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India's Functional Fungi: Evaluating the Antioxidant Activity of Methanolic Extracts of Native Medicinal and Edible Mushrooms

SHREYA CHAHAL* AND ANIL SINDHU

Department of Biotechnology, Deenbandhu Chhotu Ram University of Science and Technology, Murthal, Sonipat-131 039 (Haryana), India
**(e-mail: shreyachahal57@gmail.com; Mobile: 86858 27789)*

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ABSTRACT

Oxidative stress, caused by reactive oxygen species (ROS), compromises cellular integrity and contributes to various diseases. This research investigated the antioxidant potential of Indian cultivated fungi, including *Ganoderma lucidum*, *Hericium erinaceus*, *Agaricus bisporus, Auricularia auricular-judae, Calocybe indica, Pleurotus citrinopileatus, Cordyceps militaris* and *Pleurotus djamor*. *In vitro* antioxidant activities were assessed using ABTS and DPPH assays. Fourier-transform infrared (FTIR) spectroscopy was employed to chemically characterize the extracts and identify functional groups contributing to their bioactive profiles. Phytochemical analysis confirmed the presence of major constituents, including polyphenols, flavonoids and terpenoids. These indigenous mushrooms demonstrated strong antioxidant activity, effectively neutralizing ROS and mitigating oxidative damage. Their traditional relevance and bioactivity support their potential use in nutraceutical and pharmaceutical formulations.

Key words: Antioxidant activity, DPPH assay, ABTS assay, Indian cultivated fungi, medicinal mushrooms, reactive oxygen species

INTRODUCTION

Cells are continually exposed to reactive oxygen species (ROS), highly reactive molecules generated as natural byproducts of metabolic processes (Jomova *et al*., 2023). Although ROS are involved in cellular signalling, their excessive accumulation leads to oxidative stress, a condition in which antioxidant defenses are overwhelmed (Snezhkina *et al*., 2019). This imbalance leads to damage to biomolecules, including lipids, proteins and nucleic acids, resulting in processes such as lipid peroxidation, protein carbonylation and DNA strand breaks (Juan *et al.*, 2021). Such oxidative damage plays a critical role in the development of chronic diseases, including cancer, cardiovascular and neurodegenerative disorders and viral infections (Pizzino *et al*., 2017). To mitigate ROS-mediated damage, biological systems depend on enzymatic antioxidants such as superoxide dismutase and catalase, along with non-enzymatic antioxidants including glutathione, vitamin C and vitamin E (Seo and Choi, 2021). Although synthetic antioxidants have been widely utilized in food and pharmaceutical industries, safety concerns have increased the demand for natural alternatives.

Medicinal and edible mushrooms are promising sources of natural antioxidants due to their rich content of bioactive compounds, including phenolic acids, flavonoids, polysaccharides, sterols, and terpenoids. These compounds exert antioxidant effects via free radical scavenging, inhibition of lipid peroxidation and metal ion chelation (Muszyñska *et al*., 2020; Effiong *et al*., 2024). Their bioactivity is well documented through *in vitro* studies and is supported by traditional systems such as Traditional Chinese Medicine and Ayurveda (Zhou *et al*., 2023). Although individual mushroom species have been widely studied, comparative evaluations of antioxidant activity among Indian mushroom varieties are limited. Furthermore, few investigations have integrated spectroscopic tools to correlate molecular structure with antioxidant potential. Fourier-transform infrared (FTIR) spectroscopy provides a rapid and non-destructive method to identify functional groups associated with bioactivity, such as hydroxyl, carbonyl and aromatic moieties. The present study evaluated and compared the antioxidant potential of methanolic extracts from selected Indian mushrooms using DPPH and ABTS assays. FTIR spectroscopy was applied to identify major

functional groups and aid in structure activity relationship analyses. This approach aimed at enhancing the understanding of Indian mushrooms as natural antioxidant sources and promoting their application in nutraceutical and functional food development.

MATERIALS AND METHODS

Fresh fruiting bodies of cultivated medicinal and edible mushrooms were collected for analysis. The species included *Ganoderma lucidum*, *Hericium erinaceus*, *Agaricus bisporus*, *Auricularia auricula-judae*, *Calocybe indica*, *Pleurotus citrinopileatus* and *Pleurotus djamor*. All samples were obtained from the Regional Mushroom Research Centre, Maharana Pratap Horticultural University, Murthal, Haryana, India. The identification of each species was formally authenticated by the Regional Director of the Centre. The mushrooms were cleaned using distilled water, air-dried and subsequently stored at -12°C to maintain sample integrity for further analyses.

Cryogenic pulverization of the mushroom samples was performed using liquid nitrogen to obtain a fine powder, thereby maximizing surface area for efficient extraction. The powdered material was combined with methanol and subjected to ultrasonication on ice using a cycle of 10 seconds on and 5 seconds off for a total of 15 min at 30% amplitude. This step facilitated effective disruption of cellular structures, enhancing extraction yield. After sonication, the samples were placed in a water bath for one hour to allow complete evaporation of methanol. The resulting mixtures were centrifuged at 10,000 rpm for 10 min at 4°C to separate particulate matter. The clear supernatants were filtered through 0.22 µm membrane filters and used as crude mushroom extracts for antioxidant assays.

A suite of validated assays was employed to evaluate the antioxidant potential of the mushroom extracts. The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to assess radical scavenging activity. Kinetic measurements were conducted at 517 nm using a UV–VIS spectrophotometer over 8-10 min, capturing a total of 250 readings to generate a detailed absorbance profile.

The ABTS [2,2′ -azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] assay was conducted following standard protocols, with ABTS radicals generated before spectrophotometric evaluation at 734 nm. Absorbance was recorded for 20 sec to monitor the rapid reduction in radical concentration.

Fourier-transform infrared (FTIR) spectroscopy was performed to identify functional groups associated with antioxidant compounds. Characteristic peaks corresponding to hydroxyl, carbonyl and aromatic groups were observed, consistent with the presence of phenolics and flavonoids (Feher *et al.*, 2021).

Qualitative phytochemical screening was conducted for flavonoids, alkaloids, terpenoids, steroids, saponins, phenolics, carotenoids, tannins and coumarins using established methods (Rao *et al*., 2023). Total polyphenol content was determined using the Folin-Ciocalteu method, with visual indicators such as colour change and precipitation confirming compound presence.

All experimental procedures were meticulously performed in triplicate to ensure the robustness and reproducibility of our findings. Data obtained were subsequently expressed as the mean ± standard error (SE). To ascertain statistical significance, a one-way Analysis of Variance (ANOVA) was employed with a predetermined significance level set at $P< 0.05$.

RESULTS AND DISCUSSION

The antioxidant potential of the mushroom extracts was initially assessed using the DPPH radical scavenging assay, a widely recognized method for evaluating free radical neutralization. The baseline DPPH control remained stable throughout the 8-10 min observation period, confirming the absence of spontaneous degradation or non-specific activity (Figs. 1 and 2). In contrast, all mushroom extracts demonstrated time-dependent reductions in absorbance at 517 nm, indicating effective scavenging of DPPH radicals. Notably, extracts of *Ganoderma lucidum* (GL), *Agaricus bisporus* (AB) and *Calocybe indica* (CI) exhibited the most pronounced declines in absorbance, suggesting superior antioxidant activity relative to the other tested species.

The ABTS radical scavenging assay provided complementary evidence supporting the antioxidant activity of the mushroom extracts. A marked reduction in absorbance at 734 nm

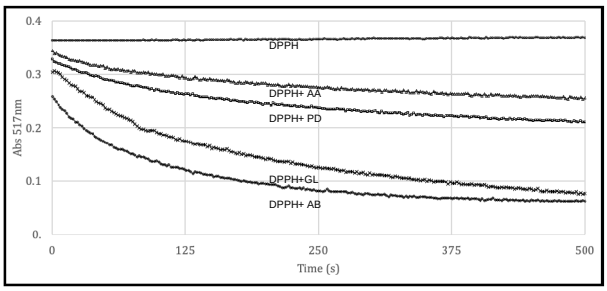

Fig. 1. Declining graph of the first four mushroom extracts, illustrating their DPPH radical scavenging activity over time.

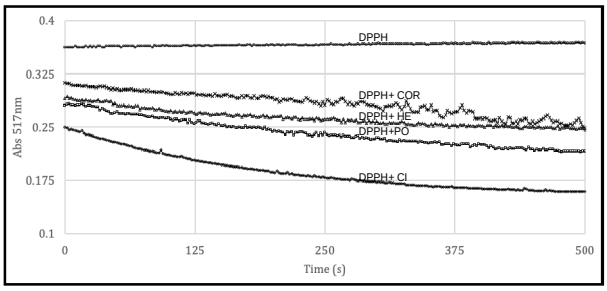

Fig. 2. Declining graph of remaining mushroom extracts, depicting their DPPH radical scavenging activity over time.

was recorded over a 20 sec interval in all treated samples, indicating effective neutralization of ABTS radicals (Fig. 3). The control (pure ABTS) remained stable throughout the measurement period, confirming the absence of non-specific degradation in the absence of antioxidant agents. Among the tested extracts, *Ganoderma lucidum* (GL), *Hericium erinaceus* (HE) and *Agaricus bisporus* (AB) exhibited the most substantial declines in absorbance, reflecting their strong radical scavenging potential. These findings align with results from the DPPH assay, further substantiating the antioxidant efficacy of these species.

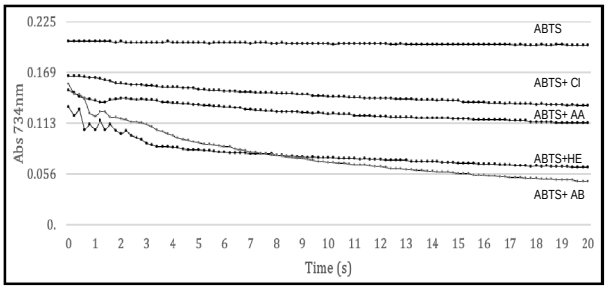

Fig. 3. Graph illustrating the ABTS radical scavenging activity of first four mushroom extracts, showing the reduction in absorbance over time.

In addition to kinetic profiling, antioxidant efficacy was quantified by calculating percentage inhibition based on reductions in absorbance at 517/ nm (DPPH; control: 0.369) and 734/ nm (ABTS; control: 0.200). The mushroom extracts exhibited varying degrees of free radical scavenging activity (Table 1). *Agaricus bisporus* (AB) and *Ganoderma lucidum* (GL) demonstrated the highest antioxidant potential, with AB achieving 84.02% inhibition in the DPPH assay and 76.0% in the ABTS assay. GL exhibited 82.08 and 98.5% inhibition, respectively. *Pleurotus djamor* (PD) and *Cordyceps militaris* (COR) showed balanced performance across both the assays (PD: 45.52%; DPPH, 71.0% ABTS; COR: 36.04% DPPH, 69.0% ABTS). *Calocybe indica* (CI) exhibited stronger inhibition in the DPPH assay (59.34%) than in ABTS (34.0%), whereas *Hericium erinaceus* (HE) showed the reverse trend (37.66% DPPH, 68.5% ABTS). *Auricularia auricula-judae* (AA) and *Pleurotus ostreatus* (PO) displayed moderate activity in both the assays. These findings underscore the diverse antioxidant capacities among cultivated Indian mushrooms and support their potential as natural sources of free radical scavengers.

Table 1. Percentage inhibition of DPPH and ABTS radicals by methanolic mushroom extracts

Mushrooms	% inhibition ABTS	% inhibition DPPH
Ganoderma lucidum	98.5	82.08
Pleurotus djamor	71.0	45.52
Auricularia auricularia-judae	43.5	32.08
Hericium erinaceus	68.5	37.66
Calocybe indica	34.0	59.34
Pleurotus ostreatus	71.0	44.17
Cordyceps militaris	69.0	36.04
Agaricus bisporus	76.0	84.02

Fourier-transform infrared (FTIR) spectroscopy was utilized to characterize the functional groups present in the mushroom extracts, offering insights into the molecular constituents contributing to antioxidant activity. Several key absorbance regions were consistently observed across all samples (Figs. 4, 5 and 6). In the Fingerprint Region (600-1500/cm), a prominent absorption band around 1051/cm indicated C–O stretching vibrations, commonly attributed to polysaccharides or alcohol groups (Yao *et al*., 2018). Additional peaks within the 1200-1500/ cm range likely reflected C–H bending and vibrational modes of aromatic rings,

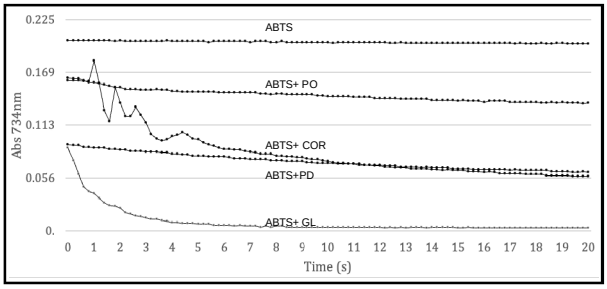

Fig. 4. Graph illustrating the ABTS radical scavenging activity of remaining mushroom extracts, showing the reduction in absorbance over time.

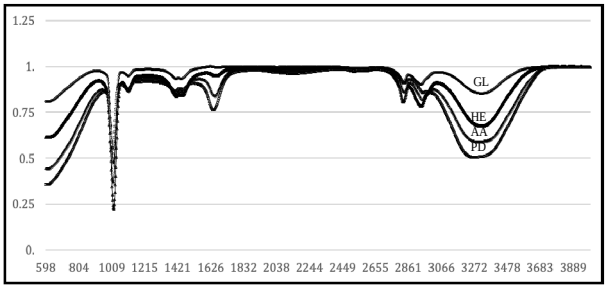

Fig. 5. FTIR spectral peaks for the first four mushroom extracts highlighting key functional groups.

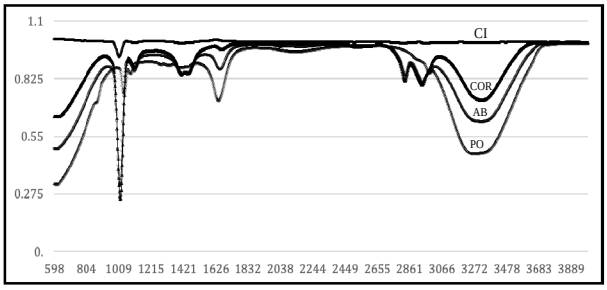

Fig. 6. An FTIR spectral peak for the remaining mushroom extracts displaying their characteristic functional groups.

characteristic of phenolic structures known for radical scavenging capacity (Alvarez *et al*., 2021). Within the Amide and Protein Region (1500-1700/cm), a strong peak near 1684/cm was detected, corresponding to the amide I band (C=O stretching), indicative of proteins or peptides (Wickramasinghe *et al*., 2023). Such macromolecules are implicated in antioxidant mechanisms via electron donation and metal ion chelation. The Hydroxyl and Amine Stretch Region (3200-3600/cm) displayed broad, intense bands associated with –OH and –NH groups (Oliveira *et al*., 2016). These functional groups are frequently found in phenolics, polysaccharides and protein derivatives, which are recognized for their hydrogen-donating and radical-neutralizing abilities (Bekiaris *et al*., 2020). Finally, absorption peaks at approximately 2861 and 2920/cm were located within the lipid or fatty acid region (2800-3000/cm), corresponding to C–H stretching in CH_2 and CH_3 groups. These signals suggested the presence of lipidic compounds, which, while not primary radical scavengers, may contribute to antioxidant defense in hydrophobic environments.

In addition to spectroscopic profiling, a comprehensive phytochemical screening was performed to identify secondary metabolites present in the mushroom extracts. The analysis encompassed a broad spectrum of bioactive compounds, including alkaloids, flavonoids, triterpenoids, steroids, carotenoids, saponins, tannins, coumarins and polyphenols (Table 2). Total polyphenolic content was quantified using the Folin-Ciocalteu method, while qualitative tests were employed for all other classes. The detection of multiple phytochemical groups across the extracts provides a biochemical basis for their antioxidant activity and suggests potential for broader therapeutic applications.

Table 2. Phytochemical test results indicating the presence (+) or absence (-) of various secondary metabolites in each mushroom extract

Mushroom	Alk	Fla	Tri	Ste	Car	Sap	Tan	Fc	Cou
Ganoderma lucidum	+	+	+	+	+	+	+	+	-
Pleurotus djamor	-	+	-	-	+	+	+	+	+
Auricularia auricularia-judae	-	+	-	-	-	+	-	+	-
Hericium erinaceus	-	+	+	-	+	-	+	+	+
Calocybe indica	+	+	-	-	+	+	-	+	-
Pleurotus ostreatus	-	+	-	-	+	+	+	+	+
Cordyceps militaris	-	+	+	+	-	+	-	+	+
Agaricus bisporus	-	+	-	-	-	+	-	+	-

Where, Alk: Alkaloids, Fla: Flavonoids, Tri: Triterpenoids, Ste: Steroids, Car: Carotenoids, Sap: Saponins, Tan: Tannins, Fc: Folin-Ciocalteu and Cou: Coumarin.

This study highlights the substantial antioxidant potential of several Indian-cultivated mushrooms, extending beyond functional assessment to elucidate underlying chemical determinants. All tested species exhibited significant radical scavenging activity in both the DPPH and ABTS assays. Notably, *Ganoderma lucidum*, *Hericium erinaceus*, *Calocybe indica* and *Agaricus bisporus* demonstrated the highest inhibition rates, indicating strong efficacy against reactive oxygen species (ROS). The observed bioactivity correlated closely with the rich phytochemical composition of the extracts, particularly the presence of phenolics, flavonoids, triterpenoids and polysaccharides. FTIR spectral data revealed diagnostic absorbance bands—broad peaks in the 3200-3500/cm region corresponding to hydroxyl (–OH) and amine (–NH) groups and distinct signals near 1684/cm indicative of amide I bonds. These functional groups are known contributors to antioxidant activity through hydrogen donation, electron transfer and metal ion chelation mechanisms. Further spectral features: Such as peaks at 1051/cm (C–O stretching), the 1200-1500/cm aromatic region and lipid-associated bands around 2861-2920/cm suggested the presence of structurally diverse bioactive constituents. The consistency between FTIR signatures and radical scavenging performance reinforced the conclusion that these compounds play a central role in antioxidant efficacy. Collectively, these findings underscore the therapeutic potential of Indian mushrooms as natural antioxidants. The convergence of phytochemical diversity, functional group architecture and assay-based potency supports their application in the development of standardized antioxidant formulations. Moreover, these results validate traditional medicinal use while also offering promising avenues for incorporation into functional foods, nutraceuticals and health supplements. To fully harness this potential, future research should include *in vivo* and clinical evaluations to verify efficacy and safety in biological systems. Investigating synergistic interactions among bioactives may further enhance therapeutic outcomes. In addition to health applications, promoting the cultivation and commercialization of these mushrooms could contribute significantly to rural economic development and strengthen India's nutraceutical sector.

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