicinal enzymes, providing insights into their clinical and biotechnological applications.

2. Classification and Nutritional Value of Mushrooms

2.1. Types of Mushrooms

Mushrooms are broadly classified into edible, medicinal, poisonous, and miscellaneous groups.

- Edible mushrooms are safe for consumption and valued for their high nutritional content. Examples include *Pleurotus spp.*, *Cantharellus spp.*, *Auricularia spp.*, *Ganoderma spp.*, *Termitomyces spp.*, *Agaricus spp.*, and *Russula spp.*
- Poisonous mushrooms are harmful when ingested and may lead to severe illness or even death. Species such as *Amanita spp.*, *Lepiota brunneoincarnata*, *Inocybe asterospora*, *Coprinus spp.*, and *Lepiota cristata* are among the toxic varieties [5,
- 6].
- Medicinal mushrooms are not always edible but are widely studied for their pharmacological potential. Notable species include *Ganoderma lucidum* and *Trametes*, which are used for their immunomodulatory and therapeutic properties.
- Miscellaneous mushrooms comprise species with limited or unknown uses, awaiting further research [7].

Globally, there are approximately 70,000 species of fungi**,** of which more than 10,000 species produce visible fruiting bodies considered as mushrooms. However, only about 100 species are experimentally grown**,** 30 cultivated commercially**,** and 6 species are produced at an industrial scale [8].

While many mushrooms are beneficial, cases of fatal poisoning are often linked to misidentification of toxic species. This underscores the need for accurate morphological and molecular identification techniques beyond traditional reliance on taste, odor, or color [9].

2.2. Nutritional Composition

Mushrooms are recognized as nutrient-dense foods due to their high content of proteins, vitamins, minerals, and dietary fibers, combined with a low level of fats and calories. Their composition makes them comparable to other high-value foods such as soybeans, beans, and corn [10].

- Proteins: Mushrooms contain 5–49% protein (dry weight)**,** providing essential amino acids.
- Minerals: Significant levels of phosphorus, iron, and potassium.
- Vitamins: Mushrooms are rich in Vitamin C, Vitamin D, and members of the B-complex family (Thiamine B1, Riboflavin B2, Pantothenic acid B3, Nicotinic acid B5). They contain 5–10 times more Vitamin B3 compared to vegetables [11].
- Other Nutrients: They consist **of** 3–8% protein, 1–2% ash, 88–90% water, 4–9% carbohydrates, and 0–3% fat on a fresh weight basis.

Due to this composition, mushrooms are recommended as functional foods and are especially beneficial for patients with diabetes, hypertension, obesity, and cardiovascular diseases. Their low caloric content, coupled with high nutritional density, positions them as a valuable dietary component worldwide.

3. Bioactive Compounds in Mushrooms

Mushrooms are rich in diverse bioactive compounds that contribute to their therapeutic value. These compounds, derived from fruiting bodies, cultured broth, and mycelium, include polysaccharides, terpenes, proteins, phenolic compounds, vitamins, and minerals. They exhibit a wide spectrum of pharmacological activities such as antioxidant, anti-inflammatory, immunomodulatory, anticancer, and antimicrobial effects [12, 13].

3.1. Polysaccharides

Polysaccharides are the most abundant and well-studied bioactive compounds in mushrooms. They display multiple biological functions, including antitumor, immunomodulatory, antioxidant, anti-inflammatory, antimicrobial, and anti-diabetic activities.

- β-glucans are the most prevalent polysaccharides, followed by heteroglycans, peptidoglycans, and polysaccharide-protein complexes.
- Their activity is influenced by structural characteristics such as molecular weight, branching patterns, and monosaccharide composition.
- Polysaccharides **exert** immunomodulatory effects by binding to receptors on immune cells (e.g., macrophages, natural killer cells), stimulating cytokine production and enhancing immune responses [14].

3.2. Terpenes and Terpenoids

Terpenes and terpenoids are important bioactive molecules derived from five-carbon isoprene units.

- They modulate the immune system by stimulating gene expression linked to immune responses.
- Terpenes possess antitumor, anti-inflammatory, and antioxidant properties**.**
- Notably, *Ganoderma* species are a rich source of triterpenes, which are widely recognized for their pharmacological activities [15].

3.3. Proteins and Lectins

Mushrooms are a significant source of biologically active proteins with diverse properties:

- Fungal Immunomodulatory Proteins (FIPs): Known for their ability to regulate immune response
- Lectins: Proteins that bind reversibly to monosaccharides or oligosaccharides on cell surfaces, facilitating cell-to-cell communication and immune recognition.
- Some mushroom proteins exhibit cytotoxic, antiproliferative, and anticancer effects. For example, ribosome-inactivating proteins (RIPs) from *Hypsizigus* species have demonstrated potent antitumor activity [16, 17].

3.4. Phenolic Compounds

Phenolic compounds, characterized by hydroxyl groups attached to aromatic rings, are widely distributed in mushrooms and exhibit strong antioxidant activity.

- They act as free radical scavengers, peroxide decomposers, and oxygen quenchers**,** thereby reducing oxidative stress [18].
- Examples include tannins, lignans, flavonoids, hydroxybenzoic acids, and oxidized polyphenols.
- In *Agaricus bisporus*, *Cantharellus cibarius*, and *Lactarius deliciosus*, phenolic compounds such as pyrogallol have shown antioxidant and anti-inflammatory activities [18].

3.5. Other Compounds

Mushrooms also contain a variety of minerals, vitamins, and specialized metabolites that contribute to human health.

- *Hericium erinaceus* produces bioactive metabolites such as dilinoleoyl-phosphatidylethanolamine, which reduces oxidative stress in the endoplasmic reticulum and shows promise in neurodegenerative disease therapy [19].
- Their composition of vitamins, proteins, and minerals makes mushrooms an ideal low-calorie functional food**,** beneficial for patients with diabetes, obesity, and cardiovascular disorders [20].

4. Pharmacological Activities of Medicinal Mushrooms

Medicinal mushrooms have been widely investigated for their therapeutic potential due to the presence of diverse bioactive compounds. Their pharmacological activities include anti-inflammatory, immunomodulatory, and antimicrobial effects, which are central to their application in disease prevention and treatment.

4.1. Anti-inflammatory Properties

Inflammation is a defense mechanism of the innate immune system against injury, infection, or harmful external stimuli such as pathogens, allergens, and ultraviolet radiation. However, chronic inflammation is associated with the development of cancer and other degenerative diseases [21].

Mushrooms exert anti-inflammatory activity primarily through cytokine modulation and the suppression of pro-inflammatory mediators. Elevated production of nitric oxide (NO) and cytokines such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin-6 (IL-6), and interleukin-1β (IL-1β) has been linked to inflammatory diseases [22].

In vitro studies demonstrate that extracts from mushrooms such as *Ganoderma applanatum* and ***Pleurotus eryngii*** inhibit cyclooxygenase-2 (COX-2) activity more effectively than COX-1. Bioactive compounds including ergosterol peroxide, ergosterol, and triterpenes have been shown to suppress LPS-induced inflammatory responses by inhibiting NF-κB activation, mitogen-activated protein kinases (MAPKs), and transcription factors. Extracts of *Ganoderma lucidum*, for instance, significantly reduce the secretion of TNF-α, IL-6, NO, and prostaglandin E2 in activated macrophages [23].

4.2. Immunomodulatory Properties

Mushrooms act as potent immunomodulators, enhancing the body's ability to combat infections, cancer, and other diseases. Immunomodulation refers to the regulation of immune responses, either by stimulating or suppressing immune activity. Key agents involved in immunomodulation include cytokines, antibodies, and vaccines [24].

Polysaccharides, especially β-glucans, play a central role in this process. They stimulate immune cells such as macrophages, natural killer (NK) cells, and lymphocytes, leading to the production of cytokines and antibodies. For example, a homogeneous polysaccharide (EPA-1) isolated from *Pleurotus eryngii* using ion-exchange and gel filtration chromatography was found to enhance cytokine production and antibody secretion, thereby regulating the immune system [25].

Medicinal mushrooms such as *Ganoderma lucidum* and *Agaricus blazei* are widely studied for their immunomodulatory and antitumor properties, with clinical trials supporting their use in complementary cancer therapy.

4.3. Antimicrobial Properties

Mushrooms also display broad-spectrum antimicrobial activity, attributed to their polysaccharides, proteins, phenolic compounds, and secondary metabolites.

- Pleurotus spp. are rich in proteins, essential fatty acids, vitamins, and polysaccharides, which contribute to their antimicrobial, anticancer, antioxidant, anti-diabetic, and hepatoprotective properties [26].
- Lentinus edodes (shiitake) exhibits strong antibacterial activity against both Gram-positive and Gram-negative bacteria due to its protein extracts.
- Methanolic extracts of mushrooms such as *Tricholoma lobayensis*, *Auricularia polytricha*, *Daedalea elegans*, *Daldinia concentrica*, and *Corilopsis occidentalis* have been shown to inhibit bacterial species including *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus cereus*, *Proteus vulgaris*, and *Escherichia coli*. However, antifungal effects were weaker, with limited activity against *Candida albicans*, *Aspergillus flavus*, and *Aspergillus niger* [27].

The minimum inhibitory concentrations (MICs) of mushroom extracts against bacteria ranged between 1.25–9.00 mg/ml, whereas for fungi, MIC values were higher (10.50–17.50 mg/ml), indicating stronger antibacterial than antifungal effects.

5. Enzymatic Potential of Mushrooms

Enzymes derived from mushrooms represent an important class of bioactive molecules with both clinical and industrial applications. Due to their catalytic efficiency and specificity, mushroom-derived enzymes are widely utilized in medicine, diagnostics, and biotechnology.

5.1. Importance of Enzymes in Medicine

Enzymes play a crucial role in maintaining metabolic balance in the human body and are widely applied in disease diagnosis and therapy. In clinical practice, enzymes such **as** acid phosphatase, alanine transaminase (ALT), aspartate transaminase (AST), creatine kinase-MB (CK-MB), and alkaline phosphatase are routinely used as biomarkers for various conditions, including liver disorders, myocardial infarction, neurodegenerative diseases, and cancer [28].

Mushroom enzymes are also investigated for their therapeutic potential. Historically, proteolytic enzymes such as pepsin were used for gastrointestinal disorders. Later discoveries revealed that enzymes could also combat infectious agents and even inhibit cancer growth [29]. These findings have expanded the application of mushroom enzymes as therapeutic agents and functional supplements.

5.2. Types of Enzymes from Mushrooms

5.2.1. Extracellular Enzymes

Basidiomycete mushrooms produce extracellular enzymes that play an essential role in biodegradation and nutrient recycling. These enzymes are widely used in waste treatment, food processing, brewing, dairy, and textile industries**.** They contribute to the breakdown of lignocellulosic biomass, converting complex polymers into simpler compounds that can be absorbed by fungal hyphae [29].

5.2.2. Fibrinolytic Enzymes

Fibrinolytic enzymes regulate the balance between coagulation and anticoagulation in blood, making them vital for cardiovascular health**.** They hydrolyze fibrin clots and prevent thrombosis, offering therapeutic potential in antithrombotic and thrombolytic therapies**.**

- Lentinus edodes: A fibrinolytic enzyme (LEFE), identified as a metalloprotease, was purified using chromatography techniques. LEFE displayed a molecular weight of ~38 kDa and was inhibited by EDTA and divalent metal ions, confirming its metalloprotease nature. It significantly prolonged prothrombin and coagulation times, demonstrating potential as an antithrombotic agent [30].
- Pleurotus eryngii: A fibrinolytic protease (~20 kDa) was isolated through gel filtration and ion-exchange chromatography. Active at acidic pH (pH 4), the enzyme inhibited fibrin clot formation and hydrolyzed fibrin polymers directly. It also showed in vivo protective effects against thromboembolism, suggesting its use in the prevention and treatment of thrombosis-related diseases [31].

5.2.3. Tyrosinase

Tyrosinase, an oxidase enzyme isolated from *Lentinus edodes* (shiitake mushroom), is involved in the biosynthesis of melanin by catalyzing the hydroxylation of monophenols and oxidation of diphenols. This enzyme has applications in cosmetics, dermatology, and pharmaceuticals**,** particularly for skin pigmentation disorders, hair and nail treatments, and anti-aging formulations (Riley, 1997; K. et al., 2014). Shiitake mushrooms also produce eritadenine**,** a compound with cholesterol-lowering effects, further supporting their role as functional foods [32].

5.2.4. Lignocellulolytic Enzymes

Lignocellulolytic enzymes degrade cellulose, hemicellulose, pectin, and lignin, making them crucial for biomass conversion and biogas production. These enzymes have significant environmental benefits**,** reducing greenhouse gas emissions and aiding in bioremediation. They catalyze hydroxylation and oxidation reactions involving phenolic compounds, contributing to the breakdown of complex organic matter [33].

5.2.5. Cellulases and Xylanases

Pleurotus ostreatus produces cellulases and xylanases, which degrade polysaccharides into simple sugars. These enzymes are widely used in biofuel production, animal feed, food industries (e.g., baking, brewing), and paper processing. Xylanases hydrolyze hemicellulose, while cellulases break down cellulose, making them essential in the conversion of plant biomass into value-added products [34].

6. Isolation Techniques of Medicinal Enzymes

The isolation and purification of enzymes from mushrooms are essential steps to study their structure, activity, and therapeutic potential. Over the years, various classical chromatographic methods as well as advanced biotechnological techniques have been employed to extract bioactive compounds from medicinal mushrooms.

6.1. Classical Techniques

Traditional methods for enzyme isolation rely on chromatography and precipitation techniques. Commonly used methods include:

- Ion-exchange chromatography (DEAE-cellulose, Q-Sepharose, SP-Sepharose): Separates enzymes based on charge differences.
- Gel filtration chromatography (Sephadex G-150): Separates proteins based on molecular size.
- Affinity chromatography (Concanavalin A-Sepharose, Affi-gel): Exploits specific binding affinities of enzymes.
- Fast Protein Liquid Chromatography (FPLC): Provides high-resolution purification under controlled conditions.

These methods are often used in combination to achieve high-purity enzyme fractions. For example, fibrinolytic enzymes from *Pleurotus ostreatus* and *Armillariella mellea* were isolated using multi-step processes involving ion-exchange, gel filtration, and FPLC.

6.2. Advanced Techniques

Recent technological innovations have enhanced enzyme isolation and single-cell analysis:

- Laser printing: A modern tool enabling the precise isolation and positioning of individual microbial cells, including fungi, for cultivation and metabolic studies.
- Microfluidics: Allows manipulation of small fluid volumes, enabling rapid screening and separation of bioactive compounds.
- Optical tweezers and multi-photon lithography: Facilitate non-invasive trapping and analysis of fungal cells and enzymes at the micro-scale [35].

These techniques offer greater precision and efficiency compared to conventional approaches, supporting advances in biotechnology, medicine, and drug discovery.

6.3. Case Studies

6.3.1. Ganoderma lucidum (Laccase)

Ganoderma lucidum produces a laccase enzyme with unique biochemical properties. The enzyme was purified using DEAE-Affi-Gel Blue Gel chromatography and Con A-Sepharose affinity chromatography. It displayed a molecular mass of ~75 kDa, higher than that of most mushroom laccases. The enzyme showed maximal activity at pH 3–5 and 70 °C, but activity was lost at alkaline pH (8–9) and after exposure to 100 °C [36].

6.3.2. Cordyceps militaris (CMase)

A fibrinolytic enzyme (CMase) from *Cordyceps militaris* was isolated by ammonium sulfate precipitation and gel filtration chromatography. It exhibited a molecular weight of 27.3 kDa and optimal activity at pH 6.0 and 25 °C. Its activity was enhanced by Fe^{2+} and Mg^{2+} ions but inhibited by Cu^{2+} and EDTA, confirming it as a metalloenzyme. CMase demonstrated strong fibrinolytic activity, making it a candidate for thrombolytic therapy [37].

6.3.3. Morchella esculenta (Polysaccharides)

Polysaccharides from *Morchella esculenta* were extracted using hot water treatment followed by deproteinization. Structural characterization by FTIR spectroscopy revealed β-glucan-type structures. The polysaccharides exhibited antioxidant activity, as well as inhibition of butyrylcholinesterase and acetylcholinesterase, suggesting potential applications in neurodegenerative disease therapy [38].

6.3.4. Auricularia polytricha (Immunomodulatory Protein)

An immunomodulatory protein (APP) was isolated from *Auricularia polytricha* using acid extraction (5% acetic acid, 2-mercaptoethanol) followed by anion-exchange chromatography (MonoQ) and ammonium sulfate fractionation**.** The protein significantly enhanced splenocyte proliferation, stimulated gamma-interferon secretion, and promoted macrophage activation without cytotoxic effects. APP was shown to increase TNF-α and nitric oxide production, confirming its potential as an immune stimulant [39].

7. Applications and Future Prospects

Medicinal mushrooms represent a valuable resource for modern medicine and biotechnology due to their wide range of therapeutic activities. The bioactive compounds they contain—particularly polysaccharides, terpenoids, phenolics, and enzymes—have shown promising results in both preclinical and clinical studies**.**

7.1. Use of Mushroom Compounds in Disease Treatment

Mushroom-derived bioactive molecules have demonstrated potential in the prevention and management of major human diseases:

- Cardiovascular Diseases: Fibrinolytic enzymes from mushrooms such as *Lentinus edodes* and *Pleurotus eryngii* prevent thrombosis by degrading fibrin clots, offering a safer alternative to synthetic anticoagulants. Eritadenine from *Lentinus edodes* reduces blood cholesterol, contributing to heart health.
- Neurodegenerative Disorders: Compounds such as dilinoleoyl-phosphatidylethanolamine from *Hericium erinaceus* reduce oxidative stress in the endoplasmic reticulum, demonstrating therapeutic potential in Parkinson’s and Alzheimer’s disease [40].
- Cancer: Mushroom polysaccharides (e.g., lentinan, polysaccharopeptides) stimulate immune responses and inhibit tumor growth. Clinical studies in Asia have already incorporated these polysaccharides as adjuvant therapies in cancer treatment [41].
- Diabetes and Metabolic Disorders: Due to their low-fat and low-calorie profile, mushrooms are suitable dietary interventions for patients with diabetes, obesity, and hypertension. Specific bioactive compounds also exhibit anti-diabetic properties by modulating glucose metabolism.

7.2. Mushrooms as Functional Foods and Nutraceuticals

Mushrooms are increasingly recognized as functional foods and nutraceuticals. Their high protein, vitamin, mineral, and dietary fiber content, combined with bioactive compounds, make them suitable for daily consumption to promote health and prevent disease. Commercial extracts from mushrooms such as *Ganoderma lucidum*, *Agaricus blazei*, and *Lentinus edodes* are marketed worldwide for their immunomodulatory and antioxidant properties.

7.3. Need for Standardization and Clinical Trials

Despite promising results, significant challenges remain:

- Variability in mushroom species, cultivation methods, and extraction processes leads to inconsistencies in bioactive compound yields.
- Many studies have been conducted using crude extracts rather than purified compounds, making it difficult to attribute activity to specific molecules.
- Rigorous clinical trials are necessary to validate therapeutic claims and ensure safety for large-scale pharmaceutical use.
- Standardized methods for isolation, characterization, and dosage determination are urgently needed to translate mushroom-derived products into approved medicines.

7.4. Potential in Biotechnology and Pharmacology

The applications of mushroom-derived compounds extend beyond medicine:

- Biotechnology: Enzymes such as lignocellulolytic enzymes, cellulases, and xylanases are employed in biomass conversion, biofuel production, food processing, and environmental remediation.

- Pharmaceutical Development: Novel proteins, immunomodulators, and small molecules from mushrooms serve as templates for drug discovery.
- Nutraceutical Industry: Functional mushroom-based supplements are increasingly incorporated into global health markets, supporting the trend toward natural, plant- and fungi-derived therapeutics.

8. Conclusion

Mushrooms, as spore-bearing fruiting bodies of macro-fungi, represent a unique group of organisms with profound nutritional and medicinal importance. They are distinct from plants and animals, yet play a vital role in human health and culture as both functional foods and sources of therapeutic compounds. Globally, only a small fraction of the vast mushroom biodiversity has been scientifically explored, but even this limited investigation has revealed significant pharmacological potential.

This review highlighted the broad spectrum of bioactive compounds found in mushrooms, including polysaccharides, terpenoids, phenolics, proteins, and enzymes, all of which contribute to their antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, anticancer, hepatoprotective, and cardiovascular benefits. Among these, enzymes such as fibrinolytic proteases, tyrosinase, and lignocellulolytic enzymes have drawn special attention due to their clinical applications in cardiovascular therapy, dermatology, and biotechnology.

The discussion of isolation techniques demonstrated that while classical chromatographic methods remain fundamental, advanced approaches such as laser printing and microfluidics are opening new avenues for precise characterization and application of mushroom-derived biomolecules. These techniques are crucial for identifying specific compounds responsible for therapeutic effects, moving beyond crude extract studies.

Mushrooms thus stand out as a promising resource for drug discovery, nutraceutical development, and industrial biotechnology. However, challenges remain in terms of standardization, compound isolation, and clinical validation. To fully harness the potential of medicinal mushrooms, future research should prioritize:

- The rigorous clinical trials to confirm efficacy and safety of bioactive compounds.
- The optimization of cultivation and extraction methods to ensure consistent yields.
- The molecular characterization of active ingredients for targeted therapeutic applications.
- Integration of mushrooms into functional food and pharmaceutical industries through evidence-based approaches.

In conclusion, mushrooms are not only valuable dietary resources but also reservoirs of novel therapeutic agents. With continued research and clinical validation, they hold the potential to contribute significantly to global healthcare, biotechnology, and pharmacology.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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