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Evaluation of antioxidant potential of four cultivated mushroom species of India

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ABSTRACT

Mushroom cultivation has got a boost over the past few years due to their nutrition, biological activities and potential to convert agro-wastes into nutritious food by eco-friendly methods. The present study was conducted with objectives to evaluate the antioxidant potential through DPPH radical scavenging activity, reducing power, total phenolic contents (TPC), non-protein thiols (NPTs), superoxide dismutase activity and ascorbate peroxidase activity of four cultivated mushrooms in India. Among all studied mushroom species *V. volvacea* (77.618%±0.378) displayed the highest DPPH radical scavenging activity in aqueous extract (WE) and *C. indica* (10.10%±0.378) displayed the lowest in methanol extract (ME). The maximum reducing activity was shown by *Calocybe indica* (0.415±0.002) in WE and minimum by *Flammulina velutipes* (0.029±0.002) in ME. The highest value of TPC was shown by *Volvariella volvacea* (2.045±0.047 µg GAE/mg DW) in WE and the minimum by *C. indica* (0.673±0.03 µg GAE/mg DW) in AE (acetone extract). Non-protein thiols (NPTs) values ranged from 1.061±0.029 to 2.360±0.016 µ moles/g DW. These cultivated mushrooms also showed good Superoxide dismutase activity (8.95±0.27-15.43±0.65 U activity/mg Protein) and Ascorbate peroxidase activity (0.677±0.14-7.07±0.58 µ moles/mg protein min^{-1}). All studied mushrooms displayed high antioxidant activities *in vitro*, hence, recommended for Indian diets and for further screening for medicinal uses.

Keywords: Antioxidant, mushroom, DPPH, total phenolic contents, superoxide dismutase, ascorbate peroxidase

Mushrooms have occupied a prominent space in human food due to their nutrition, taste, aroma and biological activities. Mushroom cultivation has gained a boost over the past few years due to their potential to convert agro-wastes into nutritious food by eco-friendly methods. The worldwide production of mushrooms is increased five times in the last 22 years and reached up to 44 million tonnes in 2023, but in India, the production is merely 0.24 million tonnes (FAOSTAT, 2023). Mushrooms contain several nutritional ingredients such as carbohydrates, proteins, minerals, vitamins, dietary fibers and low content of lipids (Kumar and Sagar, 2023a, b) besides various bioactive compounds such as alkaloids, polysaccharides, phenolic compounds, glycoproteins, steroids, ascorbic acid, carotenoids, ergothioneine, glycosides etc. (Kozarski *et al.,* 2015; Kumar, 2015; Ruthes *et al*., 2016; Sakamoto *et al*., 2020; Baosong *et al*., 2020; Abdelshafy *et al*., 2022).

Human body produces free radicals during normal metabolic processes but their production exceeds

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during the presence of some external factors like environmental pollution, smoking, alcohol, unhealthy diets and unhealthy lifestyle (Pham-Huy *et al*., 2008). Increased accumulation of free radicals in the body causes oxidative stress, which is deleterious and can damage cells or tissues. Excessive oxidative stress caused by reactive oxygen species (ROS) results in multiple disorders *i.e*., metabolic disease, cardiovascular problems, cancer, premature aging, autoimmune disorders and severe neurodegenerative disorders such as Parkinson's and Alzheimer's (Kozarski *et al.,* 2015). Our body has endogenous and exogenous types of antioxidant systems. The endogenous system includes enzymatic antioxidant (ascorbate peroxidase, catalase, glutathione peroxidase and superoxide dismutase) and non-enzymatic antioxidant (albumin, bilirubin, ceruloplasmin, coenzyme Q10, glutathione (non-protein thiol), linolenic acid, polyamides, transferrin and uric acid), while exogenous antioxidants include externally taken vitamins, minerals, carotenoids, xanthophylls, and polyphenols (Caverzan *et al.,* 2012; Kozarski *et al.,* 2015; Kuciel-Lewandowska *et al.,* 2020).

Mushrooms are the part of the human diet not only due to their nutrition, taste and aroma but also for their curative properties (Ma *et al.,* 2018). Mushrooms are well off in biologically active compounds, which make them a good antioxidant food source (Nitha *et al*., 2010; Kozarski *et al.,* 2015). The richness in bioactive compounds makes the mushrooms functional foods or nutraceuticals, which accounts for many medicinal properties such as antimicrobial, antiviral, anticancer, antidiabetic, immune-stimulating, hepatoprotective, hypolipidemic, neuroprotective, antiatherogenic and hyposensitive activities (Kozarski *et al.,* 2015; Rai *et al*., 2021; Rauf *et al*., 2023; Gebru *et al*., 2024). The dietary intake of mushrooms may contribute to boosting the antioxidant defense system of our body to regulate oxidative homeostasis.

Mushroom consumption in India is lower in comparison to developed countries due to scanty knowledge of the economic, ecological, nutritional, and health benefits of mushroom diets. The data regarding antioxidant properties of cultivated mushrooms in India is also not enough (Sudha *et al*., 2008; Jagadish *et al*., 2009; Ramkumar *et al*., 2012; Thillaimaharani *et al.,* 2013; Kumar and Priyanka, 2022), hence the present study has been taken up. The present study was conducted with objectives to compare the DPPH scavenging activity, reducing power, total phenolic contents, non-protein thiols, superoxide dismutase activity and ascorbate peroxidase activity of four cultivated mushrooms in India and to make people aware of their health benefits.

MATERIALS AND METHODS

The pure cultures of milky mushroom (*Calocybe indica* DMRO-302), winter mushroom (*Flammulina velutipes* DMRO-253), oyster mushroom (*Pleurotus florida* DMRP-136) and paddy straw mushroom (*Volvariella volvacea* DMRO-484) were obtained from Indian Council of Agricultural Research-Directorate of Mushroom Research, Solan, Himachal Pradesh, India in 2020-21. These fruiting bodies were grown under standard cultivation technologies developed by ICAR-DMR.

The sporocarps of mushrooms were dried at a constant temperature of 40-45 °C and powdered in a mixer grinder. 20g powdered samples were extracted separately in 200 ml acetone, distilled water and methanol for seven days. The mushroom extracts were filtered and dried by evaporating at 45 °C in an oven. The extracts were designated as AE (acetone extract), WE (aqueous extract) and ME (methanol extract). These extracts were stored at a temperature below 4°C and used for assaying DPPH scavenging activity and reducing power.

Scavenging activity of DPPH

DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of different mushroom extracts was estimated by the method given by Barros *et al*. (2008) with few alterations. For estimation of DPPH free radical scavenging activity, 1 ml of different concentrations of 100, 200, 300, 400 and 500µg/ml of acetone extract (AE), aqueous extract (WE) and methanol extract (ME) were taken in separate test tubes. To each test tube 1 ml of DPPH solution (0.1mM) was added, shaken vigorously and kept at a temperature of 25 °C in the dark for 30 minutes. The reduction of the DPPH was estimated by measuring the bleaching of the purple-coloured methanol solution of DPPH at 517 nm. The following formula was used to calculate the radical scavenging activity as a percentage of DPPH discolouration:

$$\% \text{ radical scavenging activity} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Where A control is the absorbance of the DPPH solution and A sample is the absorbance of the reaction mixture when an extract of a particular concentration has been added. IC_{50} value was determined from the plotted graph of radical scavenging activity against the concentration of extract by the following formula:

$$IC_{50} = \frac{50 - \text{Y intercept}}{\text{Slope}}$$

Reducing power

Reducing power evaluation was done by measuring the ability of mushroom extracts to reduce ferricyanide to ferrocyanide (Oyaizu, 1986). Concentrations of 100, 200, 300, 400 and 500 µg/ml of each extract were made. Each concentration (0.5ml) was added to 0.5ml of a 0.2M phosphate buffer (pH 6.6) and 0.5ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The reaction mixture was kept at a temperature of 50 °C for 20 minutes, followed by the addition of 0.5ml of 10% trichloroacetic acid (TCA) and centrifuged for 10 minutes at 3000 rpm. The upper layer of supernatant (0.5ml) was taken and mixed with 0.5 ml of distilled water and 0.1ml of 0.1% $FeCl_3$. The reaction mixture was kept for 10 minutes at room temperature before the absorbance was measured at 700 nm against a reagent blank. A higher absorbance indicates a higher reducing power.

Total Phenolic Contents (TPC)

The total phenolic contents of cultivated mushrooms were determined by the Folin-Ciocalteu phenol reagent method (Goldstein and Swain, 2001) with few alterations. 100 mg of each mushroom's powder was homogenized in 2 ml of 0.3N HCl in acetone, 0.3N HCl in water and 0.3N HCl in methanol, separately and centrifuged at 6000rpm for 10 minutes. Each supernatant was evaporated and the residue was dissolved in 2 ml distilled water in the case of methanol and aqueous extracts and in acetone in acetone extract. The resulting solution was added with 0.5ml of Folin and Ciocalteu's phenol reagent and shaken vigorously to mix thoroughly. After three minutes, 1ml of saturated sodium carbonate (35%) solution was added to the reaction mixture. The reaction mixture was kept in the dark for 90 minutes after which the absorbance was read at 725nm against blank. The TPC was determined through the gallic acid calibration curve. The TPC was expressed as µg of gallic acid equivalents (GAEs)/mg of DW of tissue.

Non-Protein Thiols (NPTs)

The non-protein thiols (NPTs) of mushroom samples were evaluated through the method given by Noctor and Foyer (1998). 100mg of powdered

mushroom sample was homogenized in 3ml of extraction buffer (sodium phosphate buffer 0.1M, pH 7.8 and EDTA 1mM). The homogenized mixture was centrifuged at 10,000 rpm for 10 minutes. The supernatant was used as an enzyme extract. The reaction mixture contained 2.5ml buffers (sodium phosphate buffer 0.1M, pH 7.8 and EDTA), 50µl Ellman's reagent (Dithiobisnitrobenzoic acid DNTB) 4mg/ml and 250µl enzyme extract. The absorbance was observed at 412 nm and NPTs were quantified from the standard calibration curve of reduced glutathione (GSH). The NPTs were determined using the molar extinction coefficient 13600$mM^{-1}cm^{-1}$ for DNTB.

Superoxide dismutase activity (SOD)

The superoxide dismutase activity (SOD) was quantified by measuring the potential of the mushroom sample to impede the photochemical reduction of nitro blue tetrazolium chloride (NBT) (Beauchamp and Fridovich, 1971). 100 mg of powdered mushroom tissue was homogenized with extraction buffer (2.8ml phosphate buffer 50mM, pH 7.8 and 0.2ml EDTA 0.1mM). The homogenized mixture was subjected to centrifugation at 13,000 rpm for 25 minutes at 4°C. The supernatant of centrifugation is used as an enzyme extract. Test tubes were labeled as T, S, C and B and following reaction mixtures were prepared.

Reaction	Ingredients
Test (T)	Methionine (1.5ml), Riboflavin (0.3ml), NBT (0.1ml) and enzyme extract (0.1ml)
Standard (S)	methionine (1.5ml), riboflavin (0.3ml), NBT (0.1ml), and phosphate buffer (0.1ml).
Control (C)	Methionine (1.5ml), Riboflavin (0.3ml), phosphate buffer (0.1ml), and enzyme extract (0.1ml)
Blank (B)	Methionine (1.5ml), riboflavin (0.3ml) and phosphate buffer (0.2ml).

All labeled test tubes containing reaction mixtures were illuminated for 10 minutes in an illumination chamber under a 15W fluorescent lamp. Immediately after illumination, the absorbance of all the reaction mixtures was measured at 560 nm. The unit activity of enzyme present in the extract was calculated by using the following formula and expressed as U/mg protein.

$$\text{Decrease in OD} = (S-B)-(T-C)$$

$$\text{SOD activity} = \frac{\text{Decrease in OD} \times 2}{S - B}$$

$$\frac{\text{SOD activity/}}{\text{mg of protein}} = \frac{\text{SOD activity} \times \text{Dillution factor}}{\text{Total protein (mg/µl)}}$$

Ascorbate peroxidase activity (APX)

The ascorbate peroxidase (APX) activity of mushroom samples was assayed by following the method given by Nakano and Asada (1981) with some modifications. 100 mg of powdered mushroom tissue was homogenized with 1ml of HEPES-NaOH buffer (100mM, pH 7.6) and containing 5mM ascorbate. The homogenate was centrifuged at 10,000 rpm for 5minutes at a temperature of 4°C. The supernatant served as an enzyme extract. The reaction mixture contained 1ml (50mM, pH 7.6) HEPES-NaOH buffer, 50µl (5mM) ascorbate, 50µl enzyme extract and 100µl (4mM) H_2O_2. Following the addition of substrates, a change in absorbance was recorded at 290 nm for up to 3minutes. The enzyme activity was determined using the extinction coefficient 2.8$mM^{-1}cm^{-1}$ for ascorbate.

$$\frac{\text{Enzyme activity (Units/ml enzyme)}}{} = \frac{(\Delta\text{Abs} \times \text{total assay volume} \times \text{dilution factor})}{\Delta t \times \text{extinction coeeficient} \times l \times \text{enzyme sample value}}$$

Where: ΔAbs is the change in absorbance, Δt is time in minutes and l is the path length (1 cm).

$$\text{Units/mg solid} = \frac{\text{Units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{Units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

Every experiment was performed in triplicate and results were recorded as average values (± Standard Error).

RESULTS AND DISCUSSION

Scavenging activity of DPPH

The radical scavenging activity of methanol, aqueous and acetone extracts of four cultivated mushrooms was tested against DPPH. The hydrogen atom or electron donation abilities of the corresponding extracts were estimated from the bleaching of the purple-colored methanol solution of DPPH. The decrease in absorbance was taken as a measure of the amount of radical scavenging. The results revealed that there was an increase in radical scavenging activity with an increasing concentration of extracts.

Table 1 depicts the radical scavenging activity of extracts of *C. indica, F. velutipes P. florida* and *V. volvacea*. The radical scavenging activity of extracts increased with the increase in concentration of extracts and also varied in different mushroom species. The IC_{50} values of radical scavenging activity in acetone extract and methanolic extracts were found to be higher than water extract. The minimum IC_{50} value was recorded in *Volvariella volvacea* water extract and *P. florida* methonolic extract. The DPPH radical scavenging activity was found to be solvent dependent. The lowest DPPH radical scavenging activity was observed in acetone extracts among the three extracts. Among all studied mushroom species *V. volvacea* displayed the highest DPPH radical scavenging activity in aqueous extract (WE) and *C. indica* displayed the lowest in methanol extract (ME).

Table 1. DPPH radical scavenging activity of different extracts of test mushroom species at different concentrations

Conc. of extract (µg/ml)	**DPPH radical scavenging activity %**											
	C. indica			***F. velutipes***			***P. florida***			***V. volvacea***		
	AE	**WE**	**ME**	**AE**	**WE**	**ME**	**AE**	**WE**	**ME**	**AE**	**WE**	**ME**
100	7.53±0.52	13.25±0.23	4.09±0.28	7.03±0.52	5.65±0.30	11.07±0.99	4.933±0.775	15.56±0.00	19.07±0.44	3.290**±0.508	18.39±0.26	15.31±0.36
200	10.38±0.52	23.84±1.15	5.00±0.23	10.29±0.46	11.79±0.24	17.42±0.82	8.670±0.462	25.93±0.05	27.74±0.12	4.369±0.305	35.09±0.45	24.55±0.26
300	11.94±0.45	31.24±0.18	5.63±0.38	16.52±0.53	15.25±0.37	19.49±0.66	12.905±0.215	36.25±0.47	38.98±0.36	7.228±0.447	50.72±0.34	37.67±0.35
400	15.16±0.69	38.08±0.82	8.40±0.28	21.30±0.54	19.95±0.11	23.91±0.34	14.798±0.141	45.36±0.08	52.63±0.09	11.057±0.176	66.56±0.11	48.31±0.32
500	17.55±0.57	46.25±0.35	10.10±0.38	28.48±0.80	26.03±0.19	26.64±0.75	18.585±0.285	53.97±0.43	62.42±0.68	13.970±0.308	77.62±0.38	61.20±0.64
IC_{50} value	1872.21 µg/ml	544.29 µg/ml	3197.68 µg/ml	933.02 µg/ml	1019.56 µg/ml	1124.11 µg/ml	1456.12 µg/ml	452.66 µg/ml	390.14 µg/ml	1770.32 µg/ml	304.03 µg/ml	410.99 µg/ml

The DPPH radical scavenging activity of mushroom extract may be the result of neutralization of free radicals (DPPH) either by transfer of an electron or by hydrogen atom (Naik *et al*., 2003). Alam *et al*. (2019) recorded DPPH radical scavenging activity of *C. indica* 33.71, 31.75 and 25.41% in acetone, methanol and hot water extracts, respectively at 125 ìg/ml concentrations. Nguyen *et al*. (2022) reported radical scavenging activity of 72.36% (500 µg/ml) and IC_{50} =240.11(µg/ml) in *C. indica* which was much higher than the present study. Yeh *et al*. (2014) reported 99.7 ± 3.62% DPPH scavenging activity in *Flammullina velutipes* while Ryu *et al*. (2018) reported 12.45 mg/ml IC_{50} value of aqueous extract, which was considerably below than present results. Shah *et al*. (2018) reported higher DPPH scavenging activity (67.37%) in water extract of *F. velutipes*. Banerjee *et al*. (2020) detected 84.2% DPPH scavenging activity of *F. velutipes* in methanolic extract at 1mg/ml concentration. Krsmanoviæ *et al*. (2023) reported DPPH scavenging 1.17 ± 0.181% µg/ml in ethanol extract in *F. velutipes*.

Menaga *et al*. (2013) reported the DPPH radical scavenging activity of *P. florida* in the methanolic extract was 78% at the concentration of 100 µg/ml. Prabhu and Kumuthakalavalli (2016) reported the IC_{50} values of *P. florida* and *C. indica* were 413.28 ± 5.87 ìg/ml and 588.40 ± 11.85 ìg/ml, respectively. Maji *et al*. (2018) recorded radical scavenging activity of aqueous extract at 200 ìg/ml of *C. indica* (30%) and *P. florida* (42.5%), which was confirmed in the present study. The radical scavenging activity of the *P. ostreatus* grown on different sub-strates ranged from 44.24 to 75.68% and IC_{50} values varied from 0.46-13.17 mg/ml for all samples tested (Gebru *et al*., 2024).

Mishra *et al*. (2014) reported radical scavenging activity of 33.5% in methanol extract and 14.7 in aqueous extracts at 0.5mg/ml concentration in *Volvariella volvacea* while IC_{50} values for ethanol and methanol extracts were reported to be 2.03 mg/ml and 2.05 mg/ml, respectively by Ali *et al*. (2017). Punitha and Rajasekaran (2014) reported IC_{50} values of 110.40 and 142.45 mg/ml for methanol and aqueous extracts of *V. volvacea*. Masitah *et al*. (2023) reported 32.95 ± 0.97% DPPH radical scavenging activity at 20 mg/ ml in ME of mature fruiting bodies of *V. volvaceae*.

Reducing power

The reducing activity was estimated through the reduction of Fe^{3+}/Ferricyanide complex to ferrous (Fe^{2+}). The colour of the reaction mixture changes to various shades of green and blue. A higher absorbance of the sample indicates a higher reducing power. The reducing power of all mushroom extracts increased with an increase in concentration. The reducing power assay showed the maximum activity in *Calocybe indica* followed by *Volvariella volvacea* in water extract followed by methanolic extract (Table 2). The maximum reducing activity was in *Calocybe indica* (0.415 ± 0.002) and minimum by *Flammulina velutipes* (0.029 ± 0.002).

The reducing ability of a substance may be employed as a notable indicator of its antioxidant potential (Meir *et al*., 1995). Mushrooms may have a reducing ability due to their hydrogen donating ability which reduces Fe^{3+} to Fe^{2+} (Shimada *et al*., 1992). In the present study, the reducing power of mushrooms differed with the kind of extract solvent and increased with an increase in concentration. Mishra *et al*. (2014) reported 0.41 ± 0.02, reducing powers of ME and WE of *C. indica* caps, respectively at the 2.0 mg/ml concentration. Govindan *et al*. (2016) evaluated *in vitro* and *in vivo*, the reducing of polysaccharides extracted from *C. indica*.

Table 2. Reducing power of different extracts of test mushroom species at different concentrations

Conc. of extract (µg/ml)	DPPH radical scavenging activity %											
	C. indica			*F. velutipes*			*P. florida*			*V. volvacea*		
	AE	WE	ME	AE	WE	ME	AE	WE	ME	AE	WE	ME
100	0.023 ±0.005	0.087±0.001	0.007±0.002	0.023 ±0.000	0.026±0.003	0.003±0.000	0.019 ±0.005	0.032±0.003	0.043±0.003	0.042 ±0.004	0.036±0.002	0.061±0.001
200	0.049 ±0.006	0.193±0.002	0.015±0.002	0.051 ±0.002	0.052±0.001	0.010±0.002	0.038 ±0.008	0.065±0.004	0.090±0.002	0.063 ±0.006	0.068±0.001	0.120±0.005
300	0.060 ±0.000	0.248±0.001	0.030±0.003	0.070 ±0.009	0.073±0.002	0.016±0.003	0.071 ±0.003	0.099±0.007	0.147±0.004	0.067 ±0.006	0.103±0.002	0.192±0.005
400	0.083 ±0.004	0.343±0.002	0.044±0.003	0.086 ±0.010	0.097±0.006	0.023±0.001	0.093 ±0.009	0.123±0.002	0.222±0.005	0.077 ±0.006	0.147±0.003	0.259±0.004
500	0.103 ±0.007	0.415±0.002	0.062±0.004	0.109 ±0.014	0.131±0.000	0.029±0.002	0.116 ±0.010	0.157±0.004	0.283±0.005	0.096 ±0.004	0.197±0.006	0.335±0.010

The four varieties of *F. velutipes* was recorded a reducing activity of 0.42-0.82 at the concentration of 1.2 mg /ml in methanol extraction (Zhang *et al*., 2013) which was very high than the current study while Yeh *et al*., (2014) recorded a reducing power 68.1 ± 1.07% in water extract. Ramkumar *et al*. (2012) reported similar results in methanol extract of *V. volvacea*. Menaga *et al*. (2013) found very high reducing activity (0.911 at 100 ìg/ml concentration) in methanol extract for *P. florida*. Maji *et al*. (2018) reported 1.4 at 300 ìg/ml iron reducing power of aqueous extract of *P. florida* and 1.1 at 300 ìg/ml in *C. indica* was much higher than the current study.

Total Phenolic Contents (TPC)

Phenols are the major naturally occurring antioxidant components found in mushroom extracts. Cultivated mushrooms under evaluation have a good amount of TPC in their extracts. Table 3 present the TPC of acetone, aqueous and methanol extracts of mushrooms. TPC of mushroom samples were calculated from the standard curve drawn with the help of Gallic acid. The results were expressed in µg GAE/mg dry weight (DW) of mushroom tissue. Results revealed that *P. florida* had the highest total phenol contents in all extracts followed by *C. indica* and *F. velutipes* except acetone extract in which *C. indica* had the lowest phenol content.

The antioxidant property of plant materials has a relation with their phenol contents (Velioglu *et al*., 1998). Mishra *et al*. (2014) and Prabhu and Kumuthakalavalli (2014) reported higher TPC in different mushroom what we got in the present study. Patil *et al*. (2024) reported 4.86 mg GAE/g Phenolic content in *C. indica* which is also higher than the present study. Slawinska *et al*. (2013) and Krsmanoviæ *et al*. (2023) also reported higher TPC in *F. velutipes*. Sun *et al*. (2023) reported 18.96 ± 0.12 mg GAE/g in methanol extract *F. velutipes.*

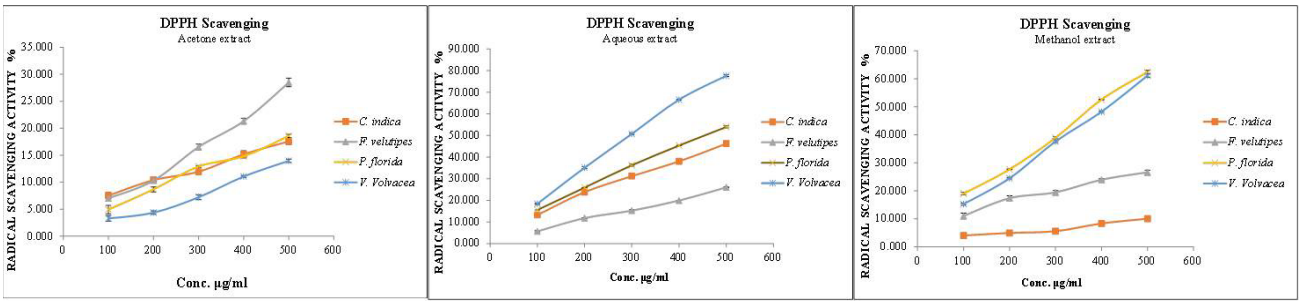

Fig. 1. DPPH radical scavenging activity (%) of the different extracts of four cultivated mushroom species at different concentrations

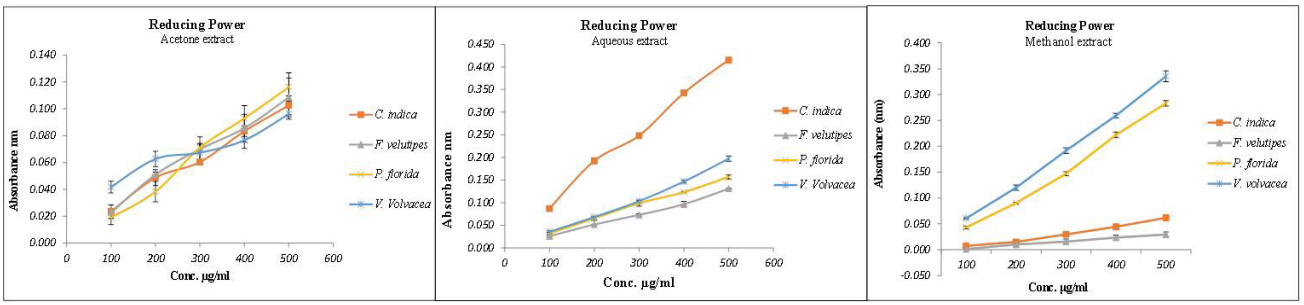

Fig. 2. Reducing powers of the different extracts of four cultivated mushroom species at different concentrations

Table 3. Total Phenol Content (TPC) of four cultivated mushroom species in different solvents.

Total phenol content (µg GAE/mg DW)	Solvent	*C. indica*	*F. velutipes*	*P. florida*	*V.volvacea*
	Acetone	0.673±0.03	1.180±0.03	1.187±0.03	1.612±0.04
	Aqueous	1.873±0.037	0.778±0.015	2.010±0.049	2.045±0.047
	Methanol	1.714±0.03	1.078±0.04	1.850±0.03	1.928±0.03

Thillaimaharani *et al*. (2013) reported 4.17 mg GAE/g while Prabhu and Kumuthakalavalli (2014) reported 38.06 ± 10.09 mg GAE/g TPC in ME of *P. florida*. Krishna *et al*. (2023) reported 84.10 ± 0.17 mg GAE/g in AE and 91.29 ± 0.46 mg GAE/g in ME of *P. florida*.

Punitha and Rajasekaran (2014) reported 53.13 mg GAE/g of ME and 36.67 mg GAE/g of WE in *V. volvacea*. Cheung, *et al*. (2003) reported the TPC to be 15mg GAE/g DW in ME and 1.315mg GAE/g DW in WE. Chanate *et al*. (2024) reported 6.78 to 33.27 mg GAE/g TPC in WE of *V. volvacea*. Masitah *et al*. (2023) reported phenols 2.15 ± 0.02 mg GAE/g DW in ME of mature fruiting bodies of *V. volvaceae*.

Non-Protein Thiols (NPTs)

The NPTs were quantified by calibrating them with a reduced glutathione (GSH) standard curve. Results were presented in µ moles/g DW of mushroom tissues. The present study showed the highest amount of NPTs in *V. volvacea* (2.360 ± 0.016 µ moles/g DW) followed by *P. florida* (1.733 ± 0.067 µ moles/g DW), *F. velutipes* (1.220 ± 0.045 µ moles/g DW) and *C. indica* (1.061 ± 0.029 µ moles/g DW). (Table 4).

Living organisms have glutathione as a major, non-protein thiol that plays a key role in endogenous antioxidant defense mechanisms and it maintains the

Table 4. Non-protein thiol content, Super oxide dismutase and Ascorbate peroxidase activity of four cultivated mushrooms

	C. indica	*F. velutipes*	*P. florida*	*V. volvacea*
Non-protein thiol contents (µ moles/g DW)	1.061±0.029	1.220±0.045	1.733±0.067	2.360±0.016
Superoxide dismutase (SOD) (U activity /mg Protein)	10.81±0.60	15.43±0.65	15.04±0.25	8.95±0.27
Ascorbate peroxidase activity (µ moles/mg protein min^{-1})	7.07±0.58	0.677±0.14	1.23±0.3	2.11±0.11

normal structure and metabolism of cells, probably through its antioxidant and detoxification activities (Gueeri, 1995). The reduced glutathione GSH/ oxidized glutathione GSSG ratio inside the cell is a noteworthy indicator of oxidative stress of an organism (Hwang *et al*., 1992). Kalaras *et al*. (2017) studied thirteen mushroom species in USA and found that the glutathione level ranged from 0.35 µ moles/g DW to 7.84 µ moles/g DW, which agreed with the present results. Selvi *et al*. (2007) reported GSH as 0.156 ± 0.025 n moles/g (fresh samples) in *C. indica* and 0.176 ± 0.025 n moles/g (fresh samples) in *P. florida*. Sudha *et al*. (2008) evaluated total glutathione (125.70 - 395.25 ìg /g DW) in *V. volvacea* raised on substrates added with different organic and inorganic supplements. Datta *et al*. (2019) reported 15.50±2.05 µM GSH in *C. indica* grown on paddy straw Substrate.

Superoxide dismutase (SOD) activity

SODs are the class of enzymes that catalyze the conversion of superoxide anions into H_2O_2 and O_2 (Zelko *et al*., 2002). The results of present study (Table 4) showed that the superoxide dismutase activity was highest in *F. velutipes* (15.43 ± 0.65 U/ mg protein) followed by *P. florida* (15.04 ± 0.25 U/ mg protein). In similar studies, Turfan *et al*. (2020) measured the SOD activity of 15 wild and cultivated mushrooms species from Turkey and found the SOD activity in the range of 19.12 ±0.06 - 58.23±0.12 U / mg protein. Cheng *et al*. (2012) reported the Superoxide dismutase activity of seventeen edible mushroom from China and found that *A. bisporus*, *V. volvacea*, *P. ostreatus* had high and *F velutipes* had low SOD activity. Dama *et al*. (2010) found that the SOD activity increases during low temperature storage. Ramkumar *et al*. (2012) reported a very high SOD activity of *V. volvacea* (23.92±0.07-29.21±0.04 U/mg protein) than the present study. Khatun *et al*. (2015) reported 347.5 ± 0.06 units $g^{"1}$ DW Superoxide dismutase (SOD) activity in *P. florida*. Kaushal *et al*. (2018) reported 22.12 - 26.70 U/mg activity with 400µl crude enzyme extract of *P. florida*. Datta *et al*. (2019) reported 21.58 ±2.21 U/mg protein activity of SOD in *C. indica* grown on paddy straw Substrate. Mao *et al*. (2024) observed 0.-0.97 U/g FW SOD activity in *Volvariella volvacea*.

Ascorbate peroxidase (APX) activity

The APX is a member of the group of haeme-containing peroxidases that are found in higher plants, green and red algae and in members of Protista. In the present study the APX activity was measured the highest in *C. indica* followed by *V. volvacea*, *P. florida* and *F. velutipes* (Table 4). Ascorbate peroxidase is a key antioxidant enzyme in plants that removes excessive H_2O_2 in the Ascorbic acid–glutathione (AsA–GSH) pathway in plant cells under normal and stress conditions (Wang *et al*., 2012). Turfan *et al*. (2020) measured the APX activity of 15 wild and cultivated mushroom species from Turkey and found the APX activity in the range of 0.201 - 2.118 U mg^{-1} protein min^{-1}, which was in agreement with the present study. Sahu *et al*. (2018) recorded APX activity in White Button Mushroom (10.07 ± 2.17 (m mol min^{-1} g^{-1} FW) and Oyster mushroom (9.09 ± 2.11 (m mol min^{-1} g^{-1} FW). Sagar and Thakur (2018) reported ascorbate peroxidase activity of *Lactarius deliciosus* (4.64 µmoles/mg protein min^{-1})

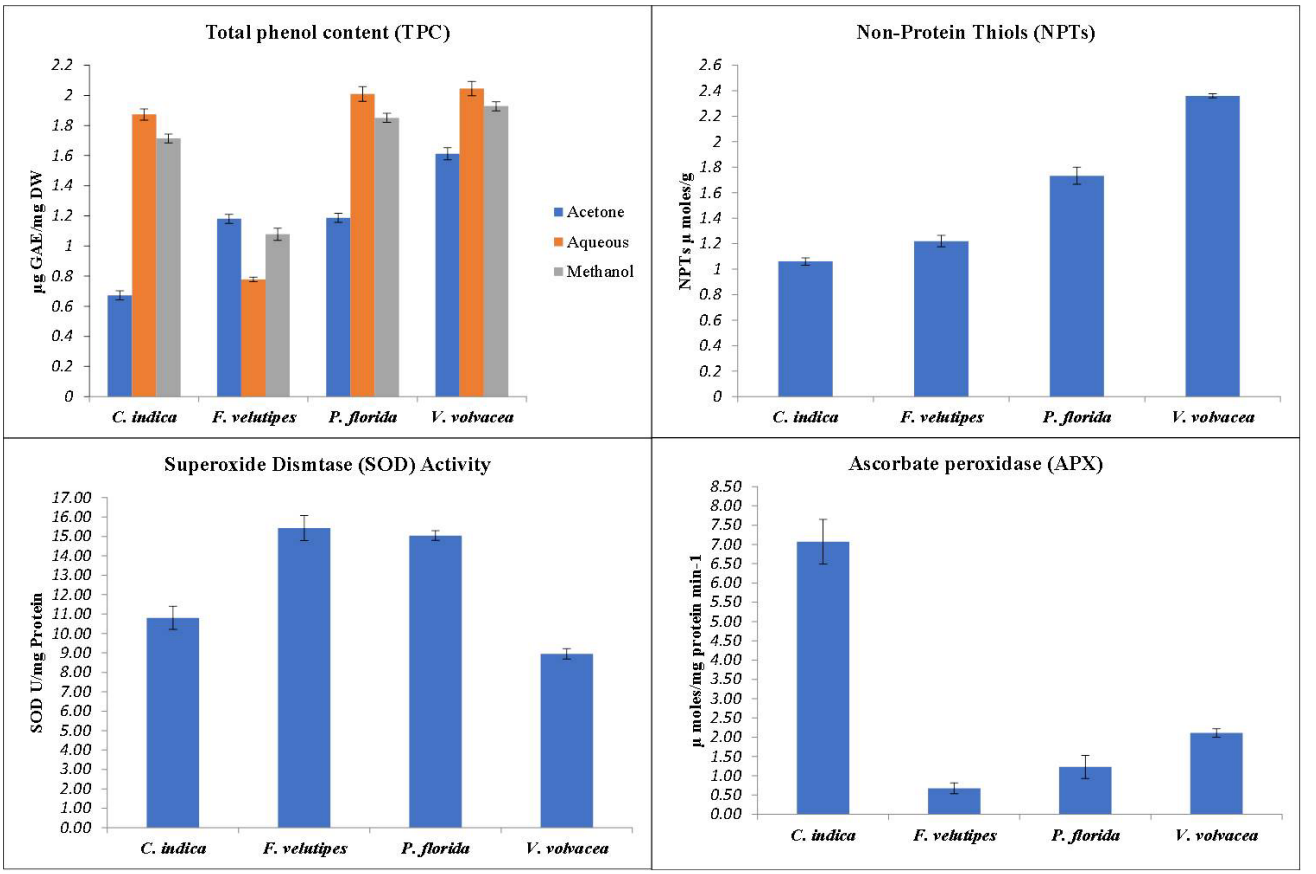

Fig. 3. TPC content, NPTs, SOD and APX activity of four cultivated mushrooms

and *L. salmonicolor* (0.76 µmoles/mg protein min-1) from Indian Himalayas. Datta *et al*. (2019) reported 3.56 ± 0.60 U/mg protein activity of Ascorbate oxidase in *C. indica* grown on paddy straw Substrate.

The present study revealed that the four cultivated mushrooms of India have high antioxidant activities in vitro and might be helpful to safeguard our body against oxidative stress and degenerative diseases. Further this study can be extended on the lines of isolation and purification of biomolecules present in these four mushrooms which can be employed as a medicine in the welfare of mankind.

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REFERENCES

1. Abdelshafy, A.M., T. Belwal, Z, Liang, L. Wang, D, Li, Z. Luo, and L. Li. 2022. A comprehen-sive review on phenolic compounds from edible mushrooms: occurrence, biological activity, application and future prospective. *Crit Rev Food Sci Nutr* **62(22):** 6204–6224.

2. Alam, N., M.M. Sikder, M.A. Karim, and S.M.R, Amin. 2019. Antioxidant and antityrosinase activities of milky white mushroom. *Bangladesh J Bot* **48(4):** 1065–1073.

3. Ali, R., M. Rava, H. Boro, and S. Das. 2017. Studies on growth, anti-oxidant properties,

minerals, macro and micro-morphological characters of *Volvariella volacea* collected from Kokrajhar, (Assam), India. *Mush Res* **26(2):** 165-174.

4. Banerjee, D.K., A.K. Das, R. Banerjee, M. Pateiro, P.K. Nanda, Y.P. Gadekar, S. Biswas, D.J. McClements, and J.M. Lorenzo. 2020. Application of Enoki mushroom (*Flammulina velutipes*) stem wastes as functional ingredients in Goat Meat Nuggets. *Foods* **9:** 432 www.mdpi.com/journal/foods.

5. Baosong, C., W. Sixian, L. Gaoqiang, B. Li, H. Ying, Z. Ruilin and L. Hongwei. 2020. Anti-inflammatory diterpenes and steroids from peels of the cultivated edible mushroom *Wolfiporia cocos*. *Phytochem Lett* **36:** 11–16. https://doi.org/10.1016/j.phytol.2020.01.005

6. Barros, L., S. Falcao, P. Baptista, C. Freire, M. Vilas-Boas, and I.C.F.R. Ferreira. 2008. Antioxidant activity of *Agaricus* sp. Mushrooms by chemical, biochemical and electrochemical assays. *Food Chem* **111:** 61–66.

7. Beauchamp, C. and I. Fridovich. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analyt Biochem* **44:** 276–287.

8. Caverzan, A., G. Passaia, S.B. Rosa, C.W. Ribeiro, F. Lazzarotto, and M. Margis-Pinheiro. 2012. Plant responses to stresses: Role of ascorbate peroxidase in the antioxidant protection. *Genet Mol Biol* **35(4):** 1011–1019.

9. Chanate, W., B. Narumon, P. Sirirat, C. Jaruwan, and W. Piyada. 2024. Comparative evaluation of chemical compositions and antioxidant activity in fruiting bodies and mycelia of *Volvariella volvacea* and *Pleurotus sajor-caju* extracts. *Int J Pharma Quality Assurance* **15(3):** 1499–1509.

10. Cheng, G.Y., J. Liu, M.X. Tao, C.M. Lu, and G.R. Wua. 2012. Activity, thermostability and isozymes of superoxide dismutase in 17 edible Mushrooms. *J Food Compos and Analysis* **26:** 136–143.

11. Cheung, L.M., P.C.K. Cheung, and V.E.C. Ooi. 2003. Antioxidant activity and total phenolics of edible mushroom extract. *Food Chem* **81:** 248–255.

12. Dama, C.L., S. Kumar, B.K. Mishra, K.B. Shukla, S. Mathur, and A. Doshi. 2010. Antioxidative enzymatic profile of mushrooms stored at low Temperature. *J Food Sci Technol* **47(6):** 650–655.

13. Datta, G.B. and A.K. Mitra. 2019. Effect of different agricultural waste substrates on the biological efficiency and therapeutic value of *Calocybe indica* (P and C), *Int J Pharma Biol Sci-IJPBSTM* **9(2):** 1157–1168.

14. Food and Agriculture Organization. 2023. FAOSTAT Statistics database 2023. Food and Agriculture Organization of the United Nations.

15. Gebru, H., G. Faye, and T. Belete. 2024. Antioxidant capacity of *Pleurotus ostreatus* (Jacq.) P. Kumm influenced by growth substrates. *AMB Expr* **14:** 73.

16. Goldstein, J.L. and T. Swain. 1963. Changes in tannins in ripening fruits. *Phytochem* **2:** 371–383.

17. Govindan, S., E.E.R. Johnson, J. Christopher, J. Shanmugam, V. Thirumalairaj, and J. Gopalan. 2016. Antioxidant and anti-aging activities of polysaccharides from *Calocybe indica* var. APK2. *Exp Toxicol Pathol* **68(6):** 329–34.

18. Gueeri, H. 1995. Influence on prolonged ethanol intake on the level and turnover of alcohol and aldehyde dehydrogenase and glutathione. *Adv Exp Med Biol* **23:** 133–134.

19. Hwang, C.J.S.A., A.J. Sinsky, and H.F. Lodish. 1992.Oxidized redox state of glutathione in endoplasmic reticulum. *Science* **257:** 1496–1502.

20. Jagadish, L.K., V.V. Krishnan, R. Shenbhagaraman, and V. Kaviyarasan. 2009.Comparative study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* (J. E. Lange) Imbach before and after boiling. *Afr J Biotechnol* **8(4):** 654–661.

21. Kalaras, M.D., J.P. Richie, A. Calcagnotto, and R.B. Beelman. 2017. Mushrooms: A rich source of the antioxidants ergothioneine and glutathione. *Food Chem* **233:** 429–433.

22. Kaushal, V., V. Thakur, M. Kumar, and A.K. Bhatt. 2018. Anti-oxidative stress enzyme from Pleurotus *sp. Adv Biotech & Micro* **10(5):** 555796.

23. Khatun, S., A. Islam, U. Cakilcioglu, P. Gulerd, and N.C. Chatterjee. 2015. Nutritional qualities and antioxidant activity of three edible oyster mushrooms (*Pleurotus* spp.) *NJAS - Wageningen J Life Sci* **72-73:** 1–5.

24. Kozarski, M., A. Klaus, D. Jakovljevic, N. Todorovic, J. Vunduk, P. Petroviæ, *et al*. 2015. Antioxidants of edible mushrooms: A review. *Molecules* **20:** 19489–19525.

25. Krishna, K. V., J.M. Murugan, H. Khan, M. Kumar, V. Veeramanikandan, A.A. Hatamleh, M.A. Al-Dosary, K. Venkatachalam, and P. Balaji. 2023. Exploring the therapeutic potential of edible *Pleurotus* mushroom species for oxidative stress and diabetes management, *Journal of King Saud University – Science* **35(9):** 102926.

26. Krsmanoviæ, N., M. Rašeta, J. Miškoviæ, K. Bekvalac, M. Bogavac, M. Karaman, and O.S. Isikhuemhen. 2023. Effects of UV stress in promoting antioxidant activities in fungal species *Òrametes versicolor* (L.) Lloyd and *Flammulina velutipes* (Curtis) Singer. *Antioxidants* **12:** 302.

27. Kuciel-Lewandowska, J., M. Kasperczak, B. Bogut, R. Heider, W.T. Laber, W. Laber, and M. Paprocka-Borowicz. 2020.The impact of health resort treatment on the nonenzymatic endogenous antioxidant system. *Oxid Med Cell Longev* **2020:** 8423105

28. Kumar, K. 2015. Role of edible mushrooms as functional foods-a review. *South Asian J Food Technol Environ* **1:** 211–218.

29. Kumar, S. and Priyanka. 2022. Studies on Antibacterial and Antioxidant Activities of *Agaricus bisporus* (J.E. Lange) Imbach. *Int J Food Nutr Sci* **11(1):** 3241–3251.

30. Kumar, S. and A. Sagar. 2023a. Nutritional analysis of cultivated mushrooms of India. *Indian J Agri Sci* **93(4):** 452–455.

31. Kumar, S. and A. Sagar. 2023b. Micronutrient status of cultivated mushrooms of India. *Mushroom Research* **32(2):** 123–131.

32. Ma, G., W. Yang, L. Zhao, F. Pei, D. Fang, and Q. Hu. 2018. A critical review on the health promoting effects of mushrooms nutraceuticals. *Food Sci Hum Well* **7(2):** 125–133.

33. Maji, P., S. Basu, B.K. Banik, and J. Ganguly. 2018. Antioxidant edible mushrooms: a green and rapid electrochemical study with the aqueous extracts. *Mod Chem Appl* **6:** 247.

34. Menaga, D., S. Rajakumar, and P.M. Ayyasamy. 2013. Free radical scavenging activity of methanolic extract of *Pleurotus florida* mushroom. *Int J Pharma Sci* **5:** 601–606.

35. Mao, M., L. Ma, N. Jiang, J. Lin, S. Qu, H. Li, P. Xu, D. Liu, and L. Hou. 2024. Effects of ^{60}Coy-irradiation combined with sodium dehydrogenate on post-harvest preservation and physiological indices of *Volvariella volvacea*. *Peer J* **12:** e18177.

36. Masitah, K.P. Candra, M.A. Masruhim, and P. Kusumaningtyas. 2023. Mycochemicals and proximate composition of two different stages in fruiting body development of *volvariella*

volvaceae growing naturally on oil palm empty fruit bunches. *J Sustain Sci Manag* **18(8):** 24–36.

37. Meir, S. J. Kanner, B. Akiri, and S.P. Hadas. 1995. Determination and involvement of aqueous reducing compounds in oxidative defence systems of various senescing leaves. *J Agric Food Chem* **43:** 1813–1819.

38. Mishra, K.K., R.S. Pal, and K.R. Arun. 2014. Antioxidant activities and bioactive compound determination from caps and stipes of specialty medicinal mushrooms *Calocybe indica* and *Pleurotus sajor-caju* (Higher Basidiomycetes) from India. *Int J Med Mush* **16(6):** 555–567.

39. Naik, G.H., K.I. Priyadarsini, J.G. Satav, M.M. Banavalikar, P.P. Sohoni, and M.K. Biyani. 2003. Comparative antioxidant activity of herbal components used in Ayurvedic medicine. *J Phytochem* **63:** 97–104.

40. Nakano, Y. and K. Asada. 1981. Hydrogen peroxide is scavenged by ascorbate-spesific peroxidase in spinach chloroplasts. *Plant Cell Physiol* **22(5):** 867–880.

41. Nguyen, Q., S.L. Lam, M. Nguyen, C. Nguyen, X.A. V. Ho, V. Nguyen, T. Tran, T. Lê, T. Tran, and H. le trung. 2022. In vitro antioxidant activity and bioactive compounds from *Calocybe indica*. *Hue University J Sci: Nat Sci* **130:** 10.26459/hueunijns.v130i1D.6348.

42. Nitha, B., De, S., S. K. Adhikari, T.P.A. Devasagayam, and K.K. Janardhanan. 2010. Evaluation of free radical scaveng-ing activity of morel mushroom, *Morchella esculenta* mycelia: a potential source of therapeutically useful antioxid. *Pharm Biol* **48(4):** 453–460.

43. Noctor, G. and C.H. Foyer. 1998. Ascorbate and glutathione: keeping active oxygen under control. *Ann Rev Plant Physiol and Plant Mol Biol* **49:** 249–279.

44. Oyaizu, M. 1986. Studies on products of browning reaction: antioxidative activities of products of browining reaction prepared from glucosamine. *Japanese J Nutri* **44:** 307–315.

45. Patil, N.K., S. Thakur, A. Bains, N. Ali, R. Arora, M.K. Parvez, G. Goksen, S. Janghu, and P. Chawla. 2024. Exploration of *Calocybe indica* mushroom phenolic acid-kidney bean protein complex: Functional properties, amino acid profiles, *in-vitro* digestibility, and application in vegan product development. *Food Chemistry* **460(1):** 140401.

46. Pham-Huy, L.A., H. He, and C. Pham-Huy. 2008. Free radicals, antioxidants in disease and health. *Int J Biomed Sci* **4(2):** 89–96.

47. Prabhu, M. and R. Kumuthakalavalli. 2014. Nutritional and phytochemical studies on *Pleurotus florida* (Mont.) Singer and *Calocybe indica* P&C. *World J Pharma Res* **3(3):** 4907–4913.

48. Prabhu, M. and R. Kumuthakalavalli. 2016. Antioxidant activity of oyster mushroom (*Pleurotus florida* [Mont.] Singer) and milky mushroom (*Calocybe indica* P&C). *Int J Curr Pharma Res* **8(3):** 48–51.

49. Punitha, S.C. and M. Rajasekaran. 2014. Free radical scavenging activity of fruiting body extracts of an edible mushroom, *Volvariella volvacea* (Bull Ex Fr) Singer. *Asian Biomed and Pharm Sci* **4(4):** 6–11.

50. Puttaraju, N.G., S.U. Venkateshaiah, S.M. Dharmesh, S.M.N. Urs, and R. Somasundaram. 2006. Antioxidant activity of indigenous edible mushrooms. *J Agric Food Chem* **54(26):** 9764–9772.

51. Rai, S.N., D. Mishra, P. Singh, E. Vamanu, and M.P. Singh. 2021. Therapeutic applica-tions of mushrooms and their biomolecules along with a glimpse of in silico approach in neurodegenerative diseases. *Biomed Pharmacotherapy* **137:** 111377.

52. Ramkumar, L. T. Ramanathan, and J. Johnprabagaran. 2012. Evaluation of nutrients,

trace metals and antioxidant activity in *Volvariella volvacea* (Bull. Ex. Fr.) Sing. *Emir J Food Agric* **24 (2):** 113–119.

53. Rauf, A., P.B. Joshi, Z. Ahmad, H.A. Hemeg, A. Olatunde, S. Naz, N. Hafeez, and J. Simal-Gandara. 2023. Edible mushrooms as potential functional foods in amelioration of hypertension. *Phytother Res* **37(6):** 2644–2660.

54. Ruthes, A.C., F.R. Smiderle, and M. Iacomini. 2016. Mushroom heteropolysaccharides: a review on their, structure and biological effects. *Carbohydr. Polym* **136:** 358–375.

55. Ryu, H.S., S.H. Kim, M.H. Jeon, and H.Y. Choi. 2018.Antioxidant and anticancer effects of water extract from *Pleurotus eryngii*, *Flammulina velutipes. Korean J Food Nutr* **31(6):** 911–918.

56. Sagar, A., and K. Thakur. 2018. Studies on antioxidant potential and nutritional component of two medicinal mushrooms from thr forests of N.W. Himalayas. *Mush Res* **27(2):** 161–170.

57. Sahu, R., P. Katre, and B. Sahu. 2018. Comparison of Bioactive Compounds and Enzymatic Antioxidant found in White Button Mushroom and Oyster Mushroom. *Int J Curr Microbiol App Sci Sp Issue* **6:** 2062–2065.

58. Sakamoto, T., A. Nishida, N. Wada, Y. Nakamura, S. Sato, T. Konishi, and S. Matsugo. 2020. Identification of a novel pyrrole alkaloid from the edible mushroom basidiomycetes-X (Echigoshirayukidake). *Molecules* **25(21):** 4879.

59. Selvi, S., P.U. Devi, S. Suja, S. Murugan, and P. Chinnaswamy. 2007. Comparison of Non-Enzymic Antioxidant Status of Fresh and Dried Form of *Pleurotus florida* and *Calocybe indica. Pakistan J Nutri* **6(5):** 468–471.

60. Shah, S.R., C.I. Ukaegbu, H.A. Hamid, and O.R. Alara. 2018. Evaluation of antioxidant and antibacterial activities of the stems of *Flammulina velutipes* and *Hypsizygus tessellates* (white and brown var.) extracted with different solvents. *J Food Measur Charact* **12:** 1947–1961.

61. Shimada, K., K. Fujikawa, K. Yahara, and T. Nakamura. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J Agric Food Chem* **40:** 945–948.

62. S³awiñska, A., K. Radzki, and J. Kalbarczyk. 2013. Antioxidant activities and polyphenolics content of *Flammulina velutipes* mushroom extracts. *Herba Polonica* **59(3):** 27–36.

63. Sudha, A., P. Lakshmanan, and B. Kalaiselvan. 2008. Antioxidant properties of paddy straw mushroom (*Volvariella volvacea* (Bull. ex Fr.)) Sing. Extraction yield, *Int J Applied Agri Res* **3(1):** 9–16.

64. Sun, M., L. Ni, Y. Huang, M. Yang, G. Cheng, M. Zhang, M. Wu, and C. Ma. 2023. Effects of different drying treatments on the microstructure, free amino acids, volatile compounds and antioxidant activity of *Flammulina velutipes* root, *Food Chemistry* **18:** 100656.

65. Thillaimaharani, K.A., K. Sharmila, P. Thangaraju, M. Karthick, and M. Kalaiselvam. 2013. Studies on antimicrobial and antioxidant properties of oyster mushroom, *Pleurotus florida*. *Int J Pharma Sci Res* **4(4):** 1540–1545.

66. Turfan, N., S. Ayan, A. Pekºen, and S.S. Akýn. 2020. Antioxidant enzyme activities of some wild and cultivated edible mushrooms in Turkey. *Int J Agric Wild Sci (IJAWS)* **6(2):** 202–209.

67. Velioglu, Y.S., G. Mazza, L. Gao, and B.D. Oomah. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J Agaric food Chem* **46:** 4113–4117.

68. Wang, L.Y., Q.Y. Zhang, F. Wang, X. Meng, and Q.W. Meng. 2012. Ascorbate plays a key role in alleviating low temperature-induced oxidative stress in *Arabidopsis*. *Photosynthetica* **50(4):** 602–612.

69. Yeh, M.Y., W.C. Ko, and L.Y. Lin. 2014. Hypolipidemic and antioxidant activity of enoki mushrooms (*Flammulina velutipes*). *BioMed Res Int* **2014:** 352385.

70. Zelko, I.N., T.J. Mariani, and R.J. Folz. 2002. Superoxide dismutase multigene family a comparison of CuZn-SOD (SOD1), Mn-SOD (SOD2) and EC-SOD (SOD3) gene structures, evolution and expression. *Free Rad Biol Med* **33:** 337–349.

71. Zhang, Z., Q. Jin, G. Lv, L. Fan, H. Pan, and L. Fan. 2013. Comparative study on antioxidant activity of four varieties of *Flammulina velutipes* with different colour. *Int J Food Sci Technol* **48:** 1057–1064.