REVIEW ARTICLE

The health-promoting potential of edible mushroom proteins

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Abstract: Edible mushrooms have been classified as "next-generation food" due to their high nutritional value coupled with their biological and functional potential. The most extensively studied and reported mushroom macromolecules are polysaccharides. However, macrofungi proteins and peptides are also a representative and significant bioactive group. Several factors such as species, substrate composition and harvest time significantly impact the mushroom protein content, typically ranging between 19 and 35% on a dry weight basis. Proteins work based on their shape and structure. Numerous extraction methods, including chemical and non-conventional, and their implications on protein yield and stability will be discussed. Beyond their biological potential, a great advantage of mushroom proteins is their uniqueness, as they often differ from animal, vegetable, and microbial proteins. According to recently published reports, the most relevant mushroom bioactive proteins and peptides include lectins, fungal immunomodulatory proteins, ubiquitin-like proteins, and proteins possessing enzymatic activity such as ribonucleases laccases, and other enzymes and ergothioneine. These are reported as antioxidant, antiviral, antifungal, antibacterial, antihypertensive, immunomodulatory, antitumour, antihypercholesterolemic or antihyperlipidemic, antidiabetic and anti-inflammatory properties, which improved proteins and peptides research interest and contributed to the increase of mushroom market value. This review provides an overview of the most relevant biochemical and biological properties of the main protein groups in edible mushrooms, explicitly focusing on their biomedical potential. Although mushrooms are a rich source of various proteins, many of these molecules have yet to be identified and characterised. Accordingly, it is crucial to identify and characterise new macromolecules of macrofungi origin, which opens an opportunity for further investigation to identify new bioactives for food, nutraceutical, or medicinal applications.

Keywords: Mushrooms; Bioactive proteins; Protein extraction; Lectins; Fungal immunomodulatory protein; Mushroom enzymes; Peptides; Ergothioneine; Health benefits.

1. INTRODUCTION

Mushrooms possess a high worldwide distribution and molecular diversity, with approximately 15,000 estimated species [1,2] and 2,000 identified species [3,4]. This large macrofungi group can be classified as edible, medicinal, poisonous, and others (with non-defined properties) [5]. Approximately 700 mushroom species are characterised as safe and possess related health benefits [2,4], thus simultaneously classified as edible and medicinal species [5]. Some examples of these species are *Agaricus bisporus*, *Lentinula edodes*, and *Pleurotus* spp., which are among the most globally cultivated mushroom species [5].

Edible mushrooms have been considered "next-generation food" due to their high nutritional value coupled with their biological and functional properties [5,6]. They are hypocaloric and a rich source of dietary fibre and digestible proteins. Mushrooms also possess essential amino acids, minerals, and vitamins for all age groups, which are often deficient in plants [6,7]. Numerous health-promoting properties such as immunomodulatory, anti-inflammatory, anticancer, antimicrobial, antiviral, and antioxidant effects have been reported and associated with mushroom species,

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specific bioactive macromolecules or macromolecule groups. Thus, these macrofungi have been used as nutrient-rich foods, supplements, nutraceuticals, and pharmaceutical products [3,6,8,9].

The most extensively studied and reviewed mushroom biomolecules are polysaccharides, which are reported as "biological response modifiers" due to their health-promoting potential. However, mushrooms also contain many proteins and peptides, which are significant bioactive nutraceuticals with health benefits [9–13]. Proteins are one of the most abundant organic macromolecules in living organisms. Polymers of amino acids are the "building blocks" of proteins, linked by amide groups acquaintance as peptide bonds. These biomacromolecules possess great nutritional value and participate in almost all biochemical reactions. Proteins work based on their shape and structure, being involved in several functions in biological systems, such as a source of energy, structural components, transporters, and enzymes, acting in the immune system and as regulators of gene expression [14,15].

In this context, this review provides an overview of the most relevant biochemical and biological properties of the main protein groups in edible mushrooms, explicitly focusing on their bioactive and biomedical potential. At the same time, the protein content present in different mushroom species, as well as several conventional and non-conventional protein extraction methodologies, will be discussed. A particular focus on key and recent literature data will be given.

2. MUSHROOM PROTEIN CONTENT

In general, mushrooms contain between 19 and 35% of protein on a dry weight basis, which is relatively high compared to most vegetable protein sources [13]. Nevertheless, the mushroom protein content differs according to numerous factors such as species, substrate composition, pileus size and harvest time [13,16]. For example, the *Pleurotus sajor-caju* mushroom is a cultivated species with one of the highest protein contents, being reported protein content of 50.29% (dry weight) [13]. The protein content of several edible and medicinal mushroom species is presented in Table 1. The range of protein content in each species corroborates that chemical composition highly depends on external parameters.

There seems to be a negative correlation between the protein content of mushrooms and the C/N ratio of the substrate [17]. In mushrooms, the chemical composition is also influenced by the composition of the substrate, the cultivation method, and the stage of harvesting [18]. Thus, it is difficult to generalise the nutritional contents of a particular mushroom [15]. For example, using a substrate where mushrooms were previously cultivated can further increase the content of bioactive compounds [13].

3. MUSHROOM PROTEIN EXTRACTION

Although mushrooms are a rich source of various proteins, many of these molecules have yet to be identified and characterised. Valuable bioactive mushroom proteins can be extracted from fruiting bodies, cultivated mycelium, or their supernatants [10]. Numerous extraction methods have been reported, and several variables play key roles in the protein yield. Solvent type and mushroom solvent ratio, extraction time, temperature, and pH are examples of critical parameters during protein extraction, purification, and concentration [15].

Several protocols are available based on some basic principles for protein yield improvement. Firstly, it is important to increase the solubility of protein to extract as much protein as possible and, at the same time, prevent artificial modifications. Also, concerning the separation of proteins and other bioactive compounds the interaction between the molecules and the denaturation state [19]. Another important issue in some mushroom species is the possible presence of molecules that interfere in protein extraction, such as carbohydrates and pigments [19,20]. Thus, to obtain a pure protein extract, it is important to remove other molecules present in the mushroom matrix. Accordingly, many protocols for protein extraction in fungi have followed these key steps [19] and will be presented in the following topics.

3.1. Conventional/ chemical extraction methods

For the recovery of high valuable mushroom compounds as proteins, solvent-assisted methods have been extensively reported [21]. The extraction methods used are typically classified according to the group of solvents applied, namely, water, organic, alkali, and acid [22].

Water extraction is a more easy, economical and green approach but typically requires high temperatures and longer treatment times. Generally, the extraction times reported varied from 1.5 to 5 hours while the temperatures from 50 to 80 ºC [21]. Kumakura et al. tested different temperatures to extract functional proteins from *Ganoderma lucidum*. In this case, 50 ºC was the optimal condition to extract functional molecules with high yield [23].

However, the application of high temperatures can destroy other valuable mushroom compounds, which are temperature sensitive, and/or impact the functional and conformational properties of proteins [21,22]. This could reduce the functionality and nutritional value of protein extracts. Although their typically high solubility and stability in an aqueous environment [22], the solubility of proteins may be inversely correlated with their molecular weight (MW), being the amino acids and peptides more soluble in water [24].

The use of organic solvents is extremely important in the presence of aromatic, hydrophobic, and/or non-polar amino acid residues in the target proteins. Some examples include ethanol, butanol, and acetone [22]. For example, the use of an organic solvent such as ethanol allows the use of lower temperatures (from 25 to 60 ºC) but generally higher extraction times and % of solvent (from 30 to 99%) [21].

Table 1. Protein content (% or g/ 100 g of dry matter) of most common mushroom species.

Mushroom species	Non-scientific name	Protein content[1]	Reference
Agaricus bisporus	Table mushroom/ button mushroom	14-26	[25–27]
Agaricus brasilienses	The sun mushroom	37	[28]
Agaricus campestris	Field mushroom/ Meadow mushroom	19	[29]
Agaricus comtulus	Mini mushroom	21	[29]
Amanita battarrae	Grey-zoned ringless amanita	17	[29]
Amanita caesarea	Caesar's mushroom	35	[30]
Armillaria mellea	Honey fungus	16	[31]
Auricula auricula	Judas's ear/ Jew's ear	11	[32]
Boletus edulis	King mushroom/ Cep/ Porcini	19	[27]
Cordyceps militaris	Caterpillar fungus	36	[33]
Flammulina velutipes	Winter mushroom/ Velvet stem/ Enoki	4-28	[25,34]
Ganoderna lucidium	Reishi/ Lingzhi	15	[13,35]
Grifola frondosa	Hen-of-the-woods/ Maitake	21	[36]
Hericium erinaceus	Lion's mane/ Hedgehog mushroom	10-29	[35,37,38]
Lentinula edodes	Shiitake	4-23	[25,35]
Lentinus polychrous	White rot fungi	18	[32]
Lentinus squarrosulus	White rot fungi	40	[32]
Pholiota nameko	Nameko/ Huagu/ Guanmaosan/ Huazimo	17	[37]
Pleurotus citrinopileatus var. cornucopiae	Golden oyster mushroom	24	[37]
Pleurotus eryngii	King trumpet/ King oyster mushroom	11-16	[25,37]
Pleurotus sajor-caju	Grey oyster mushroom	21-50	[32,39]
Pleurotus ostreatus	Oyster mushroom/ Oyster fungus	7-42	[25,35,39,40]
Pleurotus salmoneo stramineus	Pink Oyster Mushroom	27	[37]
Trametes versicolor	Coriolus versicolor/ Polyporus versicolor	4	[36]
Volvariella volvacea	Chinese/ Straw/ paddy straw mushroom	24	[35]

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Moreover, protein precipitation is also one of the most used approaches [19,41–43]. For instance, trichloroacetic acid (TCA) and TCA in acetone [19,42,43] can be used since acid, hydrophobic, or both are conditions that promote protein denaturation and concentration, and remove other macromolecules contaminants (e.g., sugars, lipids, and salts) [44]. Also, ammonium sulfate has been extensively used, promoting an alteration of protein solubility and their consequent precipitation [41]. However, beyond high costs, the total solvents remotion of mushroom extracts is difficult [21].

Although time-demanding, phenol-based extraction has been proposed as an effective protein extraction method for rich polysaccharides matrixes. This methodology is based on protein concentration and purification by dissolving these molecules in phenol [45]. A high advantage of phenol-based extraction is the minimal protein degradation [42,43,45]. In a study comparing different conventional extraction methods, TCA precipitation and phenol-based extraction showed the best yields for *Auricularia auricula* mycelia. Concerning the fruiting body, phenol-based extraction showed the best results in the proteome analysis [19]. Nevertheless, their time-consuming and toxic nature limits the use of this approach in some studies [19].

Therefore, the conventional methods for protein extraction involving temperature and solvents possess several issues. The main problems are related to the degradation and modification by temperature, pH, and/ or solvent used and incubation period, as well as low yields, increasing the interest in other extraction approaches [22].

3.2. Non-conventional methods

In the last decades, to improve extraction efficiency, increase protein yields and minimize protein degradation, other extraction methods, mainly non-thermal green technologies, have been reported [21,22].

One example is the aqueous two-phase system (ATPS), where two compounds of different natures are applied, allowing the separation, concentration, and purification of molecules [46,47]. Some advantages pointed out are low cost, rapidity, adjustable, high yield, selectivity, and biocompatibility [46,48]. Yan et al. reported a simple, fast, and efficient ATPS system to separate polysaccharide and protein fractions of *Cordyceps sinensis* [48].

Furthermore, enzyme-assisted extraction (EAE) is based on the disruption of the cell wall by enzymes (e.g., cellulases, pectinases, and hemicellulases), releasing intracellular proteins [22,49]. This approach depends on critical factors for enzymatic action, such as pH and temperature. β-glucanase is a commercial enzyme with cellulolytic activity used for this purpose in mushroom matrixes [49].

Subcritical water extraction (SWE), where critical temperature coupled with high pressure is used, has been reported for protein extraction in different matrixes. This technique improves the polysaccharides depolymerization, resulting in smaller soluble proteins [22]. In the case of mushrooms, studies have even focused more on the extraction of polysaccharides, for instance, in a study focused on the extraction of *G. lucidum*, polysaccharides were extracted by SWE [50].

Recently, novel cell disruption techniques have gained importance in the protein extraction field. Some of these approaches are microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pulsed electric field extraction (PEF), high voltage electrical discharges (HVED), and high hydrostatic pressure (HPP) [22].

Over the last two decades, MAE has played a significant role as an extraction tool for high-value and bioactive molecules in biological materials [51]. MAE is a non-contact heat source that produces thermal energy via ionic conduction between solvents and dissolved ions. This leads to continuous collisions within the matrix, causing the cells to rupture under enormous pressure and, consequently, release bioactive molecules [51–53]. In this procedure, a higher polarity of the bioactive target and extractant results in better microwave absorption, improving the extraction rate [52]. Thus, MAE has several advantages: flexibility, stability of thermolabile compounds, lower solvent consumption, less processing time, and high extraction yield. However, one of its main drawbacks is heterogeneous heating [51] MAE showed an efficient extraction of free amino acids from fungi, namely, from residues of *Fammulina velutipes* and *Lentinus edodes* [52].

Another innovative extraction technique is UAE, which is environmentally friendly, inexpensive, and an efficient alternative to conventional methods [54]. The UAE uses the cavitation effect generated by the passage of the ultrasonic wave in the solvent to extract target substances [55]. This method can be used to extract molecules with low thermal stability since UAE does not need excessive temperatures [52]. Cheung *et al.* [56] isolated the polysaccharide-protein complexes of three medicinal mushrooms with higher content, namely, *Grifola frondosa*, *Trametes versicolor* and *L. edodes*. Besides, these extracts showed higher antioxidant activity than those extracted by hot water extraction [56]. Presently, UAE efficiently extracts polysaccharides and melanin from edible mushrooms by-products [52].

The use of PEF focuses on the concept of transmembrane potential [21,57]. The combination of PEF and pressure extraction results in extracts with high protein content [21]. The use of PEF for a combined extraction of polysaccharides, polyphenols, and protein in *A. bisporus* showed a significantly increased yield [57]. Otherwise, HVED is based on the electrical breakdown in water, and their application also showed a high protein yield [21].

Finally, high hydrostatic pressure (HPP), known as “cold pasteurization”, applies isostatic pressure (varied from 100 to

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1000 MPa), and it is transmitted by the fluid (typically water), promoting the cell wall damage [22,58]. In a study focused on peptide extraction in mushrooms, the authors established a period of 10 min and a pressure of 400 MPa in the HPP method as the optimal and effective condition for maximizing the yield [59].

Although some studies have started to apply these non-conventional approaches to mushroom matrixes, the literature in this field is very scarce and limited. Most of the studies found focus on mushroom polysaccharides extraction. In this context, optimising protocols to extract proteins, peptides, and amino acids from fungi requires further investigation.

After extraction, chromatography methods are also an example of techniques extensively used to understand the profile and MW distribution of proteins extracted, identify specific proteins and peptides, as well as purify and recover target proteins or peptides [41]. Chromatographic methods are used according to the biochemical properties of each type of protein [60].

Affinity chromatography, ion exchange chromatography, and gel filtration by fast protein liquid chromatography (FPLC) are some techniques extensively used [41]. Specifically, affinity chromatography allows for high-quality and specific protein recovery. For example, in the case of lectins, a glycoprotein easily extracted from mushrooms, various affinity ligands involving mannose-, lactose- and fetuin-Sepharose and bovine submaxillary mucin (BSM)-Toyopearl are used. As a normal chromatography technique, purification steps include column washing, column length, pore size, flow rate, pH, temperature, elution buffer, collecting the correct fraction volume, calculating the protein concentration, and matrix loading [60].

4. BIOACTIVE PROTEINS OF MUSHROOMS

The great advantage of mushroom proteins is their uniqueness, as they often differ from animal, vegetable and microbial proteins. Moreover, although mushrooms are not extremophile organisms, several proteins exhibit considerable pH and thermal stability [10], representing a biotechnological advantage in the formulation of supplements, nutraceuticals, and pharmaceuticals.

Mushroom proteins are reported as possessing antioxidant, antiviral, antifungal, antibacterial, antihypertensive, immunomodulatory, antitumour, antihypercholesterolemic or antihyperlipidemic, antidiabetic, and anti-inflammatory properties [9–13]. According to recently published reports, the most relevant mushroom bioactive proteins and peptides, including lectins, fungal immunomodulatory proteins (FIPs), ubiquitin-like proteins, proteins possessing enzymatic activity such as ribonucleases, laccases, and other enzymes and ergothioneine (Figure 1) [9,11,41,61].

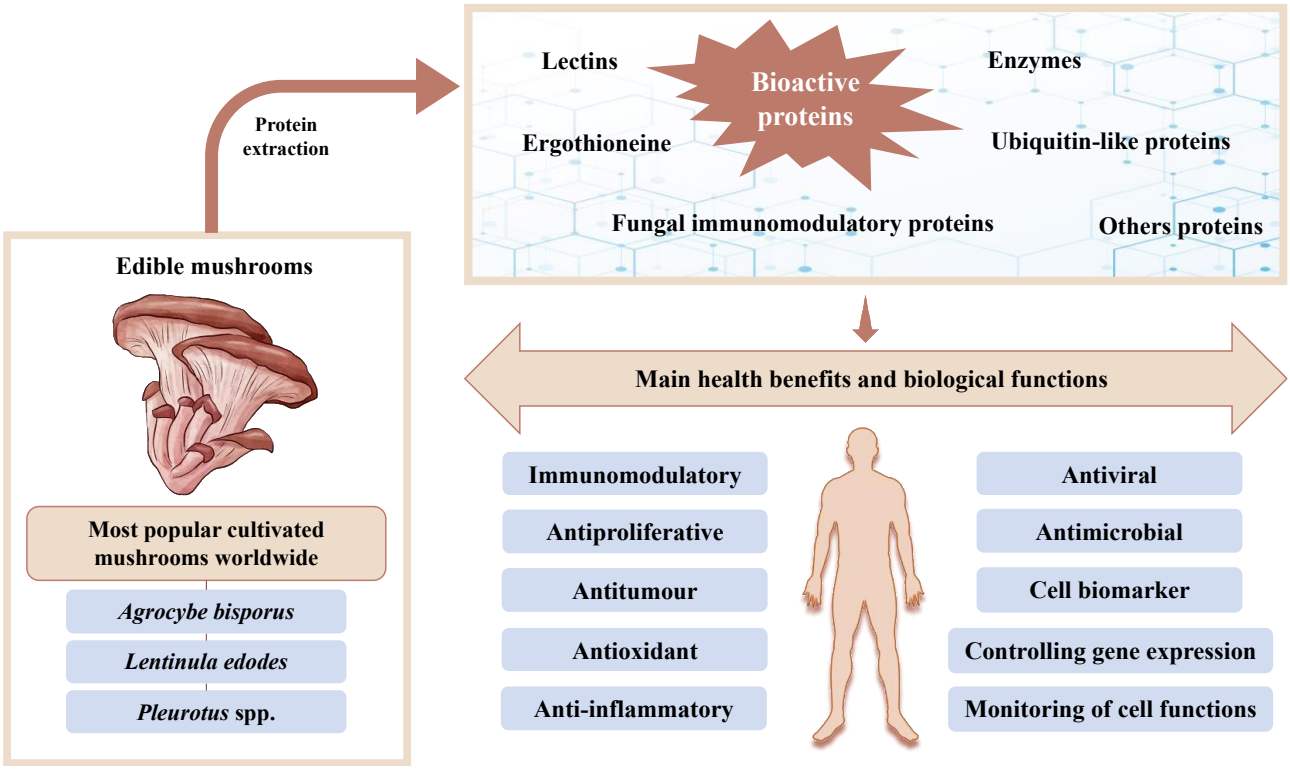

Fig. (1). The main proteins with bioactive properties from edible mushrooms and their health benefits.

4.1. LECTINS

Lectins are a group of proteins or glycoproteins of nonimmune and nonenzymatic nature, with high sequence and structural variability [60,62]. They are ubiquitous and have been isolated from various sources, including viruses, bacteria, fungi, plants, and animals [62,63]. Lectins can also be classified as protein-conjugate complexes, which, like polysaccharide-based compounds, exert immunomodulatory effects [64]. However, lectins are not immunoglobulins [61].

Lectins contain at least one noncatalytic carbohydrate-binding site (CBS), which helps recognise and reversibly bind to different carbohydrate moieties of glycoconjugate (glycoproteins and glycolipids), as well as monosaccharides and oligosaccharides, without altering their covalent structure [60,61,65]. Indeed, this unique ability of lectins to interact with cell surface carbohydrates leads to the agglutination of different cell types and precipitation of glycoconjugates, such as erythrocytes, exhibiting hemagglutination activity and often are described as hemagglutinins [41,65]. However, not all lectins are hemagglutinins, only proteins that possess the ability to agglutinate erythrocytes by the reversible binding of sugars found on the cell surface [66]. Besides, lectins may be involved in storing carbohydrates in the cell, sugar transport, and aggregation of immunoglobulins [9].

Furthermore, these lectin-carbohydrate interactions play roles in several biological processes at the cell physiology level, such as cell-cell communication, cell growth, morphogenesis and adhesion, molecular recognition and pathogenesis, scavenging of glycoconjugate in the circulatory system, cell-cell interactions in the immune system, among others [63,66]. Such proteins have been used in many therapeutical applications, including histochemical and cytochemical diagnostic tools and control drug release [66]. Thus, the study of these protein-conjugated complexes has been of increased interest in recent years due to their bioactivity with potential applications in biomedical research, including antiproliferative and antitumor, HIV reverse transcriptase (HIV-RT) inhibitory, immunomodulatory, mitogenic, and antimicrobial activities [6,65].

Mushrooms exhibit high lectin levels, being probably the most extensive protein group studied with great pharmaceutical potential [66]. For curiosity, the first mushroom lectin was reported in 1910 from *Amanita muscaria*, where lectin activity was related to the fungus's toxicity. After that, lectins from the edible fungi *Boletus edulis* (1912) and *Lactarius deliciosus* (1991) were published [62]. As reviewed by Singh *et al*. [63], about 144 lectins have been isolated and studied in edible mushrooms, e.g. *Agrocybe aegerita* [67], *Agrocybe cylindracea* [68], *Pleurotus citrinopileatus* [69], *P. ostreatus* [70], *Tricholoma mongolicum* [71] and *Volvariella volvacea* [72]. Commonly, lectins are widely distributed in the fruiting body and mycelia of mushrooms, and the amount of these proteins may vary depending on fruit-body age and season [65,66]. Moreover, distinct lectins with different biochemical properties can be isolated from a single mushroom species [66].

Mushroom lectins exhibit diverse physicochemical characteristics, varying in molecular weight, number of subunits, sugar content, and stability under different temperatures and pH values [65]. The molecular weight of mushroom glycan-binding proteins ranges between 12 and 190 kDa. Its structure consists of two to four identical or nonidentical subunits generally linked together by non-covalent bonds. Usually, lectins are stable at moderate temperatures, but lose activity at higher temperatures. These macromolecules resist gastrointestinal tract digestion, penetrate the intestinal epithelial cell monolayer, and enter undamaged blood circulation [9,65,73]. Thus, these proteins are suitable for oral administration. The carbohydrate content in lectins differs from 0 to 18%. As mentioned earlier, lectins selectively recognise carbohydrates and reversibly bind to them, assuming that the ligands are oriented in a specific mode. Some of the sugars commonly found in this interaction are glucose, lactose, fucose, turanose, raffinose, N-acetyl-D-glucosamine (GlcNAc), N-acetylgalactosamine (GalNAc), inulin or sialic acid [11,66].

It is important to know mushroom lectins' crystal structure to understand better the molecular mechanisms underlying lectin-sugar interactions and their atomic structure [60]. As reviewed by Hassan *et al.* [66], mushroom lectins have been classified into different families according to their crystal structure, including actinoporin-like folds, galectin-like folds, β-propellor folds, and β-trefoil folds. Moreover, the sequence and arrangement of amino acids in the lectin determine its specificity for CBS, consequently defining its functions and potential biological applications [60]. Mucin O-glycan-specific lectins can act as disease and cell surface biomarkers, and fucose-specific binding lectins can detect cancer cells as potential biomarkers [65]. Singh *et al*. [65] reported that the exhibited specificity of mushroom lectins towards GlcNAc could be a potential tool for analysing O-GlcNAcylated proteins. Therefore, based on their carbohydrate specificity, lectins can be used in many therapeutic applications, acting as a diagnostic tool in clinical practice, namely, in the study of cellular pathology and physiology and drug delivery [9,60].

Over the years, various researchers have been exploring mushroom lectins and reported their potential applications, especially their promising health effects. According to the up-to-date reviews by Singh *et al*. [65] and El-Maradnay *et al.* [60], mushroom lectins exhibit antiproliferative activity, mitogenic activity, immune-stimulating potential, antiviral, antioxidant, antimicrobial, and antidiabetic therapeutic effects (Figure 2). Along with this, Singh *et al*. [63] highlighted the available data from the literature about lectins from edible

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mushrooms, namely, their binding specificities, structures, and biofunctionality. As mentioned above, lectins can be used as a cytochemical and histochemical diagnostic tool. In this way, a lectin isolated from *A. bisporus* (ABL) is already commercially available. It can even be supplied in biotinylated fluorescein isothiocyanate and other forms conjugated with fluorescence markers, mainly for detection and bioanalysis purposes [61].

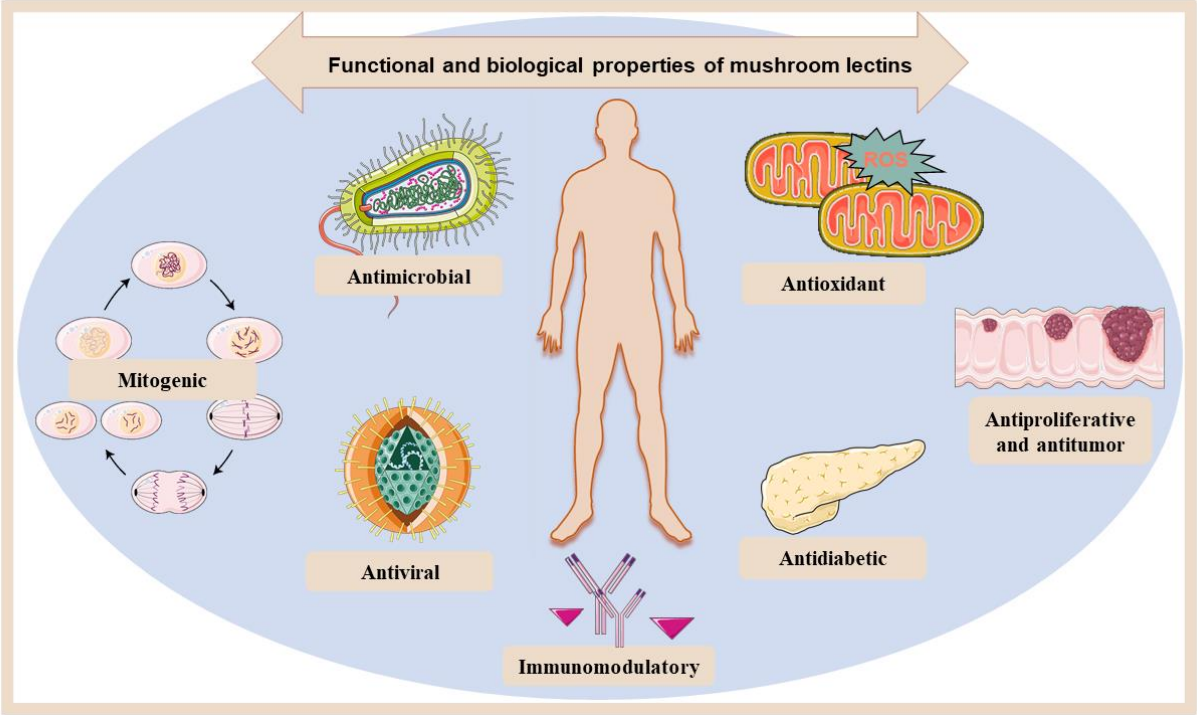

Fig. (2). Schematic illustration of bio-functional applications of lectins from edible mushrooms.

4.1.1 Immunomodulatory activity

A few mushroom lectins possess immunological activity. On the one hand, due to their specificity in binding to surface receptors of immune cells. Thus, these glycoproteins can activate various downstream signalling pathways and induce the secretion of nitric oxide (NO), NOS, interleukin factors (IL-2, IL-6, IL-1α, IL-1β and IL-10), tumour necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ) and other immune mediators against pathogens [60,62,65]. On the other hand, lectins can also induce phagocytosis and increase the secretion of toll-like receptors (TLRs), which is crucial in eliminating pathogens [60]. Consequently, mushroom lectins exert immunomodulatory effects through cytokine regulation and proliferation, mainly boosting the production of macrophage-activating factors and activating lymphocytes, the essential immune cells [64,65].

The isolated glycoproteins from *A. bisporus* (named ABL) and *Auricularia polytricha* (designed APP) can stimulate RAW264.7 macrophages by producing NO and TNF-α. These two lectins are promising candidates as immune stimulants for use in food and pharmaceuticals. They have thermal/freeze-resistant, and acid/alkali tolerance properties and are stable to dehydration [74]. Additionally, two lectins isolated from *T. mongolicum*, named TML-1 and TML-2, show *in vivo* immunomodulatory and antitumor effects through activation of the immune system without direct cytotoxic effects. These lectins stimulate the secretion of TNF-α, and NO and inhibit the growth of mouse lymphoblast-like (p815) mastocytoma by producing macrophage-activating factors, such as IFN-γ and other cytokines [75]. Moreover, El Enshasy and Hatti-Kaul [75] suggested that lectin extracted from *V. volvacea* has higher immunomodulatory activity than other known lectins such as concanavalin A. Recently, a new lectin obtained from *Latiporus sulphureus* (named LSL) was considered a natural immunopotentiator, capable of activating macrophages and inducing cell proliferation [76].

4.1.2. Antiproliferative/antitumour activity

It is well known that the tumour cell surfaces vary in the glycoconjugates composition, namely, in their carbohydrate terminal units, compared with normal cells [62]. Disaccharides such as Thomsen Friedenreich antigen (Gal/3-1,3-GalNAc or TF-antigen) are exposed in tumour cells while hidden in normal cells [60]. In this context, lectins, due to their carbohydrate specificity, present antiproliferative potential by cross-linking the altered glycans over the cancer cell surface

or through immunomodulatory effects. After this interaction of the lectin with the cancer cell, several different mechanisms, including apoptosis, are induced within the cells, leading to cell death [65]. Moreover, lectins can be used as a predictive tool and biomarker to detect metastases and diagnose different tumours. This mechanism occurs by the variation in the binding affinity of lectin to the surface N-glycan moiety expressed on the tumour cell metastasis [60].

As reviewed by Singh *et al.* [65], mushroom lectins exhibit antiproliferative effects against various cancers such as breast, colon, leukaemia, carcinoma, hepatoma, and sarcoma. Lectins from *Hericium erinaceus* [77], *Inocybe umbrinella* [78], *Pholiota adiposa* [79] and *Russula delica* [41], demonstrated potent antiproliferative activity against HepG2 and MCF-7 cells [60].

Furthermore, Rouf *et al*. [80] reported a GlcNAc specific lectin from *Psathyrella asperospora* (named PAL) that showed antiproliferative activity against human colon cancer (HT-29). The ability of PAL to inhibit the proliferation of cancer cells is due to arrest in the G2/M phase of the cell cycle. However, this effect can be halted with the addition of free GlcNAc [80].

Amongst mushrooms, the *A. aegerita* lectin (designed AAL) displays antitumor activity against various human cancer cell lines, such lines HeLa (cervical cancer), SW-480 (colon adenocarcinoma), MGC-803 (gastric adenocarcinoma), HL-60 (leukaemia), SGC-790 and BGC-823 (both of gastric cancer) and mouse sarcoma S-180 *in vivo* and *in vitro*. The antitumor effects of ALL occur due to the induction of apoptosis and DNase activities [81].

Furthermore, ABL inhibits the proliferation of human cancer Caco-2 (colon cancer), HT-29, and MCF-7 cells, probably resulting from their ability to block the transport of proteins to the nucleus. This mechanism is necessary for DNA synthesis during cell proliferation without cytotoxicity, directly blocking the internal transport of proteins in nuclear pores [61].

4.1.3. Mitogenic activity

The most significant value of the mitogenic property has been analysed in biochemical events that lead to the conversion of resting cells into actively growing cells. Some mushroom lectins have the remarkable ability to stimulate the transformation of small resting cells (lymphocytes/splenocytes) to large blast-like cells, which may undergo mitosis [62]. Usually, lymphocyte activation and proliferation begin with the binding of lectins to T-cell receptors, which activate a signalling cascade: intracellular calcium mobilisation, IL-2 gene expression and consequent cell proliferation [82].

As reviewed by Hassan *et al.* [66], lectins from *A. cylindracea* [68], *Armillaria luteovirens* [83], *B. edulis* [84], *Cordyceps militaris* [85], *Flammulina velutipes* [86], *Hygrophorus russula* [87], *P. citrinopileatus* [88] and *Xerocomus spadiceus* [89] are known to exhibit mitogenic effects in murine splenocytes. As well, a heat-stable lectin isolated by Ngai *et al*. from *Ganoderma capense* also has a potent mitogenic activity for mouse splenocytes and antiproliferative effects for HepG2 and leukaemia cells (L1210 and M1) [90]. Moreover, the *V. volvacea* lectin (named VVL) exerts mitogenic activity on T lymphocytes via a calcium-dependent pathway through the T cell receptor [91].

Although several mushroom lectins exhibit mitogenic activity, a few lectins are non-mitogenic or anti-mitogenic [66]. An example of a lectin with non-mitogenic activity is ABL, which suppresses the production of T and B lymphocyte cells through the inhibition of DNA synthesis, being an example of a lectin with non-mitogenic activity [61].

4.1.4. Antiviral activity

Recently, the antiviral activity of lectins, especially from fungi, has been a research area of great interest, although its action mechanism is not yet well understood. As a result, lectin's properties as an antiviral agent have been studied against several viruses, including herpes simplex types 1 and 2, hepatitis C, influenza A/B, Japanese encephalitis virus, HIV, SARS virus and the current SARS-CoV-2 virus by adhering specifically to viral envelope glycans, which prevents viral cell invasion [13,61]. In fact, discovering new antiviral agents is a public health emergency to overcome the present COVID-19 pandemic and prepare for the subsequent viral pandemics [60].

These proteins can inhibit viral activity by binding to the virus surface glycoprotein, blocking the host receptor, or inhibiting the viral polymerase enzyme by binding to its active site [60,92]. In addition, lectins impede the entry of many viruses, such as SARS-CoV, by binding to the spike proteins (viral envelope glycoproteins) and thus inhibiting virus-cell fusion [92].

The most studied antiviral activity of mushroom lectin is against HIV. In this context, mannose-binding lectins (MBL) are the most investigated against HIV. Their antiretroviral activity results from the binding of these molecules to the glycoproteins gp120 (rich in mannose) and gp41 surface. Consequently, this fact makes the virus easily neutralised by host antibodies and other immune system cells [93]. *Hygrophorus russula* lectin is an example of MBL, and it inhibits HIV infection by establishing a strong hydrogen bond with mannose in gp120 [87].

Moreover, lectins also act as anti-HIV by inhibiting HIV-reverse transcription (RT), a critical enzyme in the HIV life cycle [84]. According to their mechanism of action, HIV-RT inhibitors can be categorised into nucleosides (NRTIs) or non-nucleosides (NNRTIs). The NRTIs get incorporated into viral DNA, whereas NNRTIs bind directly to the enzyme (HIV-RT) [60,65]. *Boletus speciosus* lectin inhibits HIV-RT through protein-protein interaction, which belongs to the NNRTIs group [84]. In addition, Singh *et al*. [62] reported that lectins from *C. militaris* [94], *A. bisporus* [95], *I. umbrinella* [78], *S. commune* [96] and *P. citrinopileatus* [88] exhibit potential inhibition of HIV-RT activity, with a half-maximal inhibitory concentration (IC_{50}) value from highest to lowest (between 10 µM and 0.93 µM), respectively. Therefore, due to their specific binding abilities, lectins targeting HIV-RT possess a significant interest in developing anti-HIV drugs.

Furthermore, Ma *et al*. [97] described that AAL could be an effective adjuvant for the Influenza vaccine against mice's H9N2 (Influenza A virus subtype) infection. The interaction between AAL and the H9N2 virus appears to be associated with the binding of the AAL CSB to the surface glycosylated proteins, neuraminidase, and hemagglutinin.

In addition to the previously mentioned viruses, *P. ostreatus* lectin (named POL) has immunogenic activity and can be used as a safe and effective adjuvant in therapy against hepatitis B virus (HBV). POL can activate an innate immune response and stimulating the expression of the TLR6 signalling pathway in dendritic cells. In addition, POL can elicit helper follicular T cell responses to the production of HBV-specific antibodies and thereby treat chronic HBV infection [98].

Therefore, exploring further *in vivo* clinical research studies to optimise the dose, bioavailability, delivery, mechanism of action, and antiviral activity of different mushroom lectin types would be relevant. Besides, the combination of lectin therapy antiviral activity or immunomodulatory effects and available drugs should be investigated as a possible solution to control the infection and avoid the spread of viral diseases [60].

4.1.5 Antimicrobial activity

The effects of lectins on microorganisms suggest their future use as antimicrobial agents. In general, lectins interfere with microorganisms' growth, multiplication, and spread by agglutination and/or microorganism immobilisation [99]. Thus, the study and application of lectins with antimicrobial activity may be a promising strategy to overcome the problem of multiresistant strains. However, there are a small number of reports about fungal lectins exhibiting antimicrobial activity, namely, against bacteria (Gram-positive and Gram-negative) and fungi [60]. The few reported mushroom lectins that possess antibacterial and antifungal activity are presented in Table 2.

The antibacterial activity of lectins results from their ability to bind cell surface glycans present in the bacterial cell walls. Lectins bind to N-acetylmuramic acid, N-acetylglucosamine, and teichoic acid present in the cell wall of Gram-positive bacteria or to lipopolysaccharides (such lipid A) as present in the cell wall of Gram-negative bacteria. The interaction between lectins and glycans results in bacterial motility and prevents their adhesion to the surface [60,99,100]. Thus, this interaction results in bacterial agglutination or cell death by the presence of pores and bubbling on gram-positive and gram-negative bacteria cells, respectively [99,101].

A few mushroom lectins have shown antifungal effects. These glycoproteins bind to chitin or other glycans on the fungal surface, affecting nutrient absorption, fungal growth, and spore formation [99,100]. Besides, small lectins can penetrate the fungal cell surface, inhibiting several vital enzymes and altering cell wall morphogenesis [99].

Table 2. Antimicrobial activity of selected edible mushroom lectins.

Mushroom species	Lectin name	Target bacteria	MIC	Target fungi	MIC	Reference
Aleuria aurantia	AAL	N. A.	N. A.	*Mucor racemosus*	1 μM	[102]
Gymnopilus spectabilis	GSL	*Staphylococcus aureus*	-	*Aspergillus niger*	-	[100]
Sparassis latifolia	-	*Bacillus subtilis*	50 μg	*Candida albicans*	100 μg	[103]
		Escherchia coli	100 μg	*Candida catenulate*	25 μg	
		Listeria monocytogenes	100 μg	*Candida glabrata*	100 μg	
		Pseudomonas aeruginosa	50 μg	*Candida rugosa*	50 μg	
		Salmonella typhimurium	25 μg	*Fusarium oxysporum*	0.4 mg	
		Staphylococcus aureus	100 μg	*Fusarium solani*	0.4 mg	

MIC: Minimum inhibitory concentration; N.A.: Not applicable; -: Not mentioned.

4.1.5. Antioxidant activity

Antioxidants are compounds that have the potential to decrease free radical-mediated oxidative stress [65]. In this context, the antioxidant activity of mushroom lectins can be used to target various free radical-mediated diseases. Consequently, these biomolecules may even be considered an approach in cancer therapy through the inhibition of oxidative stress [60]. Amongst mushroom lectins, *Pleurotus florida* lectin (named PFL) exhibits a potent time-dependent antioxidant activity against arsenic-induced nephrotoxicity in rats [104]. For that, PFL reverses the effect of arsenic-mediated oxidative stress via upregulation of mRNA expression of the superoxide dismutase gene, which inhibits excessive NO release [105]. Indeed, mushroom lectins can protect the membrane integrity from oxidative stress since they have the potential to scavenge free radicals through interaction with the oxidative cascade, chelate metal ions, and

oxygen extinction, and prevent the membrane lipid peroxidation [65].

4.1.6. Antidiabetic activity

Diabetes is a chronic and metabolic disease characterised by high blood glucose levels resulting from insulin imbalances within the body [106]. Lectins with antidiabetic effects may be promising functional food ingredients with health benefits for both diabetes and hypercholesterolemia people. In 1975, Ewart *et al*. [107] demonstrated that, in the presence of glucose, lectins from *A. bisporus* and *A. campestris* stimulate insulin and glucagon release from isolated rat islets. This fact results from the specific interaction between mushroom lectins and their oligosaccharide receptors, leading to conformational changes in the membranes of the Langerhans islets and β-cells that enable insulin exocytosis. According to Wang *et al*. [108], ABL has exhibited hypoglycemic activity and was proposed to prevent and/or treat diabetic patients with pancreatic damage. ABL increases insulin secretion *in vivo* when added to the Langerhans islets of diabetic mice and promotes the proliferation of pancreatic β-cells, regulating cell cycle proteins [108].

4.2. FUNGAL IMMUNOMODULATORY PROTEINS

FIPs are novel bioactive proteins isolated from some medicinal and edible mushrooms, such as *G. lucidum* [109], *F. velutipes* [110], *V. volvacea* [111], among others, that have been summarised by Liu *et al.* [112]. In general, FIPs have a molecular weight of about 13 kDa and 110–114 amino acids, with high asparagine and acetylated-extreme valines concentrations [11,113]. Moreover, they are dimers with a dumbbell-shaped structure similar to the variable region of immunoglobulin heavy chains [75]. According to their conserved structure and protein identity, FIPs can be classified into five subgroups: Fve-type FIPs, Cerato-type FIPs, PCP-like FIPs, TFP-like FIPs and unclassified FIPs. This last subgroup corresponds to FIPs whose amino acid sequence has not yet been elucidated. Therefore far, as reviewed by Liu *et al*. [112], more than 38 types of FIPs have been found and identified.

FIPs-Fve is the most prominent subgroup with 29 members and is also the most studied due to its hemagglutinating, immunomodulation, and anticancer properties. The structure of this predominant group of FIPs consists of two parts, the N-terminal and C-terminal domains. The N-terminal domain starts with an N-terminalα-helix, which is a crucial structure for all Fve-type FIPs. The N-terminal domain starts with an N-terminal α-helix. On the other hand, the C-terminal domain is a sandwich-like FibronectinI II domain, consisting mainly of 7β-sheets. The Fve-type FIPs monomer assembles into a homodimeric or tetrameric structure via non-covalent interactions, such as hydrophobic interactions and hydrogen bonds. The dimerization sustained by domain switching depends mainly on the N-terminal α-helix and the β sheet. Regarding the tetramer formation, more detailed information is needed. Actually, more studies are required in order to clarify the chemical structure of FIPs [112].

As mentioned above, FIPs exhibit secondary structural similarities to the human immunoglobulin heavy chain. That explains why they show noticeable immunomodulatory effects and have been extensively studied for their biochemical and pharmacological application [4,112]. It is essential to clarify that FIPs differ from lectins due to the absence of a conjugate, even though both are well-known as immunomodulatory compounds [64].

Indeed, FIPs can promote antigen-presenting cells and release cytokines such as NO and IL-12 through TLRs binding. Moreover, by activating the phosphorylation of the p38/MAPK signalling pathway and increasing the production of nuclear factor-kappa (NF-kB). Consequently, these proteins can stimulate the differentiation of helper T cells (Th0) to Th1 or activate Th2 cells, activate lymphocyte B and macrophages, as well as produce a variety of cell factors [64,110]. Therefore, aside from immunomodulatory activity, FIPs have revealed several beneficial functions. These bioactivities include anti-allergy properties (namely, in the treatment of asthma), the ability to stimulate immune cells to produce cytokines and antitumour activities (such as inhibition of cell growth and proliferation, induction of apoptosis, among others) [4,113,114]. Nevertheless, these functions are directly related to their structural or physicochemical characteristics [113].

The first FIP identified and probably the best-known is Ling-Zhi-8 (FIP-LZ-8) from *G. lucidum*, discovered in 1989 [109]. FIP-LZ-8 consists of 110 amino acid residues and acts as an immunosuppressive agent. It has been shown to suppress diabetes in an animal model. In parallel, FIP-LZ-8 also increases skin grafts' survival in transplanted allogeneic mice with fewer nephrotoxic effects than other immunosuppressive agents such as cyclosporine A [75,109]. Moreover, FIPs isolated from *F. velutipes* (designated as FIP-fve) possess various biological activities, including agglutinating human erythrocytes and intensely stimulating T cells via cytokine regulation of p38/MAPK [110,115]. Moreover, FIP from *V. Volvacea* (named FIP-vvo) exerts its immunomodulatory properties by regulating cytokines and enhancing the transcriptional expression of IL-2, IL-4, IFN-γ, TNF-α, and IL-2 receptors. FIP-vvo triggers the proliferation of lymphocytes in human peripheral blood [111]. Additionally, two new FIPs (Fip-lti1 and Fip-lti2) isolated from *Lentinus tigrinus* protected the liver from Con A-induced necrosis by reducing serum aminotransferase (aspartate aminotransferase and alanine aminotransferase) levels, and it improved liver histology. Moreover, both Fip-lti1 and Fip-lti2 reduced the levels of proinflammatory cytokines (TNF-α, IL-1β, and IL-6) and oxidative stress [116]. All of these FIPs and the FIP-LZ-8 are examples of FIPs classified as Fve-type FIP [112].

Furthermore, *P. eryngii* protein (named PEP 1b) is an unclassified FIP suggested as an immunomodulatory. This protein can boost cellular immune responses through the proliferation of macrophages by activating the TLR4-NF-κB and MAPK signalling pathways [112]. In the same subgroup, FIP from *H. erinaceus* (designed HEP) shows

immunomodulatory activity and auxiliary antitumor effects. Additionally, this group can also act as a possible prebiotic due to the regulation of the gut microbiota. In particular, HEP exhibited an anti-inflammatory effect on inflammatory bowel disease mice.

Moreover, we demonstrated immunomodulatory activity in lipopolysaccharide-activated RAW 264.7 macrophages by declining the overproduction of cytokines in mice. Regarding regulating the composition and metabolism of the intestinal microbiota, HEP inhibited certain aerobic and microaerophilic bacteria. At the same time, HEP increased the content of some probiotic bacteria, such as *Bifidobacterium* and *Lactobacillus*, among others [3,4].

FIPs usually occur in low numbers in their native mushrooms. Therefore, the low yield of FIPs extraction is a significant limitation in their research and application. Consequently, genetic engineering techniques are rapidly being developed to increase the production of recombinant FIPs (rFIPS) in other organisms, such as the yeast *Pichia pastoris* and the bacteria *Escherichia coli*. For example, the expression of the LZ-8 gene of *G. lucidum* in *P. pastoris* produces a recombinant LZ-8 protein (rLZ-8). Despite the recombinant protein lacking the carbohydrate portion of the natural protein, it has similar bioactivity for IL - 2 induction as the natural protein [64]. Furthermore, rFIPs have higher immunomodulatory activities and induce better expression of specific cytokines than those produced naturally in mushrooms [64,75].

4.3. UBIQUITIN-LIKE PROTEINS

Ubiquitin and ubiquitin-like proteins (Ubls) are proteins covalently conjugated to ubiquitin. Consequently, these proteins share structural and evolutionary relationships with ubiquitin. The chemical structure of an Ubls is defined by a β-grab fold composed of a five-strand β sheet (that partially surrounds a central α-helix) and a short flexible C-terminal tail that typically ends with at least one glycine residue. Analogous to ubiquitin, conjugation is achieved through a cascade of activities catalysed by three different enzymes: E1 activating enzymes, E2 conjugating enzymes, and E3 ligases [117].

Ubls are signalling messengers that monitor cell functions such as cell proliferation, endocytosis, regulation of signal transcription, apoptosis, cell cycle repair, DNA repair, and immune response. In this context, dysregulation of the ubiquitin-mediated pathway has been associated with the development of several tumours. Therefore, Ubls may exhibit anticancer and anti-inflammatory activity, representing an important class of targets for human therapeutics [114,118]. Some Ubls isolated from edible mushrooms and their health benefits are shown in Table **3**.

Table 3. Selected ubiquitin-like proteins from edible mushrooms and their health function.

Mushroom species	Ubls name	Function	IC_{50}	Reference
Agrocybe aegerita	UbcA1	Proapoptotic activity in including cervical cancer cells (HeLa).	N. A.	[119]
Agrocybe cylindracea	-	Antiproliferative activity against leukaemia cell line (M1).	10 μM	[120]
		Antiproliferative activities against hepatoma cell line (HepG2).	100 μM	
		Immunomodulatory activities by stimulating the production of nitric oxide in murine peritoneal macrophages.	N. A.	
Pleurotus sajor-caju	PSULP (peptide)	Inhibition of translation within the rabbit reticulocyte lysate system.	30 nM	[121]
		Exhibits ribonuclease activity.	N. A.	
Ramaria botrytis	RBUP	Antitumour effect by inducing apoptosis.	15.93 μM	[122]
		Haemagglutinating activity.	N. A.	
		Exhibits DNAse activity.	N. A.	

Ubls: Ubiquitin-like proteins; IC_{50}: Half-maximal inhibitory concentration; N. A.: Not applicable; -: Not mentioned.

4.4. Enzymes

Several proteins are also enzymes. Mushrooms naturally produce and secrete a series of enzymes that facilitate the degradation of lignocellulosic substrates [123]. However, some of these enzymes also catalyse specific steps in metabolism and possess health-promoting effects such as antimicrobial, antiproliferative, and antiviral activities [7]. This subtopic will discuss the principal mushroom enzymes, such as ribosome-inactivating proteins (RIPs), ribonucleases, proteases, laccases, and tyrosinases, and respective examples of associated health benefits. Additionally, mushrooms' possible production of pectinases, lipases, and phytases may also be of interest to study in the future [123].

4.4.1. Ribosome-inactivating proteins

Ribosome-inactivating proteins (RIPs) are enzymes that usually have an RNA N-glycosidase domain, which

irreversibly inactive ribosomes and consequently avoid protein synthesis [41]. Indeed, RIPs are well known for their ability to inhibit translation in the cell-free rabbit reticulocyte lysate system. The RNA-glycosidase can specifically eliminate an adenine from adenosine (A4324) from 28S ribosomal RNA in rats, resulting in the arrest of protein synthesis [118]. Since the early 2000s, RIPs have been isolated from several edible mushroom species such as *F. velutipes* [124,125], *Pleurotus tuber-regium* [126], *Lyophyllum shimeiji* [114], *Hypsizigus marmoreus* [127] and *V. volvacea* [128] (Table 4).

Table 4. Some ribosome-inactivating proteins and their functions.

Mushroom species	RIPs name	Function	IC_{50}	Reference
Flammulina velutipes	Flammulin	Proapoptotic activity in HeLa (cervical cancer) cells.	N. A.	[125]
	Flammin	Inhibition of translation in a rabbit reticulocyte lysate system, without showing ribonuclease or protease activity.	1.4 nM	[124]
	Velin		2.5 nM	
Pleurotus tuber-regium	Pleuturegin	Inhibition of translation in cell-free rabbit reticulocyte lysate system.	0.5 nM	[126]
Lyophyllum shimeiji	Lyophyllin	Inhibition of translation in rabbit reticulocyte lysate.	1 nM	[114]
		Thymidine uptake by murine splenocytes.	N. A.	
		Antimitogenic activity.		
Hypsizigus marmoreus	Hypsin	Antiproliferative activity against mouse hepatoma and leukaemia and human leukaemia cells.	N. A.	[129]
	Marmorin	Prevent the proliferation of MCF-7 and HepG2 cells (human breast and liver cancer cells, respectively).	N. A.	[127]
		Stimulation of the death receptor apoptotic pathway and endoplasmic reticulum stress.		
Volvariella volvacea	Volvarin	Inhibitory action on protein synthesis in the rabbit reticulocyte lysate system.	0.5 nM	[128]

RIPs: ribosome-inactivating proteins; IC_{50}: Half-maximal inhibitory concentration; N. A.: Not applicable

4.4.2. Ribonucleases

Ribonucleases (RNases) catalyse the hydrolysis of RNA molecules into smaller components, possessing different degrees of specificity. RNA degradation controlled by RNase is a crucial factor in controlling gene expression, maturation, and turnover. Thus, RNA-degrading enzymes include angiogenic, antitumor, antiviral, or immunosuppressive activities and constitute a significant group of potential therapeutic agents [10,130]. However, the studies on the antitumor action mechanism of mushroom RNases are limited. Therefore, in the future, research in this field may be an area of great interest and may facilitate their potential therapeutic applications. These applications include the use in the areas of diagnosis and treatment of tumours and its promising use in the treatment of acquired immune deficiency syndrome (AIDS) due to its ability to inhibit HIV [114,130].

Several RNases are isolated from different edible mushroom species such as *A. cylindracea* [120], *L. shimeiji* [131], *H. marmoreus* [131], *P. ostreatus* [132,133], *Thelephora ganbajun* [134], with antitumor activity or inhibition of HIV-RT activity [130] (Table 5). For example, an RNase isolated from *A aegerita*, named AAD, inhibited the proliferation of several types of human cancer cells, including HeLa, HepG2, and SH-SY5Y (neuroblastoma) cells. ADD also induced tumour cell apoptosis by activating caspase-8, an initiator of apoptosis [135]. Recently, another RNase from *A. aegerita*, named Ageritin, was described as antiviral, antibacterial, antifungal, endonuclease, and nuclease. In parallel, Ageritin also exhibited cytotoxicity to COLO 320 (human colon carcinoma), HeLa, and Raji (lymphoma) cells through the promotion of apoptosis [136]. GLR, an RNase purified from *G. lucidum*, exerted antiproliferative activity on HT-29 and HCT116 colorectal cancer cells. This protein induces cell cycle arrest in the G1 phase through the regulation of cyclins D1 and P53 expression [137]. Besides, the RNase from *P. major-caju* shows antibacterial and antifungal activities, coupled with antiproliferative action on leukaemia and hepatoma cells and anti-mitogenic properties on mouse spleen cells [138]. In addition, according to Rezvani *et al.* [114], although several RNases have been identified from mushrooms, several still have no biological activity attributed. These mushroom species include *A. bisporus* [139], *V. volvacea* [140], *Russulus virescens* [141], *Termitomyces globulus* [142] and *P. tuber-regium* [143], among others.

Table 5. Ribonucleases from edible mushrooms with potential health benefits.

Mushroom species	RNases	Health benefits	IC_{50}	Reference
Agrocybe aegerita	AA-RNase	Antiproliferative activity against HepG2 (hepatocellular carcinoma), HeLa (cervical cancer), and SH-SY5Y (neuroblastoma).	-	[120]
	Ageritin	Antibacterial activity against *Micrococcus lysodeikticus*.	-	[136]
		Antifungal activity against *Penicillium digitatum*.	-	
		Antiviral activity against tobacco mosaic virus RNA.	-	
		Endonuclease activity against a supercoiled plasmid.	-	
		Nuclease activity against genomic DNA.	-	
		Antiproliferative activity against cervical cancer cells (HeLa).	4 nM	
		Antiproliferative activity against human colon carcinoma (COLO 320).	1.9 μM	
		Antiproliferative activity against lymphoblastoid cells (Raji).	1 μM	
Agrocybe cylindracea	AC-RNase	Antiproliferative activity against HepG2.	0.1 mM	[120]
		Antiproliferative activity against leukaemia cell line (M1).	10 μM	
		Trigger the production of nitric oxide.	N. A.	
Ganoderma lucidum	GLR-RNase	Antiproliferative activity against colorectal cancer cells (HT-29).	2.8 μM	[137]
		Antiproliferative activity against colorectal cancer cells (HCT116).	0.1 μM	
Hypsizigus marmoreus	HM-RNase	Antiproliferative activity against leukaemia cells (L1210).	60 μM	[144]
Lactarius flavidulus	LF-RNase	Antiproliferative activity against HepG2.	3.19 μM	[145]
		Antiproliferative activity against L1210.	6.52 μM	
Lyophyllum shimeiji	LS-RNase	Antiproliferative activity against HepG2.	10 μM	[131]
		Antiproliferative activity against breast cancer cells (MCF-7).	6.2 μM	
Pleurotus djamor	PD-RNase	Antiproliferative activity against HepG2.	3.9 μM	[146]
		Antiproliferative activity against MCF-7.	3.4 μM	
Pleurotus ostreatus	PO-RNase 1	Inhibition of HIV reverse transcriptase activity (HIV-RT).	200 μM	[147]
	PO-RNase 2	Antiproliferative activity against L1210.	41 μM	[133]
Pleurotus sajor-caju	PS-RNase	Antiproliferative activity against HepG2.	0.22 μM	[138]
		Antiproliferative activity against L1210.	0.1 μM	
		Antifungal activity against *Fusarium oxysporum*.	95 μM	
		Antifungal activity against *Mycosphaerella arachidicola*.	72 μM	
		Antibacterial activity against *Pseudomonas aeruginosa*.	51 μM	
		Antibacterial activity against *Staphylococcus aureus*.	34 μM	
Russula delica	RD_RNase	Antiproliferative activity against HepG2.	8.6 μM	[148]
		Antiproliferative activity against MCF-7.	7.2 μM	

Schizophyllum commune	SC-RNase	Inhibition of HIV-RT activity.	65 μM	[149]
Thelephora ganbajun	TG-RNase	Potent inhibition of HIV-RT activity.	300 nM	[134]

RNases: ribonucleases; IC_{50}: Half-maximal inhibitory concentration; N. A.: Not applicable; -: Not mentioned.

4.4.3. Proteases

Proteases are a group of enzymes that disintegrate proteins by hydrolysis of peptidic bonds [114]. These enzymes are grouped based on the nature of the functional group at the active site and the type of enzymatic mechanisms of asparagine, aspartic, cysteine, glutamic, metallo, serine, threonine, and unknown. Proteases are naturally distributed in cells and tissues and are involved in the development of physiological processes in all living organisms [150]. Therefore, they are known for their biological activities in cells, such as protein turnover, enzyme modification, germination, nutrition, regulation of gene expression, and cell death initiation, among others [114,150,151]. Besides, proteases also have important applications in several industrial fields. These include detergent, leather, and textile applications as well as pharmaceutical industries as potential drug targets or as diagnostic and prognostic biomarkers. At the same time, commercial fermentations in the food industry, including yoghurt, cheese, beer, and others, are also crucial applications of proteases [151,152].

Concerning the role of proteases in health, there is a vital balance between them and their natural inhibitors. Therefore, the disruption of this equilibrium may lead to many diseases (e.g., cancer). On the one hand, due to the vast implications of proteases in pathologic processes, the selective inhibition of proteases involved in diseases, without affecting nontarget proteases would be very promising in medical applications. Examples are mycocypines, and cysteine protease inhibitors isolated from basidiomycetes [10]. On the other hand, proteases, among other functions, include immune function. In this context, this macromolecule can act as an anticancer agent in several stages, including tumour initiation, growth and metastasis [114].

Microorganisms, such as fungi and bacteria, are the essential sources of this group of enzymes [152]. Proteases have been isolated from edible mushrooms such as *A. bisporus* [153], *Armillariella mellea* [154], *C. militaris* [155], *F. velutipes* [156], *G. frondosa* [152], *Helvella lacunosa* [157], *Lignosus rhinocerotis* [158], *Lyophyllum cinerascens* [159], *P. eryngii* [160], *P. ostreatus* [161] and *P. citrinopileatus* [162].

Pleureryn is an aspartic protease obtained from *P. eryngii*, which inhibited translation in a rabbit reticulocyte lysate system with an IC_{50} of 20 nM. Pleureryn has exhibited some inhibitory effects on HIV-RT. This suggests a suppressive action of HIV protease, an aspartic protease, on its homologous RT. Pleureryn is relatively stable to changes in temperature and pH. It exhibits an optimum pH and temperature of 5.0 and 45 ºC, respectively, maintaining its activity at high temperatures and pH 4 and 12 [160].

A protease named CMP, identified from *C. militaris*, was shown cytotoxicity effects against MCF-7 and A-549 cells, human breast and bladder cancer cells, respectively. Although, the mechanisms underlying these effects are unclear. Moreover, CMP exerted a potent antifungal activity against the growth of *Fusarium oxysporum* [155]. A protein fraction (characterised as F5) of *L. cinerascens* was identified as a serine protease capable of antitumor activity against MCF7 cells (at an IC_{50} of 3 μg/mL). These effects induced apoptosis by upregulating caspase-8 and –9 and decreasing Bcl-2 (a cellular protein that inhibits apoptosis) [158].

4.4.4. Laccases

Laccases are another widespread group of enzymes present in mushrooms. Laccases are oxidase enzymes that catalyse the oxidation of various substrates, such as phenolic and inorganic compounds, and concomitantly with the reduction of molecular oxygen in the water [41,118]. Fungal laccases are extracellular and monomeric glycoproteins with approximately 520-550 amino acids and a typical weight of about 60-70 kDa in their glycosylated form. Besides, have four copper atoms distributed between three functionally distinct sites (Cu_1, Cu_2 and Cu_3). Additionally, encompass an N-terminal signal peptide sequence of 20-22 residues. Structurally, these enzymes consist of three neatly arranged cupredoxin-like domains, each of which has β-barrel symmetry [118,163].

Mushroom laccases are associated with multiple factors such as pathogen/host interactions, lignin degradation, pigment degradation, rectification, and stress response in biotechnological and industrial applications [11]. Laccases also have pharmaceutical and medical applications. Their transversal application may be explained by their implications in the pathogenesis, immunogenesis, and morphogenesis of organisms and the metabolic turnover of complex organic substances [41]. Several enzymes with immunology effects and antiviral and antiproliferative activities have been obtained from several species of edible mushrooms (Table 6). For example, laccases isolated from oyster mushrooms exhibit health-promoting effects. Thus, the laccase identified from *P. ostreatus* showed an antiviral effect against the hepatitis C virus [164]. A laccase from *P. eryngii* also demonstrated antiviral activity against HIV by inhibiting HIV-RT [165]. As well, laccase purified from *Pleurotus cornucopiae* inhibited HIV-RT activity and the proliferation of breast cancer cells MCF-7, hepatoma cells HepG2 and leukaemia L1210 cells [166,167].

Table 6. Laccases from edible mushrooms with potential therapeutic effects.

Mushroom species	Therapeutic effects	IC_{50}	Reference
Agrocybe cylindracea	Antiproliferative activity against hepatocellular carcinoma cells (HepG2).	5.6 μM	[168]
	Antiproliferative activity against breast cancer cells (MCF-7).	6.5 μM	
	Inhibition of HIV reverse transcriptase activity (HIV-RT).	12.7 μM	
Coprinus comatus	Antiproliferative activity against HepG2.	3.46 μM	[169]
	Antiproliferative activity against MCF-7.	4.95 μM	
	Inhibition of HIV-RT activity.	5.85 μM	
Ganoderma lucidum	Inhibition of HIV-RT activity.	1.2 μM	[170]
Pleurotus cornucopiae	Antiproliferative activity against leukaemia cells (L1210).	-	[167]
	Antiproliferative activity against HepG2.	3.9 μM	[166]
	Antiproliferative activity against MCF-7.	7.6 μM	
	Inhibition of HIV-RT activity.	3.7 μM	
Pleurotus eryngii	Inhibition of HIV-RT activity.	2.2 μM	[165]
Pleurotus ostreatus	Antiviral activity against the hepatitis C virus.	-	[164]

IC_{50}: Half-maximal inhibitory concentration; -: Not mentioned.

4.4.5. Tyrosinases

Tyrosinases belong to a larger group of cooper proteins that catalyse sequential oxidation steps of mono- and di-phenolic substrates. For that, tyrosinase is also known as polyphenol oxidase (PPO) [171]. PPOs are present in animals, plants, and microorganisms, and being involved in numerous biological functions [172]. However, the biotechnological interest of this macromolecule group has focused on mushroom tyrosinases [171]. This may be explained by the fact these fungus-derived enzymes are usually associated with the formation and stability of spores, defence and virulence mechanisms, and browning and pigmentation [172].

Tyrosinases are key enzymes involved in the first step of melanin biosynthesis [10]. They catalyse the conversion of L-tyrosine to L-3,4-dihydroxyphenylalanine (known as L-DOPA), which is then converted into Dopaquinone, a melanin biosynthesis precursor [61]. Melanin is an abundant pigment in all organisms, so tyrosinase has been related to pigment deficiency disorders. Some examples include albinism and vitiligo, as well as hyperpigmentation conditions, including glaucoma and malignant melanoma [10,61]. Thus, these effects have attracted considerable scientific attention, particularly in searching for tyrosinase inhibitors for therapeutic use [10]. Along with this, the use of tyrosinase inhibitors has been of great interest in distinct industries, namely, in the cosmetic industry, in investigating skin whitening agents, and in the agri-food industry, avoiding the browning of agricultural products [16,61].

According to Ismaya *et al.* [61], PPO suppresses tumour growth and can also be used to produce L-DOPA, one of the most effective agents in treating Parkinson's disease. However, the use of tyrosinases directly in human therapy has been challenged due to their potential toxicity. They are mutagenic, and their catalysed reactions generate harmful radicals (e.g., oxy radicals, peroxides, and quinones).

The first fungal tyrosinase was extracted from the edible mushroom *A. bisporus*. The study involved mainly the enzymatic browning phenomenon during development and postharvest storage [172]. Besides, PPO has been found effective against inflammation and inflammatory diseases. Furthermore, an ethanol extract of *A. bisporus* demonstrated potent antioxidant properties, playing an essential role in anti-ageing skin. Alternatively, this extract also exhibited antibacterial activity against harmful microorganisms such as *Staphylococcus aureus* [16]. This mushroom enzyme possesses high homology to mammalian enzymes, making it appropriate as a model for studies of melanogenesis and the design and search for novel PPO inhibitors and substrates [10]. Actually, mushroom tyrosinase is commercially available and has been used in almost all studies on tyrosinase inhibition, as well as has been applied as a potent and viable inhibitor for anti-pigmentation [61].

4.5. Other proteins

In addition to the previously mentioned, several proteins obtained from mushrooms have not yet been associated with any chemical structure category. A few examples will be referenced below.

Pleurostrin and eryngin are two proteins isolated from *P. ostreatus* and *P. eryngii* mushrooms with antibacterial and

antifungal activities. Eryngin inhibited the mycelial growth in *Fusarium oxysporum* and *Mycosphaerella arachidicola*, whereas pleurostrin inhibited these two fungi and *Physalospora piricola* [10]. Another protein, nebrodeolysin, a hemolysin from *Pleurotus nebrodensis*, showed intense hemolytic activity upon rabbit erythrocytes and caused efflux of potassium ions from erythrocytes. Moreover, this hemolysin revealed potent cytotoxicity against a few human cancer cell lines Bre-04 (breast), Lu-04 (lung), HepG2 and induced apoptosis in L929 and HeLa cells. Besides, nebrodeolysin exhibited anti-HIV effects in CEM cell culture [173].

A bioactive protein, named PEP, was isolated from *P. eryngii*, which showed anti-inflammatory effects in LPS-stimulated RAW 264.7 macrophages by inhibiting the overproduction of proinflammatory mediators such as cytokines IL-1β and IL-6 and NO. These anti-inflammatory effects resulted from the suppression of inducible NOS expression and the inactivation of NF-κB and MAPK pathways [174]. This mechanism of action is important because NF-κB and MAPK are essential pathways in cancer therapy. This fact may be explained by their involvement in developing chemotherapy resistance, promoting tumour propagation, and the invasion of tumour cells [114].

4.6. Peptides

Bioactive peptides possess lower MW (<3 kDa), generally holding until 20 amino acid resides. These properties confer higher solubility and technological and functional properties, resulting in better biological properties [175]. These molecules may be associated with higher antioxidant potential due to their ability to interact and neutralize free radicals [175,176]. Some studies are focused on peptide mixtures of *G. lucidum* [177–180], *C. sinensis* [181], *Terfezia claveryi* and *A. bisporus*[182], and *Morchella esculenta* [183] and the results suggested high antioxidant capacity.

Regarding specific peptides, a peptide with the sequence WALKGYK was isolated from *Tricholoma matsutake* and showed high 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (50% at 10 mg/mL) [184]. Also, a polypeptide from *P. eriyngii* exhibited antioxidant potential through DPPH radical scavenging activity and other antioxidant methods [185]. *Kimatu* et al. [186] studied the antioxidant potential of *A. bisporus* protein hydrolysates. Several enzymes were tested, and the resultant hydrolysates were ultrafiltrated. The results indicated that the peptide fraction between 1 and 3 kDa from alcalase and pancreatin hydrolysis was very promising in terms of antioxidant activity [186].

Another bioactivity extensively associated with peptides is antihypertensive potential. High blood pressure results in several body dysfunctions and diseases, constituting a considerable problem in this century [187]. Typically, antihypertensive peptides possess between 2 and 12 amino acid resides [175,188]. Synthetic inhibitors for ACE have been associated with several side effects, which makes peptides from natural sources a promising option. Generally, peptides possess a high bioavailability and no negative impacts [187]. Competitive inhibition is the most reported mode of action for synthetic drugs and natural peptides, being characterized by competition for ACE active sites [187,189].

Two potential ACE inhibitory oligopeptides, described as AHEPVK and GPSMR, were identified from *Pleurotus cystidiosus*. Both peptides showed high ACE inhibitory effects with IC_{50} values of 62.8 and 277.5 μM, respectively. In the case of AHEPVK, its mode of action was determined, corresponding to a competitive inhibition [190]. Lau et al. isolated three peptides from *A. bisporus* with good antihypertensive potential [189]. Their sequences were AHEPVK, RIGLF and PSSNK, which exhibited IC_{50} values of 63 μM, 116 μM and 129 μM, respectively. The first two peptides exhibited a competitive mode of action, while the third one showed a non-competitive antihypertensive action [189]. Similarly, Wu et al. [191] isolated and characterized three peptides from *G. lucidum* with good antihypertensive properties. Their sequences were QLVP (IC_{50} = 127.9 μM), QLDL (IC_{50} = 151.5 μM) and QDVL (IC_{50} = 155.8 μM). In this case, QLVP showed a mixed-type mode of inhibition, while QLDL and QDVL were non-competitive and competitive, respectively [191].

Other bioactivities, such as antitumor and immunostimulatory, were also described for mushroom peptides [187]. Beyond antioxidant capacity, peptides isolated from *P. eryngii* showed antitumour effects in stomach, breast, and cervical cancer cell lines, coupled with immunostimulatory potential, promoting macrophage proliferation [185]. Additionally, peptides isolated from mushrooms also revealed activity against platelet aggregation [192] as well as antidiabetic and tyrosinase inhibitory activities [193].

4.7. Amino acids

Additionally, mushrooms are a good source of all nine essential amino acids (phenylalanine, lysine, isoleucine, leucine, valine, histidine, threonine, tryptophan, and methionine) required for human nutrition [13]. The mushroom amino acid composition is species-dependent, but generally, the limiting amino acids are sulfur-containing amino acids such as methionine and cysteine. The more abundant are arginine, aspartic acid, glutamine, glutamic acid, and valine [5,11]. Besides, substantial amounts of two unusual amino acids, γ-aminobutyric acid (GABA) and ornithine, have also been found in mushrooms [6,13]. In particular, these two amino acids are essential to human health due to their peculiar physiological activities: GABA acts as a neurotransmitter in the central nervous system by reducing neuron activity, while ornithine is a precursor in arginine synthesis [194].

In addition, amino acids are constantly assimilated to produce urea in the mushroom, contributing to its total nitrogen content [16]. Some amino acids in mushrooms also play a fundamental role at the sensory level, namely, flavour. In this sense, umami is a pleasant savoury flavour that occurs naturally in many foods, including mushrooms. Edible mushrooms have a peculiar umami taste attributed to the

presence of amino acids such as aspartic acid (Asp) and glutamic acid (Glu) and flavour 5'-nucleotides [195].

Interestingly, among the amino acids of mushrooms, ergothioneine, unusual betaine amino acid, stands out as a potent antioxidant[11,196].

4.7.1. Ergothioneine

Ergothioneine is an amino acid produced by certain fungi and bacteria to prevent oxidative stress in the mitochondria [11,197]. Ergothioneine is biologically synthesised from the amino acid histidine, with cysteine and methionine, which provide the sulphur and methyl groups, respectively. The 2-mercaptoimidazole side chain in the structure of the ergothioneine molecule is characterised by redox properties, which play an essential role in cellular function, and, consequently, participate in the protection and cellular redox homeostasis [196]. This mercapto group can be oxidized (losing an electron) to a disulfide, which could explain its ability to scavenge free radicals, being similar to an essential physiological antioxidant thiol named glutathione (GSH). Besides, the unique structure of ergothioneine makes it different from conventional sulfur-containing antioxidants, as this amino acid exists as a tautomer between its thiol and thione forms, with the latter predominating under physiological conditions. This particularity allows ERT not to oxidize and requires a more severe oxidative stress to oxidize when compared to other natural thiols [198]. This amino acid exists in an aqueous solution and at physiological pH, firstly in the thione rather than the thiol form [197].

Ergothioneine is suggested as a potent and effective antioxidant with the ability to associate with other antioxidants such as GSH and protect against mitochondrial oxidative stress. However, despite the great interest in its antioxidant potential, the knowledge about ergothioneine physiological functions is limited [198]. Indeed, it has been proposed that ergothioneine may help keep GSH levels in the presence of an oxidative charge, interacting with other cellular defence systems [11,197]. Beyond this, ergothioneine can: neutralise reactive oxygen and reactive nitrogen species (ROS/RNS); protect against ultraviolet (UV) radiation-induced damage; exhibit anti-inflammatory actions; inhibit the expression of vascular adhesion proteins; protect monocytes activity; inhibit myeloperoxidase activity; prevent lung and liver fibrosis; promote neuronal differentiation, among others cytoprotective abilities [11,212–215]. Thereby, this antioxidant, known as a longevity nutrient, plays essential physiological roles in human health and development and possibly in preventing and/or treating diseases. Some examples include neurological and cardiovascular disorders, pre-eclampsia, prevention of injury and fibrosis problems, and protection of skin cells from UV irradiation, among others [196]. However, despite the wide interest in its antioxidant potential, the physiological function and role in the disease of ergothioneine are limited.

Even though ergothioneine could accumulate in most cells and tissues in the body, early studies indicated that humans could not synthesise it. Therefore, the presence of ergothioneine in human blood depends on diet [199]. In this way, mushrooms are the primary dietary source of ergothioneine compared to any other food source. The ergothioneine is essentially accumulated in mushroom fruiting bodies and mycelia [7,11]. This amino acid can be found as a component of white button mushrooms [198]. Recently, Martinez-Medina et al. [11] reviewed that different edible mushrooms are rich in ergothioneine content, including *F. velutipes*, *L. edodes*, *P. cornucopiae* and *P. eryngii*. Besides, *A. bisporus* and *B. edulis* are also good sources of this amino acid [199,200]. Given its health benefits, ergothioneine is an important physiological antioxidant and cryoprotectant [11]. Therefore, Cheah and Halliwell [201] suggested that ergothioneine could be used to alleviate symptoms and improve the prognostic outcomes of COVID-19 patients, particularly in the elderly and those with underlying health problems.

5. CONCLUSION AND FUTURE PERSPECTIVES

Due to mushrooms' nutritional and health-promoting properties, they have been classified as "next-generation food". Although polysaccharides are the most explored bioactive group from macrofungi species, mushrooms also contain several proteins and peptides in their fruiting bodies and cultivated mycelium. Proteins are also a representative group in the mushroom structure, representing between 19 and 35% of mushroom content on a dry-weight basis. Significant biological and functional properties have been ascribed to these macromolecules, which increase their research interest and contribute to the increase of mushroom market value.

Protein is an essential macromolecule for the maintenance of body homeostasis. In this context, edible mushrooms provide a rich pool of bioactive proteins that can be used to solve biotechnological and medical problems. Additionally, bioactive mushroom proteins can also be used as nutrient-rich foods and in supplement and nutraceutical formulations. These proteins are lectins, FIPs, RIPs, enzymes (e.g., ribonucleases, laccases, tyrosinases), ergothioneine, and other proteins and peptides. The present review has summarised the possible health promotion and benefits of different protein groups in edible mushrooms.

Lectins are examples of these proteins with great potential for application in the health and medicine fields. They act as crucial tools for developing anticancer agents as well as potential biomarkers, immunomodulators, antivirals, antidiabetics, antimicrobials, and antioxidants. In the future, new strategies must be explored to maximise their bioavailability and effectiveness for pharmacological applications. Moreover, advances in genetic engineering may allow food and pharmaceutical industries to produce desirable mushroom strains on a large scale and with high quality [3]. Concerning FIPs, these proteins have a remarkable immunomodulatory activity, emphasising FIPs of the Fve type. This group of FIPs is highly interesting because of their anticancer, anti-inflammatory, and anti-allergic bioactivities. However, in general, it is still necessary to further study the biological potential of FIPs. Regarding RIPs, these can

irreversibly inactivate ribosomes and protein synthesis. At the same time, RIPs can exert antiproliferative, antimitogenic, and proapoptotic effects.

Moreover, mushrooms also contain enzymes, such as ribonuclease, tyrosinases, laccases, and proteases, which possess distinct health-promoting effects (e.g., antimicrobial, antiproliferative, and antiviral activities). Mushrooms are also a source of an important biological antioxidant, named ergothioneine, with possible health benefits and potential applications in foods. Two examples include its use as a preservative or in functional food formulations.

Although the high potential of mushroom proteins and peptides, it remains necessary to identify and characterise new macromolecules of macrofungi origin, which opens an opportunity for further investigation in this area. At the same time, it is mandatory to clarify some underlying biological mechanisms of mushroom proteins and peptides that remain unclear or unknown. Some approaches, such as genomic, transcriptomic, and proteomic at the cellular/molecular level or animal models, may allow the scientific community to overcome this gap [6,11]. Thus, in the near future, the contributions of these research studies coupled with the results of clinical trials would be helpful in the prevention and treatment of several human disorders and diseases [202], being of utmost importance for future innovations in supplements, nutraceuticals, and drug development.

LIST OF ABBREVIATIONS

Agaricus bisporus lectin (ABL); *Agrocybe aegerita* lectin (AAL); Angiotensin I-converting enzyme (ACE); Aqueous two-phase system (ATPS); Carbohydrate-binding site (CBS); Cervical cancer cells (HeLa); Caco-2 Colon cancer (Caco-2); Colon adenocarcinoma cells (SW-480); colorectal cancer cells (HCT116); Coronavirus disease 2019 (COVID-19); Enzyme-assisted extraction (EAE); Fungal immunomodulatory proteins (FIPs); Gamma-aminobutyric acid (GABA); Gastric cancer cells (SGC-790 and BGC-823); Glutathione (GSH); Glutathione peroxidase (GPx); Hepatitis B virus (HBV); High hydrostatic pressure (HPP); High voltage electrical discharges (HVED); Human breast adenocarcinoma cells (MCF-7); Human breast cancer cell (Bre-04); Human colon cancer (HT-29); Human colon carcinoma cells (COLO 320); Human hepatocarcinoma cells (HepG2); Human immunodeficiency virus (HIV); Human immunodeficiency virus reverse transcriptase (HIV-RT); Human lung cancer cell (Lu-04), Interferon-gamma (IFN-γ); L-3,4-dihydroxyphenylalanine (L-DOPA); Leukaemia cells (HL-60, L1210 and M1); Mannose-binding lectins (MBL); N-acetylgalactosamine (GalNAc); Microwave-assisted extraction (MAE); N-acetyl-D-glucosamine (GlcNAc); Neuroblastoma cells (SH-SY5Y); Nitric oxide (NO); Non-nucleoside (NNRTIs); Nucleoside (NRTIs); *Pleurotus florida* lectin (PFL); *Pleurotus ostreatus* lectin (POL); Polyphenol oxidase (PPO); Pulsed electric field extraction (PEF); Subcritical water extraction (SWE); Reactive oxygen species (ROS); Ribonucleases (Rnases); Ribosome-inactivating proteins (RIPs); Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); Tool-like (TLR); Tumour necrosis factor-α (TNF-α); Ubiquitin and ubiquitin-like proteins (Ubls); Ultrasound-assisted extraction (UAE); Ultraviolet (UV); Trichloroacetic acid (TCA).

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CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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