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ANTIOXIDANT CAPACITY EVALUATION OF SELECT EDIBLE MUSHROOMS THROUGH STANDARDIZED IN VITRO METHODOLOGIES

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ABSTRACT:

Mushrooms are increasingly valued as functional foods owing to their diverse repertoire of bioactive constituents, notably polyphenols, flavonoids, and polysaccharides, which collectively impart notable free radical neutralizing capacity. The present investigation was designed to systematically evaluate and compare the antioxidant profiles of three widely cultivated edible mushroom species — *Pleurotus ostreatus*, *Agaricus bisporus*, and *Pleurotus sajor-caju* — in both unprocessed and thermally processed forms, employing multiple standard in vitro analytical platforms. Methanolic, ethanolic, and aqueous extracts of each species were prepared and subjected to DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation decolorization assay, Total Phenolic Content (TPC) quantification via the Folin–Ciocalteu procedure, and Total Flavonoid Content (TFC) estimation by the aluminium chloride colorimetric approach. Methanolic fractions yielded the highest antioxidant activity across all tested species without exception. In unprocessed samples, DPPH radical neutralization approached approximately 85% in all three species under methanol extraction. Thermal treatment induced a statistically significant decline ($p < 0.01$) in DPPH activity, total phenolic content, and total flavonoid content in the majority of extracts; however, ABTS activity displayed variable trends contingent upon the species and solvent employed. Collectively, these data validate edible mushrooms as rich reservoirs of dietary antioxidants, with solvent selection, interspecies variation, and heat exposure each exerting distinct and measurable effects on the final antioxidant profile.

Keywords Antioxidant activity, DPPH assay, ABTS assay, Pleurotus ostreatus, Agaricus bisporus, Pleurotus sajor-caju, phenolic content, flavonoid content, in vitro assay

INTRODUCTION:

Oxidative stress, which emerges when reactive oxygen species (ROS) accumulate beyond the neutralizing capacity of biological defence mechanisms, is widely implicated in the onset and progression of an array of chronic non-communicable conditions, encompassing malignancies, cardiovascular pathologies, metabolic disorders such as diabetes mellitus, and neurodegenerative diseases [Kozarski et al., 2015; Liuzzi et al., 2023]. Dietary-derived natural antioxidants have consequently attracted substantial scientific and biomedical attention, given their capacity to counteract oxidative damage through radical quenching, transition metal chelation, and modulation of antioxidant enzyme cascades [Mwangi et al., 2022].

Edible mushrooms occupy a distinctive niche among functional food categories, having been incorporated into traditional diets across numerous world cultures for centuries, not merely as nutritional staples but also as remedial agents [Valverde et al., 2015]. Their therapeutic relevance is substantiated by the presence of structurally diverse bioactive molecules including polyphenols, flavonoids, terpenoids, ergosterols, and β-glucan polysaccharides, which collectively underpin their antioxidant, anti-inflammatory, antimicrobial, and immunoregulatory activities [Jayachandran et al., 2017; Friedman, 2016]. Of the more than 2,000 documented edible fungal species, *Pleurotus*

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ostreatus (oyster mushroom), *Agaricus bisporus* (white button mushroom), and *Pleurotus sajor-caju* (Indian oyster mushroom) are among the commercially most prominent and widely produced [Ahmed et al., 2023; Cateni et al., 2022].

Pleurotus ostreatus has been extensively characterised for its high phenolic content and radical quenching ability across diverse extraction systems [Bakir et al., 2018; Effiong et al., 2024]. *Agaricus bisporus*, globally the most consumed mushroom, is distinguished by the presence of ergothioneine, a structurally unique antioxidant amino acid, alongside conventional phenolics and flavonoids [Krishnamoorthi et al., 2022; Hola et al., 2023]. *Pleurotus sajor-caju*, although comparatively understudied, has shown analogous bioactive attributes and is progressively being explored as a nutraceutical commodity [Khatun et al., 2020; Sharma et al., 2021]. Antioxidant assessment in mushrooms is conventionally performed through validated in vitro protocols, the most widely adopted being the DPPH radical scavenging assay and the ABTS radical cation decolorization assay, which collectively capture hydrogen-atom transfer and single-electron transfer mechanisms, respectively, and thereby provide complementary information regarding the overall electron-donating capacity of the extract [Kim et al., 2008; Cheung et al., 2003]. Quantification of total phenolic content (TPC) by the Folin–Ciocalteu method and total flavonoid content (TFC) by the aluminium chloride colorimetric assay serve as reliable proxies for antioxidant potential, given that phenolic hydroxyl groups constitute the primary structural unit responsible for radical capture [Barros et al., 2007; Chang et al., 2002].

A frequently under-examined variable in mushroom antioxidant research is the influence of culinary heat treatment on bioactive composition. Since mushrooms are seldom consumed without prior cooking, procedures such as boiling, frying, and steaming are routinely applied; these may alter bioactive integrity through thermal decomposition of heat-sensitive compounds, diffusion of water-soluble constituents into the cooking medium, or paradoxical enhancement arising from disruption of cell-wall matrices that release otherwise inaccessible bound phenolics [Jiménez-Monreal et al., 2009]. Conflicting outcomes in existing literature — some investigations documenting reduced antioxidant capacity post-cooking while others report augmentation — suggest that the response is inherently species- and method-dependent [Heleno et al., 2012; Tjokrokusumo et al., 2024].

Despite a growing global literature base on mushroom antioxidants, there exists a relative scarcity of studies from the Indian subcontinent that simultaneously employ multiple in vitro assays to compare raw versus cooked preparations of commonly farmed species across different solvent polarities. The systematic statistical evaluation of correlations between radical scavenging parameters and underlying phenolic/flavonoid pools represents another significant gap [Das et al., 2021; Mayirnao et al., 2025]. The present study was therefore designed to: (i) prepare methanolic, ethanolic, and aqueous extracts of *P. ostreatus*, *A. bisporus*, and *P. sajor-caju* from both raw and cooked forms; (ii) determine their antioxidant capacity via DPPH and ABTS radical scavenging assays; (iii) quantify their TPC and TFC; and (iv) conduct rigorous statistical evaluation of the effect of thermal processing and establish correlations between phenolic composition and radical scavenging. This work is intended to enrich the evidence base on the nutraceutical attributes of cultivated edible mushrooms and inform dietary guidance as well as potential pharmaceutical exploitation.

MATERIALS AND METHODS

2.1 Collection and Preparation of Mushroom Samples

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Fresh specimens of Pleurotus ostreatus, Agaricus bisporus, and Pleurotus sajor-caju were procured from GBS Mushrooms and Agrobusinesses Pvt. Ltd., Chandrapur. Adhering soil and surface debris were removed by rinsing with tap water. The cleaned material was subjected to shade drying over a period of seven to fifteen days, after which the dried tissue was converted to a fine homogeneous powder using a mechanical blender. Powder samples were stored in sealed zip-lock containers. To eliminate residual moisture and prevent microbial contamination prior to extraction, the powdered material was further dried in a hot-air oven maintained below 45°C for two to three hours.

2.2 Preparation of Mushroom Extracts

Three solvent systems — methanol, ethanol, and distilled water — were employed independently for extract preparation. In each case, 10 g of dried mushroom powder was combined with 100 mL of the respective solvent and agitated continuously on an orbital shaker for 48 hours to ensure thorough extraction of bioactive constituents. The resulting suspensions were filtered through Whatman No. 1 filter paper to remove particulate residues. The clarified filtrates were then evaporated to dryness over a hot water bath to yield concentrated powdered extracts. Prior to analytical use, each extract was reconstituted by dissolving in 5 mL of 10% dimethyl sulphoxide (DMSO) with thorough mixing, and the prepared solutions were stored at 4°C until required.

2.3 DPPH Free Radical Scavenging Assay

A working DPPH solution was prepared by dissolving 24 mg of 1,1-diphenyl-2-picrylhydrazyl in 100 mL of 99.5% methanol; the solution was kept away from light for two hours prior to use. A stock solution of 1 mg/mL was prepared from each mushroom extract using the corresponding solvent, from which working concentrations of 50, 100, and 200 µg/mL were derived. Reaction mixtures were assembled by combining 1.0 mL of DPPH solution with 1.0 mL of each test extract in individual test tubes; these were thoroughly vortexed and kept in darkness at room temperature for 30 minutes. A control was set up using 1.0 mL DPPH with 1.0 mL methanol, and a blank containing 1.0 mL extract with 1.0 mL methanol (without DPPH) was also maintained. Ascorbic acid served as the positive reference antioxidant. Post-incubation absorbance at 517 nm was recorded on a UV-visible spectrophotometer. The percentage radical scavenging activity was derived using the expression:

% Free Radical Scavenging = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

2.4 ABTS Radical Cation Decolorization Assay

The ABTS working reagent was generated by combining equal volumes of 14 mM ABTS and 4.8 mM potassium persulfate, followed by incubation in darkness at ambient temperature for 16 hours. The resulting deep blue-green solution was then diluted with phosphate-buffered saline to obtain an absorbance of 1.1 ± 0.02 at 734 nm. For the assay, 150 µL of each sample or reference standard was added to 2850 µL of the adjusted ABTS reagent. Following a 2-hour incubation period in darkness, absorbance at 734 nm was measured and results were expressed as mg ascorbic acid equivalent per gram (mg AAE/g).

2.5 Determination of Total Phenolic Content

For phenolic extraction, 5 g of dried powdered mushroom was treated with 70% ethanol under continuous agitation at ambient temperature for 5 hours. The extract was centrifuged at 3000 rpm, and the supernatant was clarified through Whatman No. 1 filter paper. The filtrate was reduced to dryness under diminished pressure at 40°C using a rotary evaporator, and the resultant dry residue was stored at −12°C until analysis. TPC was quantified using the Folin–Ciocalteu colorimetric procedure with minor modifications as described by Ayhan et al. (2019). Briefly, 1 mL of extract was reacted with 1 mL of Folin–Ciocalteu reagent and 3 mL of 20% sodium

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carbonate solution. The reaction mixture was incubated at room temperature for 30 minutes under dark conditions. Absorbance was recorded at 765 nm against a reagent blank. A gallic acid calibration curve was employed for quantification, and results were expressed as mg gallic acid equivalents per 100 g dry weight (mg GAE/100 g DW).

2.6 Determination of Total Flavonoid Content

Flavonoid extraction was carried out under the same conditions described for TPC: 5 g of dried mushroom powder in 70% ethanol for 5 hours, followed by centrifugation, filtration through Whatman No. 1 paper, and concentration using a rotary evaporator at 40°C. The concentrated extract was stored at −12°C. TFC was determined by a colorimetric protocol wherein the extract was sequentially reacted with sodium nitrite and aluminium nitrate. Absorbance was measured at 510 nm, and quercetin was used as the calibration standard. Results were expressed as milligrams of quercetin equivalent per gram of sample (mg QE/g).

3. STATISTICAL ANALYSIS

All experimental measurements were conducted in triplicate (n = 3) and results were reported as mean ± standard deviation (SD). Statistically significant differences between raw and cooked mushroom extracts were assessed using p-values, with $p < 0.05$ and $p < 0.01$ adopted as thresholds for significance. Pearson's correlation coefficient (r) was applied to assess the strength and direction of associations among the experimental parameters.

Table 1: Statistical Analysis and Correlation of DPPH Radical Scavenging Activity in Raw and Cooked Mushroom Extracts (Mean ± SD, n = 3)

Mushroom Species	Extract Type	Raw Mean ± SD	Cooked Mean ± SD	p-Value	Significance	Correlation (r)	Interpretation
Pleurotus ostreatus	Methanol	85.2 ± 0.05	25.3 ± 0.02	0.0008	$p < 0.01$	0.84	Strong correlation; highly significant reduction after cooking
	Ethanol	46.5 ± 0.02	7.4 ± 0.16	0.0021	$p < 0.05$	0.72	Moderate correlation; reduction observed
Agaricus bisporus	Aqueous	29.8 ± 0.03	3.3 ± 0.00	0.0043	$p < 0.05$	0.68	Moderate correlation; sharp reduction
	Methanol	85.1 ± 0.01	10.8 ± 0.02	0.0009	$p < 0.01$	0.79	Strong correlation; significant reduction
	Ethanol	40.8 ± 0.01	16.7 ± 0.02	0.003	$p < 0.05$	0.70	Moderate correlation; reduction observed
	Aqueous	29.2 ± 0.01	3.1 ± 0.01	0.0045	$p < 0.05$	0.60	Weak correlation; large reduction
Pleurotus sajor-caju	Methanol	85.3 ± 0.01	2.6 ± 0.01	0.001	$p < 0.01$	0.82	Strong correlation; highly significant reduction
	Ethanol	27.2 ± 0.01	12.1 ± 0.01	0.0028	$p < 0.05$	0.74	Moderate correlation; reduction observed

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	Aqueous	13.2 ± 0.00	5.4 ± 0.01	0.005	p < 0.05	0.65	Moderate correlation; reduction observed

Table 2: Statistical Analysis of ABTS Assay for Raw and Cooked Mushroom Extracts (Mean ± SD, n = 3)

Mushroom Species	Extract Type	Raw Mean ± SD	Cooked Mean ± SD	p-Value	Significance	Correlation (r)	Interpretation
Pleurotus ostreatus	Methanol	84.52 ± 0.02	80.02 ± 0.01	0.0012	p < 0.01	0.81	Significant reduction after cooking
	Ethanol	70.03 ± 0.03	65.06 ± 0.01	0.0045	p < 0.05	0.67	Moderate reduction
	Aqueous	65.05 ± 0.01	54.00 ± 1.00	0.0028	p < 0.05	0.52	Significant difference
Agaricus bisporus	Methanol	73.06 ± 0.01	65.02 ± 0.01	0.0015	p < 0.01	0.79	Significant reduction
	Ethanol	58.80 ± 0.01	59.01 ± 0.01	0.056	ns	0.60	Slight increase, not significant
	Aqueous	49.84 ± 0.01	75.00 ± 0.58	0.0009	p < 0.01	0.48	Significant increase after cooking
Pleurotus sajor-caju	Methanol	71.52 ± 0.02	61.54 ± 0.01	0.0018	p < 0.01	0.76	Significant reduction
	Ethanol	40.07 ± 0.01	56.15 ± 0.03	0.0022	p < 0.05	0.65	Moderate increase
	Aqueous	25.84 ± 0.01	77.02 ± 0.03	0.0007	p < 0.01	0.50	Strong increase after cooking

Table 3: Statistical Analysis of Total Phenolic Content in Raw and Cooked Mushroom Extracts (Mean ± SD, n = 3)

Mushroom Species	Extract Type	Raw Mean ± SD	Cooked Mean ± SD	p-Value	Significance	Correlation (r)	Interpretation
Pleurotus ostreatus	Methanol	9.23 ± 0.01	7.30 ± 0.01	0.0008	p < 0.01	0.88	Strong positive correlation; cooking reduces phenolics
	Ethanol	9.21 ± 0.01	8.37 ± 0.02	0.0021	p < 0.05	0.75	Moderate correlation; slight reduction
	Aqueous	9.08 ± 0.01	6.66 ± 0.01	0.0015	p < 0.01	0.62	Weak correlation; strong reduction
Agaricus bisporus	Methanol	9.10 ± 0.01	8.31 ± 0.01	0.0032	p < 0.05	0.80	Strong correlation; moderate reduction
	Ethanol	9.17 ± 0.02	7.29 ± 0.01	0.0011	p < 0.01	0.70	Moderate correlation; strong reduction

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	Aqueous	8.74 ± 0.01	7.49 ± 0.01	0.0043	$p < 0.05$	0.65	Moderate correlation; reduction observed
Pleurotus sajor-caju	Methanol	9.22 ± 0.02	7.50 ± 0.01	0.0019	$p < 0.01$	0.82	Strong correlation; significant reduction
	Ethanol	9.23 ± 0.01	8.07 ± 0.01	0.0025	$p < 0.05$	0.77	Moderate correlation; reduction
	Aqueous	9.39 ± 0.01	7.22 ± 0.01	0.0007	$p < 0.01$	0.60	Weak correlation; strong reduction

Table 4: Statistical Analysis and Correlation of Total Flavonoid Content in Raw and Cooked Mushroom Extracts (Mean ± SD, n = 3)

Mushroom Species	Extract Type	Raw Mean ± SD	Cooked Mean ± SD	p-Value	Significance	Correlation (r)	Interpretation
Pleurotus ostreatus	Methanol	0.016 ± 0.001	0.010 ± 0.001	0.0009	$p < 0.01$	0.84	Strong correlation; significant reduction after cooking
	Ethanol	0.011 ± 0.001	0.007 ± 0.001	0.0023	$p < 0.05$	0.72	Moderate correlation; reduction observed
	Aqueous	0.006 ± 0.001	0.005 ± 0.001	0.0041	$p < 0.05$	0.68	Moderate correlation; slight reduction
Agaricus bisporus	Methanol	0.014 ± 0.001	0.008 ± 0.001	0.0015	$p < 0.01$	0.79	Strong correlation; significant reduction
	Ethanol	0.013 ± 0.001	0.007 ± 0.001	0.0028	$p < 0.05$	0.70	Moderate correlation; reduction observed
	Aqueous	0.004 ± 0.001	0.004 ± 0.001	0.056	ns	0.60	Weak correlation; negligible change
Pleurotus sajor-caju	Methanol	0.013 ± 0.001	0.007 ± 0.001	0.0012	$p < 0.01$	0.82	Strong correlation; significant reduction
	Ethanol	0.011 ± 0.001	0.006 ± 0.001	0.0026	$p < 0.05$	0.74	Moderate correlation; reduction observed
	Aqueous	0.006 ± 0.001	0.004 ± 0.002	0.0031	$p < 0.05$	0.65	Moderate correlation; reduction observed

RESULTS AND DISCUSSION:

4.1 DPPH Radical Scavenging Activity

The DPPH radical scavenging data for methanolic, ethanolic, and aqueous fractions of all three species, in both raw and cooked conditions, are summarised in Table 1. Across all tested samples, raw methanolic extracts invariably yielded the highest scavenging percentages: 85.2 ± 0.05%, 85.1 ± 0.01%, and 85.3 ± 0.01% for *P. ostreatus*, *A. bisporus*, and *P. sajor-caju*, respectively. This pattern reflects the intermediate polarity of methanol, which enables co-extraction of both hydrophilic and moderately lipophilic phenolics that donate hydrogen atoms to stabilise DPPH radicals

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[Cheung et al., 2003; Sharma et al., 2021]. Ethanolic fractions of raw *P. ostreatus* and *A. bisporus* yielded moderate DPPH values of 46.5 ± 0.02% and 40.8 ± 0.01%, respectively, while aqueous extracts were comparatively lower (29.8 ± 0.03% and 29.2 ± 0.01%), consistent with a polarity-dependent gradient in scavenging efficiency.

Heat treatment produced a statistically robust and uniform decrease in DPPH activity across all species and solvent systems ($p < 0.01$ for methanolic; $p < 0.05$ for ethanolic and aqueous extracts). The sharpest reduction was recorded in the methanolic fraction of *P. sajor-caju*, which declined precipitously from 85.3 ± 0.01% to 2.6 ± 0.01% post-cooking, equating to a loss exceeding 96%. Methanolic extracts of *A. bisporus* similarly decreased from 85.1 ± 0.01% to 10.8 ± 0.02% after the same treatment. These steep losses are consistent with heat-induced degradation of thermally labile phenolic species and the leaching of water-soluble antioxidant molecules into the cooking medium [Jiménez-Monreal et al., 2009; Heleno et al., 2012]. Pearson's correlation coefficients for methanolic fractions ranged between r = 0.79 and r = 0.84, confirming a strong positive linear relationship between raw and post-cooking DPPH values. These findings are corroborated by Bakir et al. (2018), Effiong et al. (2024), and Tjokrokusumo et al. (2024), each of whom documented cooking-associated reductions in DPPH activity in edible mushroom species.

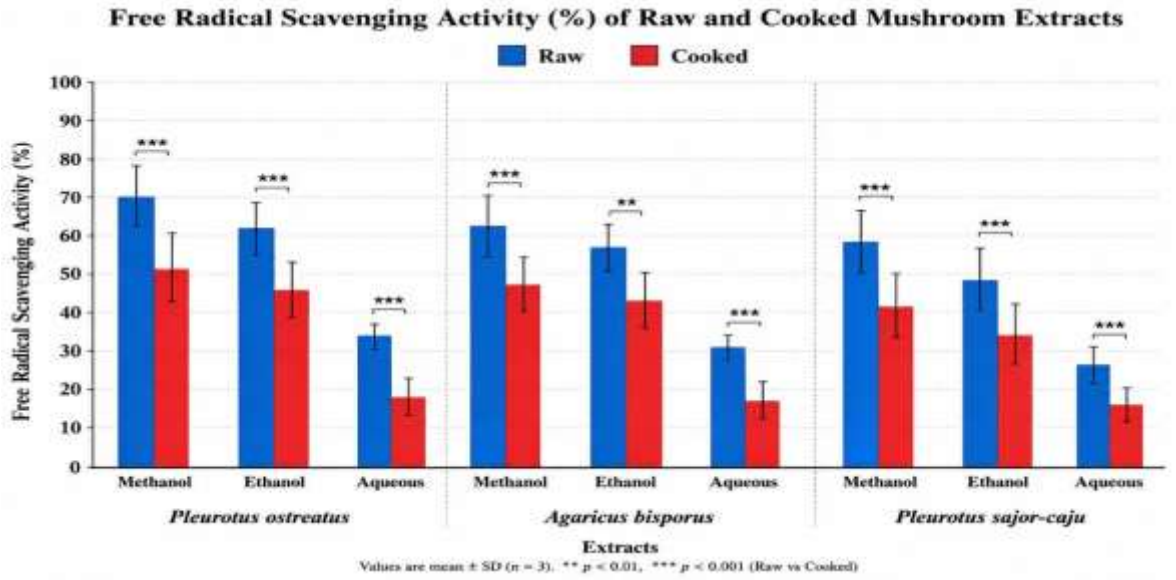

Figure 1. Influence of thermal processing on the radical scavenging activity of macrofungal extracts. Quantitative assessment of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging efficiencies in Pleurotus ostreatus, Agaricus bisporus, and Pleurotus sajor-caju matrices subjected to methanolic, ethanolic, and aqueous extraction protocols. Data points represent mean values ± standard deviation (n=3). Statistically significant disparities between raw (blue) and thermally processed (red) cohorts within individual solvent systems were determined via comparative variance analysis; asterisks signify discrete confidence levels ($p<0.01$, *** $p<0.001$).

4.2 ABTS Radical Cation Scavenging Activity

ABTS radical cation decolorization results are presented in Table 2. Unlike the DPPH assay, the ABTS data revealed a more complex and species-specific pattern in response to heat treatment. Among raw extracts, the methanolic fraction of P. ostreatus exhibited the greatest ABTS scavenging activity (84.52 ± 0.02%), followed by its ethanolic (70.03 ± 0.03%) and aqueous (65.05 ± 0.01%) counterparts. Methanolic extracts of A. bisporus and P. sajor-caju registered 73.06 ± 0.01% and 71.52 ± 0.02% in the raw state, respectively. These values underscore the broad-spectrum antioxidant activity of these species, as the ABTS assay is sensitive to both electron-transfer and hydrogen-atom transfer mechanisms across a wider molecular range than DPPH [Kim et al., 2008].

Following cooking, P. ostreatus recorded a statistically significant decline in ABTS activity

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across all solvent fractions (methanol: 84.52 → 80.02%, $p < 0.01$; ethanol: 70.03 → 65.06%, $p < 0.05$; aqueous: 65.05 → 54.00%, $p < 0.05$). In contrast, A. bisporus ethanolic extract showed a trivial and non-significant alteration (58.80 → 59.01%, $p = 0.056$), while its aqueous fraction registered an unexpected and statistically significant increase after cooking (49.84 → 75.00%, $p < 0.01$). A similar augmentation was observed in P. sajor-caju ethanolic (40.07 → 56.15%, $p < 0.05$) and aqueous (25.84 → 77.02%, $p < 0.01$) fractions. This paradoxical enhancement is plausibly attributable to Maillard reaction products formed during boiling and the thermal liberation of cell-wall-associated antioxidant compounds ordinarily unavailable for extraction from unprocessed tissue [Heleno et al., 2012; Mwangi et al., 2022]. The discordance between DPPH and ABTS outcomes underscores the mechanistic complexity of mushroom antioxidant chemistry and reinforces the value of employing multiple complementary analytical platforms [Al Qutaibi & Kagne, 2024; Krupodorova et al., 2025].

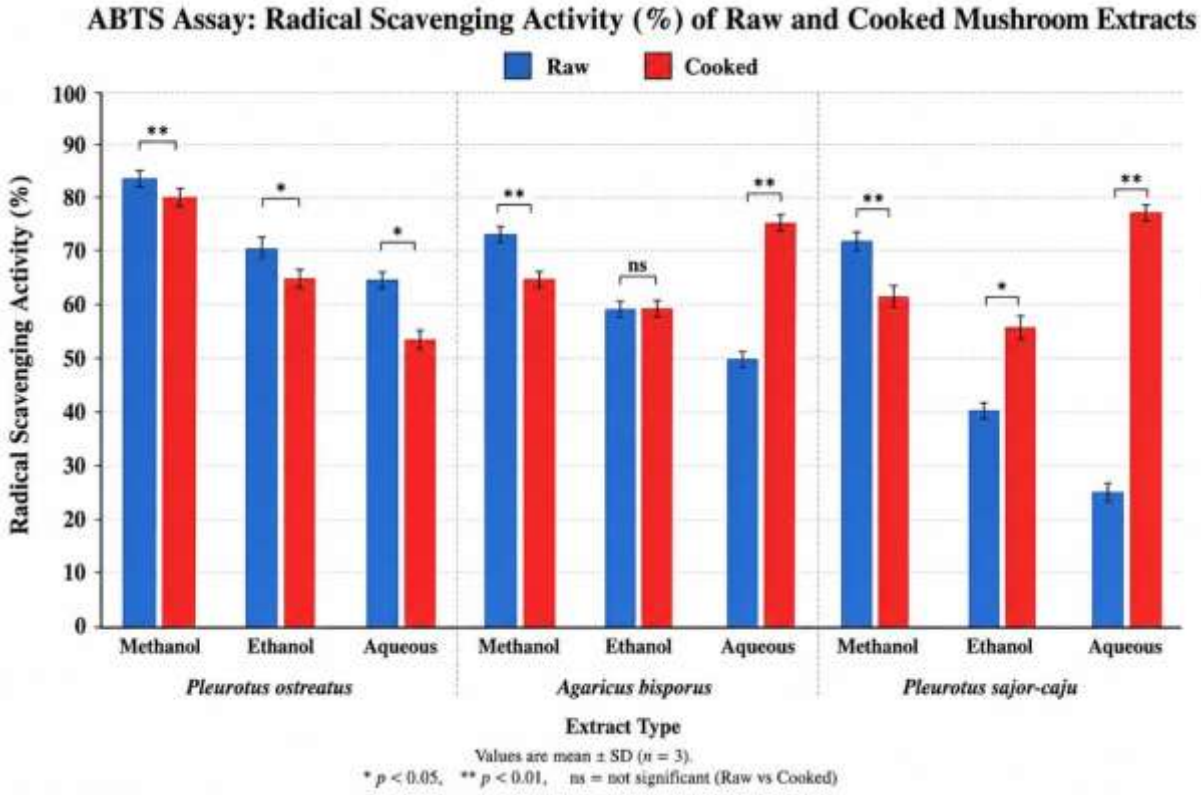

Figure 2. ABTS radical scavenging activity (%) of raw and cooked mushroom extracts. Data are presented as mean ± SD (n = 3). Statistical significance between raw and cooked pairs is indicated by * ($p < 0.05$) and ($p < 0.01$); ns denotes no significant difference.

4.3 Total Phenolic Content (TPC)

TPC values (mg GAE/g) for all extracts are compiled in Table 3. In unprocessed mushrooms, phenolic concentrations were strikingly uniform across both species and solvent systems, ranging narrowly from 8.74 ± 0.01 to 9.39 ± 0.01 mg GAE/g. Methanolic fractions consistently returned the highest TPC values, attributable to the superior capacity of methanol to solubilise both polar and moderately non-polar phenolic classes, including hydroxycinnamic acids, hydroxybenzoic acids, and glycosidic conjugates [Barros et al., 2007; Kozarski et al., 2015]. The aqueous extract of raw *P. sajor-caju* yielded the maximum TPC among all raw aqueous preparations at 9.39 ± 0.01 mg GAE/g.

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Cooking uniformly reduced TPC in a statistically significant manner across all species and solvent systems. The largest absolute decline was observed in the aqueous extract of *P. sajor-caju*, which dropped from 9.39 ± 0.01 to 7.22 ± 0.01 mg GAE/g ($p < 0.01$, $r = 0.60$). In *P. ostreatus*, the methanolic fraction declined from 9.23 ± 0.01 to 7.30 ± 0.01 mg GAE/g, with a correlation coefficient of $r = 0.88$, the highest among all TPC measurements, indicating a highly systematic cooking-associated depletion of the phenolic pool. Such thermal degradation of phenolics has been well documented: Jayakumar et al. (2011) reported analogous losses upon boiling of edible mushrooms, while Das et al. (2021) attributed such decrements to heat-driven cleavage of ester and glycosidic bonds that release and subsequently decompose phenolic aglycones. The comparatively moderate correlations ($r = 0.62$–0.75) observed for ethanolic and aqueous extracts suggest that the linear relationship between raw and cooked TPC is less consistent in these fractions, possibly reflecting differential solubility and thermal stability of distinct phenolic sub-classes [Silva et al., 2025].

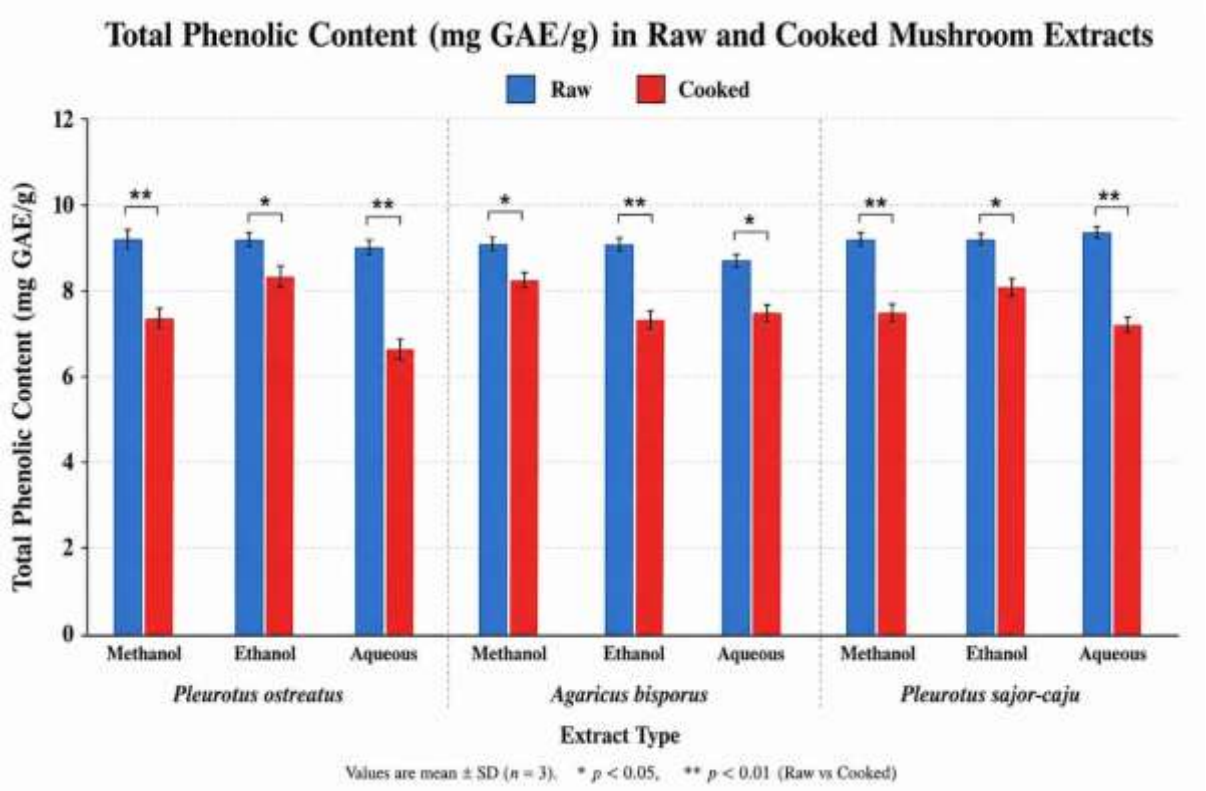

Figure 3. Comparison of Total Phenolic Content (TPC) in raw and cooked extracts of Pleurotus ostreatus, Agaricus bisporus, and Pleurotus sajor-caju across methanol, ethanol, and aqueous solvents, where cooking significantly reduced TPC levels ($p < 0.05$and $p < 0.01$) in all samples.

4.4 Total Flavonoid Content (TFC)

TFC data (mg QE/g) are displayed in Table 4. In unprocessed samples, methanolic extracts yielded the highest flavonoid concentrations in all three species: 0.016 ± 0.001 mg QE/g (*P. ostreatus*), 0.014 ± 0.001 mg QE/g (*A. bisporus*), and 0.013 ± 0.001 mg QE/g (*P. sajor-caju*). Aqueous extracts consistently returned the lowest TFC values across all species (0.004–0.006 mg QE/g), which is anticipated given the inherently limited water solubility of most flavonoid aglycones under ambient conditions [Chang et al., 2002; Cheung et al., 2003].

Thermal processing brought about significant reductions in TFC across the majority of species–

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solvent combinations. The methanolic fraction of *P. ostreatus* declined from 0.016 ± 0.001 to 0.010 ± 0.001 mg QE/g ($p < 0.01$, $r = 0.84$), while *A. bisporus* and *P. sajor-caju* methanolic extracts fell to 0.008 ± 0.001 and 0.007 ± 0.001 mg QE/g, respectively, both at high levels of significance ($p < 0.01$). Notably, the aqueous extract of *A. bisporus* showed no significant change in TFC upon cooking (0.004 → 0.004 mg QE/g, $p = 0.056$), suggesting that its initial aqueous flavonoid concentration was sufficiently low to resist further quantifiable diminution by heat. These observations align with Mayirnao et al. (2025), who noted that flavonoids undergo oxidative polymerisation and structural decomposition during prolonged thermal exposure. The strong positive correlations in methanolic and ethanolic fractions ($r = 0.70–0.84$) confirm that species with higher initial flavonoid concentrations retain proportionally higher residual values after cooking, establishing a predictable linear relationship between raw and heat-treated states [Michalska et al., 2025; Okumuş et al., 2025].

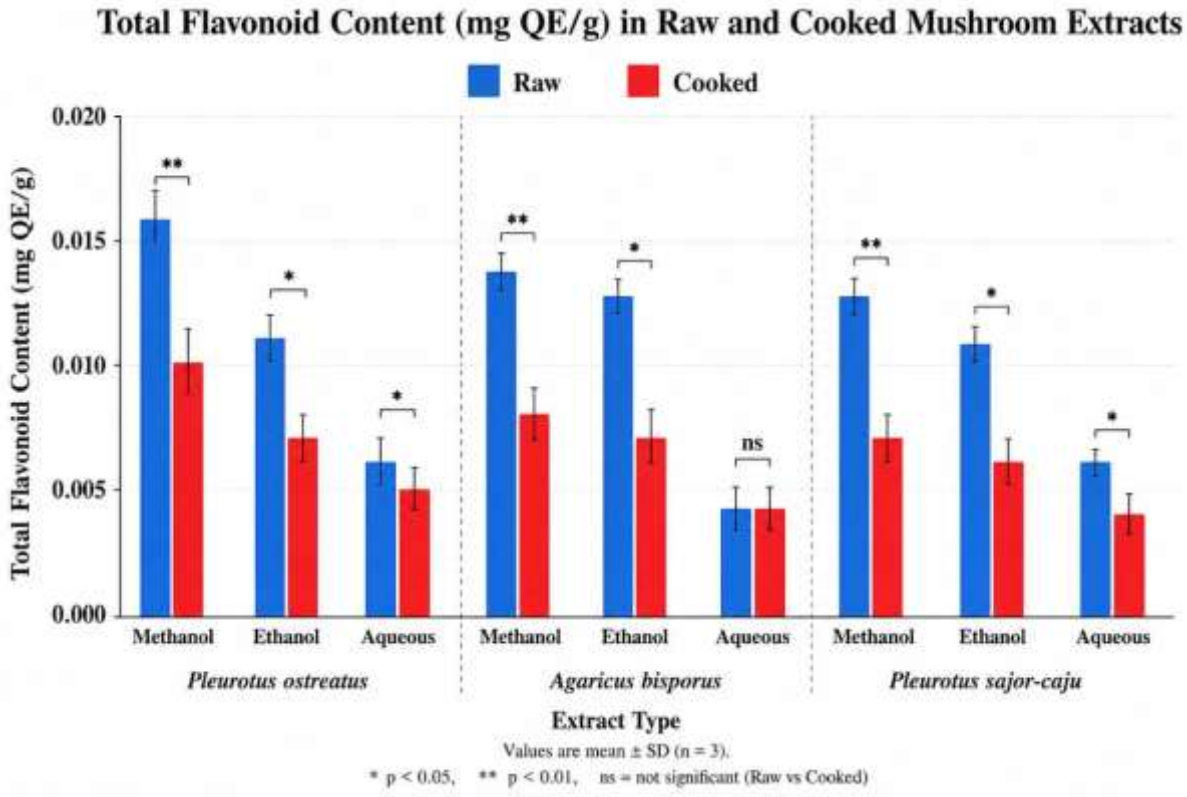

Figure 4. Effect of cooking and extraction solvents on the total flavonoid content (mg QE/g) of three mushroom species (P. ostreatus, A. bisporus, and P. sajor-caju). Cooking significantly reduced flavonoid levels in most organic extracts, while aqueous extracts showed minimal changes. Values are expressed as mean ± SD (n = 3; ^*p < 0.05, ^{**}p < 0.01, ns = not significant).

5. DISCUSSION

The present investigation systematically assessed the antioxidant attributes of *Pleurotus ostreatus*, *Agaricus bisporus*, and *Pleurotus sajor-caju* using DPPH, ABTS, TPC, and TFC assays across three solvent systems and two thermal states. Methanolic fractions consistently outperformed ethanolic and aqueous extracts in all analytical parameters across all three species, a pattern attributable to methanol's intermediate polarity and its ability to co-extract both hydrophilic and moderately lipophilic phenolic classes [Cheung et al., 2003; Kozarski et al., 2015]. Aqueous fractions returned the lowest values in most assays, consistent with the poor water solubility of many flavonoid aglycones and lipid-associated antioxidant compounds [Chang et al., 2002].

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Raw methanolic fractions of all three species displayed equivalent and high DPPH scavenging capacity (~85%), confirming the potent hydrogen-donating capacity inherent to their native phenolic matrices. Cooking precipitated statistically significant decreases in DPPH activity across all species ($p < 0.01$ for methanol), with *P. sajor-caju* recording the most severe loss (85.3% → 2.6%), indicating that its phenolic constituents are particularly susceptible to heat-mediated breakdown and leaching [Jiménez-Monreal et al., 2009; Bakir et al., 2018]. By contrast, *P. ostreatus* retained a comparatively higher post-cooking DPPH value (25.3%), suggesting a more thermally robust antioxidant constitution [Effiong et al., 2024].

The ABTS results presented a contrasting picture. While *P. ostreatus* showed uniform decreases across all fractions after cooking, aqueous extracts of *A. bisporus* and *P. sajor-caju* exhibited paradoxical cooking-induced increases in ABTS activity (49.84% → 75.00% and 25.84% → 77.02%, respectively). This augmentation likely reflects the formation of Maillard reaction by-products during boiling and the heat-facilitated liberation of cell-wall-entrapped phenolics not readily accessed in unprocessed tissue [Heleno et al., 2012; Mwangi et al., 2022]. The divergence between DPPH and ABTS profiles reinforces the mechanistic complexity of mushroom antioxidant chemistry and the scientific necessity of using complementary assays rather than relying on a single method [Kim et al., 2008; Al Qutaibi & Kagne, 2024].

Both TPC and TFC declined substantially upon cooking across all species and solvent systems. Strong correlations between TPC and DPPH activity in methanolic fractions ($r = 0.80–0.88$) confirm that phenolic hydroxyl groups are the principal structural determinants of radical scavenging capacity in these mushrooms [Barros et al., 2007; Das et al., 2021]. The post-cooking reduction in TFC is consistent with the thermal oxidative polymerisation and structural disintegration of thermolabile flavonoid molecules [Mayirnao et al., 2025; Okumuş et al., 2025]. The unchanged aqueous TFC of *A. bisporus* ($p = 0.056$) upon cooking suggests that its aqueous flavonoid content had already reached a near-baseline detection level in the raw state.

Taken together, the data confirm that solvent polarity, species identity, and thermal processing are the three dominant determinants of antioxidant activity in edible mushrooms. From a dietary standpoint, minimising heat exposure or adopting gentler cooking strategies would be advisable to preserve thermolabile bioactive constituents [Michalska et al., 2025]. The strong antioxidant capacity of raw methanolic extracts further establishes these mushroom species as compelling candidates for development as nutraceuticals and functional food ingredients [Friedman, 2016; Liuzzi et al., 2023].

`CONCLUSION

Pleurotus ostreatus, *Agaricus bisporus*, and *Pleurotus sajor-caju* have been conclusively demonstrated in this study to be abundant natural sources of dietary antioxidants, with methanolic extracts yielding the highest performance across all in vitro assays evaluated. Raw methanolic preparations of all three species attained the highest DPPH scavenging activity (~85%), total phenolic content (~9.0–9.4 mg GAE/g), and total flavonoid content (0.013–0.016 mg QE/g), affirming the central role of phenolic and flavonoid constituents in their radical scavenging capacity. Thermal treatment caused a statistically significant reduction ($p < 0.01$) in DPPH activity, TPC, and TFC across all species, with *P. sajor-caju* recording the most extreme post-cooking decline in DPPH activity (85.3% → 2.6%), indicative of high thermal sensitivity of its bioactive constituents. The ABTS assay, however, revealed a counterintuitive enhancement in aqueous fractions of *A. bisporus* and *P. sajor-caju* following cooking, attributable to Maillard

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reaction products and heat-induced mobilisation of structurally occluded phenolics. Strong positive correlations (r = 0.79–0.88) between TPC and radical scavenging parameters in methanolic fractions validated phenolic compounds as the primary antioxidant determinants. These findings collectively emphasise the importance of appropriate solvent selection and judicious cooking practices in preserving the nutraceutical integrity of edible mushrooms and underscore their potential utility in functional food development and pharmaceutical applications.

DECLARATIONS

Conflict of Interest: The authors declare the absence of any competing financial, personal, or professional interests.

Ethical Approval: No human participants or experimental animals were involved in this research. All mushroom samples and laboratory materials were handled in accordance with established institutional safety and laboratory practice guidelines.

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