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Insights Into the Cold Stress-Driven Metabolome of Edible Mushrooms from Arid Highlands of the Indian Trans-Himalayas

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Abstract

Cold desert ecosystems impose selective pressures that condition the biochemical architecture of resident macrofungi. The present study provides a comprehensive evaluation of metabolome profile of three wild edible mushrooms namely; *Flammulina filiformis*, *Agaricus campestris*, and *Cantharellus cibarius*, inhabiting the cold desert of the Indian Trans-Himalayas. Our findings highlight how these mushrooms adapt to such adverse conditions while exhibiting nutraceutical potential. Untargeted metabolome profiling identified 88 metabolites, encompassing carbohydrates, polyols, fatty acids, organic acids, amino acids, and nitrogenous compounds. Carbohydrates dominated the metabolome, with trehalose representing 13.68–18.14%, followed by glucitol (7.44–17.25%). Fatty acids constituted 23.65–29.04%, with oleic and linoleic acid predominating among monounsaturated and polyunsaturated fatty acids, respectively. Protein content was highest in *F. filiformis* (218.4 mg g^{-1} dry weight), suggesting potential utility as an alternative to animal-based protein sources. Essential amino acids including threonine and valine were enriched in *F. filiformis*. Mineral profiling revealed species-specific enrichment: *A. campestris* was richest in Na, K, Ca, Mg, Fe, Zn, and Mn; *C. cibarius* in N, Cr, Se, and Mo; and *F. filiformis* in S, Al, and Cu. Antinutritional factors remained below critical thresholds, ensuring bioavailability of essential minerals. Total phenolic content was highest in *F. filiformis*, while flavonoid and carotenoids peaked in *A. campestris*. Quantification of twelve phenolic compounds revealed high concentrations, which were corroborated by antioxidant capacities. Fourier transform infrared spectroscopy confirmed diverse functional groups, reinforcing the presence of bioactive metabolites. Collectively, these mushrooms from extreme environment are nutrient-dense, bioactive-rich resources with tangible applications in nutraceutical innovation.

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Introduction

Environmental variables play a crucial role in regulating the physiology, secondary metabolism, and ecological interactions of macrofungi or mushrooms. Unlike vascular plants, fungi lack photosynthetic machinery and do not respond to environmental pressures through anatomical modifications. However, they primarily rely on the production of specialized metabolites and enzymatic versatility to gain ecological fitness and ensure survival. Their adaptive responses involve metabolic reprogramming that results in the accumulation/ synthesis of secondary metabolites. These metabolites exhibit diverse biological activities and play essential roles in defense, environmental sensing, oxidative balance, and inter-organismal communication.[1,2]

The current trajectory of mycological research exhibits a discernible bias toward macrofungi inhabiting mesic and agriculturally modified ecosystems, thereby marginalizing the mycodiversity of high-altitude cold desert biomes.[3,4] This underrepresentation is scientifically consequential, as extremophilic fungi in arid and thermally volatile habitats, such as Kargil located in the Indian trans-Himalayan belt, are likely to possess adaptive metabolic frameworks that remain largely uncharacterized.[4,5] The sporadic nature of fungal fruiting, largely dictated by microclimatic windows renders macrofungal inventories in such regions both methodologically challenging and temporally constrained.

In cold desert ecosystems, environmental stringency, manifested in the form of persistent ultraviolet radiation, high oxidative stress, sub-zero temperatures, and episodic desiccation, acts as an overwhelming selective pressure. Thus, these factors elicit significant abiotic stress on fungal communities.[6] Such abiotic stresses are known to trigger intricate signalling cascades, including the activation of ROS-scavenging mechanisms and the transcriptional upregulation of stress-responsive genes. These pathways culminate in the biosynthesis of a diverse array of secondary metabolites, including phenolic acids, flavonoids, terpenoids, and polyketides, many of which are endowed with high antioxidant capacity, metal chelation potential, and cytoprotective properties.[7] Importantly, compounds that function as osmolytes and ROS quenchers in fungal systems demonstrate pharmacodynamic parallels in human cellular models and a translational potential that warrants deeper investigation.[7,8] Thus, fungal responses to stresses enhance therapeutic potential and render those mushrooms from stressful habitats potentially richer in bioactive compounds than their temperate counterparts.

Therefore, the systematic bioprospecting of macrofungi from cold desert habitats represents an important aspect for the recognition of bioactive scaffolds. Within this context, the present study was designed to bridge the knowledge gap by undertaking a comprehensive metabolite profiling of three wild edible macrofungi – *Flammulina filiformis* (Ge *et al*.) Wang *et al*., *Agaricus campestris* L., and *Cantharellus cibarius* Fr. – from different microhabitats in Kargil. In addition to the exhaustive elemental and nutritional analyses, parameters that serve as indicators of nutraceutical potential and adaptive biochemical response were focused on, which include untargeted metabolites screening via gas chromatography-mass spectrometry (GCMS), ultra-high performance liquid chromatography (UHPLC)-based quantification of phenolic compounds (ferulic acid, resorcinol, catechol, orcinol, vanillin, salicylic acid, cinnamic acid, butylated hydroxytoluene, pyrogallol, caffeic acid, quercetin, luteolin), total phenol content, total flavonoid content, carotenoid-based antioxidants (lycopene and β-carotene), and antioxidant assays. Moreover, functional groups were identified to reveal their chemical nature using Fourier transform infrared (FTIR) spectroscopy. By aligning with the thematic framework of biochemical analyses, the study seeks to support the inference that metabolite richness is stress induced.

Materials and Methods

Sample Collection and Identification

Three species of mushrooms were collected during the fruiting season (August, 2023) from multiple cold desert microhabitats in the Kargil, located at altitudes ranging from 2,500–4,800 m asl. Mushroom samples were morphologically characterized, followed by molecular identification (The methodology is provided in detail in the Supplementary Material).

Untargeted Metabolite Profiling

For untargeted metabolite profiling using GC-MS, 1 g each of pulverised sample were extracted in

methanol for 3 h. The crude extract was centrifuged at 8000 *g* for 10 min and the resulting supernatant was concentrated. For derivatization, MSTFA method of trimethylsilylation was followed.

The test samples were analysed using GCMS-QP2010 Ultra (Shimadzu Corp., Kyoto, Japan). The mass spectra of the detected compounds were matched against the NIST-14 (National Institute of Standards and Technology) and WILEY-08 spectral libraries for compound identification. The relative percentage composition of the compounds was estimated based on peak area measurements, and the resulting data were processed using the GC-MS Postrun Solution software (Shimadzu Corp., Kyoto, Japan).

Macronutrients

Protein content was estimated using Bradford assay. The total available carbohydrate content (TAC) and total soluble sugars (TSS) were estimated using the anthrone method and phenol-sulfuric acid method, respectively. The concentration of reducing sugars was determined following the protocol of Miller. Non-reducing sugars were quantified by subtracting the reducing sugar concentration from the TSS. Starch concentration was quantified following colorimetric anthrone method.

Mineral Composition

The samples were digested with a mixture of nitric acid, perchloric acid, and sulfuric acid (4:2:0.5, v/v/v) using a hot-block digestion system. After digestion, the samples were allowed to cool, filtered through Whatman No. 44 filter paper, and diluted to a final volume of 50 mL with Milli-Q water. The concentrations of Ca, Mg, Fe, Cu, Co, Zn, Ni, and Al were determined using an AAS ZEEnit 60 atomic absorption spectrometer (Analytik Jena GmbH, Jena, Germany); Na and K were determined using flame photometry; Cr, Se, Mn, and Mo using inductively coupled plasma-mass spectrometer (ICPMS) by Agilent Technologies (8900 ICP-MS Triple Quad, California, US); N and S using CHNS analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany). P concentration was quantified by molybdenum blue method.

Antinutrients and Mineral Bioavailability

Antinutrients

Phytic acid (PA) content was determined following the Fe(III)-sulfosalicylate method. Condensed tannins (CT) were estimated following Prussian blue assay.

Mineral Bioavailability

Bioavailability of Fe, Ca, Mg, and Zn was determined by calculating molar ratios of antinutrients-to-minerals. Critical values of 2.5 for OA/(Ca+Mg), 0.4 for PA/Fe, 0.4 for PA/Ca, and 10 for PA/Zn were considered.

Antioxidants

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method. Total flavonoid content (TFC) was quantified using the aluminium chloride colorimetric assay. Spectrophotometric estimation of ascorbic acid, α-tocopherol, and carotenoid-based antioxidants (β-carotene and lycopene) were carried out.

Quantification of Phenolic Compounds

Phenolic profiling was performed using ultra-high performance liquid chromatography (UHPLC) on an Acquity UPLC™ H-Class System (Waters Corp., Massachusetts, USA) equipped with a BEH C18 column (1.7 µm, 2.1 mm × 50 mm). Phenolics were identified by retention times of standards (ferulic acid, resorcinol, catechol, orcinol, vanillin, salicylic acid, cinnamic acid, butylated hydroxytoluene, pyrogallol, caffeic acid, quercetin, luteolin) and quantified at 280 nm using Waters Empower® 3 software (Waters Corp., Massachusetts, USA). Standard curves showed high linearity ($r^2 > 0.999$).

Antioxidant Potential

The antioxidant potential of the studied samples was evaluated through hydroxyl radical scavenging activity and reducing power assays.

Identification of Functional Groups

Fourier transform infrared spectroscopy (FTIR) was used to identify functional groups present in the sample extracts. Methanolic extracts were analysed in attenuated total reflectance (ATR)

mode. Spectra were recorded using Nicolet™ iS50 FTIR Spectrometer (Thermo Fisher Scientific™, Massachusetts, USA) over the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}. OMNIC™ Specta Software (Thermo Fisher Scientific™, Massachusetts, USA) was used to collect, display, and process data. Characteristic absorption peaks were interpreted to identify relevant functional groups. Stacked graph was plotted and interpreted using OriginPRO® v.2025b (OriginLab Corporation, Northampton, Massachusetts, USA).

The Methodology is Described in Detail in the Supplementary Material

Statistical Analysis

All experiments were performed in triplicate, and the results are presented as mean ± standard deviation (SD). Statistical significance ($p < 0.05$) was determined by one-way ANOVA with Tukey's post hoc test using SPSS v.21.0 (IBM Corporation, New York, USA).

Results

Identification and Sample Description

The species were identified as *Flammulina filiformis* (Ge *et al*.) Wang *et al., Agaricus campestris* L.*,* and *Cantharellus cibarius* Fr. (Supplementary Figure 1).

Untargeted Metabolome Profiling

Untargeted GCMS-based metabolome profiling revealed a total of 88 metabolites across the three fungal taxa, spanning saccharides and polyols, fatty acids, amino acids, non-amino N-containing compounds, as well as organic and mineral acids along with their derivatives (Table 1).

Table 1: Gas chromatography mass spectrometry-based metabolite profiling of Flammulina filiformis(Ge et al.) Wang et al., Agaricus campestris L., and Cantharellus cibarius Fr. The concentration of compounds is expressed in percentage compositions

Compound	*F. filiformis*	*A. campestris*	*C. cibarius*
Amino Acids and their Derivatives			
Essential amino acids			
Isoleucine	$0.24^{a} \pm 0.06$	$0.15^{b} \pm 0.02$	$0.13^{b} \pm 0.04$
Histidine	$0.19^{b} \pm 0.04$	Nd	$0.25^{a} \pm 0.01$
Leucine	$0.31^{a} \pm 0.01$	$0.36^{a} \pm 0.08$	$0.10^{b} \pm 0.04$
Phenylalanine	$0.19^{b} \pm 0.02$	$0.24^{a} \pm 0.02$	$0.13^{c} \pm 0.03$
Threonine	$0.54^{a} \pm 0.19$	$0.21^{b} \pm 0.03$	$0.53^{a} \pm 0.10$
Valine	$0.13^{b} \pm 0.04$	$0.37^{a} \pm 0.09$	$0.04^{c} \pm 0.02$
Non-essential amino acids			
Alanine	$0.15^{b} \pm 0.08$	$0.37^{a} \pm 0.01$	Nd
Asparagine	$0.47^{a} \pm 0.03$	Nd	Nd
Aspartic acid	$0.29^{b} \pm 0.04$	$0.69^{a} \pm 0.13$	$0.17^{c} \pm 0.05$
Glutamic acid	$0.10^{c} \pm 0$	$0.51^{b} \pm 0.07$	$0.61^{a} \pm 0.05$
Glycine	$0.02^{b} \pm 0.01$	$0.04^{a} \pm 0$	$0.03^{b} \pm 0$
Homoserine	$0.42^{a} \pm 0.09$	Nd	$0.35^{a} \pm 0.06$
Proline	$0.67^{a} \pm 0.16$	$0.25^{c} \pm 0.02$	$0.39^{b} \pm 0.04$
Serine	$0.21^{b} \pm 0.01$	$0.28^{a} \pm 0.01$	$0.16^{c} \pm 0.01$
Tyrosine	$0.11^{b} \pm 0.01$	$0.13^{a} \pm 0.01$	$0.12^{ab} \pm 0.02$
Non-protein amino acids / derivatives			
Glycyl-L-proline	$0.12^{a} \pm 0.03$	Nd	$0.07^{b} \pm 0.01$
Glycyl glycine	$0.11^{b} \pm 0.05$	$0.13^{b} \pm 0.01$	$0.23^{a} \pm 0.06$
Ornithine	$0.16^{a} \pm 0.05$	$0.18^{a} \pm 0.02$	$0.04^{b} \pm 0.01$
5-Oxoproline	$7.83^{ab} \pm 2.01$	$9.44^{a} \pm 0.61$	$6.15^{b} \pm 0.52$

Organic Acids and Derivaties			
Ascorbic acid	0.05^{b} ± 0.02	Nd	0.09^{a} ± 0
Citric acid	0.90^{a} ± 0.01	0.34^{b} ± 0.02	0.21^{c} ± 0.10
Fumaric acid	1.13^{c} ± 0.10	4.32^{a} ± 0.18	3.37^{b} ± 0.23
Glutaric acid	0.08^{b} ± 0.04	0.06^{c} ± 0.01	0.67^{a} ± 0.04
Glyceric acid	0.13 ± 0	Nd	Nd
Hydroxycinnamic acid	Nd	0.06^{b} ± 0 0.	11^{a} ± 0.04
Hydroxymalonic acid	0.26^{b} ± 0.11	0.40^{a} ± 0.10	0.15^{b} ± 0.07
Isocitric acid	1.80^{b} ± 1.03	2.84^{a} ± 0.50	1.78^{b} ± 0.93
Malic acid	4.70^{c} ± 0.87	5.79^{b} ± 0.57	7.03^{a} ± 0.59
Methylmaleic acid	0.10^{a} ± 0.06	0.07^{ab} ± 0.04	0.04^{b} ± 0.02
Methylsuccinic acid	0.12 ± 0.04	Nd	Nd
Oxalic acid	0.16^{a} ± 0.09	0.13^{ab} ± 0.06	0.10^{b} ± 0.01
Pyruvic acid	0.11^{a} ± 0.04	0.12^{a} ± 0.01	0.05^{b} ± 0
Salicylic acid	0.09^{a} ± 0	0.06^{b} ± 0.03	0.11^{a} ± 0.04
Succinic acid	1.91^{b} ± 0.76	2.63^{a} ± 0.13	1.56^{b} ± 0.22
Tartaric acid	1.00^{a} ± 0.31	Nd	0.02^{b} ± 0.01
α-Hydroxyglutaric acid	2.57^{a} ± 1.08	Nd	0.55^{b} ± 0.18
Saccharides and Polyols			
Sugars and sugar acids			
Arabinopyranose	Nd	0.05b ± 0.01	0.07^{a} ± 0.01
α-Mannobiose	1.93^{b}± 0.21	3.27^{a} ± 0.13	0.14^{c} ± 0.02
Deoxygalactose	0.10^{a} ± 0	Nd	0.10^{a} ± 0.04
Deoxyribose	0.07 ± 0.04	Nd	Nd
Erythrose	0.19^{b} ± 0.05	0.11^{c} ± 0.04	0.40^{a} ± 0.09
Erythrotetrofuranose	Nd	0.10^{a} ± 0.02	0.08^{a} ± 0.02
Fructopyranose	0.41^{a} ± 0.09	0.24^{b} ± 0.07	0.08^{c} ± 0.01
Galactose	Nd	Nd	0.02 ± 0
Gluconic acid	1.01^{c} ± 0.28	3.68^{a} ± 0.49	1.85^{b} ± 0.45
Glucose	Nd	0.08^{b} ± 0.02	0.38^{a} ± 0.17
Glyceric acid	1.55 ±0.32	Nd	Nd
Maltose	Nd	0.28^{a} ± 0.05	0.05^{b} ± 0
Talose	3.40 ± 0.37	Nd	Nd
Threose	Nd	0.04^{b} ± 0.01	0.55^{a} ± 0.11
Trehalose	18.14^{a} ± 2.56	13.68^{b} ± 1.99	17.91^{a} ± 1.85
Polyols			
Arabinitol	0.15^{b} ± 0.01	0.24^{a} ± 0.04	0.13^{b} ± 0.05
Fucitol	Nd	Nd	0.16 ± 0.07
Glucitol	7.44^{c} ± 0.16	13.71^{b} ± 1.15	17.25^{a} ± 2.51
Gluconolactone	0.21^{b} ± 0.07	Nd	0.40^{b} ± 0.12
Glycerol	4.72^{b} ± 0.70	2.07^{c} ± 0.02	6.31^{a} ± 0.46
Mannitol	0.13^{b} ± 0.03	0.23^{a} ± 0.10	Nd
Myo-inositol	0.78^{b} ± 0.34	0.41^{c} ± 0.17	2.72^{a} ± 0.21
Meso-erythritol	0.32^{b} ± 0.10	Nd	1.35^{a} ± 0.20
Ribitol	2.47^{a} ± 0.41	0.94^{b} ± 0.37	2.26^{a} ± 0.21

Fatty Acids			
Saturated Fatty Acids			
Arachidic acid	$0.34^{b} \pm 0.13$	$0.44^{ab} \pm 0.18$	$0.60^{a} \pm 0.18$
Myristic acid	$1.08^{a} \pm 0.27$	$0.51^{b} \pm 0$	$0.32^{c} \pm 0.17$
Palmitic acid	$5.73^{a} \pm 0.33$	$5.60^{a} \pm 0.40$	$5.69^{a} \pm 0.34$
Margaric acid	$0.12^{a} \pm 0.04$	$0.10^{ab} \pm 0.07$	$0.04^{b} \pm 0.03$
Lignoceric acid	Nd	Nd	0.25 ± 0.12
Pentadecanoic acid	Nd	$0.43^{a} \pm 0.24$	$0.33^{a} \pm 0.13$
Stearic acid	$3.76^{a} \pm 0.65$	$2.96^{b} \pm 0.23$	$2.80^{b} \pm 0.75$
Pimelic acid	0.14 ± 0.04	Nd	Nd
Unsaturated Fatty Acids			
Dehydroergosteryl benzoate	0.08 ± 0.01	Nd	Nd
Eicosatrienoic acid	$0.15^{b} \pm 0.07$	Nd	$0.36^{a} \pm 0.13$
Ergosterol	$2.44^{a} \pm 0.15$	$2.19^{a} \pm 0.34$	$0.95^{b} \pm 0.17$
Linoelaidic acid	$0.15^{c} \pm 0.09$	$0.28^{b} \pm 0.08$	$1.74^{a} \pm 0.09$
Linoleic acid	$5.45^{c} \pm 0.55$	$7.63^{b} \pm 0.91$	$11.00^{a} \pm 1.27$
Oleic acid	$4.18^{a} \pm 0.31$	$2.61^{c} \pm 0.27$	$3.42^{b} \pm 0.62$
Palmitelaidic acid	$0.22^{b} \pm 0.13$	$0.90^{a} \pm 0.34$	$0.19^{b} \pm 0.08$
Ricinoleic acid	3.26 ± 0.91	Nd	Nd
Stigmasterol	1.94 ± 0.63	Nd	Nd
Non -Amino Nitrogen-Containing-Compounds			
Adenosine	$1.19^{a} \pm 0.18$	$0.84^{b} \pm 0.35$	$0.89^{b} \pm 0.43$
Dihydrouracil	$0.77^{a} \pm 0.21$	$0.30^{b} \pm 0.15$	$0.83^{a} \pm 0.18$
Ethanolamine	Nd	$0.13^{b} \pm 0.06$	$0.25^{a} \pm 0$
Methyluridine	Nd	0.21 ± 0.10	Nd
Methyladenosine	Nd	0.14 ± 0.09	Nd
N-acetylglucosamine	0.25 ± 0.01	Nd	Nd
Niacin	$0.24^{b} \pm 0.08$	$0.47^{a} \pm 0.06$	$0.51^{a} \pm 0.15$
Pantothenic acid	$0.04^{b} \pm 0.01$	$0.06^{b} \pm 0.03$	$0.34^{a} \pm 0.06$
Uracil	$0.14^{b} \pm 0$	$0.18^{a} \pm 0.01$	$0.08^{c} \pm 0.01$
Uridine	$1.07^{b} \pm 0.05$	$0.31^{c} \pm 0.24$	$2.31^{a} \pm 0.92$
Mineral Acid			
Phosphoric acid	$1.13^{a} \pm 0.45$	$1.27^{a} \pm 0.48$	$0.57^{b} \pm 0.45$

Nd: Not detected

Carbohydrates constituted the dominant fraction of the metabolome (39.13–51.21%) in all species analyzed. Trehalose emerged as the most abundant metabolite, contributing 18.14% of the total metabolite pool in *F. filiformis* and 13.68% in *A. campestris*. Gluconic acid (1.01–3.66%) exhibited highest concentration amongst the sugar acids. Glucitol represented the principal polyol, with concentrations ranging from 7.44–17.25%, followed by glycerol (2.07–6.31%), and ribitol (0.94–2.47%) in relative abundance. Fucitol, mannitol, and meso-erythritol were detected sporadically.

Lipid-derived metabolites formed a substantial component of the metabolome. The total fatty acid content (TFAC) ranged from 23.65–29.04% of the metabolome. Saturated fatty acids contributed between 36.22% (*C. cibarius*) and 42.45% (*A. campestris*) of the TFAC. Unsaturated fatty acids (57.54–63.77% of TFAC) were predominantly

represented by oleic acid and linoleic acid. Ergosterol concentration ranged from 0.95–2.44% of the total metabolites. Ricinoleic acid, dehydroergosteryl benzoate, and stigmasterol were detected only in *F. filiformis*.

Organic acids accounted for 15.11–16.76% of the compound profile, with malic, fumaric, isocitric, and succinic acids in this order are the major contributors and were consistently represented across all species. The highest concentration of malic acid was registered in *C. cibarius* (7.04%). Non-amino N-containing compounds occurred in comparatively lower abundance relative to carbohydrates and fatty acids. Adenosine, dihydrouracil, and uridine dominated the profile. Niacin (0.24–0.51%) and pantothenic acid (0.04–0.34%) are two B vitamins consistently observed in all the species.

Essential amino acids, including isoleucine, histidine, leucine, phenylalanine, threonine, and valine, accounted for 1.18–1.60% of the total compounds. Amongst the non-essential amino acids, proline, aspartic acid, and glutamic acid were most abundant. 5-Oxoproline represented the most dominant amino acid derivative overall, peaking at 9.44% in *A. campestris*. Additional derivatives include glycyl-L-proline, glycyl glycine, and ornithine were also detected.

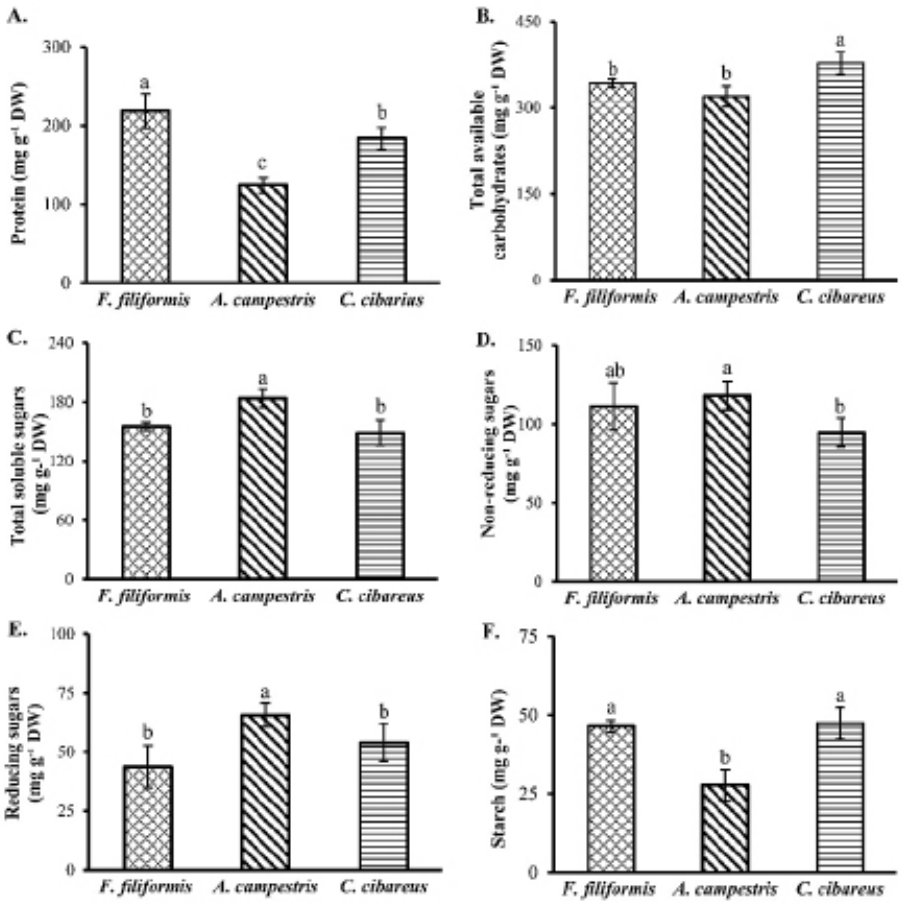

Fig. 1: Bar graphs showing concentrations of macronutrients – (A) total protein content (B) total available carbohydrates, (C) total soluble sugars, (D) non-reducing sugars, (E) reducing sugars, and (F) starch in *Flammulina filiformis* (Ge *et al*.) Wang *et al*., *Agaricus campestris* L., and *Cantharellus cibarius* Fr. Values represent mean ± SD (n=3). Different letters on error bars indicate significant differences at p ≤ 0.05, derived from one-way ANOVA.

Macronutrients

F. filiformis contained the highest total protein (218.4 mg g^{-1} DW), whereas *A. campestris* recorded the lowest (124.3 mg g^{-1} DW). Total available carbohydrate (TAC) content was highest in *C. cibarius*. *A. campestris* registered the highest concentration of TSS, followed by *F. filiformis* and *C. cibarius*. A similar pattern was observed for the concentration of non-reducing sugars. In terms of reducing sugars, *A. campestris* accounted for the highest concentration. Starch content was highest in *F. filiformis* and closely followed by *C. cibarius* (Figure 1A–F).

Mineral Composition

Macro- and trace element composition varied across species (Table 2). *A. campestris* exhibited dominance in the majority of the analyzed minerals, showing the highest concentrations of Na, K, Ca, Mg, Fe, Ni, Zn, and Mn. In contrast, *C. cibarius* was enriched in N, Cr, Se, and Mo. *F. filiformis*, on the other hand, exhibited the highest contents of S, Al, and Cu.

Table 2: Mineral composition and antinutrient-to-mineral molar ratios of *Flammulina filiformis* (Ge *et al.*) Wang *et al.*, *Agaricus campestris* L., and *Cantharellus cibarius* Fr.

Elements	*F. filiformis*	*A. campestris*	*C. cibarius*
Macro-elements (mg g^{-1} DW)			
N	$4.45^{b} \pm 0.86$	$4.95^{b} \pm 0.94$	$6.84^{a} \pm 0.91$
S	$0.79^{a} \pm 0.35$	$0.78^{a} \pm 0.26$	$0.75^{a} \pm 0.19$
P	$0.13^{a} \pm 0$	$0.10^{b} \pm 0$	$0.05^{c} \pm 0.01$
Na	$0.23^{b} \pm 0.02$	$0.26^{a} \pm 0.01$	$0.19^{c} \pm 0.01$
K	$13.31^{b} \pm 1.71$	$16.51^{a} \pm 1.28$	$16.49^{a} \pm 0.07$
Ca	$0.40^{b} \pm 0.10$	$0.95^{a} \pm 0.01$	$0.30^{c} \pm 0.02$
Mg	$0.35^{c} \pm 0.02$	$0.54^{a} \pm 0.01$	$0.52^{b} \pm 0.01$
Trace elements (µg g^{-1} DW)			
Al	$52.90^{a} \pm 14.97$	$25.72^{b} \pm 9.30$	$24.43^{b} \pm 8.93$
Cu	$20.37^{a} \pm 0.76$	7.36c ± 1.79	$14.18^{b} \pm 0.72$
Fe	$87.22^{b} \pm 5.94$	$449.51^{a} \pm 51.31$	$66.70^{c} \pm 17.14$
Ni	$16.15^{b} \pm 1.60$	$21.12^{a} \pm 1.06$	$11.20^{c} \pm 3.01$
Zn	$14.19^{b} \pm 0.48$	$27.35^{a} \pm 4.58$	$11.22^{c} \pm 0.56$
Cr	$16.41^{b} \pm 1.83$	$26.50^{a} \pm 2.94$	$29.65^{a} \pm 1.29$
Mn	$30.68^{c} \pm 3.40$	$51.72^{a} \pm 0.74$	$47.65^{b} \pm 2.29$
Se	$1.99^{c} \pm 0.84$	$2.37^{b} \pm 0.26$	$7.59^{a} \pm 0.91$
Mo	$6.65^{b} \pm 0.33$	$5.12^{c} \pm 0.59$	$12.22^{a} \pm 0.46$
Antinutrient-to-mineral molar ratio			
OA/(Ca+Mg)	0.15	0.62	0.87
PA/Fe	0.33	0.27	0.23
PA/Ca	0.61	0.15	0.13
PA/Zn	2.60	4.09	2.56

OA - oxalic acid; PA - phytic acid.

Values represent mean ± SD (n=3). Different letters within a row represent significant differences at $p \leq 0.05$, derived from one-way ANOVA.

Antinutrients and Mineral Bioavailability

Antinutritional Factors

The highest concentration of CT was observed in *C. cibarius*. *F. filiformis* exhibited the highest PA contents while *A. campestris* had comparatively low concentration (Figure 2A–B).

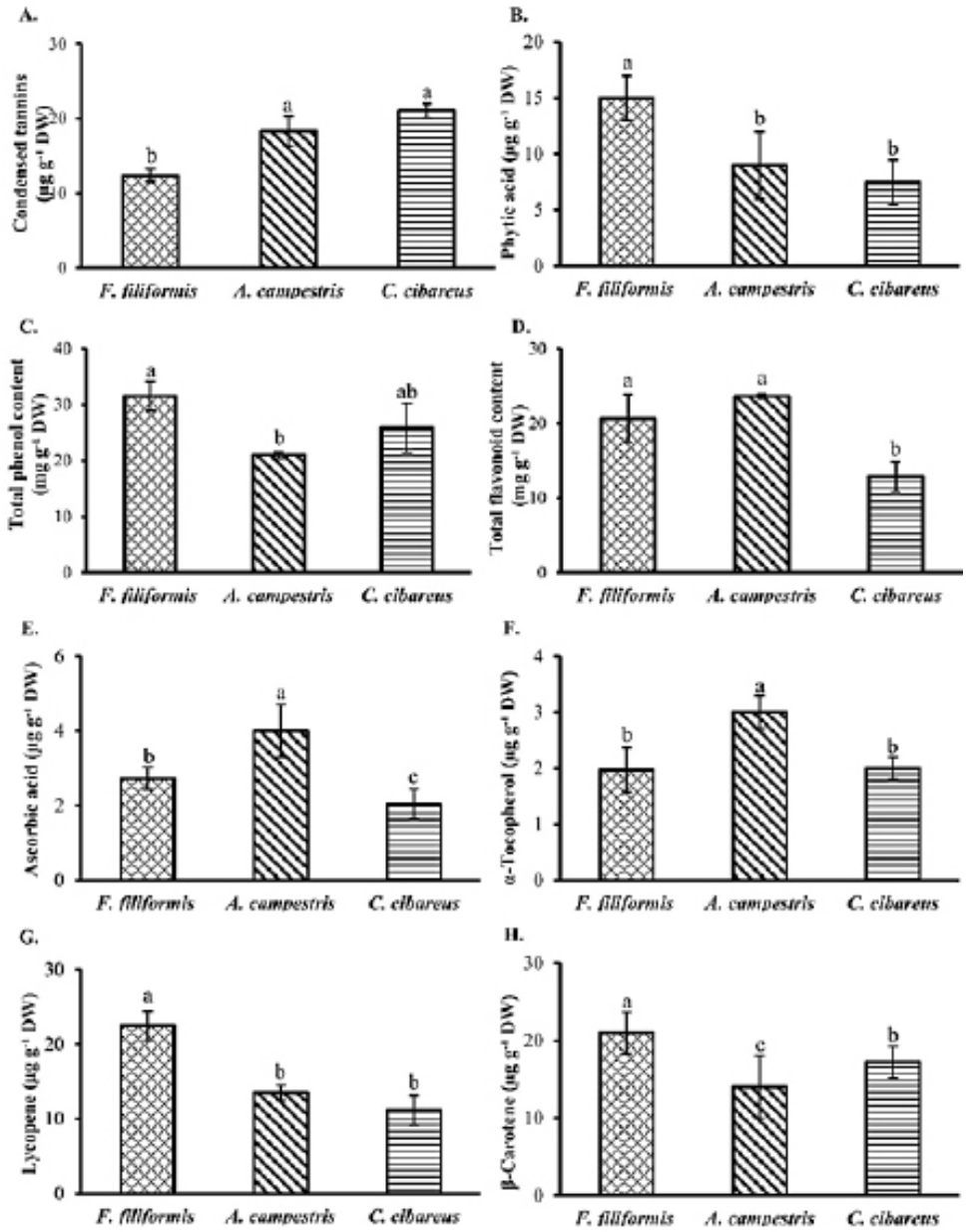

Fig. 2: Bar graphs showing concentrations of antinutrients – (A) condensed tannins, (B) phytic acid; and non-enzymatic antioxidants – (C) total phenol content, (D) total flavonoid content, (E) ascorbic acid, (F) α-tocopherol, (G) lycopene, and (H) β-carotene, in *Flammulina filiformis* (Ge *et al.*) Wang *et al*., *Agaricus campestris* L., and *Cantharellus cibarius* Fr. Values represent mean ± SD (n=3). Different letters on error bars indicate significant differences at p ≤ 0.05, derived from one-way ANOVA.

Bioavailability

The oxalic acid/(Ca+Mg) ratio remained below 2.5 for all species, with *F. filiformis* showing the lowest (0.41) and *C. cibarius* the highest (2.18). The PA/Fe ratio was well under the 0.4 threshold, lowest in *A. campestris* (0.10). The ratios of PA/Ca and PA/Zn were also within safe limits, the latter being lowest in *C. cibarius* (1.17) (Table 2).

Antioxidants

Total phenolic content (TPC) was highest in *F. filiformis*. *A. campestris* had the highest concentration of total flavonoid content (TFC), lycopene, and β-carotene (Figure 2C–H).

Quantification of Phenolic Compounds

Phenolic compounds quantified using UHPLC revealed that *A. campestris* exhibited highest concentrations of catechol and quercetin amongst the three mushrooms, while *F. filiformis* was rich in resorcinol, ferulic acid, and luteolin. *C. cibarius* showed elevated concentrations of pyrogallol, cinnamic acid, and vanillin. Guaiacol was registered only in *A. campestris*, while orcinol and salicylic acid were shared across taxa but varied in abundance (Table 3)

Table 3: Ultra-high performance liquid chromatography-based quantification of phenolic compounds in *Flammulina filiformis* (Ge *et al*.) Wang *et al*., A*garicus campestris* L., and *Cantharellus cibarius* Fr. The concentration of compounds is expressed in µg g^{-1} dry weight

Compound	*F. filiformis*	*A. campestris*	*C. cibarius*
Ferulic acid	$9.07^{a} \pm 0.91$	$2.25^{b} \pm 0.11$	$8.87^{a} \pm 1.03$
Resorcinol	$15.21^{a} \pm 0.08$	$12.04^{b} \pm 2.18$	$6.19^{c} \pm 0.44$
Catechol	$22.38^{c} \pm 0.40$	$54.34^{a} \pm 9.63$	$26.11^{b} \pm 1.15$
Orcinol	$6.29^{a} \pm 0.14$	$6.59^{a} \pm 1.97$	$3.76^{b} \pm 0.13$
Vanillin	$3.17^{c} \pm 0.67$	$5.02^{b} \pm 0.05$	$9.10^{a} \pm 0.28$
Guaiacol	Nd	4.19 ± 0.04	Nd
Salicylic acid	$10.67^{a} \pm 0.33$	$8.29^{b} \pm 1.07$	$4.58^{c} \pm 0.07$
Quercetin	$7.96^{b} \pm 0.66$	$16.09^{a} \pm 0.01$	$1.84^{c} \pm 0.18$
Cinnamic acid	$12.33^{b} \pm 1.42$	$6.35^{c} \pm 0.27$	$14.67^{a} \pm 2.57$
Butylated hydroxytoluene	$3.03^{c} \pm 1.19$	$7.98^{a} \pm 0.09$	$3.51^{b} \pm 0.07$
Pyrogallol	$7.76^{b} \pm 1.98$	Nd	$46.57^{a} \pm 2.18$
Caffeic acid	$1.71^{b} \pm 0.09$	Nd	$2.12^{a} \pm 0.07$
Luteolin	$13.90^{a} \pm 0.21$	$13.04^{b} \pm 0.32$	$4.99^{c} \pm 0.19$

Nd: not detected

Values represent mean ± SD (n=3). Different letters within a row represent significant differences at $p \leq 0.05$, derived from one-way ANOVA

Antioxidant Potential

In the H_2O_2 scavenging assay, *C. cibarius* showed the strongest antioxidant activity with the lowest IC_{50} value (8.03 mg mL^{-1}), followed by *A. campestris* (9.07 mg mL^{-1}) and *F. filiformis* (9.12 mg mL^{-1}). In the potassium ferricyanide assay, *F. filiformis* (5.46 mg mL^{-1}) and *A. campestris* (5.67 mg mL^{-1}) had the greatest reducing power, while *C. cibarius* recorded a higher IC_{50} (8.42 mg mL^{-1}).

Identification of Functional Groups

FTIR spectral analysis revealed the presence of diverse functional groups in all the studied mushrooms (Table 4). A broad absorption band between 3648–419 cm^{-1} was consistently observed in *F. filiformis*, *A. campestris*, and *C. cibarius* (Supplementary figure 2). Strong O–H stretching bands appeared at 3648 and 3345 cm^{-1}, while C–H vibrations of alkanes were observed at 2977 and

1453 cm^{-1}. A sharp band at 1790 cm^{-1} corresponded to C=O stretching of conjugated anhydrides. Peaks at 1085 and 1043 cm^{-1} suggested C–O, C–F, S=O, and CO–O–CO linkages. The band at 877 cm^{-1} indicated aromatic C–H bending, and the low-frequency band at 419 cm^{-1} represented M–O stretching.

Table 4: Assignment of characteristic absorption bands based on Fourier transform infrared spectroscopy in *Flammulina filiformis* (Ge *et al*.) Wang *et al*., *Agaricus campestris* L., and *Cantharellus cibarius* Fr

Peak (cm^{-1})	Wavenumber (cm^{-1})	Vibration Type	Functional Group
3648	3700–3584, 3700–3500	Stretch (strong, sharp)	O–H (alcohol, phenol)
3345	3550–3200	Stretch (strong, broad)	O–H (alcohol, phenol)
2977	3000–2840	Stretch (medium)	C–H (alkane)
2800	3200–2700, 3000–2800	Stretch (weak, broad)	O–H (alcohol, phenol)
1790	1815–1785, 1800–1770	Stretch (strong, sharp)	C=O (conjugated anhydride)
1453	1465–1450	Stretch (medium)	C–H (alkane)
1085	1400–1000, 1150–1085, 1124–1087, 1085–1050	Stretch (strong, sharp)	C–F (fluoro compound), C–O (aliphatic ether, alcohol)
1043	1400–1000, 1150–1085, 1070–1030, 1050–1040	Stretch (strong, sharp)	C–F (fluoro compound), C–O (aliphatic ether, alcohol), S=O (sulfoxide), CO–O–CO (anhydride)
877	900–650, 900–860, 900–860	Bend (out-of-plane, strong)	C–H (aromatic substitution), C–H (substituted benzene)
419	600–400	Stretch (strong)	M–O (inorganic metal-oxygen)

Discussion

Environmental stress such as low temperatures, desiccation, high UV radiation, and poor soil nutrient status characterizes cold desert ecosystems and impose selective pressures on native organisms.[1,2] The study presents evidence that the biochemical richness in wild edible mushrooms namely, *Flammulina filiformis, Agaricus campestris,* and *Cantharellus cibarius,* inhabiting the cold arid ecosystem of Kargil, located in the trans-Himalayan belt of India, is tightly coupled with their environmental context.

The dismissal of wild mushrooms as mere culinary novelties fails to acknowledge their adaptive biochemistry, which is often their survival mechanism in extreme environments. Their metabolic profiles reveal strategies that augment their functional value, both nutritionally and pharmacologically.[6] The nutritional profile of the three wild mushroom species offers critical insights into their potential roles in human health and diet. The heightened concentration of total carbohydrates and soluble sugars in *A. campestris* underscores its suitability as an energy-rich dietary component. This finding is buttressed by previous studies highlighting mushrooms as reliable sources of prebiotic carbohydrates that support gut microbiota and metabolic health.[9] *C. cibarius* exhibited slightly elevated starch content and a balanced profile of reducing and non-reducing sugars. Its nutritional consistency can be strategically valuable where uniform dietary supplementation is desired. Interestingly, the relatively low content of reducing sugars across all three species suggests that these mushrooms could support stifling postprandial glycaemic spikes, thus being beneficial for diabetic or low-glycaemic-index diets. In addition, non-reducing sugars, which are found in appreciable amounts, are associated with immune-modulating properties and act synergistically with other bioactive components like β-glucans to promote health.[10]

The preeminence of trehalose, glucitol, and ribitol indicates strong osmoregulatory and cryoprotective mechanisms across these mushrooms. Trehalose stabilizes proteins and membranes under stress. Moreover, it is increasingly recognized for its metabolic roles in modulating glucose homeostasis and gut microbiota health.[11] Polyols including glucitol, noted for its low glycemic impact, further enhance the nutritional profile of the investigated mushrooms.[12] Fungi generally have low lipid content yet are rich in unsaturated fatty acids, particularly linoleic and oleic acids, which support membrane fluidity under cold stress.[13] The studied species similarly showed MUFA and PUFA predominance over saturated fatty acids. The study of wild mushrooms by Ayaz *et al*[14] corroborates these findings, indicating consistent unsaturated lipid profiles regardless of habitat. This trait aids survival and also yield functional food benefits through cardiovascular health support.

Organic acids (~15–17% of the metabolite profile), including malic, fumaric, isocitric, and succinic acids, indicates active TCA cycles adapted for efficient energy production in severe conditions. Comparable acid profiles have been reported in other edible mushrooms such as *Pleurotus ostreatus* and *Macrolepiota procera*, where TCA intermediates also confer immunomodulatory and flavour-enhancing functions.[15]

The investigated mushrooms also represent a valuable source of vitamins and provitamins/vitamin-like metabolites namely ascorbic acid, pantothenic acid, niacin, myo-inositol, and ergosterol. Ascorbic acid (vitamin C), though not universally abundant in fungi, has been reported in modest amounts in mushrooms.[8] It is known to contribute in antioxidant defense and protection against oxidative stress induced by the harsh high-altitude environment.[16] Pantothenic acid (vitamin B5) is consistently present in mushrooms, supporting coenzyme A synthesis and thereby energy metabolism;[17] its dietary contribution may be significant in regions with limited crop diversity. Niacin (vitamin B3) is one of the most abundant vitamins in mushrooms such as *Lentinula edodes* and *Grifola frondosa*.[18] It is an essential vitamin for NAD/NADP-dependent redox processes, and has been highlighted as a key nutritional advantage of wild fungi, potentially counteracting pellagra risk in resource-limited diets.[17] Myo-inositol (constituted 0.78–1.72% of the metabolite pool), essentially a polyol and recognised as vitamin B8, has been reported to support cardiovascular health, immunity, sugar metabolism, reproductive function, and has neuroprotective effects; therefore, its presence in mushrooms offer additional functional benefits. Ergosterol, the principal fungal sterol, is a structural membrane component. It serves as a precursor of vitamin D2 upon UV exposure; given the strong insolation of Kargil, mushroom-derived ergosterol can be efficiently converted to vitamin D2.[19] Ergosterol concentration ranged between 0.95–2.44% in the studied mushrooms, making these fungi a natural dietary source of this otherwise scarce micronutrient. Thus, the vitamin composition of these mushrooms emphasizes their relevance as functional foods capable of supporting nutritional assortment in cold desert communities.

There is a growing nutritional demand in regions where plant protein accessibility is limited. *F. filiformis* exhibited the highest protein content (21.8%), reaffirming the notion that mushrooms can serve as sustainable alternatives to animal protein (~17–25%).[20] The bequest of such nutrient-rich fungi from wild ecosystems could serve as a foundation for developing future nutraceutical products. Essential amino acids constitute a substantial proportion of the total amino acid pool, emphasizing the nutritional relevance of these species. Threonine was the most dominant among the essential amino acids. Isoleucine, leucine, and valine emphasize their metabolic importance, as branched-chain amino acids are known to influence glucose metabolism and modulate immune responses.[21] Considering the high incidence of protein-energy malnutrition among populations residing in arid highlands, wild mushrooms provide a valuable source of essential amino acids for dietary supplementation. In areas where protein intake is predominantly meat-based, they serve as a nutritionally rich, vegetarian-compatible alternative.

Among non-proteinogenic amino acids, 5-oxoproline was consistently prominent across all three species. As a key intermediate of the γ-glutamyl cycle, 5-oxoproline plays an important role in glutathione biosynthesis, one of the principal cellular antioxidant systems. Elevated 5-oxoproline may represent increased glutathione turnover, indicating an enhanced antioxidant defense capacity that helps mushrooms mitigate oxidative damage caused

by stress-induced ROS.[22] These benefits are especially pertinent for populations residing in high-altitude environments where dietary diversity is constrained.[23]

The occurrence of adenosine, uracil, and uridine in the mushroom taxa invites meaningful extrapolation. Adenosine, uracil, and uridine, as fundamental nucleobases and nucleosides, contribute to cellular energy metabolism, nucleotide biosynthesis, and neuronal function, with emerging evidence suggesting their role in supporting cognitive resilience and synaptic plasticity.[24] Together, these metabolites enhance the nutritional and functional value of the surveyed mushrooms.

From enzymatic catalysis to maintenance of electrolyte balance, minerals are indispensable nutrients that orchestrate a broad spectrum of physiological processes. The elemental profiling revealed interspecific variation in both macro- and trace-elemental concentrations. Among macro-elements, N and K were consistently abundant, while microelements such as Fe, Zn, Cu, and Se exhibited pronounced species-specific accumulation. Nitrogen was the highest in *C. cibarius* (6.84 mg g^{-1} DW), a finding consistent with earlier studies linking high N content with protein-rich mushroom species.[25] Elevated N is nutritionally significant, as mushroom proteins contain essential amino acids in favourable proportions. The S concentrations, although comparable among species, emphasize the contribution of S-containing amino acids, are vital for enzymatic activation and redox balance. Phosphorus, which is indispensable for energy metabolism through ATP and for nucleic acid synthesis, was highest in *F. filiformis* (0.13 mg g^{-1} DW), aligning with reports that P in mushrooms is predominantly present as easily metabolizable organic phosphates.[26]

Electrolyte-regulating minerals namely, Na, K, Ca, and Mg, displayed variation in their concentrations. The concentration of Na ranged from 0.19 mg g^{-1} DW in *C. cibarius* to 0.26 mg g^{-1} DW in *A. campestris*. Mushrooms are generally considered to contain low Na and the concentrations registered in this study are comparable with those reported in wild mushrooms from the Mediterranean regions.[27] Conversely, K concentrations were markedly elevated in *A. campestris* (16.51 mg g^{-1} DW) and *C. cibarius* (16.49 mg g^{-1} DW), consistent with the well-established feature of mushrooms being K-rich and Na-poor, a ratio that supports cardiovascular health and osmotic regulation.[28] *A. campestris* exhibiting the highest Ca (0.95 mg g^{-1} DW) and Mg (0.54 mg g^{-1} DW), reinforce their value in skeletal metabolism and neuromuscular function.

Oligoelements revealed striking patterns. Fe, a central component of haemoproteins, reached exceptionally high concentration in *A. campestris* (449.51 µg g^{-1} DW), surpassing values reported in *Coprinus comatus* and *Boletus* sp.[29] Given the widespread occurrence of Fe-deficiency anaemia among populations in high-altitude regions, this enrichment positions *A. campestris* as a potential dietary intervention.[30] In contrast, *F. filiformis* and *C. cibarius* had comparatively lower Fe contents, though still within nutritionally significant ranges. Zn and Cu, cofactors for various of metalloenzymes, also varied: *A. campestris* contained the highest Zn (27.35 µg g^{-1} DW), while *F. filiformis* was enriched in Cu (20.37 µg g^{-1} DW). Both elements are vital for immune modulation, collagen synthesis, and oxidative defense.[31] Mn and Ni were most elevated in *A. campestris*, while *C. cibarius* recorded the highest Cr (29.65 µg g^{-1} DW) and Mo (12.22 µg g^{-1} DW). The latter is of catalytic importance, as molybdoenzymes support detoxification and N-metabolism.[32] Se concentrations revealed the most notable divergence: *C. cibarius* exhibited an exceptional Se content of 7.59 µg g^{-1} DW, well above the range typically reported for cultivated mushrooms i.e., 1–8.5 µg g^{-1} DW.[33] Se bioactivity includes immune regulation, thyroid hormone metabolism, and neuroprotection, and higher intake has been inversely linked to risks of cardiovascular disease and cognitive decline.[32] Thus, *C. cibarius* emerges as a valuable dietary source of Se in Se-deficient regions.

While mineral enrichment is advantageous, their nutritional utility can be modulated by the presence of antinutritional factors. Oxalic acid (OA), PA, and CT are the most relevant antinutrients in mushrooms.[34] In the studied samples, OA/(Ca+Mg) ratio remained below the critical threshold of 2.5,[35] ranging from 0.15 in *F. filiformis* to 0.87 in *C. cibarius*. This suggests negligible sequestration of divalent cations thereby ensures adequate bioavailability of Ca and Mg. Similarly, PA/Fe ratios

(0.23–0.33) were substantially lower than inhibitory thresholds (>1), indicating minimal impairment of Fe absorption. The PA/Zn ratios (ranging 2.56–4.09) also remained far below the cut-off value of 10,[36] with *C. cibarius* demonstrating most favourable values for Zn bioavailability. As such, these ratios imply that despite the presence of antinutrients, mineral bio-accessibility remains high. The potential assimilation of these minerals from the mushrooms would be favourable in populations where mineral deficiencies are prevalent, such as in regions where dietary diversification is limited, and fortification programmes are economically unfeasible.

Another nutritional dimension pertains to mineral toxicity thresholds. Elements such as Cu, Fe, Mn, Zn, Se, and Cr exert toxic effects when consumed in excess. However, the concentrations observed in these mushrooms are substantially lower than established tolerable upper intake levels (UL).[37-39] The high Fe in *A. campestris* translates to a nutritionally beneficial at the same time, non-toxic intake, provided consumption remains within dietary range recommended by the Institute of Medicine (2001).[39] Similarly, the elevated Se in *C. cibarius* is advantageous without breaching toxicity risk, as it remains below the UL of 400 µg day^{-1} for adults.[40] These findings support the growing recognition of mushrooms as functional foods that contribute both macro- and micro elements essential for human health and maintain safety margins below toxicological limits in the interim.

The TPC and TFC, most pronounced in *F. filiformis*, mirror patterns observed in other wild mushrooms, where phenolic abundance strongly correlates with antioxidant capacity.[41] These compounds, alongside notable carotenoids such as β-carotene and lycopene, contribute synergistically to radical-scavenging activity, protecting cellular structures from oxidative damage. Such bioactive profiles hold nutritional and therapeutic value, given the role of oxidative stress in chronic diseases and neurodegeneration.[41,42] The convergence of high phenolic, flavonoid, and carotenoid concentrations positions *F. filiformis* as a promising candidate for functional foods and nutraceuticals aimed at oxidative stress mitigation. These observations are also substantiated in the antioxidant assays. The low IC_{50} values observed confirm the high free radical scavenging activity. The correlation between TPC and TFC values and antioxidant activity support the established relationship between phenolic richness and oxidative stress mitigation. These observations align with prior reports on stress-induced polyphenol synthesis in medicinal mushroom, *Inonotus obliquus,* grown under UV stress.[43]

In addition, the UHPLC profiling revealed compound-specific enrichment. Catechol was the most abundant phenolic overall. Its strong radical-scavenging and metal-chelating activity is consistent with high antioxidant performance reported for *Agaricus* species.[9] Resorcinol was highest in *F. filiformis*, where it may contribute antimicrobial and anti-inflammatory benefits.[44] Flavonoids were also prominent: quercetin peaked in *A. campestris*, while luteolin was abundant in *F. filiformis,* and followed closely by *A. campestris*. Both flavonoids are potent antioxidants with anti-inflammatory and vascular-protective functions.[44] The findings of the study surpass those reported for several wild European mushrooms.[45] A notable feature was the detection of pyrogallol in *C. cibarius* (46.57 µg g^{-1} DW). Although pyrogallol exhibits strong antioxidative capacity, it may also exert pro-oxidant effects depending on cellular context.[46] This dual activity warrants further pharmacological evaluation. Ecologically, the production of these metabolites serves as a buffer against cold-induced oxidative stress. Cold desert soils are typically poor in organic matter, and fungi compete aggressively for C and N. Under such conditions, secondary metabolites provide self-protection and a competitive edge. The environmental filtering theory posits that stress-tolerant species exhibit convergent traits, such as increased investment in protective metabolites.[2,47] This pattern is clearly observable in the metabolomic profile of the studied mushrooms.

The FTIR spectrum of the mushrooms demonstrates the presence of multiple biomolecular classes. Broad O–H stretching peaks around 3648 and 3345 cm^{-1} indicate hydroxyl groups from alcohols and/or phenols,[48] consistent with high polyols and polyphenolic content found in mushrooms. The aliphatic C–H stretch at 2977 cm^{-1} and C–H bending at 1453 cm^{-1} suggest lipid and hydrocarbon chain components.[49] A sharp C=O stretch at 1790 cm^{-1} implies conjugated carbonyl types or esterified compounds, which are related to phenolic esters or lipid oxidation products.[50] In the fingerprint

region, strong peaks at 1085 cm^{-1} and 1043 cm^{-1} corresponding to C–O stretching in aliphatic ethers and alcohols are typical of polysaccharides/ polyols and carbohydrate structures. The band at 877 cm^{-1} is characteristic of out-of-plane C–H bending associated with substituted aromatic rings, supporting the presence of aromatic phenolics. The absorption at 419 cm^{-1} signals metal-oxygen stretching, suggesting inorganic minerals are incorporated into the fungal matrix.[49] Absorption peaks observed in the 1000–400 cm^{-1} region has been reported to primarily attributed to polysaccharides, including β-D-glucans and the pyranose ring vibrations of glucose. Additionally, modelling based on mid-infrared spectra in *Boletus* sp. has shown strong correlations between spectral regions and glucan and phenolic content.[48] These biomolecules are contributors to the nutraceutical properties of mushrooms. From the spectral signatures, it can be inferred that these mushrooms are rich in polysaccharides, polyols, phenolic compounds, lipids, and mineral elements, among others. This finding consolidates the metabolic heterogeneity demonstrated in the study.

Conclusion

The present study highlights the diverse metabolites and bioactive potential of three wild edible mushrooms, *Flammulina filiformis* (Ge *et al*.) Wang *et al*., *Agaricus campestris* L*.,* and *Cantharellus cibarius* Fr., inhabiting the cold desert ecosystems of Kargil in the Indian Trans-Himalayas, where environmental extremities such as low temperatures, high UV exposure, and nutrient-scarce soils act as strong drivers of secondary metabolite synthesis. The comprehensive metabolome profiling revealed a rich chemical repertoire, with saccharides, polyols, and unsaturated fatty acids dominating the metabolome. Protein content was notably high in *F. filiformis*, enriched with essential amino acids, while non-proteinogenic amino acids such as 5-oxoproline indicate enhanced oxidative defense. Comprehensive mineral profiling revealed species-specific enrichment: *A. campestris* was abundant in macro- and trace elements (Na, K, Ca, Mg, Fe, Zn, Mn), *C. cibarius* in Se, Cr, and Mo, and *F. filiformis* in S, Cu, and Al. Antinutritional factors remained below inhibitory thresholds, ensuring high bioavailability of essential minerals and safe dietary incorporation. Strong antioxidant capacity, evidenced by high total phenolic, flavonoid, and carotenoid content, alongside low IC_{50} values in radical-scavenging assays, positions these mushrooms as potent functional foods. Phenolic compounds, including resorcinol, catechol, quercetin, luteolin, and pyrogallol, further enhance their pharmacological relevance, supporting oxidative stress mitigation, immune modulation, and cardiovascular protection. Spectral evidence from FTIR validates the presence of polysaccharides, polyols, phenolics, lipids, and minerals herein. Collectively, these findings demonstrate that wild mushrooms from cold deserts represent a convergence of adaptive metabolite diversity and nutritional richness which contributes to the nutraceutical potential of the mushrooms. Their integration into diets or nutraceutical formulations offers sustainable solutions for protein-energy and micronutrient deficiencies. Current bottlenecks include the lack of bioassay-guided fractionation, compound isolation, and structural elucidation, which are essential to ascertain mechanistic pathways of action for lead and/or novel compounds.

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Conflict of Interest

The author(s) do not have any conflicts of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

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Not Applicable

Author Contributions

- **Rupam Kapoor:** Conceptualization, Funding Acquisition, Project administration, Resources, Supervision
- **Hom-Singli Mayirnao:** Investigation, Formal Analysis, Writing – Original Draft, Writing – Review and Editing
- **Surinder Kaur:** Supervision; Writing – Review and Editing
- **Yash Pal Sharma:** Funding Acquisition, Resources.

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Supplementary Data