Exploring the therapeutic potential of edible mushrooms: antioxidant and anti-inflammatory properties of *Agaricus bisporus* and *Pleurotus ostreatus* extracts

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Received: September 19, 2025 DOI: 10.14295/bjs.v5i1.826

Accepted: December 30, 2025 URL: https://doi.org/10.14295/bjs.v5i1.826

Abstract

This study comparatively analyzed the protein profiles, antioxidant potential, and anti-inflammatory activities of *Agaricus bisporus* (button mushroom) and *Pleurotus ostreatus* (oyster mushroom). Proteins were extracted, purified via ammonium sulfate precipitation and dialysis, and quantified using the Lowry assay. SDS-PAGE analysis revealed distinct protein bands, particularly in the 11-17 kDa range, within fractions exhibiting the most promising bioactivities. Both mushroom species demonstrated significant total antioxidant capacity (TAC) via the phosphomolybdate assay, with notably high values observed in the *P. ostreatus* 30% (1.52 mg mL AAE^{-1}), *P. ostreatus* 70% (0.85 mg mL AAE), and *A. bisporus* 70% (1.6 mg mL AAE^{-1}) precipitation fractions, as well as crude extracts. For anti-inflammatory activity, evaluated by red blood cell (RBC) hemolysis inhibition, all extracts and fractions showed anti-hemolytic effects. The *P. ostreatus* 70% (72.15% inhibition), *P. ostreatus* 30% (69.62% inhibition), and *A. bisporus* 70% (68.35% inhibition) precipitation fractions displayed the highest efficacy. While oyster mushroom crude extract yielded a higher protein concentration (9.516 mg mL^{-1}) than *A. bisporus* mushroom (6.516 mg mL^{-1}), the study's focus remained on the functional activities of specific fractions. This research underscores the significant potential of both *A. bisporus* and *P. ostreatus* as natural sources of antioxidants and anti-inflammatory agents. The strong correlation between these high bioactivities and the presence of specific protein bands, particularly in the 11-17 kDa range within the most active fractions, emphasizes the crucial role of their protein components. Further investigation is warranted to isolate, characterize, and elucidate the mechanisms of action of these specific bioactive proteins for potential applications in functional foods, nutraceuticals, and pharmaceuticals.

Keywords: *Agaricus* genus, button mushroom, edible mushrooms, *Pleurotus* genus, protein extraction.

Explorando o potencial terapêutico de cogumelos comestíveis: propriedades antioxidantes e anti-inflamatórias de extratos de *Agaricus bisporus* e *Pleurotus ostreatus*

Resumo

Este estudo analisou comparativamente os perfis proteicos, o potencial antioxidante e as atividades anti-inflamatórias de *Agaricus bisporus* (cogumelo-botão) e *Pleurotus ostreatus* (cogumelo-ostra). As proteínas foram extraídas, purificadas por precipitação com sulfato de amônio e diálise e quantificadas pelo método de Lowry. A análise por SDS-PAGE revelou bandas proteicas distintas, especialmente na faixa de 11–17 kDa, presentes nas frações que exibiram as atividades biológicas mais promissoras. Ambas as espécies de cogumelos demonstraram capacidade antioxidante total (CAT) significativa pelo ensaio do fosfomolibdato, com valores notavelmente elevados observados nas frações de precipitação a 30% de *P. ostreatus* (1,52 mg mL EAA^{-1}), a 70% de *P. ostreatus* (0,85 mg mL EAA^{-1}) e a 70% de *A. bisporus* (1,6 mg mL EAA^{-1}), bem como nos extratos brutos. Quanto à atividade anti-inflamatória, avaliada por meio da inibição da hemólise de eritrócitos (RBC),

todos os extratos e frações apresentaram efeito anti-hemolítico. As frações de precipitação a 70% de *P. ostreatus* (72,15% de inibição), a 30% de *P. ostreatus* (69,62% de inibição) e a 70% de *A. bisporus* (68,35% de inibição) apresentaram as maiores eficácias. Embora o extrato bruto de *P. ostreatus* tenha apresentado maior concentração proteica (9,516 mg mL^{-1}) em comparação ao de *A. bisporus* (6,516 mg mL^{-1}), o foco do estudo concentrou-se nas atividades funcionais de frações específicas. Esta pesquisa destaca o significativo potencial de *A. bisporus* e *P. ostreatus* como fontes naturais de antioxidantes e agentes anti-inflamatórios. A forte correlação entre as elevadas atividades biológicas e a presença de bandas proteicas específicas, particularmente na faixa de 11–17 kDa nas frações mais ativas, enfatiza o papel crucial de seus componentes proteicos. Investigações adicionais são necessárias para isolar, caracterizar e elucidar os mecanismos de ação dessas proteínas bioativas específicas, visando aplicações potenciais em alimentos funcionais, nutracêuticos e produtos farmacêuticos.

Palavras-chave: gênero *Agaricus*, cogumelo-botão, cogumelos comestíveis, gênero *Pleurotus*, extração de proteínas.

1. Introduction

Edible mushrooms have been recognized for centuries as valuable sources of nutrition and medicinal compounds (Kumar et al., 2021; Asssemie; Abaya, 2022; Ionescu et al., 2025). Among the most popular and widely cultivated species are the button mushroom (*Agaricus bisporus*) and the oyster mushroom (*Pleurotus ostreatus*), both renowned for their culinary versatility and potential therapeutic properties (Niedermeyer et al., 2005; Araújo et al., 2025; Jiang et al., 2025). Edible mushrooms represent a valuable source of nutrients and bioactive compounds, characterized by a favorable nutritional profile. Specifically, they exhibit a high protein content, featuring a comprehensive array of essential amino acids, while maintaining a low lipid content (Barros et al., 2008; Losoya-Sifuentes et al., 2025). Furthermore, mushrooms provide substantial carbohydrates and dietary fiber, alongside nutritionally relevant levels of vitamins (B1, B2, B12, C, and D) and minerals (Ca, K, Mg, Na, P, Cu, Fe, Mn, and Se) (Mattila et al., 2001; Singh et al., 2025).

Recent investigations have elucidated that numerous bioactive compounds derived from mushrooms exhibit a diverse range of health-promoting activities. These include, but are not limited to, anti-inflammatory, antitumor, antibacterial, antioxidant, and antiviral effects, alongside antiallergic, antiatherogenic, hypoglycemic, and hematological properties (Cheung, 2008; Zhang et al., 2025; Bai et al., 2025). Consequently, there is a growing interest in identifying novel natural sources of bioactive compounds that can modulate and enhance human physiological systems. This has led to an increased focus on the functional components of edible mushrooms. Within this context, various mushroom-derived substances possessing potent bioactivities have been isolated and disseminated globally (Mattila et al., 2001; Zhang et al., 2025; Ma et al., 2025).

The medicinal properties of *P. ostreatus* are attributed to its bioactive compounds, which include polysaccharides, proteins, peptides, glycoproteins, nucleosides, terpenoids, lectins, and phenolic compounds. These compounds have been shown to exhibit a wide range of biological activities, including antioxidant, anti-inflammatory, antidiabetic, antiviral, antibacterial, and anticancer properties (Deepalakshmi; Sankaran, 2014; Irfan et al., 2022; Waktola; Temesgen, 2020; Masri et al., 2017). *Agaricus bisporus* contains secondary metabolites with significant antioxidant and anticancer activities. These compounds help in neutralizing free radicals and may reduce the risk of cancer development (Zhang et al., 2024). The mushroom diet also promotes increased fecal excretion of cholesterol and bile acids, contributing to its hypocholesterolemic effects and potential cardiovascular benefits (Goyal; Grewal 2024).

This study will thus provide a detailed comparative analysis of the protein profiles, antioxidant potential, and health-promoting activities of *Agaricus bisporus* and *Pleurotus ostreatus*, providing a basis for future research.

2. Materials and Methods

2.1 Sample collection

Specimens of two distinct fungal fruiting bodies were procured from a local market in Indore. Upon acquisition, the samples were individually contained within sterile plastic bags. They were then subjected to rigorous washing with sterile distilled water. Subsequently, under aseptic conditions, each fruiting body was longitudinally sectioned using a sterile scalpel. Based on comprehensive morphological analysis, supplemented by molecular characterization, the specimens were identified as *P. ostreatus* and *A. bisporus*.

2.2 Extraction of mushrooms' protein

For protein extraction, 4.5 g of each mushroom sample (*P. ostreatus* and *A. bisporus*) was pulverized using a mortar and pestle and subsequently homogenized in lysis buffer. The lysis buffer consisted of 10 mM EDTA, 0.1% SDS, and 0.4% β-mercaptoethanol, all dissolved in 0.1 M phosphate buffer at pH 7.4. The homogenate was incubated for a period of 5-15 min, followed by centrifugation (Petrovska et al., 2004). The resulting supernatant, containing the crude protein extract, was collected.

2.3 Protein purification

Protein purification was conducted using a two-step process: Ammonium Sulfate Precipitation (ASP) and Dialysis.

a) Ammonium sulfate precipitation (ASP)

ASP was employed to fractionate proteins based on their differential solubility in increasing concentrations of ammonium sulfate. This technique leverages the "salting out" effect, whereby high salt concentrations decrease protein solubility by competing for water molecules, leading to protein precipitation (Wingfield, 1998). Fractionation was performed at three distinct saturation levels: 30%, 50%, and 70%, with all procedures maintained at 4 °C.

b) Dialysis

Dialysis was performed to remove residual ammonium sulfate from the precipitated protein pellets. Each pellet was suspended in an appropriate dialysis buffer and placed in dialysis tubing. The tubing was immersed in a large volume of dialysis buffer, which was regularly exchanged over a 24-hour period (Andrew et al., 2001). This process facilitated the removal of ammonium sulfate through osmotic diffusion, resulting in a purified protein solution.

2.4 Total antioxidant capacity (TAC) by phosphomolybdate assay

The Total Antioxidant Capacity (TAC) of the mushroom extracts was determined using a phosphomolybdate assay (Patel et al., 2024). This assay is based on the reduction of Mo (VI) to Mo (V) by antioxidants in an acidic medium, resulting in the formation of a bluish-green complex. The intensity of this color, measured spectrophotometrically, is proportional to the antioxidant capacity. A standard curve was generated using ascorbic acid (1 mg mL). Absorbance was measured at 765 nm. The ascorbic acid standard curve ranged from 25 µg to 125 µg. Results were expressed as Ascorbic Acid Equivalents (AAE) per mg of extract^{-1}.

2.5 Investigation for anti-inflammatory property

This assay evaluates the protective effect of mushroom extracts against heat-induced erythrocyte membrane destabilization and subsequent hemolysis (Patel et al., 2023). Erythrocyte rupture, observed when a red blood cell (RBC) suspension is heated at 54 °C, is inhibited by antioxidants that stabilize the membrane. The extent of hemolysis is determined by measuring the absorbance of released hemoglobin. A lower absorbance indicates greater inhibition of hemolysis, reflecting the protective effect of the extract or standard.

2.6 Protein quantification

Protein quantification was performed using the Lowry assay (Patel et al., 2024). A standard calibration curve was generated using Bovine Serum Albumin (BSA) at concentrations ranging from 100 µg to 500 µg. The absorbance was recorded at 670 nm. Protein concentrations in the *P. ostreatus* and *A. bisporus* extracts were subsequently determined by comparison to this calibration curve. The resulting absorbance values were plotted against corresponding BSA concentrations to construct the calibration curve.

2.7 Protein profiling using SDS-PAGE

Protein samples were subjected to SDS-PAGE, following Laemmli's method (Laemmli, 1970), to determine their molecular weight profiles. Equal protein quantities, along with an 11-100 kDa molecular weight marker, were loaded onto 13% polyacrylamide gels. The gels consisted of a 13% separating gel (pH = 8.8) and a 4% stacking gel (pH = 6.8), both containing polyacrylamide, bisacrylamide, and SDS. Polymerization was initiated using ammonium persulfate (APS) and TEMED. The separating gel was cast first, followed by the stacking gel, which

included wells for sample loading. Samples were prepared by adding a loading dye containing bromophenol blue, SDS, glycerol, and β-mercaptoethanol, followed by boiling. The tracking dye, bromophenol blue, was monitored to determine the completion of the run. Following electrophoresis, the gels were stained with Coomassie Brilliant Blue R-250 for 1 h and subsequently destained using a solution of 30% methanol and 10% acetic acid.

3. Results

3.1 Extracts' yield

This table provides a preliminary overview of the differences between *P. ostreatus* and *A. bisporus* mushrooms in terms of product yield and appearance (Table 1).

Table 1. Preliminary overview of product yield and appearance for *P. ostreatus* and *A. bisporus* mushrooms.

Sample	Volume obtained	Appearance	Percentage yield
P. ostreatus	21 mL	White & semi-transparent	84%
A. bisporus	23 mL	Light yellow & semi-transparent	92%

Note: Expressed on a wet weight basis. Source: Authors, 2025.

3.2 Protein purification

This experiment details the purification of proteins from two mushroom species, *P. ostreatus* and *A. bisporus*, utilizing a combination of ammonium sulfate precipitation (ASP) and dialysis. The goal was to isolate and partially purify proteins from these mushrooms, leveraging differences in protein solubility and size. Three different concentrations of ammonium sulfate were employed: 30%, 50%, and 70%. These percentages represent the saturation level of ammonium sulfate in the solution (Table 2).

Table 2. Protein fractionation from *P. ostreatus* and *A. bisporus* mushroom extracts using ammonium sulfate precipitation.

Ammonium sulfate concentration	Description of Fractionation
30%	Precipitated the least soluble proteins, forming the first fraction.
50%	Precipitated proteins with intermediate solubility, yielding the second fraction.
70%	Precipitated the most soluble proteins, resulting in the third and final fraction.

Source: Authors, 2025.

3.3 Total antioxidant capacity (TAC)

The phosphomolybdate assay was used to determine the TAC. This assay measures the reduction of phosphomolybdate by antioxidants in the sample, resulting in the formation of a blue-green phosphomolybdenum complex. A calibration curve of ascorbic acid is constructed by plotting the absorbance at 670 nm against known concentrations of ascorbic acid (Figure 1). The resulting straight-line equation is used to determine the TAC of the mushroom extracts.

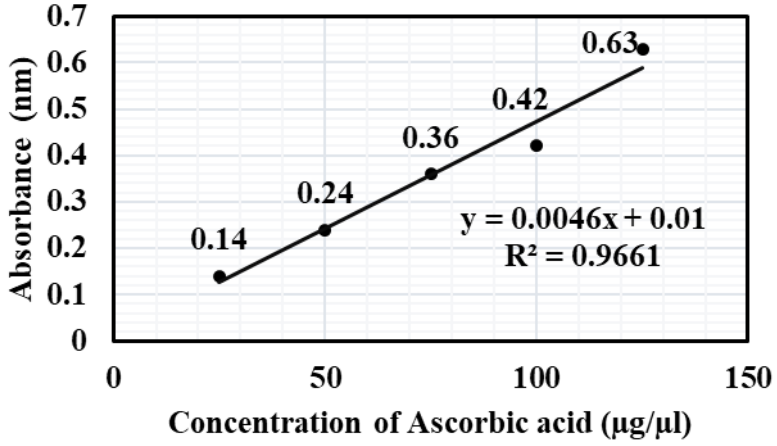

Figure 1. Standard curve of ascorbic acid for total antioxidant capacity (TAC) determination using the phosphomolybdate assay. Source: Authors, 2025.

Table 3 shows variations in TAC among the different extracts and fractions. The crude extracts generally exhibited higher TAC compared to the fractionated samples. The results indicate that both oyster and button mushroom extracts possess antioxidant activity, as evidenced by their ability to reduce phosphomolybdate. The different TAC values suggest variations in the antioxidant potential of the extracts and fractions, possibly due to differences in the composition and concentration of antioxidant compounds. The high TAC values indicate that these extracts can effectively reduce reactive oxygen species (ROS), peroxides, and superoxide radicals, which are implicated in oxidative stress.

Table 3. Total antioxidant capacity (mg mL^{-1}) of crude and fractionated *P. ostreatus* and *A. bisporus* mushroom extracts determined by the phosphomolybdate assay.

S.no.	Sample	Absorbance at 765 nm	Total Antioxidant Capacity
1.	*P. ostreatus* crude	0.18	1.84 mg mL
2.	*P. ostreatus* 30% precipitation fraction	0.15	1.52 mg mL
3.	*P. ostreatus* 50% precipitation fraction	0.12	1.15 mg mL
4.	*P. ostreatus* 70% precipitation fraction	0.09	0.85 mg mL
5.	*A. bisporus* crude	0.19	1.95 mg mL
6.	*A. bisporus* 30% precipitation fraction	0.13	1.3 mg mL
7.	*A. bisporus* 50% precipitation fraction	0.18	1.8 mg mL
8.	*A. bisporus* 70% precipitation fraction	0.16	1.6 mg mL

Source: Authors, 2025.

3.4 In-vitro anti-hemolytic activity

The experiment employed a hemolysis assay, a standard method for evaluating the protective effect of substances against cell membrane damage. Red blood cells (RBCs) served as a model system, as their lysis is measured by the release of hemoglobin, which absorbs light. Crude extracts of *P. ostreatus* and *A. bisporus* mushrooms were analyzed, along with their fractionated components (30%, 50%, and 70% precipitation) obtained from ammonium sulfate precipitation. A known anti-inflammatory drug was also included as a positive control. All mushroom extracts and their fractions were tested at a concentration of 20 µL, while the drug was used at 100 µL. The extent of hemolysis was quantified by measuring the absorbance of the supernatant at a specific wavelength (nm), which is proportional to the amount of released hemoglobin.

Table 4. Absorbance (nm) values of supernatant in the anti-hemolytic assay, demonstrating the protective effect of crude and ammonium sulfate fractionated *P. ostreatus* and *A. bisporus* mushroom extracts on red blood cell lysis.

Extracts	**Concentration (µL)**	**Absorbance (nm)**
Control	20 µL	0.79
Drug	100 µL	0.33
P. ostreatus crude	20 µL	0.23
P. ostreatus 30% precipitation fraction	20 µL	0.24
P. ostreatus 50% precipitation fraction	20 µL	0.27
P. ostreatus 70% precipitation fraction	20 µL	0.22
A. bisporus crude	20 µL	0.20
A. bisporus 30% precipitation fraction	20 µL	0.53
A. bisporus 50% precipitation fraction	20 µL	0.28
A. bisporus 70% precipitation fraction	20 µL	0.25

Source: Authors, 2025.

All the mushroom extracts and fractions showed lower absorbance values compared to the control, suggesting they inhibited RBC lysis to some degree (Table 5). All mushroom extracts and fractions showed positive hemolysis inhibition (Table 5), indicating their anti-inflammatory potential. Among these, the *P. ostreatus* 70% precipitation fraction exhibited the highest inhibition at 72%. The *P. ostreatus* 30% precipitation fraction also showed strong activity with 69% inhibition, closely followed by the Button 70% precipitation fraction at 68%. These results collectively suggest that these specific fractions are particularly promising sources of anti-inflammatory compounds.

Table 5. Percent inhibition of red blood cell (RBC) hemolysis by crude extracts and ammonium sulfate precipitation fractions (30%, 50%, and 70%) of *P. ostreatus* and *A. bisporus* mushrooms, indicating their anti-inflammatory potential.

Sample	**% Hemolysis inhibition**
P. ostreatus crude	70.88%
P. ostreatus 30% precipitation fraction	69.62%
P. ostreatus 50% precipitation fraction	65.82%
P. ostreatus 70% precipitation fraction	72.15%
A. bisporus crude	74.68%
A. bisporus 30% precipitation fraction	32.91%
A. bisporus 50% precipitation fraction	64.55%
A. bisporus 70% precipitation fraction	68.35%

Source: Authors, 2025.

3.5 Protein estimation

The Folin-Lowry test Figure 2 yielded a positive result for both *P. ostreatus* and *A. bisporus* mushroom extracts, indicated by the development of a blue color. This confirms the presence of proteins in both samples.

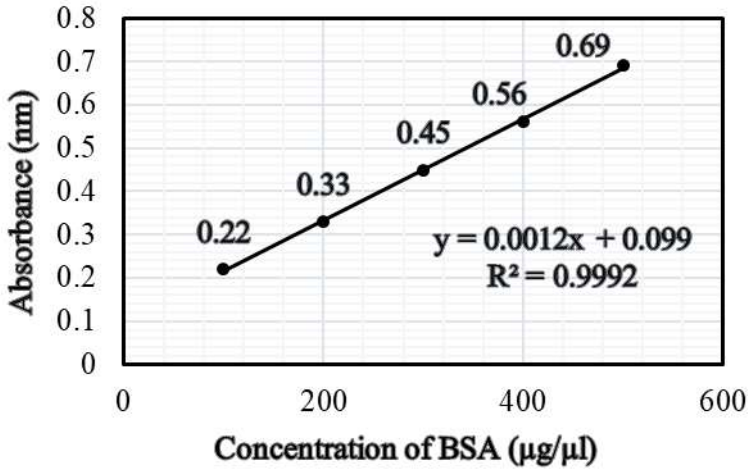

Figure 2. Standard curve of Bovine Serum Albumin (BSA) for protein quantification using the Lowry assay. Source: Authors, 2025.

The results in Table 6 indicate that *P. ostreatus* mushroom extracts generally have a higher protein concentration compared to *A. bisporus* mushroom extracts. The "crude" extracts (*P. ostratus* Crude and *A. bisporus* Crude) represent the initial extracts, while the 30%, 50%, and 70% ammonium sulfate precipitation fractions represent further processed or diluted samples. The decreasing protein concentrations in these fractions suggest that the processing steps (e.g., fractionation, purification) resulted in a reduction of protein content.

Table 6. Protein concentrations (mg mL^{-1}) of crude and fractionated *P. ostreatus* and *A. bisporus* mushroom extracts determined by the Lowry assay.

S.no.	Sample	Absorbance at 670 nm	Amount of protein
1.	*P. ostreatus* crude	0.67	9.516 mg mL
2.	*P. ostreatus* 30% precipitation fraction	0.55	7.516 mg mL
3.	*P. ostreatus* 50% precipitation fraction	0.46	6.016 mg mL
4.	*P. ostreatus* 70% precipitation fraction	0.38	4.68 mg mL
5.	*A. bisporus* crude	0.49	6.516 mg mL
6.	*A. bisporus* 30% precipitation fraction	0.67	4.75 mg mL
7.	*A. bisporus* 50% precipitation fraction	0.39	2.425 mg mL
8.	*A. bisporus* 70% precipitation fraction	0.22	1.008 mg mL

Source: Authors, 2025.

3.6 SDS-PAGE analysis

Fractions exhibiting high anti-inflammatory activity in the *in-vitro* anti-hemolytic activity assay were specifically selected for SDS-PAGE analysis. SDS-PAGE was then employed to analyze the protein profile, a technique that separates proteins based on their molecular weight. The protein profiles obtained from both *A. bisporus* and *P. ostreatus* mushroom extracts revealed distinct and well-resolved bands across a wide range of molecular weights. Specifically, in the *P. ostreatus* 30% precipitation fraction, the *P. ostratus* 70% precipitation fraction, and the *A. bisporus* 70% precipitation fraction, distinct protein bands were observed, all falling within the 11-17 kDa range (Figure 3). This indicates successful extraction of intact proteins and provides clear evidence of the diverse protein composition within both mushroom species. The presence of these prominent bands, particularly in the 11-17 kDa range, suggests that the integrity of the proteins was largely maintained throughout the extraction and purification process, allowing for effective visualization and characterization on the gel. This analysis confirms that valuable protein content, suitable for further investigation, was isolated from both *A. bisporus* and *P. ostreatus*.

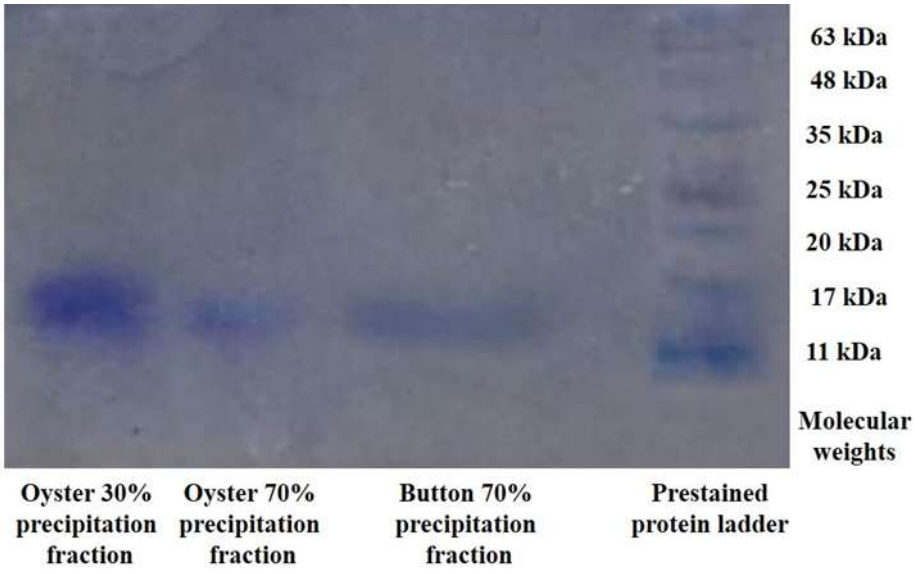

Figure 3. SDS-PAGE analysis of anti-inflammatory active fractions from *Pleurotus ostreatus* and *Agaricus bisporus* mushrooms (*P. ostreatus* 30% precipitation fraction, *P. ostreatus* 70% precipitation fraction & *A. bisporus* 70% precipitation fraction) along with the prestained protein ladder with indicated molecular weights (kDa). Source: Authors, 2025.

4. Discussion

In this study, we conducted a comparative analysis of the protein profiles, antioxidant capacity, and anti-inflammatory activities of *A. bisporus* and *P. ostreatus*. The initial extraction yielded a higher protein volume and percentage yield for A. bisporus, indicating greater extraction efficiency under the applied conditions. However, protein quantification using the Lowry assay revealed a higher protein concentration in the crude extract of *P. ostreatus*, highlighting the influence of extraction and purification steps on both protein yield and final concentration.

The total antioxidant capacity (TAC) assay confirmed that both species exhibit strong antioxidant potential. The 30% and 70% precipitation fractions of *P. ostreatus* and the 70% fraction of *A. bisporus*, as well as the crude extracts, displayed the highest TAC values. These results suggest the presence of highly effective antioxidant constituents capable of scavenging free radicals and mitigating oxidative stress. Variations observed among fractions likely reflect differences in the distribution and concentration of these bioactive compounds. These findings are consistent with previous studies highlighting the antioxidant properties and functional potential of edible mushrooms (Cheung, 2008; Zhang et al., 2024).

Anti-inflammatory activity, assessed by erythrocyte hemolysis inhibition, demonstrated that all extracts and fractions effectively protected red blood cells against lysis. Among the fractionated samples, the *P. ostreatus* 70% precipitation fraction showed the highest inhibition (>70%), followed by the *P. ostreatus* 30% fraction (≈ 69%) and the *A. bisporus* 70% fraction (≈68%). These results support the traditional medicinal use of mushrooms and reinforce their potential as natural sources of anti-inflammatory agents. The observed activities are consistent with previous reports describing anti-inflammatory compounds in mushrooms (Cheung, 2008; Deepalakshmi; Sankaran, 2014; Masri et al., 2017; Waktola; Temesgen, 2020; Irfan et al., 2022; Michalska et al., 2025).

SDS-PAGE analysis revealed well-resolved and distinct protein profiles for both species, characterized by sharp bands across a wide molecular weight range, indicating efficient extraction and preserved protein integrity. Of particular interest was the consistent presence of prominent protein bands in the 11–17 kDa range in the fractions exhibiting the highest anti-inflammatory activity (*P. ostreatus* 30% and 70%, and *A. bisporus* 70%). The clear visualization of these low-molecular-weight proteins, together with their strong bioactivities, supports their potential role as key contributors to the observed anti-inflammatory effects and identifies them as promising targets for future biochemical and functional investigations.

The differences observed in protein composition, antioxidant capacity, and anti-inflammatory activity between A.

bisporus and *P. ostreatus* likely reflect inherent variations in their chemical profiles, including the nature and abundance of specific bioactive compounds. The biological activities of *P. ostreatus* have been attributed to polysaccharides, proteins, and phenolic compounds, whereas *A. bisporus* is known to contain secondary metabolites with antioxidant and anticancer properties (Barros et al., 2008; Deepalakshmi; Sankaran, 2014; Irfan et al., 2022; Zhang et al., 2024). Additionally, the high nutritional value of both species—characterized by high-quality proteins, essential amino acids, vitamins, and minerals—may further contribute to their functional properties (Mattila et al., 2001; Rocha et al., 2025; Kumari et al., 2025; El-Maradny et al., 2025). Collectively, these results provide a strong foundation for future studies focused on isolating and characterizing the identified bioactive proteins, elucidating their mechanisms of action, and validating their efficacy through in vivo models.

5. Conclusions

This study makes a significant contribution to the growing body of evidence supporting the nutritional and medicinal value of edible mushrooms. Importantly, our results strongly indicate that the antioxidant and anti-inflammatory activities observed in *Agaricus bisporus* (button mushroom) and *Pleurotus ostreatus* (oyster mushroom) are largely associated with their protein components, as demonstrated by the distinct protein profiles obtained through SDS-PAGE. The 70% and 30% precipitation fractions of *P. ostreatus* and the 70% precipitation fraction of *A. bisporus* were particularly promising, exhibiting high anti-hemolytic activity with up to 70% inhibition and distinct protein bands in the 11–17 kDa range.

Therefore, *Agaricus bisporus* and *Pleurotus ostreatus* represent promising sources of natural antioxidants and anti-inflammatory agents, with potential applications in functional foods, nutraceuticals, and pharmaceutical products. Further studies are required to fully elucidate their therapeutic potential, particularly through the isolation, identification, and characterization of low-molecular-weight bioactive proteins, as well as through in vivo investigations aimed at translating these findings into measurable health benefits for human populations.

6. Acknowledgments

We acknowledge the research institutes Mata Jijabai Girls College, Indore, India, and the Institute of Innovative Learning and Research Academy, Indore, Madhya Pradesh, India.

7. Authors' Contributions

Preeti Shrivastava: conceptualization, methodology, investigation, writing – original draft, writing – review & editing, project administration, publication. *Nirali Ali*: methodology, investigation, writing – original draft. *Sanjana Patel*: writing – original draft, writing – review & editing, and publication. *Rashmi Limaye*: methodology, investigation, writing – original draft, and publication. *Payal Puri*: conceptualization, methodology, investigation, writing – original draft, writing – review & editing, and publication.

8. Conflicts of Interest

No conflicts of interest.

9. Ethics Approval

Not applicable.

10. References

Andrew, S. M., Titus, J. A., & Zumstein, L. (2001). Dialysis and concentration of protein solutions. *Current Protocols in Toxicology*, 10, A-3H. https://doi.org/10.1002/0471140856.txa03hs10

Araújo, P. L., Araújo, E. S., Barreto, E. M. A., Alves, J. L. B., Souza, K. M., Freire, M. P. L., & Souza, R. M. P. (2025). *Pleurotus* mushrooms in nutrition and health: Clinical and preclinical insights for nutraceutical development. *Comprehensive Reviews in Food Science and Food Safety*, 24(5), e70279. https://doi.org/10.1111/1541-4337.70279

Assemie, A., & Abaya, G. (2022). The effect of edible mushroom on health and their biochemistry. *International*

Journal of Microbiology, 1. https://doi.org/10.1155/2022/8744788

Bai, Y., Liang, F., Yang, Y., Guan, L., & Ma, H. (2025). Polysaccharides from edible fungi spent mushroom substrates: A review of their extraction, purification, structural characteristics, and biological activities. *International Journal of Biological Macromolecules*, 330(1), 147925. https://doi.org/10.1016/j.ijbiomac.2025.147925

Cheung, P. C. K. (2008). Mushrooms as Functional Foods. Wiley, A John Wiley & Sons, INC., Publication, 227 p.

Deepalakshmi, K., & Sankaran, M. (2014). *Pleurotus ostreatus*: an oyster mushroom with nutritional and medicinal properties. *Journal of Biochemical Technology*, 5(2), 718-726.

El-Maradny, Y. A., Abouakkada, A. S., Abbass, A. A. G., Abaza, A. F., & El-Fakharany, E. M. (2025). Prebiotic properties and antioxidant effect of crude extracts and polysaccharides from *Agaricus bisporus* and *Pleurotus ostreatus* mushrooms. *Scientific Reports*, 15. https://doi.org/10.1038/s41598-025-16152-9

Goyal, R. K., & Grewal, R. B. (2024). Effect of feeding *Agaricus bisporus* (white button) mushroom on serum and liver cholesterol and excretion of cholesterol and bile acids in rats. *Journal of Scientific Research*, 16(2), 579-587. https://doi.org/10.3329/jsr.v16i2.70098

Ionescu, M., Dincă, M-N., Ferdes, M., Zăbavă, B-Ș., Paraschiv, G., & Moiceanu, G. (2025). Proteins from edible mushrooms: Nutritional role and contribution to well-being. *Foods*, 14(18), 3201. https://doi.org/10.3390/foods14183201

Irfan, F. H., Shafiq, S. A., Afroz, S., Saleem, A., & Ammar, M. (2022). Medicinal importance of *Pleurotus* species (Oyster Mushroom): A review. *Interdisciplinary Journal and Hummanity*, 1(5), 29-34.

Jiang, N., Xu, S., & Li, C. (2025). Research progress on pharmacological activity of *Agaricus bisporus*: A review. *Foods Bioscience*, 73, 107763. https://doi.org/10.1016/j.fbio.2025.107763

Kumar, K., Mehra, R., Guiné, R. P. F., Lima, M. J., Kumar, N., Kaushik, R., Ahmed, N., Yadav, A. N., & Kumar, H. (2021). Edible mushrooms: A comprehensive review on bioactive compounds with health benefits and processing aspects. *Foods*, 10(12), 2996. https://doi.org/10.3390/foods10122996

Kumari, M., Poonia, M. K., & Pawariya, V. (2025). Button (*Agaricus bisporus*) and oyster mushrooms (*Pleurotus ostreatus*): Cultivation and its value addition: A overview. *International Journal of Horticulture and Food Science*, 7(5), 38-43. https://www.doi.org/10.33545/26631067.2025.v7.i5a.294

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680-685. https://doi.org/10.1038/227680a0

Lindequist, U., Niedermeger, T. H. J., & Jülich, W-D. (2005). The pharmacological potential of mushrooms. *Evidence-Based Complementary and Alternative Medicine*, 2, 285-299. https://doi.org/10.1093/ecam/neh107

Losoya-Sifuentes, C., Cruz, M., Rocha-Pizaña, M. del R., Loredo-Treviño, A., & Belmares, R. (2025). Edible mushrooms: a nutrient-rich ingredient for healthier food products – A review. *Current Nutrition Reports*, 14. https://doi.org/10.1007/s13668-024-00605-0

Ma, G., Li, X., Tao, Q., Ma, S., Du, H., Hu, Q., & Xiao, H. (2025). Impacts of preparation technologies on biological activities of edible mushroom polycaccharides – novel insights for personalized nutrition achievement. *Critical Reviews in Food Science and Nutrition*, 65(15), 2898-2920. https://doi.org/10.1080/10408398.2024.2352796

Masri, H. J., Maftoun, P., Malek, R. A., Boumehira, A. Z., Pareek, A., Hanapi, S. Z., Ling, O. M., & El Enshasy, H. A. (2017). The edible mushroom *Pleurotus* spp.: II. Medicinal Values. *International Journal of Biotechnology for Wellness Industries* 6(1), 1-11. https://doi.org/10.6000/1927-3037.2017.06.01.1

Mattila, P., Könkö, K., Eurola, M., Pihlava, J-M., Astola, J., Vahteristo, L., Hietaniemi, V., Kumpulainen, J., Valtonen, M., & Piironen, V. (2001). Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. *Journal of Agricultural and Food Chemistry*, 49(5), 2343-2348. https://doi.org/10.1021/jf001525d

Michalska, A., Sierocka, M., Drzewiecka, B., & Świeca, M. (2025). Antioxidant and anti-inflammatory properties of mushroom-based food additives and food fortified with them-current status and future perspectives. *Antioxidants*, 14(5), 519. https://doi.org/10.3390/antiox14050519

Patel, S., Dey, R., Verma, K., Deshbhratar, R., Maru, K. K., Sharma, P., Limaye, R., & Puri, P. (2023). Comparative analysis of antioxidant and anti-inflammatory activities of red, blue, and black tea for health benefits. *Brazilian Journal of Science*, 2(4), 76-89. https://doi.org/10.14295/bjs.v2i4.299

Patel, S., Sharma, P., Supat, M., Mali, S., Bafna, A., Rangwala, T., Limaye, R., & Puri, P. (2024). Investigation of antioxidant potential of black beans due to phytochemical and globulin content for nutraceutical application. *Defence Life Science Journal*, 9, 326-334.

Petrovska, B. B., Panov, S., Zafirovska, D. R., & Kulevanova, S. (2004). Electrophoretic study of mushroom proteins. *Journal of Food Agriculture and Environment*, 2, 148-152.

Rocha, E. M., Ávila, S., Lima, J. J., Silva, R. S. A., Mathias, Á. L., Bacila, D. M., & Jorge, R. M. M. (2025). Nutritional value and antioxidant activity of *Agaricus bisporus*, *Lentinula edodes* and *Pleurotus ostreatus* mushrooms. *Anais da Academia Brasileira de Ciências*, 97(4), e20241454. https://doi.org/10.1590/0001-3765202520241454

Santos-Buelga, M. T., Cruz, T., Baptista, P., Estevinho, L. M., & Ferreira, I. C. F. R. (2008). Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food and Chemistry Toxicology*, 46(8), 2742-2747. https://doi.org/10.1016/j.fct.2008.04.030

Singh, A., Saini, R. K., Kumar, A., Chawla, P., & Kaushik, R. (2025). Mushrooms as nutritional powerhouses: A review of their bioactive compounds, health benefits, and value-added products. *Foods*, 14(5), 741. https://doi.org/10.3390/foods14050741

Waktola, G, & Temesgen, T. (2020). Pharmacological activities of oyster mushroom (*Pleurotus ostreatus*). *Novel Research in Microbiology Journal*, 4, 688-695.

Wingfield, P. (1998). Protein precipitation using ammonium sulfate. *Current Protocols in Protein Science*, 13, A-3F. https://doi.org/10.1002/0471140864.psa03fs13

Zhang, S., Gantumur, M-A., He, Y., Bilawal, A., Jiang, Z., & Yang, Y. (2025). Characterization and comparison of chemical compositions and biological activities of an edible mushroom (*Lactarius volemus*) of three different sizes. *Food Chemistry*, 464(2), 141690. https://doi.org/10.1016/j.foodchem.2024.141690

Zhang, M., Zhao, L., Tang, F., Gao, J., & Qi, J. (2024). Chemical structures, biological activities, and biosynthetic analysis of secondary metabolites from *Agaricus* mushrooms: A review. *Journal of Agricultural and Food Chemistry*, 72(22), 12387-12397. https://doi.org/10.1021/acs.jafc.4c01861

Funding

Not applicable.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.