

# Innovations in Extraction and Application of Antioxidants from *Agaricus bisporus* for Sustainable Development

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**Abstract:** The increasing demand for natural antioxidants has intensified research into edible mushrooms as sustainable sources of bioactive compounds. *Agaricus bisporus* (white button mushroom) is widely consumed and recognized for its nutritional and medicinal attributes, particularly its antioxidant potential. The present study aimed to evaluate and compare the antioxidant activity of *A. bisporus* extracts obtained using different solvent systems, with an emphasis on environmentally benign extraction approaches. Cold solvent extraction was employed using methanol, distilled water, and chloroform, minimizing thermal degradation and energy consumption. Antioxidant capacity was assessed using the phosphomolybdate assay, with ascorbic acid as the reference standard. The methanolic extract exhibited the highest total antioxidant capacity (TAC), recording 43.09 µg at 24 h and 42.09 µg at 48 h, while aqueous extracts demonstrated increased antioxidant activity with prolonged extraction, reaching a maximum TAC of 42.45 µg at 72 h. In contrast, chloroform extracts showed minimal antioxidant activity across all time points. These findings confirm that polar, low-toxicity solvents are more effective for extracting antioxidant compounds from *A. bisporus*, aligning with principles of green chemistry and sustainable development. The study highlights the potential of *A. bisporus* as a renewable, eco-friendly source of natural antioxidants suitable for functional food and nutraceutical applications, supporting sustainable resource utilization and reduced dependence on synthetic antioxidants.

**Keywords:** *Agaricus bisporus*; natural antioxidants; green extraction; phosphomolybdate assay; sustainable development; edible mushrooms

## 1. Introduction

Oxidative stress, caused by an imbalance between reactive oxygen species (ROS) and the antioxidant defense system, plays a critical role in the onset of several chronic and degenerative diseases, including cancer, diabetes, cardiovascular disorders, and neurodegenerative conditions (Rajasekar & Jeyaprakash, 2015). Antioxidants neutralize free radicals and prevent oxidative damage by donating electrons or hydrogen atoms, thereby maintaining cellular homeostasis (Shimada *et al.*, 1992). Although synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been widely used in the food industry, their potential carcinogenic effects have raised safety concerns, leading to increased interest in natural antioxidant sources (Kim *et al.*, 2011).

Edible mushrooms have gained significant attention due to their dual role as nutritious foods and sources of bioactive compounds with therapeutic potential (Singh, 2017). Among them, *Agaricus bisporus* is the most extensively cultivated mushroom globally and is valued for its pleasant flavor, high digestibility, and rich nutritional profile (Usman *et al.*, 2021). This species contains a variety of antioxidant compounds, including phenolics, flavonoids, ergothioneine, vitamins, and selenium, which contribute to its health-promoting properties (Mattila *et al.*, 2001; Dubost *et al.*, 2007).

Several studies have reported strong correlations between phenolic content and antioxidant activity in *A. bisporus*, suggesting that phenolic compounds are the primary contributors to its antioxidant potential (Barros *et al.*, 2008;

Kim, 2008). Methanolic and ethanolic extracts of *A. bisporus* have been shown to exhibit higher antioxidant activities compared to non-polar solvent extracts, emphasizing the importance of solvent selection in extraction efficiency (Ramirez-Anguiano *et al.*, 2007; Jagadeeshraman *et al.*, 2018). From a sustainability perspective, extraction techniques that employ low energy input, reduced toxicity, and minimal environmental impact are essential for developing eco-friendly antioxidant products (El-Ramady *et al.*, 2022).

The present study was designed to evaluate the antioxidant activity of *A. bisporus* using different solvent systems through a cold extraction method, emphasizing sustainability and green chemistry principles. The specific objective was to compare methanolic, aqueous, and chloroform extracts in terms of total antioxidant capacity using the phosphomolybdate assay.

## 2. Materials and Methods

### Sample Collection

Fresh fruiting bodies of *Agaricus bisporus* (400 g) were procured from a local market in Bilaspur, Chhattisgarh, India, and stored at 4 °C prior to analysis to preserve bioactive compounds (Singh, 2017).

### Sample Preparation and Extraction

The mushroom samples were washed thoroughly to remove surface impurities and air-dried. Dried samples were finely powdered and subjected to cold solvent extraction to reduce thermal degradation and energy consumption. Five grams of

mushroom powder were soaked separately in 150 mL of methanol, distilled water, and chloroform for 72 h. The mixtures were centrifuged at 12,000 rpm for 15 min, filtered using Whatman No. 4 filter paper, and stored at  $-20^{\circ}\text{C}$  until analysis.

### Determination of Antioxidant Activity

Total antioxidant capacity (TAC) was evaluated using the phosphomolybdate assay, which measures the reduction of Mo(VI) to Mo(V) by antioxidant compounds, forming a green phosphate/Mo(V) complex (Rajasekar & Jeyaprakash, 2015). The reagent solution consisted of sodium phosphate (28 mM), ammonium molybdate (4 mM), and sulfuric acid (0.6 mM). Mushroom extracts (0.1 mL) were mixed with 1 mL of reagent solution and incubated at  $90\text{--}95^{\circ}\text{C}$  for 60 min. After cooling, absorbance was measured at 695 nm using a UV-Vis spectrophotometer. Ascorbic acid was used as the standard.

### Results and Discussion

The antioxidant activity of *A. bisporus* extracts varied significantly depending on solvent polarity and extraction duration. Methanolic extracts demonstrated the highest TAC values at 24 h (43.09  $\mu\text{g}$ ) and 48 h (42.09  $\mu\text{g}$ ), indicating efficient extraction of phenolic antioxidants in polar organic solvents. These findings are consistent with earlier reports highlighting methanol as an effective solvent for antioxidant extraction from mushrooms (Barros *et al.*, 2008; Jagadeeshraman *et al.*, 2018).

Aqueous extracts showed a progressive increase in antioxidant activity with time, reaching a maximum TAC of 42.45  $\mu\text{g}$  at 72 h. This suggests that prolonged extraction enhances the solubilization of water-soluble antioxidant compounds, supporting the feasibility of water-based, non-toxic extraction systems for sustainable applications (El-Ramady *et al.*, 2022).

In contrast, chloroform extracts exhibited negligible antioxidant activity, with TAC values remaining below 8  $\mu\text{g}$  across all time points. This low efficiency is attributed to the inability of non-polar solvents to extract phenolic compounds, which are the primary antioxidants in *A. bisporus* (Barros *et al.*, 2008; Kim, 2008).

From a sustainability standpoint, methanolic and aqueous extractions align with green chemistry principles by offering higher extraction efficiency with reduced environmental impact compared to non-polar solvents. The results reinforce the potential of *A. bisporus* as a renewable source of natural antioxidants suitable for functional foods and nutraceuticals.

**Table 1:** Absorbance values of ascorbic acid standard

Concentration (%)	OD at 695 nm
0	0
20	0.228
40	0.454
60	0.69
80	0.907
100	1.14

**Table 2:** Total Antioxidant Capacity of *Agaricus bisporus* extracts

Time (h)	Extract	OD 695 nm	TAC ( $\mu\text{g}$ )
24	Methanol	0.474	43.09
24	Chloroform	0.001	0.09
24	Aqueous	0.304	27.63
48	Methanol	0.463	42.09
48	Chloroform	0.043	3.90
48	Aqueous	0.428	38.90
72	Methanol	0.335	30.45
72	Chloroform	0.082	7.45
72	Aqueous	0.468	42.45

### 3. Conclusion

The present study demonstrates that *Agaricus bisporus* is a potent source of natural antioxidants, with extraction efficiency strongly influenced by solvent polarity and extraction duration. Methanolic extraction yielded the highest antioxidant activity, while aqueous extraction emerged as a promising eco-friendly alternative when extended extraction times were employed. These findings support the use of green extraction strategies to harness bioactive compounds from edible mushrooms, contributing to sustainable development through reduced reliance on synthetic antioxidants, lower toxicity, and energy-efficient processing. Future research should focus on scaling up green extraction techniques and exploring the application of *A. bisporus* antioxidants in functional food and pharmaceutical formulations.

### References

- Barros, L., Baptista, P., Estevinho, L. M., & Ferreira, I. C. F. R. (2008). Effect of fruiting body maturity stage on chemical composition and antioxidant activity of *Agaricus bisporus*. *Journal of Agricultural and Food Chemistry*, 56, 8759–8764.
- Chu, M., Khan, R. D., Zhou, Y., Agar, O. T., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. (2023). LC-ESI-QTOF-MS/MS characterization of phenolic compounds in commercial mushrooms and their antioxidant activities. *Processes*, 11(6), 1711.
- Dubost, N. J., Ou, B., & Beelman, R. B. (2007). Quantification of polyphenols and ergothioneine in cultivated mushrooms. *Journal of Agricultural and Food Chemistry*, 55, 9059–9064.
- El-Ramady, H., Abdalla, N., Badgar, K., Llanaj, X., Törös, G., Hajdú, P., ... & Prokisch, J. (2022). Edible mushrooms for sustainable and healthy human food: nutritional and medicinal attributes. *Sustainability*, 14(9), 4941.
- Jagadeeshraman, S., (2018). Antioxidant and phytochemical properties of *Agaricus bisporus*. *Journal of Food Science*, 83, 152–160.
- Kim, I. S., Yang, M. R., Lee, O. H., & Kang, S. N. (2011). Antioxidant activities of natural extracts. *International Journal of Molecular Sciences*, 12, 4120–4131.
- Mattila, P., Könkö, K., Euroola, M., Pihlava, J. M., Astola, J., Vahteristo, L., ... & 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.
- [8] Rajasekar, R., & Jeyaprakash, K. (2015). In vitro antioxidant activity of *Agaricus bisporus*. *World Journal of Pharmaceutical Research*, 4(12), 1528–1541.
- [9] Singh, R. (2017). A review on nutritional and medicinal benefits of mushrooms. *IOSR Journal of Pharmacy and Biological Sciences*, 12(1), 107–111.
- [10] Usman, M., Murtaza, G., & Ditta, A. (2021). Nutritional and medicinal value of *Agaricus bisporus*. *Applied Sciences*, 11(13), 5943.