Antioxidant properties and electrochemical behavior of cultivated commercial Indian edible mushrooms

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Abstract Methanolic extracts of cap and stipe of commercially obtained mushrooms *Agaricus bisporus*, *Hypsizygus ulmarius*, and *Calocybe indica* were analyzed for their antioxidant activity in different chemical systems including reducing power, free radical scavenging, ferric reducing antioxidant power (FRAP), superoxide scavenging, peroxide scavenging, metal chelating activities and electrochemical behavior. Scavenging effects on 2,2-diphenyl-1-picrylhydrazyl radicals were moderate (43.5–59.0%) at 1.5 mg/ml. Chelating effects on ferrous ions were moderate to excellent (40.6–96.1%) at 20 mg/ml. At 12 mg/ml, the reducing powers were excellent (2.54–1.71). FRAP results were in the range 2.15–0.98 at 16 mg/ml. The ability to scavenge H_2O_2 was moderate to excellent (48.9–97.7%) at 1.5 mg/ml. At 10 mg/ml, *Agaricus bisporus* cap proved to be better at scavenging superoxide radicals than others. Similar electrochemical responses of all extracts suggested similar electroactive chemical composition. The total phenols in the extracts ranged from 14.73–26.72 mg/g. The total flavonoid content ranged from 1.12–2.17 μ g/g.

Keywords *Calocybe indica* · *Hypsizygus ulmarius* · *Agaricus bisporus* · Antioxidant activity · Total phenol content · Total flavonoid content

Introduction

Oxidation is an important process through which energy is produced in biological systems. However, there are many

reactive oxygen species and free radicals that are associated or formed as a result of the oxidation process. These reactive species often cause cell death and are involved in other degenerative processes associated with ageing. Reactive oxygen species (ROS) along with free radicals are also found to play a role in functional changes associated with diseases like cancer, rheumatoid arthritis, cirrhosis etc. Cells are equipped with enzymes like superoxide dismutase, catalase and also chemicals like vitamin E, vitamin C, polyphenols, carotinoids and glutathione (Niki et al. 1994). Antioxidant containing natural foods can however be used to reduce the oxidative damage.

Mushrooms have long been used as food because of their unique taste and subtle flavor. Experimental evidence indicates that mushrooms contain many biologically active components that offer health benefits and protection against degenerative diseases (Barros et al. 2008). Mushrooms are rich sources for compounds like lectins, terpenoids, beta-glucans, ascorbic acid, tocopherols, carboxylic acids and various dietary fibers (Parslew et al. 1999; Mau et al. 2001; Wasser and Weis 1999; Wasser 2002). *Agaricus bisporus* (button mushroom) is the most widely cultivated and consumed mushroom species in India (Venkateshwarlu et al. 1999). *Calocybe indica* (milky mushroom) is a well recognized tropical edible mushroom and promising for cultivation in India (Purkayastha and Chandra 1976). *Hypsizygus ulmarius* (elm oyster mushroom) is a high yielding mushroom for which commercial cultivation technology has been released and is gaining popularity.

Antioxidant activities of the methanolic extract of *Agaricus bisporus* have been studied earlier (Mahfuz et al. 2007). There has been no report on the *in vitro* antioxidant studies done on *Calocybe indica* and *Hypsizygus ulmarius*. Hence, it was considered useful to evaluate the antioxidant

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activities of these commonly consumed mushrooms, using various chemical and electrochemical methods using standard equivalent references.

Materials and methods

Mushroom samples

Three commercially cultivated mushroom species in India, namely, button mushroom (*Agaricus bisporus*), elm oyster mushroom (*Hypsizygus ulmarius*) and milky mushroom (*Calocybe indica*) were analysed for their antioxidant studies. Fresh materials of the three species were obtained from the local market in Bangalore city, Karnataka state, South India. The taxonomical identification was done at Mushroom Lab, Indian Institute of Horticultural Research, Bangalore (Karnataka state, India). Voucher specimens of the three species were deposited at the herbarium of Department of Biosciences, Sri Sathya Sai Institute of Higher Learning.

Sample preparation

The caps and stipes of the fresh mushrooms were separated and dried in oven at 38 °C for 48 h. The dried material was ground into a coarse powder using mortar and pestle. Dried powders, one hundred grams each, were defatted by refluxing with petroleum ether (60–80 °C) for 6 h. The defatted material was then dried and extracted with 95% ethanol by refluxing for 6 h. The extracts were filtered through Watman No. 4 filter paper. The residue was extracted with additional two portions of ethanol for complete extraction. The combined ethanolic extracts were evaporated to dryness at 40 °C on a rotary evaporator and stored at 4 °C till further analysis. The dried extracts were redissolved in methanol to a concentration of 20 mg/ml and used for analysis.

Reagents and chemicals

L-Ascorbic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX), BHT butylatedhydroxytoluene (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, quercetin, nitroblue tetrazolium salt (NBT), Horseradish peroxidase (HRP) type II, ferrozine, methionine, riboflavin, ethylenediaminetetraacetic acid (EDTA) and homovanillic acid (HVA) were all purchased from Sigma-Aldrich Chemicals (India) Ltd. All other chemicals used are of analytical grade. HPLC grade used was purchased from Merck India. Water was purified using Millipore Direct Q₃ and used for preparation of reagents.

Chemical assays

DPPH radical scavenging activity

Various concentrations of the methanolic extracts of mushrooms (0.25–1.5 mg/ml, 2.5 ml) were mixed with methanolic solution containing DPPH radicals (0.1 mM, 0.5 ml). The mixture was shaken vigorously and left to stand in dark for 30 min. The reduction in the DPPH radical concentration was determined by measuring the absorbance at 517 nm (Hitachi spectrophotometer, U-2001). Methanol was taken as blank and DPPH solution without the extracts was taken as control (Aquino et al. 2001). The percentage of DPPH scavenged was calculated using the equation: % Scavenged = $[(A_C - A_S) / A_C] \times 100$, where A_C is the absorbance of control, and A_S is the absorbance of solution containing sample extracts. TROLOX was used as standard.

Ferrous ion chelating activity

The chelating of ferrous ions by the mushroom extracts was estimated by the method of Tuba et al. (Ak and Gulcin 2008). The Fe²⁺ chelating ability was monitored by measuring the absorbance of the ferrous iron–ferrozine complex at 562 nm. Briefly, methanolic mushroom extracts (2–20 mg/ml, 0.4 ml) were added to a solution of 2 mM FeCl₂ (0.2 ml). The reaction was initiated by adding 5 mM ferrozine (0.4 ml). The total volume was adjusted to 4 ml with methanol. The mixture was shaken vigorously and left at room temperature for 10 min. The absorbance of the solutions was measured spectrophotometrically at 562 nm (Hitachi U-2001). The percentage of chelation was calculated by using the equation: % Chelation = $[(A_C - A_S) / A_C] \times 100$, where A_C is the absorbance of control, and A_S is the absorbance of solution containing sample extracts. Control contains only FeCl₂ and ferrozine. Ascorbic acid was used as standard.

Reducing power

The reducing power was measured by the method of Oyaizu (1986). Methanolic extracts of mushroom (2–12 mg/ml, 2.5 ml) were mixed with sodium phosphate buffer (0.2 M, pH 6.5, 2.5 ml) and potassium ferricyanide [K₃Fe(CN)₆] (1%, 2.5 ml). The mixture was then incubated at 50 °C for 20 min. Trichloroacetic acid (10% w/v, 2.5 ml) was then added and the mixture was centrifuged at 3000 rpm for 10 min (REMI R25). To the supernatant layer (2.5 ml), 2.5 ml of deionised water and ferric chloride (0.1%, 0.5 ml) were added, and the absorbance was measured at 700 nm (Hitachi U-2001). Higher absorbance indicates better reducing power. TROLOX was used as standard.

Ferric reducing antioxidant power (FRAP)

In FRAP method, the complex formed when ferric tripyridyl triazene (Fe^{3+} TPTZ) complex was reduced to the ferrous (Fe^{2+}) ion is determined using UV-Vis Spectrophotometer (Hitachi U-2001). The oxidant in the FRAP assay was prepared by mixing TPTZ (10 mM in 40 mM HCl, 2.5 ml), acetate buffer (0.3 M pH 3.6, 25 ml), and 2.5 ml of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (20 mM). To 3600 μl of freshly prepared FRAP reagent, 360 μl of water and 120 μl of mushroom extracts (2–20 mg/ml) were added. The mixture was then incubated at 37 °C for 30 min. The absorbance was measured spectrophotometrically at 595 nm. Higher absorbance indicates better ferric reducing ability of the extracts. BHT was used as standard (Benzie and Strain 1996; Pulido et al. 2000).

H_2O_2 scavenging activity

This assay is based upon the spectrofluorometric determination of hydrogen peroxide scavenging activity (Pazdzioch-Czochra and Widenska 2002). Briefly, to 0.5 ml of phosphate buffer (25 mM, pH 7.5), H_2O_2 (1 mM, 0.2 ml) and 0.1 ml of the mushroom extracts (1–6 mg/ml) were added. The mixture was vortexed and incubated for 5 min at 20 °C. After incubation, HVA (1.25 mM, 0.1 ml) and Horseradish peroxidase (10 U, 0.1 ml) were added, mixed and incubated for another 5 min at 20 °C. The fluorescence intensity of the dimer formed was measured at an excitation wavelength of 315 nm and emission wavelength of 425 nm using luminescence spectrophotometer (Perkin Elmer, LS55). In order to nullify the fluorescence interference due to extracts, various concentrations of the extracts in the absence of H_2O_2 were taken as blank. TROLOX (0.2–1.6 mM) was taken as standard.

Superoxide scavenging activity

This assay is based on the capacity of the extracts to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) in the riboflavin–light–NBT system. The method used by Martinez et al. (2001) was followed after modification. Each 3 ml reaction mixture contained sodium phosphate buffer (200 mM, pH 7.8, 0.5 ml), methionine (104 mM, 0.25 ml), riboflavin (8 μM , 0.5 ml), EDTA (100 μM , 0.5 ml), NBT (600 μM , 0.25 ml) and 1 ml test sample solution. The production of blue formazan was followed by monitoring the increase in absorbance at 560 nm after 40 min illumination from a fluorescent lamp. The percentage of superoxide scavenged is calculated using the equation: % Scavenged = $[(A_C - A_S)/A_C] \times 100$, where A_C is the absorbance of control, and A_S is the absorbance of solution containing sample extracts. TROLOX was used as standard.

Determination of total phenolic content and total flavonoid content

Total phenolic content was measured at 20 mg/ml concentration of the extracts. To 0.1 ml of mushroom extracts in 13% HCl/MeOH (60:40, v/v), 2 ml of 2% sodium carbonate was added. The mixture was incubated at room temperature for 3 min. Folin ciocalteu reagent (0.1 ml) was added to the mixture. After 30 min absorbance was measured at 750 nm (Mau et al. 2002). Gallic acid was used as standard. The results were expressed as mg of gallic acid equivalents (GAEs) per gram of mushroom extract.

Total flavonoid content was measured at 20 mg/ml concentration of the extracts. To 1 ml of mushroom extracts, 1 ml of 10% AlCl_3 , potassium acetate (1 M, 0.1 ml) and 3.8 ml of MeOH were added and the mixture was incubated for 40 min at room temperature. Then absorbance was measured at 415 nm (Öztürk et al. 2007). The results were expressed as μg of quercetin equivalents (CEs) per gram of mushroom extract.

Electrochemical behavior

Instrumental setup

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were measured using IVUM COMPACTSTAT potentiostat/galvanostat equipped with a closed standard three electrode cell. A glassy carbon (BAS, $\text{Ø}=0.3$ cm) electrode was used as working electrode. Pt foil was used as counter electrode and all the potentials measured refer to Ag/AgCl 3 M HCl reference electrode. Prior to the analysis, the working electrode was polished in an aqueous suspension of 0.3 μm alumina on a polishing pad. It was then rinsed with deionised water. After every analysis the electrode was sonicated in 6 M HCl and then in methanol for 2 min. (Barros et al. 2008).

Procedure

All the extracts were studied in methanol/acetate buffer 0.1 M (pH 4)/ NaClO_4 (70:28:2) solution. The concentration of the mushroom extracts was set between 1 and 12 mg/ml. Gallic acid (0.02–0.2 mg/ml) was used as standard for calibration. All the solutions (10 ml) were prepared freshly and analysed immediately. All the electrochemical responses were measured immediately after the working electrode is immersed. This is done to minimize the adsorption of the species onto the surface of the electrode. The antioxidative power of the extracts was evaluated by using DPV with the operating conditions set to 60 mV pulse amplitude and 0.030 Vs^{-1} as scan rate. For each extract, current density (E/V) was plotted as a function of

Fig. 1 Effects of the methanolic extracts of stipe and cap from edible mushrooms on (i) scavenging 2, 2-diphenyl-1-picrylhydrazyl radicals, (ii) chelating with ferrous ions. Each value is expressed as mean $\pm$ standard deviation ($n=3$)

mushroom extract concentration and compared with that of gallic acid which was used as standard.

Statistical analysis

For the methanolic extracts of mushrooms, three samples were analysed and all assays were carried out in triplicate. The experimental results were analysed using one-way ANOVA (analysis of variance) followed by student's *t*-test to determine the least significant difference at $\alpha=0.05$. This analysis was done using Microcal Origin ver. 6.0 software.

Discussions and results

DPPH radical scavenging activity

The methanolic extracts of the three commercially cultivated mushrooms showed increasing scavenging effect with increased concentration. The activity was moderate (43.5–59.0%) even at low concentration of 1.5 mg/ml (Fig. 1). However the scavenging effect of

TROLOX at 6 $\mu\text{g/ml}$ was 65.1%. The methanolic extracts showed excellent scavenging activity at 1.5 mg/ml compared to several commercial mushrooms reported by Yang et al. (2002). Of the three species studied, *Hypsizygus ulmarius* cap exhibited maximum scavenging activity (59.0%) followed by *Agaricus bisporus* cap (58.2%). Least activity was exhibited by *Calocybe indica* cap (43.5%). Interesting observation was that the stipe of *Calocybe indica* exhibited more scavenging activity than that of its cap.

Ferrous ions chelating activity

Chelating effects of methanolic extracts from the three commercially cultivated mushrooms on ferrous ions increased with the increased concentrations and were 40.6–96.1% at a concentration of 20 mg/ml (Fig. 1). However the chelating effect of L-ascorbic acid was 87.8% at 0.5 $\mu\text{g/ml}$ concentration. The study of the chelating effects on the ferrous ions is beneficial since ferrous ions are the most active pro-oxidants in the food system (Yamaguchi et al. 1988). Among the methanolic extracts of the three mushrooms, *Agaricus bisporus* cap exhibited excellent chelating ability (96.1%)

Fig. 2 (i) Reducing powers and (ii) Ferric reducing antioxidant power (FRAP) of the methanolic extracts of stipe and cap from edible mushrooms. Each value is expressed as mean $\pm$ standard deviation ($n=3$)

Fig. 3 Scavenging effects of the methanolic extracts of stipe and cap from edible mushrooms on H_2O_2 . Each value is expressed as mean $\pm$ standard deviation ($n=4$)

whereas *Hypsizygus ulmarius* and *Calocybe indica* exhibited moderate chelating ability (44.5–58.5% and 41.9–40.6% respectively). The results showed that *Agaricus bisporus* has good metal chelating ability whereas *Hypsizygus ulmarius* and *Calocybe indica* have moderate metal chelating ability.

Reducing power

Reducing power of the methanolic extracts of the three mushrooms were excellent and increased steadily with the increased concentration (Fig. 2). At 12 mg/ml the reducing powers were 2.54–1.71. However the reducing power of TROLOX at 150 μ g/ml was 2.01. These values were found to be much higher than the reducing powers of the methanolic extracts of the commercial mushrooms reported by Yang et al. (2002). The reducing power of *Agaricus bisporus* is close to the value reported by Barros et al. (2008). The high reducing power exhibited by the extracts might be indicative of the hydrogen donating ability of the active species present in the extracts (Shimada et al. 1992). Results show that *Hypsizygus ulmarius* stipe and cap exhibited excellent reducing power (2.53 and 2.46) followed by *Agaricus bisporus* cap (2.18) and *Calocybe indica* stipe (2.05). *Calocybe indica* cap exhibited least reducing power (1.71). Interesting observation is that the stipe of *Calocybe indica* has more reducing power than that of its cap.

Ferric reducing antioxidant power (FRAP)

All the methanolic extracts showed increased FRAP with the increase in concentration (Fig. 2). At 16 mg/ml

concentration, the FRAP values measured as absorbance at 595 nm were in the range 2.15–0.98. However the FRAP of Trolox at 100 μ g/ml concentration was 0.85. Results showed that *Hypsizygus ulmarius* cap exhibited excellent FRAP compared to other extracts. *Agaricus bisporus* stipes exhibited least FRAP activity.

H_2O_2 scavenging activity

A simple, sensitive spectrofluorimetric method described by Pazdzioch-Czochra and Widenska (2002) was employed to evaluate the peroxide scavenging activity of the extracts of the three mushrooms. All the extracts showed increasing scavenging effect with increased concentration. The activity ranged from 36.8–76.1% at concentration of 2.0 mg/ml (Fig. 3). Of the three species studied, *Agaricus bisporus* cap exhibited excellent scavenging activity (76.1%) followed by *Calocybe indica* cap (63.6%). Least activity was exhibited by *Hypsizygus ulmarius* stipe (36.8%). Results showed that *Agaricus bisporus* cap has excellent H_2O_2 scavenging activity.

Superoxide scavenging activity

The superoxide scavenging activities of the methanolic extracts of the mushrooms at 10 mg/ml concentration were assayed by the non-enzymatic riboflavin–light–NBT system. TROLOX (5–25 μ g/ml) was taken as standard. At 10 mg/ml concentration the superoxide scavenging activity expressed in terms of % scavenging activity, ranged from 87.1–7.6% (Fig. 4). Superoxide

Fig. 4 Scavenging effects of the methanolic extracts of stipe and cap from edible mushrooms on superoxide radicals at 10 mg/ml concentration. Each value is expressed as mean $\pm$ standard deviation ($n=3$)

Table 1 Content of total flavonoids and total phenols of methanolic extracts of stipe and cap from edible mushrooms

Compound (concentration)	<i>H.ulmarius</i> (cap)	<i>H.ulmarius</i> (stipe)	<i>A.bisporus</i> (cap)	<i>A.bisporus</i> (stipe)	<i>C.indica</i> (cap)	<i>C.indica</i> (stipe)
Total flavonoids ($\mu\text{g/g}$)	1.622 $\pm$ 0.006a	1.116 $\pm$ 0.007b	2.173 $\pm$ 0.007c	1.533 $\pm$ 0.005d	1.306 $\pm$ 0.001e	1.844 $\pm$ 0.010f
Total phenols (mg/g)	26.72 $\pm$ 0.05a	22.67 $\pm$ 0.02b	21.17 $\pm$ 0.01c	14.73 $\pm$ 0.08d	18.53 $\pm$ 0.02d	19.80 $\pm$ 0.02d

^a Each value is expressed as mean $\pm$ standard deviation ($n=3$). Means with different letters within a row are significantly different ($P<0.05$).

scavenging activities could not be studied over a range of concentration of extracts because of the interference of reactive species present in the extracts with the superoxide radical. The results showed that *Agaricus bisporus* has significantly stronger superoxide scavenging power compared to *Hypsizygus ulmarius* and *Calocybe indica*.

Antioxidant components

Total phenols form the major antioxidant components found in the methanolic extracts of the commercial mushrooms. Phenols such as tocopherols, BHT and gallate are found in mushrooms and are known to be effective antioxidants (Yang et al. 2002). The total phenol and flavonoid content of all the extracts are summarized in Table 1. The highest content of total phenols in *Hypsizygus ulmarius* cap might account for the better results found in DPPH scavenging ability, FRAP, Reducing power and peroxide scavenging ability. The highest content of total flavonoids in *Agaricus bisporus* cap might account for better results found in ferrous ion chelation and superoxide scavenging activities. The total phenols in the extracts ranged from 14.73–26.72 mg/g. Similarly the total flavonoid content ranged from 1.12–2.17 $\mu\text{g/g}$. Total flavonoid content of *Agaricus bisporus* is similar to the value reported by Barros et al. (2008). However the total phenolic content is much more than the value reported. This may be because of the difference in the extraction procedures employed in preparation of the extracts.

Table 2 Electrochemical results from differential pulse voltammetry of the methanolic extracts of stipe and cap from edible mushrooms

Sample	$E_{1/2}/\text{V}$	Slope/ $\mu\text{Acm}^{-2} \text{mg}^{-1} \text{ml}$
<i>H.ulmarius</i> (cap)	0.89	2.09
<i>H.ulmarius</i> (stipe)	0.89	1.31
<i>A.bisporus</i> (cap)	0.91	1.00
<i>A.bisporus</i> (stipe)	0.89	0.72
<i>C.indica</i> (cap)	0.83	0.62
<i>C.indica</i> (stipe)	0.91	0.71

Evaluation of antioxidant properties by electrochemical techniques

Cyclic voltamograms showed electrochemical behavior of all the mushroom extracts. The oxidation potentials were

Fig. 5 (i) Differential pulse voltammograms of 12 mg/ml methanolic extracts of stipe and cap from edible mushrooms in methanol/acetate buffer 0.1 M (pH 4)/ NaClO_4 (70:28:2) solutions. **a** *H.ulmarius* (cap), **b** *H.ulmarius* (stipe), **c** *A.bisporus* (cap), **d** *A.bisporus* (stipe), **e** *C.indica* (cap), **f** *C.indica* (stipe). (ii) Differential pulse voltammograms of gallic acid in methanol/acetate buffer 0.1 M (pH 4)/ NaClO_4 (70:28:2) solutions at different concentrations (mg/ml). (iii) Variation of the peak current density (E/V) in DPV voltammograms, with methanolic extract concentrations of stipe and cap from edible mushrooms

Table 3 The IC₅₀ values of the methanolic extracts of stipe and cap from edible mushrooms for various antioxidant assays

	IC ₅₀ values (mg/ml)				
	DPPH scavenging activity	Reducing power	Chelation with ferrous ions	FRAP	H ₂ O ₂ Scavenging activity
<i>H.ulmarius</i> (cap)	1.242	4.390	14.493	9.007	2.920
<i>H.ulmarius</i> (stipe)	1.556	4.980	21.161	20.317	2.995
<i>A.bisporus</i> (cap)	1.230	5.470	9.440	11.446	0.908
<i>A.bisporus</i> (stipe)	1.428	7.910	14.817	21.114	2.770
<i>C.indica</i> (cap)	1.648	6.870	21.932	21.448	0.993
<i>C.indica</i> (stipe)	1.229	4.895	24.147	12.308	3.252

more positive than that of standards, around 0.91 V. The results indicate that under the electrochemical conditions used, neither of the standards is present in the mushroom extracts. Similarities in the oxidation potential of the extracts (Table 2) indicate that all the different species of mushrooms under study have similar composition in respect to electroactive species.

Cyclic voltammetry has limited application for solutions containing organic substances as they tend to get adsorbed on to the electrode surface and so cannot give quantitative measurements. Differential pulse voltammetry can be used to overcome this restraint and obtain quantitative information. In this technique the current is measured before and after the potential pulse application (Barros et al. 2008).

Figure 5(i) shows the differential pulse voltamograms for the mushroom extracts. All the extracts except that of *Calocybe indica*, have two peaks. The peak at 0.9 V is common for all the extracts. Figure 5(ii) shows differential pulse voltamograms at several concentrations (0.02–0.20 mg/ml) of gallic acid. An increase in peak current with the increase in concentration was observed leading to a linear relation between the two parameters. The same behavior was found for all the mushroom extracts although the slopes for the plots of peak current density (E/V) vs extract concentration differed (Fig. 5(i & iii) and Table 2).

Although there are many factors like diffusion coefficient of the electroactive species and electron transfer kinetics effecting the peak current density, *j*, direct correlation between the slope of the plot and the total phenolics present in the extracts was observed. This was also reflected in the results from the most of chemical assays studied thereby emphasizing the fact that cyclic voltammetry and differential pulse voltammetry can be used in evaluation of mushroom antioxidant properties (Barros et al. 2008).

Conclusions

In this study we have found that all the three commercially grown mushrooms exhibited moderate to high antioxidant

activities. All the activities increased steadily with increase in the concentration. *Hypsizygus ulmarius* cap has excellent DPPH radical scavenging, peroxide scavenging, FRAP and reducing power abilities. This may be attributed to its highest total phenol content. The excellent ferrous ion chelation and superoxide scavenging abilities exhibited by *Agaricus bisporus* cap may be attributed to its highest flavonoid content. Of all the assays studied, total phenol content of the extracts showed good correlation with chelation with ferrous ions and peroxide scavenging abilities. Total flavonoid content of the extracts showed good correlation with DPPH scavenging, FRAP and reducing power abilities. The IC₅₀ values were calculated from the plots for each assay and are summarised in Table 3. *Calocybe indica* stipe has more activity compared to the cap. It is an important observation as the stipe is generally discarded in Indian palate.

Agaricus bisporus is the major mushroom variety grown in India but the non availability of compost and its low temperature requirement is limiting its cultivation to the colder and hilly regions of India. *Calocybe indica* is an indigenous mushroom and only variety which can grow at temperate conditions (28–32 °C) (Pandey and Tewari 1993). The cultivation of *Calocybe indica* and *Hypsizygus ulmarius* is gaining importance in South India because of the availability of substrate (paddy straw) in large quantities and also because of the climate favoring the requirement. Unfortunately, there are no statistics available regarding the production of these mushrooms in India. The results indicate that *Hypsizygus ulmarius* is a promising mushroom with excellent antioxidant potential and *Calocybe indica* has moderate antioxidant activity. The chemical characteristics of the antioxidative components in these extracts will have to be further investigated.

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