

Nutrient and antioxidant profile of four species of wild mushrooms from cold-desert with implications for human dietary and supplement use

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ABSTRACT

Extreme environmental conditions in cold deserts modulate the biochemical profile of organisms thriving therein, translating into nutritional benefits for humans. This study assessed nutritional composition and antioxidant potential of four wild edible mushroom species—*Volvariella bombycina*, *Tricholoma cingulatum*, *Coprinus comatus*, and *Bovista plumbea*—from the Trans-Himalayan region of Kargil. Metabolic profiling identified sugars, polyols, organic acids, and fatty acids, with sugars and their derivatives dominating the metabolite composition (33.40–50.90 %). Trehalose (13.68–27.93 %) and glucitol (3.71–10.33 %) were especially abundant. Linoleic acid (2.50–7.63 %) was the most prevalent essential polyunsaturated fatty acid. Total protein content ranged from 122.50–312.01 mg/g dry weight, with various essential amino acids detected. The mushrooms demonstrated high bioavailability of minerals—Fe, Cu, Mg, and Ca—imputable to low antinutrient concentrations. *C. comatus* exhibited the highest enzymatic activities for superoxide dismutase, peroxidase, and glutathione peroxidase, while *B. plumbea* demonstrated the highest catalase activity and concentrations of non-enzymatic antioxidants including phenolics, flavonoids, ascorbic acid, and α -tocopherol. *V. bombycina* exhibited maximum carotenoid-based antioxidants. Antioxidant assays indicated *C. comatus* as the strongest radical scavenger (IC₅₀=7.92) and *V. bombycina* with the highest reducing power (IC₅₀=5.71). The study highlights the potential of these mushrooms as a source of nutraceuticals and dietary supplements.

1. Introduction

Cold-desert ecosystems are typified by extreme environmental conditions including high-altitude hypoxia, intense solar radiation, low temperatures, and limited water availability, that challenge the survival of most life forms. In response to these extreme conditions, organisms inhabiting cold deserts have evolved various physiological and biochemical adaptations that enable their survival (Dhakar & Pandey, 2020). The environmental pressures induce the biosynthesis of antioxidants and other protective compounds to a higher concentration which in turn enhance their resilience against the stresses imposed by the cold-desert habitat (Juurakko & Walker, 2021). Wild mushrooms, as one of the key fungal components of these ecosystems, exemplify such adaptations through their roles in nutrient cycling and symbiotic association. Thus, these macrofungi support the limited vegetation that characterizes these regions. Kargil, situated in the Trans-Himalayan belt

of northern India, represents a cold-desert environment. The biochemical traits that equip mushrooms to persist in inhospitable environments, such as those found in Kargil, remain underexplored and poorly understood despite their ecological significance (Mayirnao et al., 2023).

Mushrooms have garnered increasing scientific attention for their diverse nutritional properties. Beyond their nutritional value, mushrooms are recognized for synthesizing a wide array of secondary metabolites (SM) with biofunctional activities. Amongst these SM, phenolic compounds, flavonoids, terpenoids, alkaloids, and polysaccharides are of significant interest (Mayirnao et al., 2025). Phenolic compounds and flavonoids have been well-documented for their antioxidative properties, which can neutralize reactive oxygen species (ROS) and thereby mitigate oxidative stress. Oxidative stress, characterized by an imbalance between the excessive generation of ROS and the insufficient activity of endogenous antioxidant systems, is a key factor in the development of chronic diseases, including carcinogenesis,

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neurodegeneration, and cardiovascular dysfunction (Abdelshafy et al., 2022).

Mushrooms are also valued for their potential nutraceutical applications, referring to their role in providing health benefits beyond basic nutrition, through bioactive compounds that help in the prevention or treatment of diseases. Analyzing the full potential of mushrooms as sources of nutraceuticals requires insights into their functional constituents. The biochemical characteristics of the mushroom species native to Kargil is a relatively untrodden subject in this regard (Mayirnao et al., 2023). Although the biochemical and therapeutic properties of well-studied mushrooms such as *Agaricus*, *Pleurotus*, *Cordyceps*, and *Ganoderma* are widely documented (Tarafder et al., 2024), this work stands as the first to provide an exhaustive biochemical and metabolomic profile of four lesser-studied mushrooms belonging to the order Agaricales—*Volvariella bombycina* (Schaeff.) Singer, *Tricholoma cingulatum* (Almfelt) Jacobasch, *Coprinus comatus* (O.F. Müll.) Pers., and *Bovista plumbea* Pers.—all of which are native to Kargil.

The study was conducted with an aim to elucidate the biochemical profile of wild mushrooms in cold desert conditions so as to assess their potential applications in developing strategies for enhancing human nutrition and health. A comprehensive nutritional analysis was performed, incorporating gas chromatography-mass spectrometry (GCMS)-based metabolites profiling, spectrophotometric analysis of nutrient composition, non-enzymatic antioxidant content, antioxidative enzyme activities, in vitro antioxidant assays, as well as elemental analysis using atomic absorption spectroscopy (AAS), inductively coupled plasma-mass spectrometry (ICPMS), flame photometry, and spectrophotometry.

2. Materials and methods

2.1. Sample identification

Systematic collection of mushrooms was undertaken in September 2022 from Drass in Kargil district (34°30' N, 76°10' E), Ladakh, India at an altitude of 3276 above mean sea level. The mushrooms were collected at their mature developmental stage. Specimens were cleaned with distilled water to remove dirt and debris. Field equipment such as digital camera, scale, trowel, heater, and heat convector card board, were used; and chemical reagents namely, 30 % potassium hydroxide, 10 % ferrous sulphate, 2 % phenol, and guaiacol were used for biochemical tests. Collection of samples was made in the morning and field characteristics of mushrooms were recorded in the data sheet. Simultaneously, biochemical spot tests were performed on the surface and context of pileus and stipe in freshly collected samples and other necessary processing such as heat-drying and packaging for preservation were carried out. Macromorphological features of the fresh basidiocarps were examined, followed by molecular identification of the species (Supplementary Material).

2.2. Gas chromatography-mass spectrometry based metabolite profiling

Volatile and non-volatile compounds in the mushroom extracts were characterized using GC-MS using MSTFA (N-Methyl-N-(trimethylsilyl) trifluoroacetamide) method of derivatization with ribitol as internal standard (Mayirnao et al., 2023). GC-MS analysis was performed on a Shimadzu-QP-2010 Plus GC system fitted with a DB-5 capillary column (Shimadzu, Kyoto, Japan).

2.3. Macronutrients

Total carbohydrate content (TAC) was performed following Anthrone method, total soluble sugars (TSS) was estimated using phenol-sulphuric acid method, reducing sugars and non-reducing sugars were measured following DNS method, and total protein content was determined using Bradford's method. The detailed methodology is given in Supplementary Material.

2.4. Mineral composition

Samples were digested using a tri-acid mixture of nitric acid, perchloric acid, and sulfuric acid (4:2:0.5) on a hot block digestion system. The digested samples were cooled, and the final volume was adjusted to 50 mL with Milli-Q water following filtration using Whatman filter paper no.44. Ca, Mg, K, Fe, Cu, Co, Zn, Ni, and Al were quantified using Atomic Absorption Spectrometer (PerkinElmer, Waltham, Massachusetts, USA) following AOAC methods (detailed in Supplementary Material); Na and K concentrations were determined using flame photometer (ThermoFisher Scientific, Hampton, New Hampshire, USA). Cr, Se, Mn, and Mo using ICPMS by Agilent Technologies (8900 ICP-MS Triple Quad, California, USA); and N and S using CHNS analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany).

2.5. Antinutrients and mineral bioavailability

Antinutritional factors, including phytic acid (PA) and condensed tannins (CT), were quantified using spectrophotometric methods (Supplementary Material). The molar ratios of antinutrients [PA, CT, and oxalic acid (OA)] to minerals were also calculated. Critical values of 2.5, 0.4, 0.4, and 10 were used for OA/(Ca+Mg), PA/Fe, PA/Ca, and PA/Zn, respectively (Oberleas & Harland, 1981; Hassan et al., 2014).

2.6. Enzymatic antioxidants

Activities of enzymatic antioxidants namely superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and glutathione peroxidase (GPx) were evaluated using the methods outlined by Venkidasamy et al. (2019).

2.7. Non-Enzymatic antioxidants

Non-enzymatic antioxidants namely total phenols, total flavonoids, ascorbic acid, α -tocopherol, β -carotene, and lycopene along with antioxidant potential were evaluated using the methods outlined by Mayirnao et al. (2023). Folin-Ciocalteu colorimetric method was used for determination of total phenols. The total flavonoid content was determined by aluminium chloride colorimetric method.

2.8. Antioxidative potential

Assays of hydroxyl radical scavenging activity and hydrogen peroxide scavenging activity (detailed in Supplementary Material) were performed to determine the antioxidant potential in the studied samples.

2.9. Statistical analysis

All the analyses were performed in triplicates. The data were analyzed using one-way ANOVA to compare whether the means of the samples are significantly different. The results are expressed as mean \pm standard deviation (SD). Statistical analyses were carried out with SPSS software version 21.0 (IBM Corporation, New York, United States). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Mushroom identification

Using morphological characterization and molecular tools, the four species of mushrooms were identified as *Volvariella bombycina* (Schaeff.) Singer, *Tricholoma cingulatum* (Almfelt) Jacobasch, *Coprinus comatus* (O. F. Müll.) Pers., and *Bovista plumbea* Pers. (Fig. 1 of Supplementary Material). The detailed diagnoses are given in the supplementary material 1.

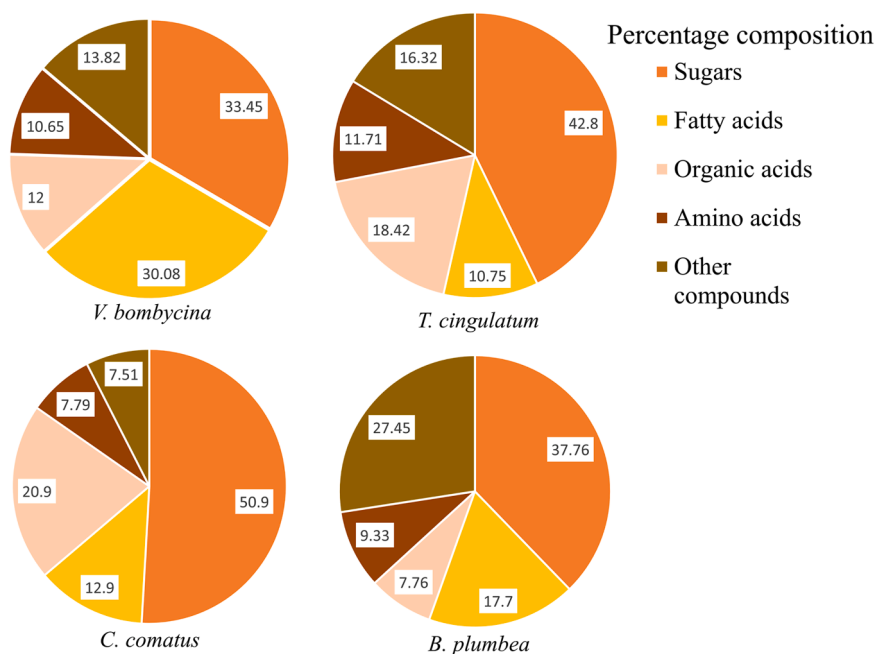


Fig. 1. Pie charts categorizing the major classes of compounds identified through untargeted gas chromatography-mass spectrometry metabolite profiling in *Volvariella bombycina*, *Tricholoma cingulatum*, *Coprinus comatus*, and *Bovista plumbea*.

3.2. GC-MS-based untargeted metabolome profiling

A comprehensive metabolomic analysis via untargeted GC-MS led to the identification and quantification of 92 different compounds in the studied mushroom species: *V. bombycina*, *T. cingulatum*, *C. comatus*, and *B. plumbea* (Table 1). The resulting data provides a detailed biochemical profile encompassing different classes of compounds, broadly classified into amino acids and their derivatives; nitrogenous compounds (other than amino acids); sugars and their derivatives; fatty acids and their derivatives; organic acids and their derivatives; as well as mineral acid (Fig. 1).

Sugars and their derivatives form the largest fraction of the compound profiles in the examined mushrooms. Trehalose was the most dominant, representing 27.93 % and 23.21 % of the total composition in *V. bombycina* and *C. comatus*, respectively. The sugar alcohol composition revealed glucitol as the predominant sugar alcohol, with concentrations ranging from 3.36 % to 10.33 %. Glycerol was also present in relatively high and consistent concentrations (4.07 % to 6.19 %). In contrast, mannitol and meso-erythritol were detected only in low and sporadic amounts. Ribitol showed moderate variability, with concentrations ranging from 1.84 % to 2.91 %.

Following sugars, fatty acids and organic acids constitute substantial portions of the profiles. The total fatty acid content (TFAC) ranged from 19.58 % to 22.26 %. Saturated fatty acids constituted up to 20.69 % (*B. plumbea*) to 43 % (*V. bombycina*) of TFAC while monounsaturated fatty acids (MUFA) constituted 36.47 % - 43.37 % of TFAC with oleic acid being the most abundant MUFA. Notably, the concentration polyunsaturated fatty acids (PUFA) were also elevated (13.52 % - 40.69 % of TFAC) in the cold-desert mushrooms. The most prominent PUFA was linoleic acid and the highest level was detected in *B. plumbea* (40.69 % of TFAC). Organic acids constitute 9.9 % to 11.61 % of the entire metabolite profile. Maleic, fumaric, and succinic acids contributed significantly to the profile across all the species studied.

Amino acids and nitrogenous compounds, though present, were less dominant compared to sugars and fatty acids. The amino acid profile of the four mushroom species revealed variations in essential and non-essential amino acids. Amongst the essential amino acids, leucine, isoleucine, phenylalanine, threonine, and valine were quantified in varying concentrations across the species. *B. plumbea* exhibited the

highest concentration of isoleucine (1.05 %) and valine (1.37 %). Conversely, *V. bombycina* presented the lowest concentrations of essential amino acids, with leucine (0.23 %) and threonine (0.36 %) being the most prominent. *T. cingulatum* demonstrated significant concentration of threonine (2.22 %) and valine (1.12 %).

In non-essential amino acids, alanine and glycine showed considerable variation. *T. cingulatum* and *B. plumbea* were found rich in alanine (2.64 % and 2.37 %, respectively) and glycine (2.11 % and 2.64 %, respectively). In contrast, *C. comatus* exhibited the lowest concentrations of alanine (0.12 %) and glycine (0.92 %). Notably, aspartic acid and glutamic acid were exclusive to *T. cingulatum* and *B. plumbea*, with *B. plumbea* having a higher concentration of these amino acids.

An amino acid derivative, 5-oxoproline, was the most abundant derivative across all species, with *V. bombycina* showing the highest concentration (11.35 %). Additionally, acetyl lysine, glycyL glutamic acid, and glycyL proline were present in *T. cingulatum* and *B. plumbea*, with these species displaying the most diverse profiles. The presence of homoserine and ornithine was registered in *B. plumbea*.

3.3. Carbohydrates

The highest TAC was registered in *V. bombycina* (Fig. 2A), followed by *T. cingulatum*. In terms of sugars, *C. comatus* exhibited a balanced distribution between total soluble sugars and reducing sugars, whereas *B. plumbea* had lower concentrations of non-reducing sugars compared to other species (Fig. 2B-D).

3.4. Protein

Tricholoma cingulatum was found notable for its highest total protein content amongst the tested samples, reaching 312 mg g⁻¹ DW, while *V. bombycina* had the lowest protein content (122.50 mg g⁻¹ DW) (Fig. 2E).

3.5. Elemental analysis

The elemental analysis of the four mushroom species revealed significant variations in both macro and trace element concentrations (Table 2). *B. plumbea* exhibited the highest N content at 28.27 mg g⁻¹

Table 1

Metabolic profiles of *Volvariella bombycina*, *Tricholoma cingulatum*, *Coprinus comatus*, and *Bovista plumbea* using gas chromatography-mass spectrometry. The concentrations of metabolites are expressed in percentage compositions.

| Compounds | <i>V. bombycina</i> | <i>T. cingulatum</i> | <i>C. comatus</i> | <i>B. plumbea</i> |
|--------------------------------------|---------------------------|---------------------------|----------------------------|---------------------------|
| AMINO ACIDS | | | | |
| <i>Essential amino acids</i> | | | | |
| Isoleucine | 0.19 ^c ± 0.01 | 0.59 ^b ± 0.14 | 0.22 ^c ± 0.06 | 1.05 ^a ± 0.28 |
| Leucine | 0.23 ^b ± 0.01 | 0.44 ^a ± 0.08 | 0.28 ^a ± 0.02 | 0.36 ^a ± 0.08 |
| Lysine | nd | 0.65 ± 0.04 | nd | nd |
| Phenylalanine | 0.19 ^c ± 0.01 | 0.58 ^b ± 0.15 | 0.20 ^c ± 0.03 | 1.03 ^a ± 0.22 |
| Threonine | 0.36 ^b ± 0.02 | 2.22 ^a ± 0.45 | 0.42 ^b ± 0.23 | 2.51 ^a ± 0.7 |
| Valine | 0.10 ^c ± 0.06 | 1.12 ^a ± 0.09 | 0.74 ^b ± 0.10 | 1.37 ^a ± 0.15 |
| <i>Non-essential amino acids</i> | | | | |
| Alanine | 0.31 ^b ± 0.04 | 2.64 ^a ± 0.59 | 0.12 ^c ± 0.04 | 2.37 ^a ± 0.71 |
| Asparagine | nd | 0.14 ^b ± 0.01 | nd | 0.66 ^a ± 0.02 |
| Aspartic acid | nd | 0.26 ^b ± 0.07 | 0.31 ^b ± 0.13 | 0.69 ^a ± 0.13 |
| Glutamic acid | nd | 0.98 ^a ± 0.35 | nd | 0.61 ^a ± 0.23 |
| Glycine | 0.17 ^c ± 0.00 | 2.11 ^a ± 0.78 | 0.92 ^b ± 0.09 | 2.64 ^a ± 0.34 |
| Proline | 0.15 ^b ± 0.06 | 0.59 ^a ± 0.28 | 0.41 ^a ± 0.20 | 0.25 ^b ± 0.09 |
| Serine | 0.16 ^b ± 0.02 | 1.00 ^a ± 0.14 | 0.19 ^b ± 0.07 | 1.14 ^a ± 0.33 |
| Tyrosine | 0.10 ^b ± 0.00 | nd | 0.11 ^a ± 0.01 | nd |
| <i>Non-proteinogenic amino acids</i> | | | | |
| Acetyl lysine | nd | 0.21 ^a ± 0.03 | nd | 0.23 ^a ± 0.06 |
| Ergothioneine | 0.10 ^b ± 0.00 | 0.16 ^b ± 0.03 | 0.12 ^b ± 0.03 | 0.75 ^a ± 0.17 |
| Glycyl glycine | nd | nd | nd | 0.13 ± 0.01 |
| Glycyl proline | 0.13 ^b ± 0.04 | 0.81 ^a ± 0.16 | 0.15 ^b ± 0.05 | 0.18 ^b ± 0.01 |
| Homoserine | nd | nd | nd | 0.13 ± 0.05 |
| Ornithine | nd | nd | nd | 0.16 ± 0.05 |
| 5-Oxoproline | 11.35 ^a ± 1.37 | 7.95 ^b ± 0.04 | 9.56 ^a ± 1.39 | 7.13 ^{ab} ± 0.78 |
| OTHER N-CONTAINING COMPOUNDS | | | | |
| Adenosine | 0.58 ^{ab} ± 0.01 | 0.27 ^b ± 0.10 | 0.89 ^a ± 0.43 | 0.76 ^a ± 0.29 |
| Dodecanamide | 0.10 ^a ± 0.00 | nd | 0.05 ^b ± 0.01 | nd |
| Ethanolamine | nd | 1.36 ^a ± 0.28 | nd | 0.21 ^b ± 0.04 |
| Methyladenosine | 1.71 ^a ± 0.28 | 0.19 ^c ± 0.01 | nd | 0.73 ^b ± 0.09 |
| Niacin | 0.18 ^b ± 0.03 | nd | 0.23 ^b ± 0.10 | 0.84 ^a ± 0.35 |
| Phosphinoline ethanamine | 0.11 ^b ± 0.03 | nd | 0.13 ^b ± 0.06 | 0.46 ^a ± 0.08 |
| Uracil | nd | 0.59 ^c ± 0.22 | 1.43 ^a ± 0.06 | 1.08 ^b ± 0.04 |
| Urea | nd | 1.88 ^b ± 0.12 | nd | 5.84 ^a ± 2.11 |
| Uridine | 0.73 ^c ± 0.57 | 1.04 ^b ± 0.17 | 2.61 ^a ± 0.51 | 0.62 ^c ± 0.19 |
| SUGARS & THEIR DERIVATIVES | | | | |
| <i>Sugars</i> | | | | |
| Arabinopyranose | nd | nd | 0.15 ^a ± 0.01 | 0.12 ^a ± 0.04 |
| Deoxygalactose | nd | 0.04 ^{bc} ± 0.01 | 0.08 ^b ± 0.04 | 0.30 ^a ± 0.11 |
| Erythrose | 0.28 ^b ± 0.01 | 0.20 ^c ± 0.05 | 0.24 ^{bc} ± 0.14 | 0.55 ^a ± 0.01 |
| Fructose | 0.08 ^c ± 0.01 | 1.55 ^a ± 0.41 | 0.58 ^b ± 0.05 | 1.04 ^a ± 0.27 |
| Fructopyranose | 0.22 ^b ± 0.02 | 0.42 ^a ± 0.06 | nd | 0.09 ^c ± 0.03 |
| Galactose | nd | nd | 0.38 ^a ± 0.17 | 0.13 ^b ± 0.01 |
| Maltose | nd | nd | nd | 0.38 ± 0.001 |
| Talose | 1.39 ^{ab} ± 0.64 | 0.86 ^b ± 0.06 | 3.68 ^a ± 1.49 | nd |
| Trehalose | 27.93 ^a ± 0.09 | 18.67 ^b ± 0.69 | 23.21 ^{ab} ± 6.86 | 13.68 ^c ± 1.99 |
| Xylulose | nd | 0.25 ^a ± 0.08 | nd | 0.05 ^b ± 0.001 |
| α-mannobiose | nd | 0.28 ^b ± 0.01 | 0.67 ^a ± 0.43 | nd |
| <i>Sugar acids</i> | | | | |
| Deoxytetronic acid | nd | 0.22 ± 0.04 | nd | nd |
| Gluconic acid | 0.56 ^c ± 0.06 | 1.55 ^b ± 0.26 | 0.85 ^c ± 0.45 | 2.33 ^a ± 0.64 |
| Glyceric acid | 1.26 ^a ± 0.03 | 0.62 ^b ± 0.23 | 1.78 ^a ± 0.39 | 0.55 ^b ± 0.18 |
| Hydroxyglutaric acid | 0.05 ^c ± 0.00 | 0.29 ^a ± 0.10 | 0.21 ^a ± 0.03 | 0.14 ^b ± 0.01 |
| Ribonic acid | nd | 0.19 ^a ± 0.06 | nd | 0.13 ^b ± 0.05 |
| Xylonic acid | nd | 0.52 ^a ± 0.11 | 0.07 ^b ± 0.04 | 0.08 ^b ± 0.01 |
| <i>Sugar alcohols</i> | | | | |
| Erythritol | 0.16 ^c ± 0.00 | 1.28 ^a ± 0.37 | 0.40 ^b ± 0.22 | 0.24 ^{bc} ± 0.09 |
| Glucitol | 7.36 ^b ± 1.07 | 10.33 ^a ± 2.42 | 3.71 ^c ± 1.15 | 6.23 ^b ± 0.66 |
| Glycerol | 2.19 ^c ± 0.88 | 5.71 ^a ± 0.45 | 5.97 ^a ± 1.91 | 4.07 ^b ± 0.32 |
| Mannitol | 0.14 ^b ± 0.00 | nd | nd | 0.19 ^a ± 0.01 |
| Meso-erythritol | nd | 0.05 ^b ± 0.03 | nd | 0.20 ^a ± 0.08 |
| Myo-inositol | 0.45 ^b ± 0.05 | 1.05 ^a ± 0.20 | nd | 1.20 ^a ± 0.26 |
| Ribitol | 2.01 ^a ± 0.05 | 2.36 ^a ± 0.18 | 2.91 ^a ± 1.38 | 1.84 ^{ab} ± 0.37 |
| FATTY ACIDS & THEIR DERIVATIVES | | | | |

(continued on next page)

Table 1 (continued)

| Compounds | <i>V. bombycina</i> | <i>T. cingulatum</i> | <i>C. comatus</i> | <i>B. plumbea</i> |
|----------------------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Saturated fatty acids | | | | |
| Arachidic acid | 0.22 ^a ± 0.03 | nd | 0.60 ^a ± 0.18 | 0.09 ^c ± 0.00 |
| Lignoceric acid | 0.21 ^a ± 0.10 | nd | 0.25 ^a ± 0.12 | 0.07 ^b ± 0.01 |
| Margaric acid | 0.05 ^a ± 0.03 | nd | 0.04 ^a ± 0.00 | nd |
| Myristic acid | 0.69 ^a ± 0.08 | 0.65 ^a ± 0.03 | 0.32 ^b ± 0.27 | 0.17 ^b ± 0.04 |
| Palmitic acid | 5.59 ^a ± 0.24 | 3.70 ^b ± 0.66 | 5.68 ^a ± 0.34 | 2.46 ^b ± 0.69 |
| Stearic acid | 2.64 ^a ± 0.56 | 1.80 ^{ab} ± 0.41 | 2.80 ^a ± 0.77 | 1.09 ^c ± 0.28 |
| Mono unsaturated fatty acids | | | | |
| Oleic acid | 2.96 ^b ± 0.41 | 6.05 ^a ± 0.50 | 3.42 ^b ± 1.62 | 6.12 ^a ± 0.46 |
| Palmitelaidic acid | 0.16 ^{ab} ± 0.05 | 0.22 ^a ± 0.11 | 0.19 ^{ab} ± 0.08 | 0.26 ^a ± 0.08 |
| Linoelaidic acid | 2.05 ^a ± 1.00 | nd | 1.74 ^a ± 1.03 | nd |
| Monooleoylglycerol | 1.37 ^a ± 0.02 | 1.47 ^a ± 0.14 | nd | 0.65 ^b ± 0.01 |
| Monopalmitin | 0.26 ^b ± 0.00 | 0.39 ^a ± 0.11 | 0.43 ^a ± 0.24 | 0.24 ^b ± 0.06 |
| Ricinoleic acid | 2.66 ^a ± 1.00 | nd | 2.34 ^a ± 0.28 | nd |
| Poly unsaturated fatty acids | | | | |
| Eicosatrienoic acid | 0.12 ^b ± 0.02 | nd | 0.36 ^a ± 0.22 | nd |
| Linoleic acid | 2.50 ^c ± 0.41 | 5.30 ^b ± 0.08 | 3.00 ^c ± 1.27 | 7.63 ^a ± 0.91 |
| Linoleoylglycerol | 0.33 ^b ± 0.21 | nd | 1.09 ^a ± 0.81 | nd |
| Other fatty acid derivatives | | | | |
| Azelaic acid | nd | 1.12 ^a ± 0.34 | 0.77 ^b ± 0.26 | 0.45 ^c ± 0.001 |
| Hydroxyandrosterone | 0.15 ^a ± 0.01 | nd | 0.13 ^a ± 0.04 | nd |
| Pimelic acid | 0.12 ^a ± 0.04 | nd | 0.13 ^a ± 0.05 | nd |
| β-sitosterol | 0.28 ^a ± 0.00 | nd | 0.31 ^a ± 0.05 | nd |
| Stigmasterol | 0.71 ^a ± 0.00 | 0.11 ^c ± 0.00 | 0.33 ^b ± 0.13 | 0.21 ^b ± 0.09 |
| Suberic acid | 0.13 ^a ± 0.02 | nd | 0.19 ^a ± 0.08 | nd |
| Tetracosane | 1.71 ^a ± 0.00 | nd | 0.65 ^b ± 0.09 | 0.04 ^c ± 0.00 |
| ORGANIC ACIDS & THEIR DERIVATIVES | | | | |
| Ascorbic acid | 0.62 ^a ± 0.05 | nd | nd | 0.05 ^b ± 0.02 |
| Citric acid | 1.24 ^a ± 0.33 | 1.77 ^a ± 0.47 | 1.80 ^a ± 0.63 | 0.65 ^b ± 0.16 |
| Ethylmalonic acid | nd | 0.29 ^a ± 0.11 | nd | 0.11 ^b ± 0.04 |
| Fumaric acid | 2.22 ^c ± 0.36 | 1.15 ^b ± 0.13 | 2.94 ^a ± 0.44 | 0.99 ^b ± 0.04 |
| Hydroxyglutaric acid | 0.19 ^a ± 0.00 | nd | nd | 0.12 ^b ± 0.03 |
| Hydroxymalonic acid | 0.13 ^c ± 0.01 | 0.34 ^a ± 0.04 | 0.19 ^{bc} ± 0.12 | 0.15 ^{bc} ± 0.07 |
| Maleic acid | 4.39 ^b ± 0.52 | 4.31 ^b ± 0.64 | 4.80 ^b ± 0.87 | 6.05 ^a ± 0.72 |
| Methylmaleic acid | 0.06 ^d ± 0.00 | 0.07 ^c ± 0.02 | 0.10 ^b ± 0.00 | 0.12 ^a ± 0.01 |
| Methyl succinic acid | 0.07 ^b ± 0.00 | 0.23 ^a ± 0.04 | nd | nd |
| Pyruvic acid | nd | 0.22 ^a ± 0.001 | nd | 0.11 ^b ± 0.04 |
| Oxalic acid | 0.16 ^a ± 0.05 | 0.15 ^a ± 0.06 | 0.20 ^a ± 0.09 | 0.12 ^a ± 0.04 |
| Salicylic acid | 0.09 ^a ± 0.00 | nd | 0.09 ^a ± 0.00 | nd |
| Shikimic acid | nd | 0.37 ± 0.10 | nd | nd |
| Succinic acid | 1.11 ^a ± 0.05 | 1.00 ^a ± 0.17 | 1.58 ^a ± 0.81 | 1.59 ^a ± 0.57 |
| Mineral acid | | | | |
| Phosphoric acid | 1.21 ^a ± 0.09 | 0.60 ^b ± 0.10 | 1.27 ^a ± 0.48 | 1.13 ^a ± 0.45 |

nd : Not detected.

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

DW. An elevated S and P concentrations were also found in *B. plumbea*. In contrast, *T. cingulatum* demonstrated the highest K content at 31.20 mg g⁻¹ DW.

In trace elements, *C. comatus* stood out with a remarkably high Fe concentration at 841.65 µg g⁻¹ DW. Although *B. plumbea* and *T. cingulatum* also contained substantial Fe concentration, they were significantly lower than that of *C. comatus*. *T. cingulatum* had the highest Cu concentration measuring 53.95 µg g⁻¹ DW. The presence of Ni and Zn across all species, with *T. cingulatum* showing relatively higher Zn concentrations, emphasized the consistent trace element composition among these mushrooms.

3.6. Antinutritional factors

In terms of antinutritional factors, *T. cingulatum* showed the highest concentration of PA and CT. *V. bombycina* shows relatively low concentration of antinutritional factors (Fig. 2A–B).

3.7. Antinutrient to mineral ratios

The antinutrient to mineral molar ratios for oxalic acid (OA) and phytic acid (PA) were assessed to determine their impact on mineral bioavailability (Table 2). The OA/(Ca+Mg) ratio, indicative of binding potential of oxalic acid to Ca and Mg, was below the critical threshold of 2.5 in all four species. *T. cingulatum* exhibited the lowest ratio (0.40). Although *C. comatus* had the highest ratio (2.22), it remained within the acceptable limit.

The PA/Fe ratio revealed all mushrooms were well below the critical value of 0.4. *V. bombycina* exhibited the lowest ratio (0.09). Similarly, the PA/Ca ratio was below the critical threshold of 0.4 for all species, with *C. comatus* having the lowest ratio (0.09). The PA/Zn ratio was also below the critical value of 10 for all species, with *C. comatus* showing the lowest ratio (1.20).

3.8. Non-enzymatic antioxidants

The highest concentration of TPC was observed in *B. plumbea* (47.65

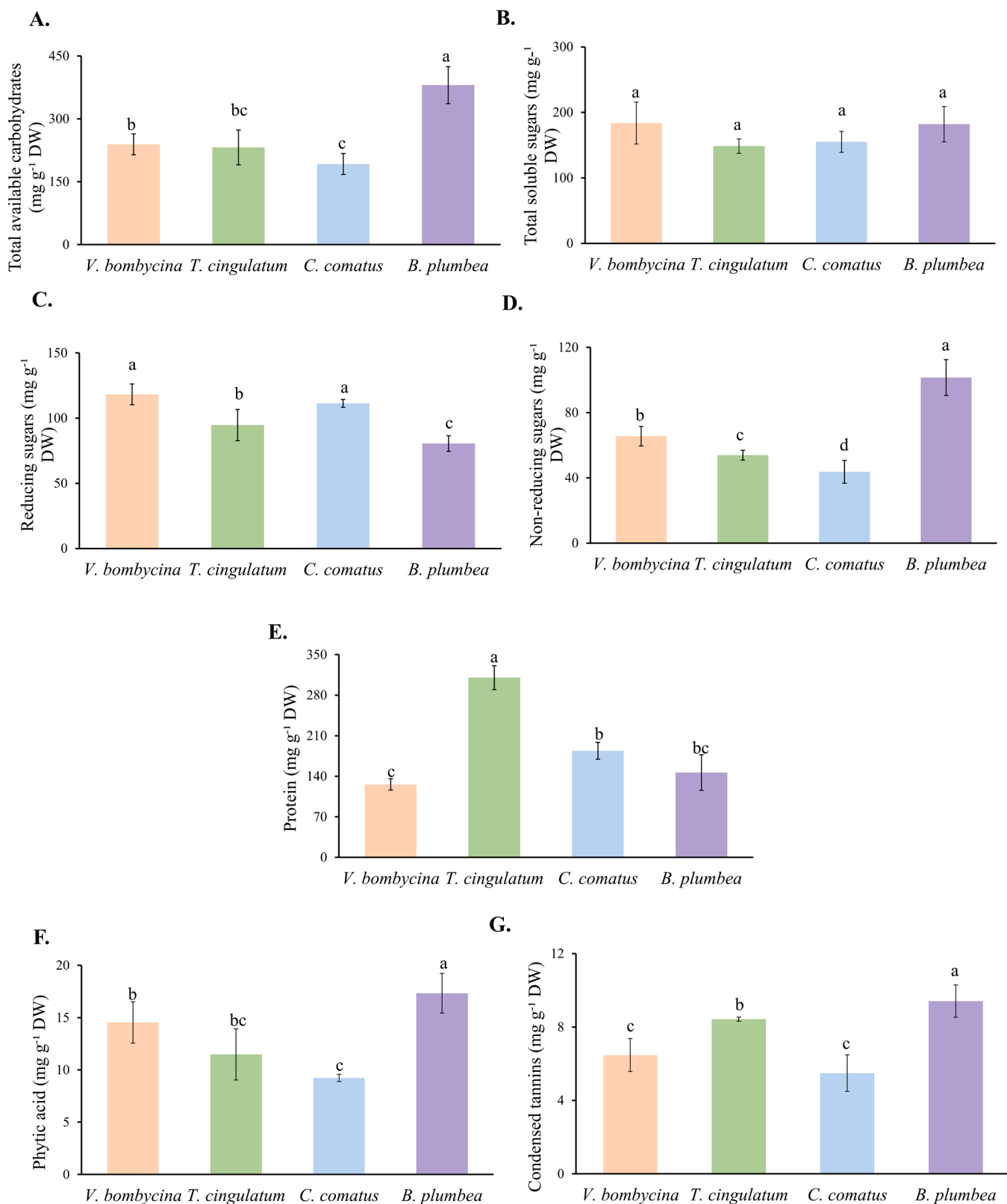


Fig. 2. Bar graphs representing concentrations of macronutrients – (A) total available carbohydrates, (B) total soluble sugars, (C) reducing sugars, (D) non-reducing sugars, and (E) total protein content; and antinutrients – (F) phytic acid, (G) condensed tannins, in *Volvariella bombycina*, *Tricholoma cingulatum*, *Coprinus comatus*, and *Bovista plumbea*. Values represent mean \pm SD ($n = 3$). Different letters on error bars represent significant difference at $p \leq 0.05$, derived from one-way ANOVA.

Table 2

Elemental composition and molar ratios of antinutrients to essential minerals in *Volvariella bombycina*, *Tricholoma cingulatum*, *Coprinus comatus*, and *Bovista plumbea*.

| Elements | <i>V. bombycina</i> | <i>T. cingulatum</i> | <i>C. comatus</i> | <i>B. plumbea</i> |
|----------------------------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Macro-elements (mg g⁻¹ DW) | | | | |
| N | 5.64 ^c ± 0.46 | 12.84 ^b ± 0.35 | 5.66 ^c ± 1.74 | 28.27 ^a ± 4.09 |
| S | 0.86 ^c ± 0.26 | 1.02 ^b ± 0.17 | 0.70 ^{bc} ± 0.23 | 2.88 ^a ± 0.06 |
| P | 0.24 ^c ± 0.00 | 0.94 ^a ± 0.03 | 0.32 ^b ± 0.06 | 0.35 ^b ± 0.02 |
| Na | 38.00 ^a ± 12.12 | 22.01 ^b ± 3.83 | 46.00 ^a ± 13.86 | 10.02 ^c ± 6.01 |
| K | 20.98 ^b ± 0.88 | 31.20 ^a ± 0.75 | 15.18 ^c ± 0.36 | 10.08 ^d ± 0.12 |
| Ca | 0.40 ^d ± 0.08 | 0.68 ^c ± 0.12 | 1.71 ^a ± 0.04 | 0.98 ^b ± 0.02 |
| Mg | 0.43 ^d ± 0.00 | 0.67 ^a ± 0.01 | 0.48 ^c ± 0.001 | 0.51 ^b ± 0.01 |
| Trace elements (µg g⁻¹ DW) | | | | |
| Al | 31.37 ^{ab} ± 7.44 | 39.07 ^a ± 3.26 | 27.02 ^b ± 3.95 | 41.23 ^a ± 10.12 |
| Cu | 32.86 ^b ± 2.14 | 53.95 ^a ± 2.40 | 11.35 ^d ± 1.65 | 25.79 ^c ± 1.44 |
| Fe | 56.65 ^d ± 2.18 | 223.05 ^c ± 38.74 | 841.65 ^a ± 17.26 | 537.45 ^b ± 16.07 |
| Ni | 23.15 ^a ± 3.64 | 22.41 ^a ± 3.93 | 16.70 ^{ab} ± 3.07 | 16.10 ^b ± 2.86 |
| Cr | 19.14 ^b ± 1.82 | 30.92 ^a ± 2.94 | 34.60 ^a ± 3.29 | 33.39 ^a ± 3.18 |
| Mn | 35.79 ^d ± 3.41 | 55.60 ^c ± 5.75 | 123.55 ^a ± 11.77 | 60.34 ^b ± 5.30 |
| Se | 22.21 ^c ± 2.36 | 26.43 ^b ± 1.09 | 84.72 ^a ± 9.02 | 10.25 ^d ± 2.81 |
| Mo | 25.66 ^b ± 4.73 | 61.22 ^a ± 5.91 | 16.81 ^c ± 3.52 | 32.91 ^b ± 3.21 |
| Zn | 15.27 ^c ± 0.09 | 30.58 ^a ± 4.77 | 13.63 ^d ± 0.80 | 20.13 ^b ± 0.16 |
| Antinutrient to mineral molar ratio | | | | |
| OA/(Ca+Mg) | 1.21 | 0.40 | 2.22 | 1.19 |
| PA/Fe | 0.09 | 0.16 | 0.34 | 0.16 |
| PA/Ca | 0.17 | 0.13 | 0.09 | 0.17 |
| PA/Zn | 2.78 | 6.33 | 1.20 | 3.74 |

OA - oxalic acid; PA - phytic acid.

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

mg g⁻¹ DW), followed by *T. cingulatum* (38.33 mg g⁻¹ DW) (Fig. 3A). A similar trend was observed in the TFC (Fig. 3B).

The ascorbic acid and α-tocopherol concentrations were found highest in *B. plumbea* (Fig. 3C–D).

V. bombycina had the highest concentration of carotenoid-based antioxidants namely, lycopene and β-carotene (Fig. 3E–F).

3.9. Enzymatic antioxidants

C. comatus exhibited the highest activity of SOD (190.71 nkat g⁻¹ protein FW). Complementing this, *B. plumbea* showed the highest CAT activity (391.16 µkat g⁻¹ protein FW). Notably, *C. comatus* also led in POD and GPx activities with 496.76 µkat g⁻¹ protein FW and 1409.94 µkat g⁻¹ protein FW, respectively (Fig. 3G–J).

3.10. Antioxidant potential

The antioxidant potential was evaluated using H₂O₂ scavenging assay and potassium ferricyanide reducing power assay (Fig. 4). *C. comatus* exhibited the strongest antioxidant activity against H₂O₂ (Fig. 4), with the lowest IC₅₀ value (7.92 mg mL⁻¹). *V. bombycina* and *T. cingulatum* followed closely with similar IC₅₀ values (8.99 and 9.03 mg mL⁻¹, respectively).

In potassium ferricyanide assay, *T. cingulatum* (5.52 mg mL⁻¹) and *V. bombycina* (5.71 mg mL⁻¹) displayed the highest antioxidant capacity, reflecting their strong reducing power. Conversely, *C. comatus* and *B. plumbea* showed higher IC₅₀ values (8.49 and 9.66 mg mL⁻¹, respectively). *B. plumbea* shows relatively lower antioxidant activity in both assays.

4. Discussion

Cold deserts impose environmental challenges that exert strong selective pressures on living organisms in driving the evolution of specialized biochemical adaptations for survival. The biochemical characterization of four wild edible mushrooms from in cold-arid conditions of Kargil desert – *Volvariella bombycina*, *Tricholoma cingulatum*, *Coprinus comatus*, and *Bovista plumbea* revealed insights into potential applications in nutraceutical and functional food industries.

The metabolic profiling conducted in the study identified a broad spectrum of compounds across the four species. The dominance of sugars and sugar derivatives in their profiles is noteworthy. Sugars, such as trehalose, have been reported to play critical roles in osmoprotection in addition to having multifarious health-promoting effects in humans. Besides functioning as an energy source, trehalose promotes gut health by fostering beneficial microbiota and modulates glucose homeostasis, thereby serving as a therapeutic agent for diabetes (Chen & Gibney, 2023). Glucitol, glycerol, and ribitol, classified as sugar alcohols (polyols), are other crucial osmoprotectants that were found in significant concentrations in the studied mushrooms. Presence of these polyols enhances the overall nutritional profile, as they are associated with low glycaemic index and metabolic health benefits (Zhang & Wang, 2017). Notably, all four mushrooms were quite abundant in glucitol, a versatile low-calorie sugar, known for its effectiveness in regulating epidemic obesity and diabetes.

Fatty acids play vital roles in maintaining cell membrane integrity under cold stress. Saturated fatty acids including palmitic and stearic acids, and MUFA such as oleic acid provide structural integrity to cellular membranes thereby maintaining cellular functions during temperature fluctuations (Ghaffari et al., 2023). Along with contributing to cell integrity, oleic acid supports cardiovascular health, reduces inflammation, enhances insulin sensitivity, and supports cognitive functions. Additionally, the presence of stigmasterol further enhances membrane stability (Valitova et al., 2024). Moreover, stigmasterol exhibits structural similarity to cholesterol. By competing with dietary cholesterol for intestinal absorption, stigmasterol lowers low-density lipoprotein cholesterol and promotes high-density lipoprotein cholesterol (Valitova et al., 2024). Linoleic acid, an essential PUFA, is critical for maintaining both cardiovascular health and cellular structure, while also serving as a precursor for bioactive lipid mediators such as prostaglandins, which are involved in regulating inflammation, immune responses, and cardiovascular functions (Ghaffari et al., 2023). In cold environments where low temperatures lead to increased rigidity of cell membranes, high PUFA content reiterates the findings that these species have modulated their lipid metabolism to preserve membrane fluidity facilitating the transport of nutrients across cell membranes (Juurakko & Walker, 2021). Therefore, the predominance of unsaturated fatty acids (MUFA and PUFA) over saturated fatty acids in these mushrooms implicated that they can serve as a well-balanced source of dietary fatty acids and can be used in the development of functional foods aimed at improving cardiovascular health.

The organic acids concentration in the studied mushroom ranges from 9.90 % to 11.61 % of the total metabolite profile. The presence of organic acids, including maleic, citric, succinic, oxalic, and fumaric acids, emphasizes the importance of efficient energy production. These organic acids are intermediates of the tricarboxylic acid cycle (TCA), which is central to cellular respiration and energy generation. A similar profile of organic acids was reported in other wild edible mushrooms such as *Macrolepiota procera*, *Pleurotus ostreatus*, and *Boletus badius*

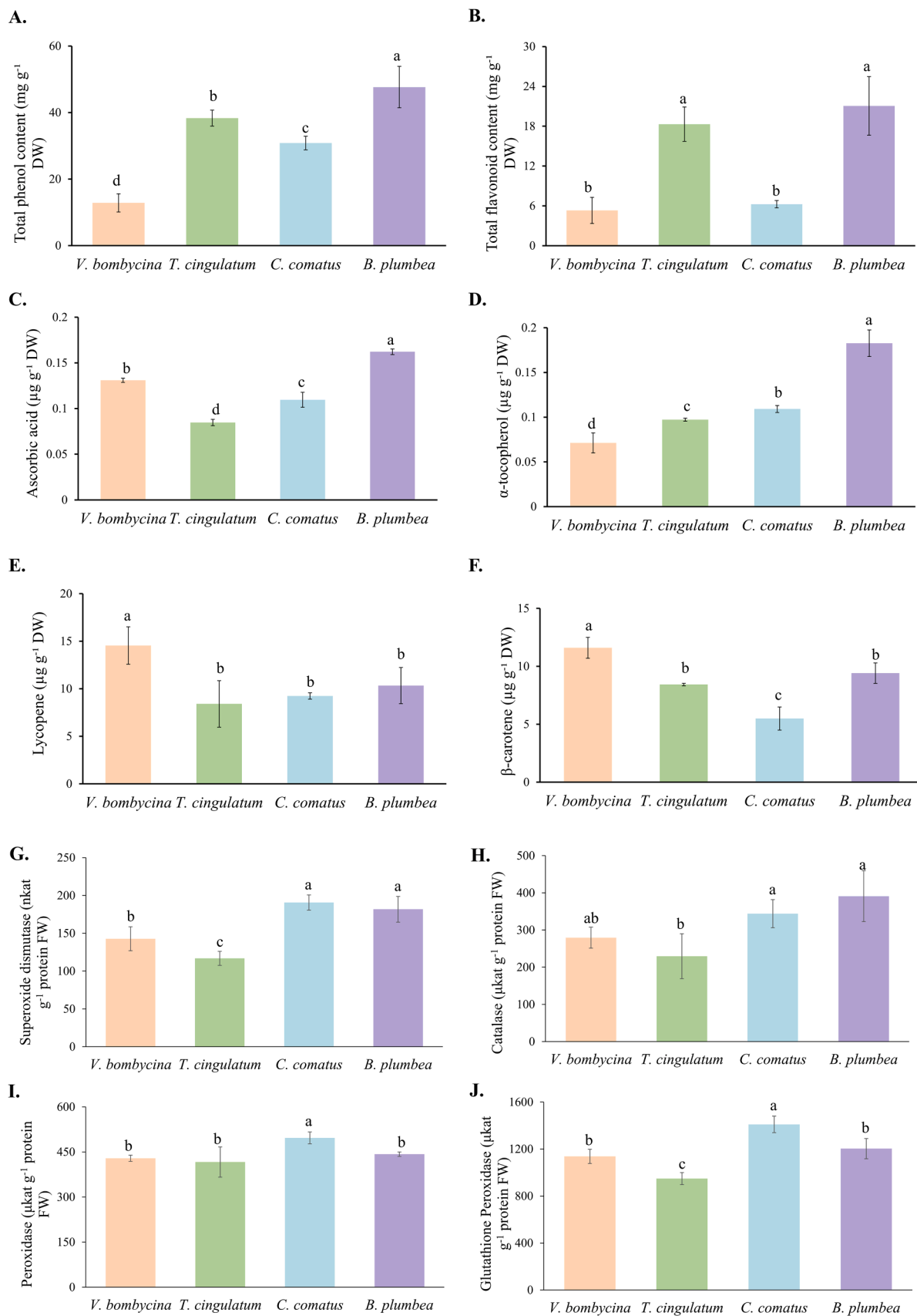


Fig. 3. Bar graphs representing concentrations of non-enzymatic antioxidants – (A) total phenol content, (B) total flavonoid content, (C) ascorbic acid, (D) α-tocopherol, (E) lycopene, and (F) β-carotene; and activities of enzymatic antioxidants – (G) superoxide dismutase, (H) catalase, (I) peroxidase, and (J) glutathione peroxidase, in *Volvariella bombycina*, *Tricholoma cingulatum*, *Coprinus comatus*, and *Bovista plumbea*. Values represent mean ± SD (n = 3). Different letters on error bars represent significant difference at $p \leq 0.05$, derived from one-way ANOVA.

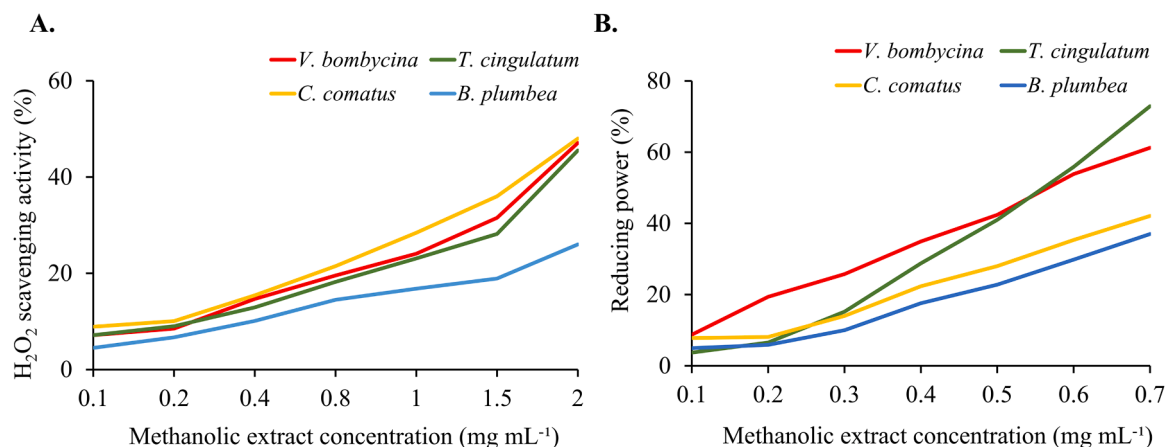


Fig. 4. Line graphs representing (A) hydrogen peroxide scavenging activity (B) and reducing power of methanolic extracts of *Volvariella bombycina*, *Tricholoma cingulatum*, *Coprinus comatus*, and *Bovista plumbea*.

(Magdziak et al., 2017). There is increasing evidence that TCA cycle metabolites play a significant role in the regulation of immune responses in addition to their role as non-volatile flavour components (Choi et al., 2021).

It is well established that protein is an essential macronutrient and the presence of essential amino acids is one of the key quality determinants of protein. The amino acid profiles of the mushrooms reveal that they are rich in leucine, isoleucine, phenylalanine, threonine, and valine, especially in *T. cingulatum* and *B. plumbea*, which also happen to have higher protein concentrations. The essential amino acid composition of these mushrooms comprised of 42 % to 55 % of the total amino acid content, which is comparable to study by Diez and Alvarez (2001). As these essential amino acids cannot be naturally synthesised by the body, they must be obtained through diet to support muscle growth, immune function, and metabolism. Amongst the essential amino acids quantified, threonine was the most abundant. The high concentration of branched-chain amino acids (BCAAs) namely, isoleucine and valine, were also registered. The BCAAs play roles in regulating glucose metabolism and immune responses (Zhang et al., 2017). Given the prevalence of protein-energy malnutrition in high-altitude and cold-desert populations, the presence of these essential amino acids in wild mushrooms offers a promising avenue for addressing dietary protein deficiencies. Their availability in wild mushrooms is particularly important in regions where the primary protein sources are meat-based, as they offer a vegetarian-friendly alternative.

Ergothioneine, another naturally occurring amino acid and an antioxidant, is known to play a major role in preserving mitochondrial bioenergetics, modulating inflammatory cascades, and enhancing immunomodulatory pathways (Servillo et al., 2017). The high ergothioneine content in these mushrooms indicates its potential as a therapeutic food for oxidative stress mitigation. In amino acid derivatives, 5-oxoproline stands out as the major constituent. A key intermediate in the γ -glutamyl cycle, 5-oxoproline is vital for glutathione synthesis, a major antioxidant in cells. Elevated 5-oxoproline concentration points to an increased glutathione turnover, suggesting that these mushrooms bolster their oxidative defense mechanisms to combat stress-induced ROS (Liu et al., 2014). These amino acids contribute to the overall metabolic flexibility of the mushrooms.

In addition to amino acids and their derivatives, N-containing compounds such as uridine and adenine, were found in significant concentrations. Uridine is a pyrimidine nucleoside which is integral to RNA synthesis and modulates cellular growth and protein synthesis. Studies have shown that uridine enhances cognitive functions, promotes synaptic membrane synthesis and plays a critical role in neuroprotection in neurodegenerative diseases like Parkinson's and Huntington's disease (Garcia-Gil et al., 2018). Adenine, a fundamental purine nucleotide

involved in critical processes of ATP synthesis and cellular energy transfer, was detected in elevated concentration suggesting an enhanced ATP production and nucleotide turnover, both of which are crucial for energy-demanding environments like cold deserts (Hara & Kondo, 2015).

Minerals are essential nutrients that regulate key physiological functions in the body. The elemental analysis of the mushrooms revealed significant variations in macro and trace element concentrations, which are indicative of their nutritional potential. Fe, Zn, and Cu were amongst the most noteworthy elements detected. On comparison with a previous study by Dimopoulou et al. (2022), the concentrations of Fe in *C. comatus* and *B. plumbea* were found to be notably higher. The high Fe content in *C. comatus* is particularly significant, as Fe deficiency is a pervasive nutritional issue, especially in high-altitude regions where anaemia is common. Fe is essential for oxygen transport, DNA synthesis, and energy metabolism, and its availability in edible mushrooms provides a valuable dietary source of this important mineral (Puig et al., 2017). Zn and Cu, both of which play essential roles in immune functions, enzyme activities, and antioxidant defenses, were also present in substantial amounts. Zn is a cofactor for numerous enzymes involved in DNA repair, protein synthesis, and immune responses, while Cu is necessary for maintaining connective tissue integrity and facilitating Fe absorption (Wang et al., 2020).

Mushrooms are generally low in Na and rich in K, which makes them a healthy choice for maintaining balanced electrolytes and supporting cardiovascular health. The concentration of Na in the studied mushrooms ranged from 10.01 to 40.0 mg g⁻¹ DW, which is relatively lower than that reported for wild mushrooms from southeastern Spain (Haro et al., 2020). Amongst the studied mushrooms, *T. cingulatum* was found to be the highest in K concentrations. K helps regulate cellular osmotic balance, enzyme activation, and osmoregulation, especially in environments with limited water availability (Hasanuzzaman et al., 2018). In addition to K, elevated concentrations of S in these mushrooms also contribute to their nutritional profile. The high concentration of S highlights its importance in protein synthesis, enzyme activation, and antioxidant defense. Meanwhile, P plays a key role in energy transfer (via ATP), maintaining cell membrane structure, and nucleic acid formation (Bhatla & Lal, 2023).

Cultivated edible mushrooms tend to be deficient in Se, with concentrations typically ranging from 1 μ g g⁻¹ to 8.5 μ g g⁻¹ DW. However, Se content has been reported to be higher (12 μ g g⁻¹ to 200 μ g g⁻¹ DW) in certain wild-grown mushrooms (Kora, 2020). Notably, the mushrooms from Kargil examined in the present study exhibited a high Se content, placing them within the elevated range. Se is an essential trace mineral known for bioactivities, such as supporting immune function, thyroid health, and heart health (Ferreira et al., 2021). The decrease in the risk

of certain cancers and cognitive decline, attributed to the anti-inflammatory properties of Se, has been documented (Ferreira et al., 2021). With a large portion of the global population experiencing sub-optimal Se intake, the Se-rich mushrooms from Kargil offer a prospective solution for addressing Se-deficiency. In addition, Mo, Mn, and Cr concentrations observed in the tested samples are significant. Mo supports detoxification and metabolic balance through molybdoenzymes, while Mn acts as a cofactor for antioxidant enzymes and is an important mineral for bone and glucose metabolism. Cr, on the other hand, plays a key role in glucose and lipid regulation by enhancing insulin sensitivity (Malik et al., 2023). Thus, mushrooms serve as a natural source of essential elements that could potentially address widespread micronutrient deficiencies.

Antinutritional factors are compounds in foods that impair nutrient bioavailability and inhibit metabolic utilization (Ali et al., 2022). Although antinutritional factors including OA, PA, and condensed tannins were present in the studied mushrooms, their concentrations were below critical thresholds, suggesting minimal impact on mineral bioavailability. OA forms insoluble complexes with Ca and Mg and reduce their bioavailability (Ali et al., 2022). However, the low OA/(Ca+Mg) ratio across all species indicates a reduced risk of Ca and Mg sequestration thereby, enhancing their bioavailability for human absorption. Also, *C. comatus* had a relatively higher OA/(Ca+Mg) ratio of 2.22, however, still lower than the threshold of potential interference.

Phytic acid is known to chelate essential minerals including Fe, Zn, and Ca, and reduce their bioavailability by forming insoluble complexes in the gastrointestinal tract (Oberleas & Harland, 1981). The PA/Fe ratio was the lowest in *V. bombycina* (0.09), indicating enhanced Fe absorption potential. The PA/Zn ratio for all the species remained well below the critical threshold of 10, with *C. comatus* exhibiting the lowest ratio (1.20), suggesting that Zn bioavailability would not be significantly compromised by the PA content.

Condensed tannins have protein-complexing ability that cause inhibition of digestive enzymes such as trypsin, alpha-amylase, and lipase. Therefore, they interfere with gastrointestinal digestion and absorption of nutrients. It has been reported that metallic ions including Cr^{+6} , Fe^{+3} , and Cu^{+2} are reduced to Cr^{+3} , Fe^{+2} , and Cu^{+1} , respectively, when exposed to tannin solutions. The reduction lowers the mineral bioavailability and absorption (Serrano et al., 2009). Meanwhile, concentrations of CT in the analyzed mushrooms were <10 % of the dry matter, which is insufficient to significantly hinder mineral absorption (Woldegiorgis et al., 2015). Overall, the low concentrations of these antinutritional factors relative to the high concentrations of essential minerals emphasize the nutritional value of the studied mushrooms as dietary supplements in regions where mineral deficiencies are prevalent.

Some essential trace elements, such as Cu, Fe, Mn, Zn, Se, Co, and Cr, can become potentially toxic to humans if consumed in excess. The toxicity arises when their intake exceeds the body's ability to properly regulate and eliminate them, resulting in accumulation and subsequent harmful effects (Malik et al., 2023). The established Upper Intake Levels (UL) for these minerals are given in Supplementary Material Table 1. Cu and Zn, vital for enzymatic processes, were present in beneficial amounts without posing a risk of toxicity (Wang et al., 2020). Fe and Mn also remained below toxic levels, offering support for oxygen transport and bone health (Islam et al., 2023). Se and Cu were found in trace amounts, contributing to antioxidant defenses and vitamin B12 synthesis, respectively, while remaining within safe limits (Islam et al., 2023). Although no UL have been established for Cr, the Cr concentrations posed no risk of toxicity. Thus, the mineral content of these cold-desert mushrooms corresponds with human nutritional needs and offers essential trace elements in beneficial concentrations without exceeding the established UL.

Oxidative stress is a major challenge for organisms living in cold-desert regions due to the high-intensity ultraviolet radiation and environmental stress leading to the production of ROS (Dhakar & Pandey, 2020). The studied mushrooms exhibited significant non-enzymatic

antioxidant concentrations, as evidenced by high total phenolic content and total flavonoid content in their fruiting bodies. Phenolic compounds are well-known for their ability to scavenge free radicals and prevent oxidative damage to cellular structures. Flavonoids, a diverse group of polyphenols with documented anti-inflammatory, antiviral, and anticancer properties in addition to their antioxidant property (Abdelshafy et al., 2022), were found in abundance in *C. comatus*. These compounds offer protection against environmental stress and potential health benefits for consumers.

Although ascorbic acid and tocopherol are vitamins, their potent antioxidant properties make them integral to the defense against ROS and subsequently enhances the resilience of mushrooms to oxidative-stress-prone habitats (Kasote et al., 2015). While mushrooms are not as abundant in ascorbic acid as citrus fruits, they still provide a decent amount. α -Tocopherol, a lipid-soluble antioxidant, provides protection specifically within cellular membranes by neutralizing lipid peroxides and preventing lipid peroxidation (Kasote et al., 2015). This is imperative in cold environments where lipid membranes are susceptible to oxidative damage due to fluctuating temperatures.

Lycopene and β -carotene are two carotenoids examined in the mushrooms studied. Lycopene, a potent scavenger of singlet oxygen, is highly effective in protecting cellular structures from oxidative stress under high UV radiation, which is common in cold deserts. β -carotene, a precursor of vitamin A, contributes to the antioxidant defense by scavenging free radicals. It also supports visual health and immune functions in humans (Tufail et al., 2024). As a fat-soluble carotenoid, β -carotene integrates into lipid membranes and enhances the capacity of mushrooms to maintain membrane integrity under stress.

Antioxidant enzymes including SOD, CAT, POD, and GPx play a critical role in mitigating oxidative stress by neutralizing ROS, thereby protecting cellular structures from damage (Garcia-Caparrós et al., 2021). SOD is one of the primary antioxidant enzymes responsible for catalyzing the dismutation of the superoxide radical (O_2^-) into oxygen and H_2O_2 . Based on their metal cofactors, SOD is classified into three types: Cu/Zn-SOD, Mn-SOD, and Fe-SOD. In Cu/Zn-SOD, Cu acts as the redox-active centre, alternating between Cu^{2+} and Cu^+ states, while Zn serves a structural role that stabilizes the enzyme, regulate metal ion concentrations, and protect against metal-induced oxidative stress (Rajput et al., 2021). In the mushrooms studied, the mineral profiles exhibited high concentrations of both Cu and Zn, which corroborated with the high enzymatic activities (ranged from 116.93 to 190.71 nkat g^{-1} protein in FW) suggesting that Cu/Zn-SOD plays a significant role in defending against oxidative stress. The highest Fe and Mn concentrations observed in *C. comatus* aligned with its peak SOD activity, implying that Fe and Mn dependency could be a feature of this species' antioxidant response alongside signifying an evolutionary adaptation to ROS management, where Fe and Mn are used both for enzymatic catalysis and maintenance of cellular redox balance. In cold desert environments, where oxidative stress is prevalent, the activities of SOD provide these mushrooms with an adaptive advantage. The relationship between SOD activity and Fe availability is significant for human health as well. Given that Fe deficiency is a widespread nutritional issue, the consumption of Fe-rich mushrooms will enhance endogenous antioxidant defenses by promoting the activity of Fe-dependent enzymes.

Catalase is another major antioxidant enzyme that requires Fe as a cofactor in the form of a heme prosthetic group (Fe^{3+}) in addition to Mn and dissociates H_2O_2 into water and oxygen (Garcia-Caparrós et al., 2021; Rajput et al., 2021). The observed high CAT activity in *B. plumbea* despite lower concentrations of Fe and Mn suggests that the enzyme may not be strictly dependent on these metals for optimal function. It is possible that CAT in this species utilizes alternative cofactors or possesses an enzyme structure that enables efficient activity without a strong reliance on Fe and Mn. In contrast, the elevated concentrations of Fe and Mn in *C. comatus* could be more related to oxidative stress response or other cellular functions rather than CAT activity itself.

Peroxidases are a diverse group of enzymes that reduce peroxides

using various electron donors. Similar to CAT, PODs dissociate H₂O₂, however they differ in their substrate specificity and reliance on metal cofactors. In addition to Fe, PODs require metals Cu and Mn for their activity (De Oliveira et al., 2021). The maximal PODs activity was detected in *C. comatus*, which correlates with its elevated concentrations of Cu and Mn. It is plausible that the enzymatic activity is predominantly facilitated by these trace elements alternatively suggesting that enzymatic efficiency is due to its preference for Cu and Mn over Fe.

Glutathione peroxidase catalyzes the reduction of H₂O₂ and lipid peroxides using glutathione as an electron donor, which is critical for maintaining redox balance (De Oliveira et al., 2021). GPx activity is dependent on the availability of Se, wherein Se is incorporated into the active site of the enzyme as the amino acid, selenocysteine. In humans, dietary Se is essential for the activity of GPx, and the presence of Se-rich mushrooms contribute to improving antioxidant defenses in populations with low Se intake (Rajput et al., 2021). Overall, the studied enzymes rely heavily on their metal cofactors such as Fe, Cu, Zn, Se, and Mn for their catalytic activity. Thus, the availability of these essential minerals is one of the key determinants for the functionality and efficiency of the antioxidant systems.

In order to correlate the antioxidants with antioxidative capacity, the study also demonstrated the radical scavenging activity using assays such as DPPH, wherein *C. comatus* exhibited the strongest overall antioxidant activity, with the lowest IC₅₀ value. The reducing power assay further confirmed the strong antioxidant potential of these mushrooms. This suggests a potent ability of the mushroom to neutralize ROS, which could be leveraged in the development of functional foods aimed at mitigating oxidative damage. Similar to the study of Ali et al. (2024) on *Volvariella* sp., *V. bombycina* demonstrated substantial antioxidant potential with strong electron-donating capacities, along with *T. cingulatum*. This supports the use of these mushrooms in nutraceutical formulations designed to enhance human antioxidant defenses.

Conclusion

The present investigation elucidates the nutritional and antioxidative properties of wild mushrooms native to the cold-desert biome of Kargil namely, *Volvariella bombycina*, *Tricholoma cingulatum*, *Coprinus comatus*, and *Bovista plumbea*. The study highlights the abundance of these mushrooms in nutritional constituents such as proteins, amino acids, sugar alcohols, and polyunsaturated fatty acids. Although *T. cingulatum* has the highest concentration of total protein, *B. plumbea* accounts for the highest concentration of amino acids. The significant concentrations of minerals in the studied mushrooms, such as Fe, Zn, Cu, Se, and Mn, which act as cofactors of various antioxidant enzymes, suggest that these species have evolved mechanisms to efficiently utilize these elements in their antioxidant defense systems. Notably, the low antinutrient-to-mineral molar ratios observed in the studied mushrooms suggests minimal interference with the bioavailability of these minerals. The enhanced mineral profile of the mushrooms was accompanied by elevated concentrations of antioxidants, including polyphenols, flavonoids, and carotenoids, all of which are integral to mitigating oxidative stress induced by harsh environmental conditions. The concentration of antioxidative compounds is further corroborated by low IC₅₀ that inferred their potent antioxidative capacity. Overall, the studied mushrooms hold promise for populations in nutrient-scarce regions as well as those exposed to oxidative stress including inhabitants of high-altitude areas. The nutrient density and antioxidant defenses suggest plausible biochemical signatures of the mushrooms. Their survival and function in exigent environments highlight their significance in sustaining cold-desert ecosystems. Moreover, exploiting the potential of mushrooms from cold-desert in the formulation of functional foods and dietary supplements offers a sustainable approach to addressing micronutrient deficiencies. Future research should prioritize the exploration of the therapeutic potential of these mushrooms in the prevention and management of oxidative stress-associated pathologies. It is important to

note that this study did not include mushrooms from regions with moderate growth conditions as controls. Future studies may benefit from comparative analyses between mushrooms from different climatic regions to further validate these findings.

Consent for publication

All authors have read the manuscript and approved for publication.

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CRediT authorship contribution statement

Hom-Singli Mayirnao: Writing – review & editing, Writing – original draft, Resources, Investigation, Formal analysis, Data curation. **Pooja Jangir:** Writing – review & editing, Visualization. **Karuna Sharma:** Writing – review & editing, Visualization. **Surinder Kaur:** Writing – review & editing, Supervision. **Yash Pal Sharma:** Resources, Funding acquisition. **Rupam Kapoor:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2025.101023.

Data availability

No data was used for the research described in the article.

References

- Abdelshafy, A. M., Belwal, T., Liang, Z., Wang, L., Li, D., Luo, Z., & Li, L. (2022). A comprehensive review on phenolic compounds from edible mushrooms: Occurrence, biological activity, application and future prospective. *Critical Reviews in Food Science and Nutrition*, 62(22), 6204–6224. <https://doi.org/10.1080/10408398.2021.1898335>
- Ali, A., Devarajan, S., Manickavasagan, A., & Ata, A. (2022). Antinutritional factors and biological constraints in the utilization of plant protein foods. In *Plant protein foods* (pp. 407–438). Springer International Publishing. https://doi.org/10.1007/978-3-030-91206-2_14.
- Ali, S., Yousaf, N., Usman, M., Javed, M. A., Nawaz, M., Ali, B., & Ahmed, A. E. (2024). *Volvariella volvacea* (Paddy straw mushroom): A mushroom with exceptional medicinal and nutritional properties. *Heliyon*, 10(21), Article e39747. <https://doi.org/10.1016/j.heliyon.2024.e39747>
- Bhatla, S.C., & Lal, M.A. (2023). Sulfur, phosphorus, and iron metabolism. In *Plant physiology, development and metabolism* (pp. 335–359). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-99-5736-1_12.

- Chen, A., & Gibney, P. A. (2023). Dietary trehalose as a bioactive nutrient. *Nutrients*, 15 (6), 1393. <https://doi.org/10.3390/nu15061393>
- Choi, I., Son, H., & Baek, J. H. (2021). Tricarboxylic acid (TCA) cycle intermediates: Regulators of immune responses. *Life (Chicago, Ill. : 1978)*, 11(1), 69. <https://doi.org/10.3390/1ife11010069>
- De Oliveira, F. K., Santos, L. O., & Buffon, J. G. (2021). Mechanism of action, sources, and application of peroxidases. *Food Research International*, 143, Article 110266. <https://doi.org/10.1016/j.foodres.2021.110266>
- Dhakar, K., & Pandey, A. (2020). Microbial ecology from the Himalayan cryosphere perspective. *Microorganisms*, 8(2), 257. <https://doi.org/10.3390/microorganisms8020257>
- Diez, V. A., & Alvarez, A. (2001). Compositional and nutritional studies on two wild edible mushrooms from northwest Spain. *Food Chemistry*, 75(4), 417–422. [https://doi.org/10.1016/S0308-8146\(01\)00229-1](https://doi.org/10.1016/S0308-8146(01)00229-1)
- Dimopoulou, M., Kolonas, A., Mourtakos, S., Androutsos, O., & Gortzi, O. (2022). Nutritional composition and biological properties of sixteen edible mushroom species. *Applied Sciences*, 12(16), 8074. <https://doi.org/10.3390/app12168074>
- Ferreira, R. L. U., Sena-Evangelista, K. C. M., De Azevedo, E. P., Pinheiro, F. I., Cobucci, R. N., & Pedrosa, L. F. C. (2021). Selenium in human health and gut microflora: Bioavailability of seleno compounds and relationship with diseases. *Frontiers in Nutrition*, 8, Article 685317. <https://doi.org/10.3389/fnut.2021.685317>
- García-Caparrós, P., De Filippis, L., Gul, A., Hasanuzzaman, M., Ozturk, M., Altay, V., & Lao, M. T. (2021). Oxidative stress and antioxidant metabolism under adverse environmental conditions: A review. *The Botanical Review*, 87, 421–466. <https://doi.org/10.1007/s12229-020-09231-1>
- García-Gil, M., Camici, M., Allegrini, S., Pesi, R., Petrotto, E., & Tozzi, M. G. (2018). Emerging role of purine metabolizing enzymes in brain function and tumors. *International Journal of Molecular Sciences*, 19(11), 3598. <https://doi.org/10.3390/ijms19113598>
- Ghaffari, M., Gholizadeh, A., Rauf, S., & Shariati, F. (2023). Drought-stress induced changes of fatty acid composition affecting sunflower grain yield and oil quality. *Food Science & Nutrition*, 11(12), 7718–7731. <https://doi.org/10.1002/fsn3.3690>
- Hara, K. Y., & Kondo, A. (2015). ATP regulation in bioproduction. *Microbial Cell Factories*, 14, 1–9. <https://doi.org/10.1186/s12934-015-0390-6>
- Haro, A., Trecastró, A., Lara, L., Fernández-Figueras, I., Nieto, R., & Seiquer, I. (2020). Mineral elements content of wild growing edible mushrooms from the southeast of Spain. *Journal of Food Composition and Analysis*, 91, Article 103504. <https://doi.org/10.1016/j.jfca.2020.103504>
- Hasanuzzaman, M., Bhuyan, M. B., Nahar, K., Hossain, M. S., Mahmud, J. A., Hossen, M. S., & Fujita, M. (2018). Potassium: A vital regulator of plant responses and tolerance to abiotic stresses. *Agronomy*, 8(3), 31. <https://doi.org/10.3390/agronomy8030031>
- Hassan, L. G., Abdulmumin, U., Umar, K. J., Ikeh, O. P., & Aliero, A. A. (2014). Nutritional and anti-nutritional composition of *Strychnos innocua* Del. (Monkey Orange) Fruit pulp grown in Zuru, Nigeria. *Nigerian Journal of Basic and Applied Sciences*, 22(1–2), 33–37. <https://doi.org/10.4314/njbas.v22i1.6>
- Islam, M. R., Akash, S., Jony, M. H., Alam, M. N., Nowrin, F. T., Rahman, M. M., & Thiruvengadam, M. (2023). Exploring the potential function of trace elements in human health: A therapeutic perspective. *Molecular and Cellular Biochemistry*, 478 (10), 2141–2171. <https://doi.org/10.1007/s11010-022-04638-3>
- Juurakko, C. L., & Walker, V. K. (2021). Cold acclimation and prospects for cold-resilient crops. *Plant Stress*, 2, Article 100028. <https://doi.org/10.1016/j.stress.2021.100028>
- Kasote, D. M., Katyare, S. S., Hegde, M. V., & Bae, H. (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International Journal of Biological Sciences*, 11(8), 982. <https://doi.org/10.7150/ijbs.12096>
- Kora, A. J. (2020). Nutritional and antioxidant significance of selenium-enriched mushrooms. *Bulletin of the National Research Centre*, 44, 1–9. <https://doi.org/10.1186/s42269-020-00289-w>
- Liu, Y., Hyde, A. S., Simpson, M. A., & Barycki, J. J. (2014). Emerging regulatory paradigms in glutathione metabolism. *Advances in Cancer Research*, 122, 69–101. <https://doi.org/10.1016/B978-0-12-420117-0.00002-5>
- Magdziak, Z., Siwulski, M., & Mleczeek, M. (2017). Characteristics of organic acid profiles in 16 species of wild growing edible mushrooms. *Journal of Environmental Science and Health*, 52(10), 784–789. <https://doi.org/10.1080/03601234.2017.1356676>. Part B.
- Malik, D., Narayanasamy, N., Pratyusha, V. A., Thakur, J., & Sinha, N. (2023). Microminerals and toxic heavy metals. In *Textbook of nutritional biochemistry* (pp. 447–504). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-19-4150-4_12
- Mayirnao, H. S., Gupta, S., Thokchom, S. D., Sharma, K., Mehmood, T., Kaur, S., & Kapoor, R. (2023). Nutritional assessment of *Lactarius drassinus* and *L. controversus* from the cold desert region of the Northwest Himalayas for their potential as food supplements. *Journal of Fungi*, 9(7), 763. <https://doi.org/10.3390/jof9070763>
- Mayirnao, H. S., Sharma, K., Jangir, P., Kaur, S., & Kapoor, R. (2025). Mushroom-derived nutraceuticals in the 21st century: An appraisal and future perspectives. *Journal of Future Foods*, 5(4), 342–360. <https://doi.org/10.1016/j.jfutfo.2024.07.013>
- Oberleas, D., & Harland, B. F. (1981). Phytate content of foods: Effect on dietary zinc bioavailability. *Journal of the American Dietetic Association*, 79(4), 433–436.
- Puig, S., Ramos-Alonso, L., Romero, A. M., & Martínez-Pastor, M. T. (2017). The elemental role of iron in DNA synthesis and repair. *Metallomics : Integrated biometal science*, 9(11), 1483–1500. <https://doi.org/10.1039/c7mt00116a>
- Rajput, V. D., Harish, Singh, R. K., Verma, K. K., Sharma, L., Quiroz-Figueroa, F. R., & Mandzhieva, S. (2021). Recent developments in enzymatic antioxidant defence mechanism in plants with special reference to abiotic stress. *Biology*, 10(4), 267. <https://doi.org/10.3390/biology10040267>
- Serrano, J., Puupponen-Pimiä, R., Dauer, A., Aura, A.-M., & Saura-Calixto, F. (2009). Tannins: Current knowledge of food sources, intake, bioavailability and biological effects. *Molecular Nutrition & Food Research*, 53(S2), S310–S329. <https://doi.org/10.1002/mnfr.200900039>
- Servillo, L., D'Onofrio, N., & Balestrieri, M. L. (2017). Ergothioneine antioxidant function: From chemistry to cardiovascular therapeutic potential. *Journal of Cardiovascular Pharmacology*, 69(4), 183–191. <https://doi.org/10.1097/FJC.0000000000000464>
- Tarafder, E., Nizamani, M. M., Karunarathna, S. C., Das, D., Zeng, X., Rind, R. A., & Tian, F. (2024). Advancements in genetic studies of mushrooms: A comprehensive review. *World Journal of Microbiology and Biotechnology*, 40(9), 275. <https://doi.org/10.1007/s11274-024-04079-8>
- Tufail, T., Bader Ul Ain, H., Noreen, S., Ikram, A., Arshad, M. T., & Abdullahi, M. A. (2024). Nutritional benefits of lycopene and beta-carotene: A comprehensive overview. *Food Science & Nutrition*, 12(11), 8715–8741. <https://doi.org/10.1002/fsn3.4502>
- Valitova, J., Renkova, A., Beckett, R., & Minibayeva, F. (2024). Stigmasterol: An enigmatic plant stress sterol with versatile functions. *International Journal of Molecular Sciences*, 25(15), 8122. <https://doi.org/10.3390/ijms25158122>
- Venkidasamy, B., Karthikeyan, M., & Ramalingam, S. (2019). Methods/protocols for determination of oxidative stress in crop plants. *Reactive Oxygen, Nitrogen and Sulfur Species in Plants: Production, Metabolism, Signaling and Defense Mechanisms*, 421–435. <https://doi.org/10.1002/9781119468677.ch17>
- Wang, J., Xiao, J., Zhang, J., Chen, H., Li, D., Li, L., & Luo, Y. (2020). Effects of dietary Cu and Zn on the accumulation, oxidative stress and the expressions of immune-related genes in the livers of Nile tilapia (*Oreochromis niloticus*). *Fish & Shellfish Immunology*, 100, 198–207. <https://doi.org/10.1016/j.fsi.2020.03.012>
- Woldegiorgis, A. Z., Abate, D., Haki, G. D., & Ziegler, G. R. (2015). Major, Minor and toxic minerals and anti-nutrients composition in edible mushrooms collected from Ethiopia. *International Journal of Processing Technology*, 6, Article 1000430. <https://doi.org/10.4172/2157-7110.1000430>
- Zhang, G., & Wang, Q. (2017). Functional carbohydrates: Development, characterization, and biomanufacturing of sugar alcohols. In *Functional carbohydrates* (pp. 269–298). CRC Press. <https://doi.org/10.1201/9781315371061>
- Zhang, S., Zeng, X., Ren, M., Mao, X., & Qiao, S. (2017). Novel metabolic and physiological functions of branched chain amino acids: A review. *Journal of Animal Science and Biotechnology*, 8, 1–12. <https://doi.org/10.1186/s40104-016-0139-z>