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Antioxidant Activity of Indigenous Edible Mushrooms

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The current study was undertaken to measure the antioxidant potential from water and methanolic extracts of fruiting bodies of 23 species of mushrooms naturally grown in different geographic locations of India. The antioxidant ability of each species was analyzed for the total antioxidative status, employing multimechanistic antioxidative assays such as inhibition of lipid peroxidation, determination of reducing power, and free radical scavenging ability, in addition to determination of total phenolics and identification of phenolic acids by HPLC analysis, because the phenolics are known to contribute largely to antioxidant potential. The antioxidant potential of these varieties of mushrooms was determined by summing the antioxidative activity (AOA) of each variety by varied antioxidant assays followed by determining the relative percent of AOA defined as the "antioxidant index" (AI). On the basis of the AI, the mushroom species were graded as very high, high, moderate, and low. *Termitomyces heimii* was identified as the best variety, which showed 100% AI with 37 mg of phenolics/g of sample, 418 units of reducing power ability (RPA)/g, and an IC_{50} of ~1.1 mg (dry weight)/mL, free radical scavenging activity (FRS) in the *water extract* followed by 11.2 mg of phenolics/g, 275 units of RPA/g, and an IC_{50} of ~2.7 mg (dry weight)/mL of FRS in the *methanolic extract*. Following *T. heimii*, *Termitomyces mummiformis* exhibited an AI of 86% within the "very high" group. Potent inhibitions of lipid peroxidation of ~100 and 69% was also observed in *T. heimii* and *T. mummiformis*, respectively. Water extracts ranged from 34 to 49% and methanolic extracts varied from 20 to 32% on dry weight of mushroom fruiting body. Total phenolic compounds were higher in the water extracts (2−37 mg/g) than in methanolic extract (0.7−11.2 mg/g). The AOA measured in the water extract was better than that from the methanolic extract. HPLC analysis of phenolic acids in the two mushroom species, namely, *T. heimii* and *T. mummiformis*, displaying maximum AOA potential indicated a preponderance of tannic acid, gallic acid, protocatacheuic acid, and gentisic acid. Studies thus provide the precise antioxidant status of 23 indigenous species of mushrooms, which can serve as a useful database for the selection of mushrooms for the function of preparation of mushroom-based nutraceutics.

KEYWORDS: Mushroom water extract (WE); mushroom methanolic extract (ME); phenolics; antioxidant activity; gallic acid equivalent; butylated hydroxyanisole equivalent; grading of mushrooms

INTRODUCTION

The consumption of plant foods, such as fruits, vegetables, red wines, and juices, provides protection against various diseases, including cancer and cardio- and cerebrovascular diseases (*1*). This protection can be explained by the capacity of antioxidants in the plant foods (*2−4*) to scavenge free radicals, which are responsible for the oxidative damage of lipids, proteins, and nucleic acids (*1*, *5*). As carcinogenic and toxic properties have been reported for some synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylated hydroxyquinine (TBHQ) at higher levels (*6*), recent research on the potential applications of natural antioxidants from herbs and spices for stabilizing foods against oxidation and as nutraceutics has received much attention (*7*).

Phytochemicals, especially phenolics in fruits and vegetables, are suggested to be the major bioactive compounds for health benefits. Phenolics are one of the groups of nonessential dietary components that have been associated with the inhibition of atherosclerosis and cancer. The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals (*8−10*).

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Table 1. Geographical Location and Natural Habitat of the Mushroom Species Studied for Antioxidant Potential

sample	mushroom species	natural habitat	geographic location
1	*Termitomyces mummiformis* Heim.	symbiotic (in association with termite nests)	forests of Himachal Pradesh
2	*Morchella angusticeps* PK.	saprophytic	forests of Himachal Pradesh
3	*Sparassis crispa* Wulf fr.	saprophytic	forests of Himachal Pradesh
4	*Cantharellus cibarius* Fr.	saprophytic	forests of Himachal Pradesh
5	*Lentinus squarrulosus* Mont.	saprophytic	forests of Kerala
6	*Hydnum repandum*	saprophytic	forests of Himachal Pradesh
7	*Pleurotus sajor-caju* (Fr.) Singer	saprophytic (on dead trunks)	forests of Kerala
8	*Geastrum arinarius* Lloyd.	saprophytic	forests of Madhya Pradesh
9	*Termitomyces heimii* Natarajan	symbiotic (in association with termite nests)	forests of Himachal Pradesh
10	*Lactarius sanguifluus* Peck.	saprophytic (coniferous wood land especially pine)	forests of Himachal Pradesh
11	*Macrolepiota procera* (Scop. ex Fr.) Sing.	saprophytic (on soil, pastures, lawns, in woods)	forests of Madhya Pradesh
12	*Russula brevepis* Peck	saprophytic (under *Picea smithiana* and *Pinus wallichiania*)	forests of Himachal Pradesh
13	*Morchella conica* Pers.	saprophytic (on sandy loam rich in organic substances in deodar forest)	forests of Himachal Pradesh
14	*Termitomyces shimperi* Heim.	symbiotic (in association with termite nests)	forests of Himachal Pradesh
15	*Boletus edulis* Bull. Fr.	saprophytic (on ground in open forests)	forests of Himachal Pradesh
16	*Lactarius deliciosus* (L. ex Fr.) S.F. Gray	saprophytic (coniferous wood land especially pine)	forests of Himachal Pradesh
17	*Cantharellus clavatus* Fr.	saprophytic (on the ground under *Picea smithiana*	forests of Madhya Pradesh
18	*Termitomyces tylerance* Heim.	symbiotic (in association with termite nests)	forests of Himachal Pradesh
19	*Auricularia polytricha* (Mont.) Sacc.	saprophytic (dead branches of *Ficus benghalensis*)	forests of Himachal Pradesh
20	*Lentius sajor-caju* (Fr.) Fr.	saprophytic (decaying plants of *Euphorbia royleana*)	forests of Kerala
21	*Helvella crispa* Scop. Fr.	saprophytic (under the shade of trees, damp places in pine forests)	forests of Himachal Pradesh
22	*Termitomyces microcarpus* (Berk. & Br.) Heim.	symbiotic (in association with termite nests)	forests of Kerala
23	*Pleurotus djamor* Sacc.	saprophytic (dead trunks of living trees)	forests of Kerala

Table 2. Yield of Mushroom Extracts in Water and Methanol

		yield of extract[a] (g/100 g of dry mushroom)	
sample	mushroom species	water extract	methanolic extract
1	*Termitomyces mummiformis*	41 ± 2.40	32 ± 2.07
2	*Morchella angusticeps*	48 ± 2.54	30 ± 2.40
3	*Sparassis crispa*	41 ± 1.58	30 ± 2.58
4	*Cantharellus cibarius*	42 ± 1.58	32 ± 2.07
5	*Lentinus squarrulosus*	40 ± 1.58	30 ± 2.07
6	*Hydnum repandum*	38 ± 1.58	28 ± 1.92
7	*Pleurotus sajor-caju*	42 ± 1.58	32 ± 2.07
8	*Geastrum arinarius*	38 ± 1.14	28 ± 1.58
9	*Termitomyces heimii*	48 ± 1.30	28 ± 1.81
10	*Lactarius sanguifluus*	46 ± 1.58	28 ± 1.58
11	*Macrolepiota procera*	48 ± 1.58	22 ± 1.64
12	*Russula brevepis*	40 ± 2.07	20 ± 2.58
13	*Morchella conica*	36 ± 1.58	26 ± 1.30
14	*Termitomyces shimperi*	42 ± 1.58	22 ± 1.58
15	*Boletus edulis*	34 ± 1.92	28 ± 1.92
16	*Lactarius deliciosus*	47 ± 1.64	29 ± 1.78
17	*Cantharellus clavatus*	41 ± 1.64	24 ± 2.07
18	*Termitomyces tylerance*	40 ± 2.23	28 ± 1.58
19	*Auricularia polytricha*	43 ± 2.07	26 ± 1.78
20	*Lentius sajor-caju*	49 ± 2.07	26 ± 1.64
21	*Helvella crispa*	45 ± 1.92	24 ± 2.07
22	*Termitomyces microcarpus*	41 ± 1.58	20 ± 1.92
23	*Pleurotus djamor*	40 ± 2.07	21 ± 1.87

[a] Each value is the mean of three replicate determinations ± standard deviation.

Mushrooms have long been appreciated for their flavor and texture as vegetables. Mushrooms provide a wealth of protein, fiber, vitamins, and minerals. On average, dried mushrooms contain ∼22% protein, which includes most of the essential amino acids, ∼5% fat, mostly in the form of linoleic acid (the essential fatty acid not synthesized in the human body), ∼63% carbohydrates including fiber, and ∼10% minerals as ash and are a good source of several vitamins including thiamin, riboflavin, niacin, and biotin (*11*).

Table 3. Total Phenolic Content in Selected Dietary Mushroom Varieties

		phenolic content[a] (mg/g)	
sample	mushroom species	water extract	methanolic extract
	Very High		
1	*Termitomyces heimii*	37.0	11.2
2	*Helvella crispa*	35.0	4.0
	High		
3	*Termitomyces tylerance*	18.0	7.8
4	*Lactarius sanguifluus*	17.8	7.3
5	*Morchella conica*	16.9	4.6
6	*Termitomyces mummiformis*	19.2	2.2
7	*Pleurotus sajor-caju*	14.3	7.4
8	*Termitomyces shimperi*	15.2	4.8
	Moderate		
9	*Lentinus squarrulosus*	15.0	4.0
10	*Boletus edulis*	10.2	8.4
11	*Pleurotus djamor*	13.3	3.6
12	*Macrolepiota procera*	10.2	6.0
13	*Cantharellus clavatus*	13.5	2.2
14	*Morchella angusticeps*	13.1	2.6
15	*Termitomyces microcarpus*	7.0	4.4
16	*Lactarius deliciosus*	7.4	4.8
17	*Geastrum arinarius*	4.8	5.5
	Low		
18	*Hydnum repandum*	7.4	2.6
19	*Lentius sajor-caju*	6.5	3.0
20	*Sparassis crispa*	5.5	1.7
21	*Russula brevepis*	5.5	0.7
22	*Auricularia polytricha*	3.2	2.3
23	*Cantharellus cibarius*	2.0	2.8

[a] Each value is the mean of three replicate determinations ± standard deviation.

In this scenario, the antioxidants present in dietary mushrooms are of great interest as possible protective agents to help the human body reduce oxidative damage without any interference (*12*). Now they are recognized as functional foods and as a source of physiologically beneficial components (*13*). Mush-

Table 4. Determination of Comparative Reducing Power Ability in Selected Dietary Mushroom Varieties[a]

		total reducing power ability[b] (mg of GAE/g of sample)		total reducing power ability[b] (units/g of sample)	
sample	mushroom species	WE	ME	WE	ME
1	*Termitomyces heimii*	10.20 a ± 0.19	6.55 b ± 0.31	418 a	275 b
2	*Helvella crispa*	6.30 b ± 0.44	8.71 b ± 0.33	264 b	366 b
3	*Termitomyces tylerance*	6.00 c ± 0.20	4.95 c ± 0.29	252 b	208 c
4	*Lactarius sanguifluus*	5.40 b ± 0.22	5. 7 b ± 0.34	224 c	238 b
5	*Morchella conica*	3.90 c ± 0.25	8.62 b ± 0.38	164 c	362 b
6	*Termitomyces mummiformis*	10.22 a ± 0.19	2.98 c ± 0.50	420 a	258 c
7	*Pleurotus sajor-caju*	2.80 c ± 0.34	4.95 c ± 0.43	116 c	208 c
8	*Termitomyces shimperi*	4.90 c ± 0.29	8.71 b ± 0.27	208 c	366 b
9	*Lentinus squarrulosus*	6.10 b ± 0.27	3.00 c ± 0.31	260 b	126 c
10	*Boletus edulis*	7.30 b ± 0.48	10.8 a ± 0.27	308 b	453 a
11	*Pleurotus djamor*	4.80 c ± 0.28	1.9 d ± 0.20	204 c	82 d
12	*Macrolepiota procera*	2.81 c ± 0.23	7.1 b ± 0.19	116 c	298 b
13	*Cantharellus clavatus*	5.36 b ± 0.29	9.10 b ± 0.31	224 c	381 b
14	*Morchella anguisticeps*	7.56 b ± 0.45	2.42 d ± 0.31	312 b	102 d
15	*Termitomyces microcarpus*	5.60 b ± 0.28	4.60 c ± 0.31	236 c	192 c
16	*Lactarius deliciosus*	9.50 b ± 0.42	7.70 b ± 0.34	396 b	323 b
17	*Geastrum arinarius*	2.88 c ± 0.44	2.70 c ± 0.31	120 c	112 c
18	*Hydnum repandum*	2.20 d ± 0.28	1.98 d ± 0.33	92 d	83 d
19	*Lentius sajor-caju*	2.90 c ± 0.36	2.10 d ± 0.31	124 c	87 d
20	*Sparassis crispa*	3.90 c ± 0.33	2.0 d ± 0.34	164 c	84 d
21	*Russula brevepis*	3.60 b ± 0.41	0.95 d ± 0.19	152 c	40 d
22	*Auricularia polytricha*	5.60 c ± 0.31	1.71 d ± 0.34	236 c	72 d
23	*Cantharellus cibarius*	2.70 c ± 0.50	3.36 c ± 0.28	108 c	141 c

[a] On the basis of reducing power ability, mushroom species are classified as follows: very high > 10; high = 5–10; moderate = 2.5–5; low < 2.5 mg of GAE/g sample. [b] Each value is the mean of three replicate determinations ± standard deviation. Means with different letters are significantly different ($p < 0.05$).

rooms have been shown to boost heart health; lower the risk of cancer; promote immune function; ward off viruses, bacteria, and fungi; reduce inflammation; combat allergies; and help balance blood sugar levels and support the body's detoxification mechanism (*14*, *15*). Mushrooms have been also shown to accumulate a variety of secondary metabolites including phenolic compounds, polypeptides, terpenes, steroids, etc. Mushroom phenolics have been found to be an excellent antioxidant and synergist (*16*). Furthermore, several companies are developing capsules from combinations of mushrooms, and these capsules, although expensive, have been shown to be health beneficial, including fighting against cancer (*17*).

In fact, mushrooms in nature are ecofriendly agents, as they represent the fewest of the microbial world gifted with the unique ability to biodegrade lignin, and their artificial culturing falls under the key concept of biodegradation and biotransformation of natural lignocellulosic wastes (*18*−*22*) into directly palatable edible forms of food often praised and priced as items of "food delicacy' because of their characteristic biting texture and flavor (*23*).

Indeed, this study is of great significance because multiple varieties of mushrooms exist in different parts of India; selection of mushroom variety becomes an important aspect to gauge the health benefits. Therefore, in the current investigation, 23 varieties of edible mushrooms, which occur commonly in hilly and tribal regions of India, were collected and screened for total phenolics; their antioxidant potentials were studied by comparing free radical scavenging activity, reducing power ability, and inhibition of lipid peroxidation in both water and methanolic extracts. The constituent phenolic acids were quantitated by HPLC. On the basis of antioxidant potentials from all different assays, the studied mushroom species have been categorized as very high, high, moderate, and low antioxidant activity sources, which would enable mushroom users to select species for further purposes of health promotion.

MATERIALS AND METHODS

Samples/Materials Collection. Twenty-three species of mushroom fruiting bodies collected from natural growth in different geographic locations of the country were procured. The complete names of these species, their geographical locations, and natural habitats are presented in **Table 1**.

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2-thiobarbituric acid (TBA), Folin−Ciocalteu reagent, ferrous sulfate, ascorbic acid, phenolic acid standards such as gallic acid, tannic acid, caffeic acid, coumaric acid, ferulic acid, gentisic acid, protocatechuic acid, syringic acid, vanillic acid, and cinnamic acid, synthetic antioxidants, namely, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), were purchased from Sigma Chemical Co. (St. Louis, MO). The HPLC column (Shimpak C_{18}) was obtained from Shimadzu Corp., Tokyo, Japan. Other chemicals such as ferric chloride, trichloroacetic acid, sodium carbonate, and reagents used in these experiments were of the highest quality, and only HPLC grade solvents were employed for HPLC analysis.

Preparation of Aqueous and Solvent Extracts of Mushrooms. All of the procured, selected, dried varieties of edible mushrooms were cleaned to remove any residual compost/soil and subsequently air-dried in the oven at 50 °C for about 3 h. All of the dried mushrooms were ground to fine powder (ca. 1 mm size) and stored in airtight plastic bags in desiccators at room temperature for further analysis. One gram of each of dried mushroom sample was mixed with 10 mL of boiled water or 10 mL of methanol. Samples were stirred for 15 min for effective extraction and centrifuged at 2000*g* for 15 min. Supernatants were referred to as water extract (WE) and methanolic extract (ME), respectively, and stored at 4 °C until the completion of the analysis. The yield of extraction was expressed as percent on a dry weight basis.

Estimation of Total Phenolics. The total phenolic contents of WE and ME of all of the mushroom samples were determined colorimetrically using the Folin−phenol method (*15*). A sample aliquot of 100 μL was added to 900 μL of water, 1 mL of Folin−Ciocalteu reagent, and 2 mL of 10% sodium carbonate solution, mixed in a cyclo mixer, and incubated for 1 h at room temperature. The absorbance was measured at 765 nm with a Shimadzu UV−visible spectrophotometer. The standard curve was drawn using 10−100 μg of gallic acid. The

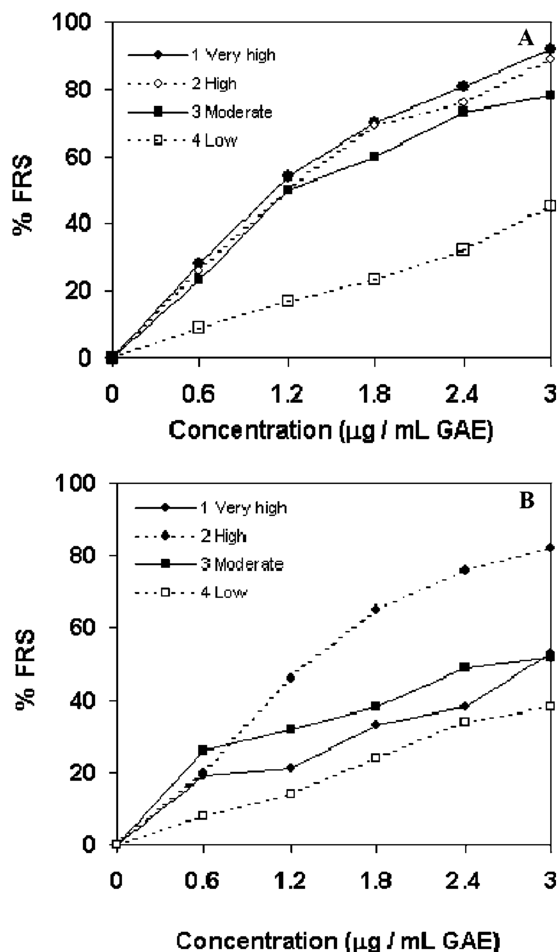

Figure 1. Free radical scavenging (FRS) activity of different doses of very high to low mushroom varieties: (**A**) water extracts of 1, *Termitomyces heimii*; 2, *Boletus edulis*; 3, *Cantharellus clavatus*; 4, *Macrolepiota procera*; (**B**) methanol extracts of 1–4. Correlation coefficient, *R* value, was found to be ∼0.95 in (**A**) and 0.96 in (**B**), indicating the concentration dependence.

total phenolic content was expressed as gallic acid equivalent (GAE) in milligrams per gram of sample.

Measurement of Antioxidant Properties. *Reducing Power Ability (RPA).* The reducing power of WE and ME of all the mushroom varieties was determined according to the method of Yen and Chen (*24*). Amounts of 5−25 μL of mushroom extracts were mixed with an equal volume of 0.2 M phosphate buffer, pH 6.6, and 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. An equal volume of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000*g* for 10 min. The upper layer of the solution was mixed with distilled water and 0.1% $FeCl_3$ in a ratio of 1:1:2 (v/v/v), and the absorbance was measured at 700 nm. Increase in the absorbance of the reaction mixture indicated increased reducing power. A higher absorbance indicates a higher reducing power. Gallic acid in the range of 2−10 μg was used as control.

Free Radical Scavenging (FRS) Activity. The effect of WE and ME of the mushroom species on DPPH radical was estimated according to the method of Lih-Shiuh et al. (*25*). Amounts of 5−25 μL of mushroom extracts were mixed with 100 mM Tris-HCl buffer (800 μL, pH 7.4). One milliliter of 500 μM DPPH in ethanol was added to the sample to a final concentration of 250 μM. The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{scavenging effect (\%)} = \frac{1 - \text{absorbance of sample at 517 nm}}{\text{absorbance of control at 517 nm}} \times 100$$

An antioxidant value of 100% indicates the strongest antioxidant activity. The EC_{50} value (milligrams of extract per milliliter) is the effective concentration at which DPPH radicals were scavenged by 50% and was obtained by interpolation from linear regression analysis.

BHA (2−10 μg) was used as the control.

Inhibition of Lipid Peroxidation in Rat Liver Homogenate. The inhibition of lipid peroxidation of rat liver homogenate was assayed according to the method described by Ng et al. (*26*) with some modifications. Liver tissues obtained from normal Wister rats were homogenized with a Polytron homogenzier in ice-cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 10% (w/v) homogenate. The homogenate was centrifuged at 3000*g* for 10 min, and an aliquot (0.5 mL) of supernatant was mixed with the extracts of various concentrations, followed by the addition of 0.1 mL of 10 μM $FeSO_4$ and 0.1 mL of 0.1 mM L-ascorbic acid. The mixture was incubated at 37 °C for 1 h. The reaction was terminated by adding 0.5 mL of trichloroacetic acid followed by 0.38 mL of thiobarbituric acid and heating at 100 °C for 20 min. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the absorbance of the supernatant containing the thiobarbituric acid-reactive substances (TBARS) was measured at 532 nm in a spectrophotometer. BHA was used as control. The percent inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \frac{A - A_1}{A} \times 100$$

A is the absorbance of the control, and A_1 is the absorbance of the test sample.

HPLC Analysis of Mushroom Phenolics in WE and ME. Phenolic acids of WE and ME extracts were analyzed according to the method of Wulf and Nagel (*27*), on a reversed phase Shimpak C_{18} column (4.6 × 250 mm), using a diode array detector (operating at 280 nm). The Shimpak C_{18} HPLC column was obtained from Shimadzu Corp.. A solvent system consisting of water/acetic acid/methanol (80:5:15) (v/v/v) was used as mobile phase at a flow rate of 1 mL/min. Phenolic acid standards such as gallic acid, tannic acid, caffeic acid, *p*-coumaric acid, ferulic acid, gentisic acid, protocatechuic acid, syringic acid, vanillic acid, and cinnamic acid were used for the identification of phenolic acids present in WE and ME. Quantitation of phenolic acids was achieved by the absorbance recorded in the chromatograms related to external standards at 320 nm (*28*).

Antioxidant Index (AI). This factor was employed to relatively grade the mushroom samples analyzed on the basis of their antioxidant potential. AI represents the average of the results based on the three methodologies employed for estimation of antioxidant activity, namely, TRP, FRS, and ILP. The highest average found was considered to be equivalent to 100, so that the others fell below this value and, accordingly, all were based on a numerical scale (**Table 8**), the mushroom species being classified into very high, high, moderate, and low antioxidant species.

Statistical Analysis. All data are expressed as mean ± standard deviation. Each value is a mean of three replicate determination. Analysis of variance was performed by ANOVA procedures. Duncan's new multiple-range test was used to determine the difference of means, and $p < 0.05$ was considered to be statistically significant. The correlation coefficient, *R*, between phenolics and antioxidant activity was determined using origin 6.1 SPSS (version 10 for Windows XP, SPSS Inc.).

RESULTS

Yield of Mushroom Extracts. The yields of water extracts (WE) and methanolic extracts (ME) of the mushroom fruiting bodies are presented in **Table 2**. The yields of WE were relatively higher compared to ME of any particular species under

Table 5. Free Radical Scavenging Activity in Selected Dietary Mushroom Samples[a]

		FRS activity[b] (mg of BHA/g of sample)		IC_{50} value[b] (mg/mL)	
sample	mushroom species	WE	ME	WE	ME
1	*Termitomyces heimii*	1.26 a	0.55 b	1.10 ± 0.31	2.70 ± 0.34
2	*Helvella crispa*	0.80 b	0.50 c	1.80 ± 0.34	3.00 ± 0.31
3	*Termitomyces tylerance*	0.56 c	1.20 a	2.76 ± 0.34	1.24 ± 0.32
4	*Lactarius sanguifluus*	0.66 b	0.44 c	2.73 ± 0.43	3.40 ± 0.31
5	*Morchella conica*	0.30 c	0.94 b	5.00 ± 0.31	1.60 ± 0.33
6	*Termitomyces mummiformis*	1.26 a	0.41 c	1.18 ± 0.29	3.70 ± 0.32
7	*Pleurotus sajor-caju*	0.83 b	0.60 b	1.80 ± 0.31	2.50 ± 0.37
8	*Termitomyces shimperi*	0.70 b	0.83 b	2.10 ± 0.34	1.80 ± 0.31
9	*Lentinus squarrulosus*	0.88 b	0.40 c	1.70 ± 0.32	3.80 ± 0.35
10	*Boletus edulis*	1.12 a	1.10 a	1.30 ± 0.34	1.40 ± 0.31
11	*Pleurotus djamor*	0.49 c	0.79 b	3.00 ± 0.31	1.90 ± 0.31
12	*Macrolepiota procera*	0.40 c	0.40 c	3.70 ± 0.34	3.70 ± 0.29
13	*Cantharellus clavatus*	0.98 b	0.81 b	1.51 ± 0.34	1.86 ± 0.34
14	*Morchella anguisticeps*	0.88 b	0.73 b	1.65 ± 0.32	2.09 ± 0.26
15	*Termitomyces microcarpus*	0.29 c	0.94 b	2.80 ± 0.31	1.60 ± 0.31
16	*Lactarius deliciosus*	0.71 b	0.29 c	2.10 ± 0.31	5.20 ± 0.31
17	*Geastrum arinarius*	0.37 c	0.44 c	3.60 ± 0.34	3.40 ± 0.31
18	*Hydnum repandum*	0.80 b	0.10 d	1.86 ± 0.31	14.50 ± 0.38
19	*Lentius sajor-caju*	0.14 d	0.80 b	10.9 ± 1.42	1.86 ± 0.32
20	*Sparassis crispa*	0.80 b	0.33 c	1.89 ± 0.36	4.54 ± 0.30
21	*Russula brevepis*	0.79 b	0.94 b	1.80 ± 0.34	1.60 ± 0.36
22	*Auricularia polytricha*	0.72 d	1.15 d	9.70 ± 0.42	13.0 ± 0.38
23	*Cantharellus cibarius*	0.23 c	0.17 d	6.40 ± 0.34	8.67 ± 0.30

[a] On the basis of IC_{50} values mushroom species are classified as follows: very high = 1–1.2; high = 1.2 – 2.5; moderate = 2.5–6.0; low = 6.0–10 mg of BHA equiv/g of sample. [b] Each value is the mean of three replicate determinations ± standard deviation. Means with different letters are significantly different ($p < 0.05$).

study. WE ranged from 34 to 49 g/100 g of dry weight (dw) and ME from 20 to 32 g/100 g of dw. Mushrooms are reported to contain more polar constituents (*29*). *Boletus edulis* and *Lentinus sajor-caju* recorded the minimum and maximum WE. Similarly, *Helvella crispa* and *Pleurotus sajor caju* yielded minimum and maximum ME. The yields of WE and ME are comparable with those of mushrooms such as *Agrocybe cylindracea* (*30*), *Dictyophora indusiata*, *Grifolia frondosa*, *Hericium erinaceus* and *Tricholoma giganteum* (*31*), and *Flammulina velutipes* and *Pleurotus cystidiosus* (*32*).

Total Phenolic Content. Total phenolics are the major naturally occurring antioxidant components found in WE and ME of several mushroom species (*30*−*36*). Accordingly, the phenolics concentration of all the mushroom varieties both in WE and in ME were estimated and expressed as milligrams of GAE per gram of dry mushroom. WE yielded more phenolics relative to ME for any given species. A wide range of phenolics contents varying from 2 to 37 mg/g and from 0.7 to 11.2 mg/g were observed in WE and ME, respectively. The 23 mushroom species could be categorized into three groups on the basis of their phenolics content as low-, moderate-, and high-phenolic species. Accordingly, *Termitomyces heimii* and *Helvella crispa* ranked as high-phenolics species (**Table 3**).

Reducing Power Ability. Reducing power abilities of all 23 varieties have been screened and are expressed as milligrams of GAE per gram of sample as well as absorbance units per gram of mushroom samples. The activity range varied from 2.2 to 10.22 mg of GAE/g of sample, which corresponds to 92−420 units/g of sample. Hence, an average of low, moderate, high, and very high categories could be observed. In WE, indeed, of 23 samples only 2 (*Termitomyces mummiformis* and *Termitomyces heimii*, ∼8% of the total) belong to the very high category; whereas 5 and 6 samples constituting percentages of 39 and 49, respectively, belong to the high and moderate categories, and the remaining sample with 4% of the total belongs to the low category. In ME also, only one species (*Boletus edulis*, ∼4% of the total) belongs to the very high category. Totals of 8 and 6 samples constituting percentages of 35 and 29%, respectively, belong to the high and moderate categories, and the remaining 7 samples with 32% of the total belong to the low category. The data thus suggest that the reducing power ability in the mushroom could be due to phenolic acids. The results are supported by the correlation

Table 6. Inhibition of Lipid Peroxidation from WE and ME of Selected Mushroom Samples[a]

		inhibition of lipid peroxidation[b] (hm of MDA/mg of phenolics)	
sample	mushroom species	WE	ME
1	*Termitomyces heimii*	32.0 a ± 0.22	128.0 c ± 0.42
2	*Helvella crispa*	32.0 a ± 1.41	132.0 c ± 0.62
3	*Termitomyces tylerance*	63.0 b ± 0.32	98.5 b ± 0.42
4	*Lactarius sanguifluus*	87.5 b ± 0.29	176.5 c ± 0.50
5	*Morchella conica*	65.0 b ± 0.37	277.0 d ± 0.60
6	*Termitomyces mummiformis*	44.5 a ± 0.38	396.0 d ± 0.43
7	*Pleurotus sajor-caju*	86.0 b ± 0.31	171.0 c ± 0.74
8	*Termitomyces shimperi*	51.5 b ± 0.71	194.0 c ± 0.57
9	*Lentinus squarrulosus*	40.0 a ± 0.49	325.0 d ± 0.35
10	*Boletus edulis*	49.0 a ± 0.34	111.0 c ± 0.77
11	*Pleurotus djamor*	70.5 b ± 0.70	293.5 d ± 0.41
12	*Macrolepiota procera*	109.5 c ± 0.34	187.5 c ± 0.87
13	*Cantharellus clavatus*	44.5 a ± 0.31	355.5 d ± 0.50
14	*Morchella anguisticeps*	57.5 b ± 0.45	374.0 d ± 0.73
15	*Termitomyces microcarpus*	93.5 a ± 0.35	227.0 d ± 0.58
16	*Lactarius deliciosus*	58.0 b ± 0.54	230.0 d ± 0.48
17	*Geastrum arinarius*	215.0 d ± 0.42	226.5 d ± 1.19
18	*Hydnum repandum*	147.0 c ± 0.55	448.0 d ± 1.00
19	*Lentius sajor-caju*	176.0 c ± 0.70	408.0 d ± 0.74
20	*Sparassis crispa*	181.0 c ± 0.49	605.5 d ± 0.46
21	*Russula brevepis*	271.0 d ± 0.37	705.0 d ± 0.57
22	*Auricularia polytricha*	335.5 d ± 0.87	500.0 d ± 0.74
23	*Cantharellus cibarius*	571.5 d ± 0.47	486.5 d ± 0.75

[a] On the basis of the inhibition of lipid peroxidation mushroom species are classified as follows: very high < 50; high = 50–100; noderate = 100–200; low > 200 nm of MDA formed/mg of phenolics. [b] Each value is the mean of three replicate determinations ± standard deviation. Means with different letters are significantly different ($p < 0.05$).

Table 7. HPLC Profile of Phenolic Acids of Mushroom Species

mushroom species		phenolic acids[a] (mg/g)									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Termitomyces heimii	WE[b]	15.54	4.07	11.10	1.48	0.37			3.70	0.37	0.37
	ME[c]	2.31	0.52	5.39	0.55	tr	tr	0.55	0.22	tr	1.43
Termitomyces mummiformis	WE	10.56	5.76	0.58	1.92		0.19				0.19
	ME	0.68	0.66	0.22	0.48	tr	0.02	tr	tr	tr	0.13
Boletus Edulis	WE	9.59		0.30				0.20	0.10		
	ME	4.08	tr	3.92	tr	tr	tr	tr	tr	tr	tr
Lactarius deliciosus	WE	5.92	0.14	0.07	1.05	tr	tr	tr	tr	0.14	tr
	ME	3.26	tr	1.53							
Pleurotus sajor-caju	WE	0.42	8.00	2.58	0.86	0.29				1.86	0.42
	ME	0.98	3.85	1.75	0.28	0.14	tr	tr	tr	tr	tr
Hydnum repandum	WE	0.21	4.17	0.51							2.51
	ME	0.02	1.56	0.41	0.36	0.05	0.02				0.18
Lentinus squarrulosus	WE		10.2	3.75	0.45	0.45		0.15			
	ME	1.40	1.92	0.40	0.11					0.08	0.08
Sparassis crispa	WE		3.00	1.33	0.72				0.45		
	ME		1.25	0.08						0.36	0.01
Morchella conica	WE	4.05	12.85								
	ME		2.70	0.79	0.28	0.10	0.04	0.09	0.56	0.04	
Russula brevepis	WE	0.11	3.90	0.60	0.66	0.16	0.07	tr	tr	tr	tr
	ME	0.45	0.18	0.05					0.02		
Geastrum arinarius	WE	3.84	0.96								
	ME	3.08							2.42		
Cantharellus cibarius	WE	1.40	0.60								
	ME	1.90						0.06		0.84	
Lactarius sangifluus	WE	5.78	2.70	5.08	1.05	0.72	0.37	0.30	0.20	0.18	1.22
	ME	4.31	1.80	0.71	0.07	0.01	0.13	0.11	0.04	0.01	0.11
Macrolepiotaprocera	WE	4.63	3.40	0.74	0.44	0.13	0.14	tr	0.11	0.41	tr
	ME	3.80	1.70	0.23	0.01	0.01	tr	0.10	0.05	0.01	tr
Cantherallus clavatus	WE	4.45	2.38	3.57	1.12	0.80	tr	tr	tr	tr	0.90
	ME	0.68	0.43	0.70	0.14	0.06	0.03	0.02		0.10	0.04
Auricularia polytricha	WE	1.72	1.04	0.31	0.03	0.07	tr	tr	tr		
	ME	2.17	0.04	0.01	0.06	0.02					
Pleurotus djamor	WE	4.45	2.38	3.57	1.12	0.80	tr	tr	tr	tr	0.90
	ME	1.36	0.51	0.84	0.01	tr	0.18	0.03	0.10	0.05	tr
Lentinus sajor caju	WE	1.81	1.21	3.08	tr	tr	0.15	tr	0.19		
	ME	1.34	1.51	0.03	tr	0.04	0.04	0.04	tr	tr	tr
Termitomyces tylerance	WE	tr	6.00	11.60	tr	tr	tr	0.28			
	ME	2.75	4.58	tr	0.16	tr	0.25	0.12	tr		
Morchella anguiticeps	WE	8.63	3.20	0.94	tr	tr	0.15	tr	tr	tr	
	ME	1.38	0.89	0.16	0.05	tr	tr	0.03			
Termitomyces Microcarpus	WE	tr	2.52	1.22	1.80	0.43	0.46	0.18	tr	0.12	tr
	ME	2.21	1.50	0.29	0.08	0.17	tr	0.15	tr	tr	tr
Helvella crispa	WE	tr	10.70	18.48	4.89	0.58	tr	tr	tr	tr	tr
	ME	9.98	tr	tr	tr	0.12	tr	tr	tr		
Termitomyces shimperi	WE		10.40	3.75	0.45	0.45		0.15			
	ME	1.60	1.92	0.40	0.12					0.36	0.40

[a] **1**, tannic acid; **2**, gallic acid; **3**, protocatechuic acid; **4**, gentisic acid; **5**, vanillic acid; **6**, syringic acid; **7**, caffeic acid; **8**, coumaric acid; **9**, ferulic acid; **10**, cinnamic acid. [b] Water extract. [c] Methanol extract.

coefficient (R) of ~0.95 between increased levels of phenolics and higher levels of reducing power ability in WE (80%) than in ME (45%). The water extract of mushrooms revealed better antioxidative reducing power ability **(Table 4)**.

Scavenging Activity of DPPH Radical. Free radical scavenging has been known as an established phenomenon in inhibiting lipid oxidation, which otherwise can be deleterious to the cellular components and hence cellular function. Concentration-dependent scavenging activity was observed in all samples; two mushroom samples representing very high and low scavenging activities each are depicted in **Figure 1**. **Table 5** depicts the scavenging activity of the DPPH. FRS activity was compared with the standard phenolic, BHA, which gave an IC_{50} of ~1.5 μg of equivalent phenol. A 1−10-fold variation in the FRS activity was observed from source to source per gram of sample. FRS activity is expressed as IC_{50} (inhibitory concentration to inhibit 50% of FRS activity), as well as milligram BHA equivalents per gram of sample. WE *T. heimii*, *T. mummiformis*, and *B. edulis* expressed very high activity (13% of the total), offering 1.26, 1.26, and 1.12 mg of BHA equiv/g of sample, respectively. About 11 samples (48% of total samples) expressed high activity, about 7 samples (30% of total) moderate, and 2 samples (9% of total) low scavenging activity. Although only 13% of samples showed very high activity, *Cantharellus cibarius* with an IC_{50} of 0.23 mg/mL showed 10-fold lower activity compared to the collected sample *Termitomyces heimii*. In ME also, *Boletus edulis* and *Termitomyces tylerance* expressed very high activity. About 10, 8, and 3 species (44, 35, and 13% of total samples) expressed high, moderate, and low activity, respectively. In both extracts *C. cibarius* expressed the lowest activity.

Inhibition of Lipid Peroxidation. Lipid peroxidation is a major cause of food deterioration, affecting color, flavor, texture, and nutritional value. Besides, it has been suggested that oxidative modification of low-density lipoproteins (LDLs) may play a role in the development of atherosclerosis, and the oxidative modification depends on a common initiating step— the peroxidation of polyunsaturated fatty acid components in

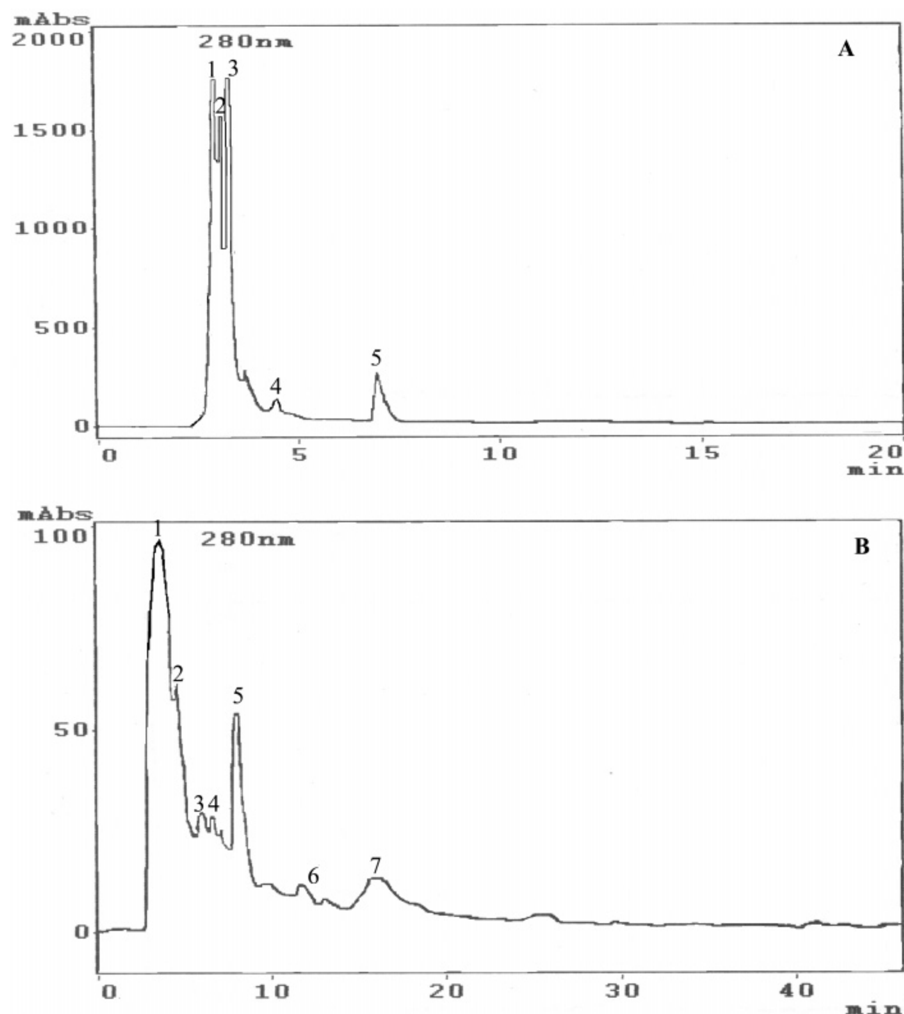

Figure 2. HPLC chromatogram of phenolic components in *Cantharellus clavatus* (**A**) water extract (peaks: 1, tannic acid; 2, gallic acid; 3, protocatechuic acid; 4, gentisic acid; 5, vanillic acid) and (**B**) methanolic extract (peaks: 1, tannic acid; 2, gallic acid; 3, protocatechuic acid; 4, gentisic acid; 5, vanillic acid; 6, syringic aicd; 7, caffeic acid).

the LDLs. The generated lipid peroxides further act on the cell/cellular components, leading to both structural and functional damage of the biomolecule as well as the cellular structure. Mushrooms are considered to be good phenolic antioxidants to inhibit lipid peroxidation (*24*). Malondialdehyde (MDA) is the secondary byproduct, which is released during the lipid peroxidation. A decrease in the production of MDA in turn symbolizes the inhibition of lipid peroxidation. The mushroom extracts used here have a stronger antioxidative action in both the water and methanolic extracts. Furthermore, a wider range of activity, $a < 50$, $b > 50$ to ≤ 100, $c > 100$ to ≤ 200, and $d > 200$ nm of BHA/g of phenol, was observed (**Table 6**). The results also corroborated with their phenolic content. A correlation coefficient (R) of 0.922 was established between the phenolic content and the antioxidative ability.

HPLC Analysis. Because antioxidant activity is mostly a function of the constituent phenolics, in the current study, the quantitative spectrum of phenolic acids in WE and ME of all 23 mushroom species was determined. **Table 7** implies the phenolic acids (milligrams per gram) present in the selected varieties. Variations in the net antioxidant activity might be due to the total effect of different phenolic acids. Irrespective of the very high or high or moderate or low antioxidant expressing species, most of them showed gallic acid/tannic acid contents in addition to gentisic acid, protocatechuic acid, and coumaric acid. **Figure 2** represents two chromatograms of *Cantharellus clavatus* as an example of mushroom phenolic profile.

Antioxidant Index. On the basis of the AI, an average of all three antioxidant assays, namely, TRP, FRS, and ILP, the studied mushroom species were classified into very high, high, moderate, and low antioxidant potential varieties. Thus, two species, *T. hemii* and *T. mummiformis*, ranked very high, three species, *B. edulis*, *L. deliciosus*, and *M. angusticeps*, ranked high, 15 species ranked moderate, and the remaining 3 ranked low (**Table 8**).

A strong correlation with phenolics and all of the assay models showed similar values, indicating the antioxidant potentials of mushroom variety for their health beneficial properties. Apparently, the mushroom WE and ME used in this work may play an important role in protection against damage to cell membrane function.

DISCUSSION

Earlier, Cheung et al. (*37*) had reported the antioxidant activity of mushroom extracts with stronger inhibition of lipid peroxidation occurring at high concentrations of the extracts in most cases. The possible mechanism of antioxidant activity of mushroom extracts includes scavenging of free radicals possibly through hydrogen-holding capacity and oxidation by peroxy radicals. In this study, by all assays *T. heimii* and *T. mummi-*

Table 8. Grading of Selected Mushroom Samples for Total Antioxidant Activity

mushroom species	rel % FRS activity	rel % RP ability	rel % ILP	av of total AOX activity (rel % = antioxidant index)	conclusion
Termitomyces heimii	100	40	100	100	1, very high
Termitomyces mummiformis	100	100	69	86	2, very high
Boletus edulis	88	73	57	71	3, high
Lactarius deliciosus	56	95	54	68	4, high
Morchella anguisticeps	70	76	50	63	5, high
Cantharellus clavatus	78	54	46	58	6 moderate
Lentinus squarrulosus	69	61	45	56	7, moderate
Helvella crispa	63	63	45	56	7, moderate
Lentius sajor-caju	11	29	41	51	8, moderate
Lactarius sanguifluus	52	54	39	49	9, moderate
Termitomyces tylerance	44	60	38	48	10, moderate
Termitomyces shimperi	55	49	37	46	11, moderate
Sparassis crispa	63	39	37	46	11, moderate
Russula brevepis	63	36	37	46	11, moderate
Pleurotus sajor-caju	65	28	35	44	12, moderate
Pleurotus djamor	39	48	33	41	13, moderate
Hydnum repandum	63	22	32	40	14, moderate
Termitomyces microcarpus	18	56	27	34	15, moderate
Auricularia polytricha	06	56	24	30	16, moderate
Morchella conica	23	39	24	30	16, moderate
Macrolepiota procera	32	28	23	29	17, low
Geastrum arinarius	29	29	23	29	17, low
Cantharellus cibarius	18	27	18	23	18, low

[a] The average of FRS, RP, and ILP of *T. heimii* is based on 100; relative AI of other mushroom species was compared, and the species were graded into very high, high, moderate, and low antioxidant potentials.

formis were found to be very high antioxidant varieties. The water extract was found to possess better antioxidant activities ($p < 0.05$), and a general correlation between higher antioxidant activity and a larger amount of total phenolics was found in mushroom extracts.

Several references are available on the insignificant quantities of ascorbic acid, β-carotene, and tocopherols in WE and ME of mushroom fruiting bodies as naturally occurring antioxidant components (*30*, *33*). However, in all such cases phenolics predominated the WE/ME. Accordingly, the present work was focused to draw a correlation between the phenolics and antioxidant activity. A general correlation coefficient (R) of about 0.95 was found at $p < 0.05$ between total phenolics and antioxidant assays conducted.

Our HPLC analysis indicated different types of phenolic acids present in them. Because they have different activities, they may be responsible for low antioxidative or high antioxidative properties of mushrooms. The relatively higher content of phenolics in WE might explain the high antioxidant property similar to that obtained in *Ganoderma tsuge* (*2*). Positive correlations were established between total phenolic content in the mushroom extract and their antioxidant activities, similar to those observed in *Lentinus edodes* and *Volvariella volvacea* (*38*). Better performance of WE than ME in the current study is similar to the results of these two mushrooms. AI was evolved as an average of different assays for AOA, namely, FRS, TRP, and ILP activities, for each of the species studied. On the basis of the AI, the studied mushroom species could be classified into four categories: very high, high, moderate, and low (**Table 8**). This gives an indication for selection of mushroom variety for further applications. It is interesting to observe so much of varietal differences. For example, *T. heimii* gave a good antioxidant activity followed by *T. mummiformis*; however, another species of the same genus, *Termitomyces microcarpus*, showed poor activity. The result hence may indicate that the level of phenolic antioxidants expressed in mushrooms may be dependent very much on the location, also, on whether the species has been exposed to stress conditions, etc. Therefore, this study may increase the understanding of the selection of mushroom sources for exploration of bioactivity. The present study accounts for the antioxidant activity of water/methanolic extracts of mushrooms with respect to phenolic compounds. However, this does not preclude the possibility of antioxidant activity of nonphenolic compounds such as ascorbic acid and that of selenium.

Despite the identification of large numbers of mushrooms for medicinal properties, only a few have been utilized or studied. These include, mainly, *Ganoderma* species (rieshi or lingzki), *Lentinus edodes* (shiitake), *Polyporus umbellate*, and *Grifola frondosa* (maitake) (*17*). In the current study, therefore, we evaluated a number of mushroom species for their total antioxidant capacities on the basis of multimechanistic antioxidant assays to find useful applications against complicated oxidative stress conditions in the body. The current information on the variety enriched with antioxidant potential would be the choice of selection for commercialization and further exploration for human health benefits. Future studies on the estimation of levels of active components other than antioxidants will also be useful in grading mushroom varieties for bioactivity because there may be heterogeneity in the distribution of active components responsible for different therapeutic effects.

ABBREVIATIONS USED

LDL, low-density lipoproteins; DPPH, 2,2-diphenyl-1-picrylhydrazyl; HPLC, high-performance liquid chromatography; BHA, butylated hydroxyanisole; TBHQ, *tert*-butylated hydroxyquinine; BHT, butylated hydroxytoluene; GAE, gallic acid equivalent; TCA, trichloroacetic acid; TBA, thiobarbituric acid; TBARS, thiobarbituric acid-reactive substances; BHAE, butylated hydroxyanisole equivalents.

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LITERATURE CITED

(1) Halliwell, B.; Gutteridge, J. M. C. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.* **1984**, 219−224.

(2) Bimla, N.; Punita, B. Aluminium-induced imbalance in oxidant and antioxidant determinants in brain regions of female rats: protection by centrophenoxine. *Toxicol. Mech. Methods* **2006**, *16*, 21−25.

(3) Collins, A. Antioxidant intervention as a route to cancer prevention. *Eur. J. Cancer* **2005**, *41*, 1923−1930.

(4) Niki, E.; Shimski, H.; Mino, M. Antioxidantism, free radicals and biological defence. *Biochem. J.* **1994**, 134−139.

(5) Chang, S. T.; Hayes, T. H. *Health Benefits of Mushrooms*; American Academic Press of Nutrition: 1978; pp 137−143.

(6) Butylated hydroxyl anisole (BHA). *Sixth Annual Report on Carcinogen*; 1991.

(7) Zhoua, K.; Yub, J. J.; Liangli. ESR determination of the reactions between selected phenolic acids and free radicals of transition metals. *Food Chem.* **2006**, *94* (5), 446−457.

(8) Mallavadhani, U.; Sudhakar, A.; Sathyanarayana, K. V. S.; Mahapatra, A.; Li, W.; Richard, B. Chemical and analytical screening of some edible mushrooms. *Food Chem.* **2006**, *95*, 58−64.

(9) Carmen, H. W.; Xin, D. Chromatographic and electrophoretic methods for Lingzhi pharmacologically active components. *J. Chromatogr.* **2004**, *812*, 241−257.

(10) John, S. Medicinal mushrooms: their therapeutic properties and current medical usage with special emphasis on cancer treatments. *Med. Mushrooms Cancer* **2005**.

(11) Mattila, P.; Sounpa, K.; Piironen, V. Functional properties of edible mushrooms. *J. Nutr.* **2000**, *16*, 7−8.

(12) Adams, A. K.; Wermuth, E. O. Antioxidant vitamins and the prevention of coronary heart disease. *Am. Family Physician* **1999**, *60*, 895−905.

(13) Wasser, S. P.; Weis, A. L. Medicinal properties of substances occurring in higher basidiomycetes mushrooms. *Int. J. Med. Mushrooms* **1999**, *1*, 31−62.

(14) Ada, M. A.; Wong, H. K.; Jiang, B. Fibrinolytic enzymes in Asian traditional fermented foods. *Food Res. Int.* **2005**, *38*, 243−250.

(15) Lo, K. M.; Cheung, P. C. K. Antioxidant activity of extracts from the fruiting bodies of *Agrocybe aegerita* var. Alba. *Food Chem.* **2005**, *89*, 533−539.

(16) Li, L.; Ng, T. B.; Zhao, L. Antioxidant activity with content of phenolics in extracts from the culinary-medicinal abalone mushroom *Pleurotus abalones*, Chen et Chang (*Agaricomycetideae*). *Int. J. Med. Mushrooms* **2005**, 237−242.

(17) Mau, J. L.; Tsai, S. Y.; Tseng, Y. H.; Huang, S. J. Antioxidant properties of hot water extracts from *Ganoderma tsugae* Murrill. *LWT−Food Sci. Technol.* **2005**, *38*, 589−587.

(18) Rajarathnam, S.; Bano, Z. *Pleurotus* mushrooms. Part IA. Morphology, life cycle, taxonomy, breeding and cultivation. *CRC Crit. Rev. Sci. Nutr*. **1988**, *26* (2), 157−223.

(19) Bano, Z.; Rajarathnam, S. *Pleurotus* mushrooms. Part II. Chemical composition, nutritional value, post-harvest physiology, preservation and role as human food. *CRC Crit. Rev. Sci. Nutr.* **1988**, *27* (2), 87−158.

(20) Rajarathnam, S.; Bano, Z. *Pleurotus* mushrooms. Part III. Biotransformation of natural lignocellulosic wastes: commercial applications and implications. *CRC Crit. Rev. Sci. Nutr*. **1989**, *28* (1), 31−113.

(21) Rajarathnam, S.; Bano, Z. Biological utilization of the fruiting fungi. In *Advances in Applied Mycology*; Dekker: New York, 1990; Vol. 3, pp 241−392.

(22) Rajarathnam, S.; Shashirekha, M. N.; Bano, Z. Biopotentalities of the Basidiomacromycetes. In *Advances in Applied Microbiology*; Academic Press: San Diego, CA, 1992; Vol. 37, pp 234−361.

(23) Rajarathnam, S.; Shashirekha, M. N.; Bano, Z. Biodegradative and biosynthetic capacities of mushrooms: present and future strategies. *Crit. Rev. Biotechnol.* **1998**, *18* (2, 3), 91−236.

(24) Yen, G. C.; Chen, H. Y. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.* **1995**, *43*, 27−32.

(25) Lai, L.-S.; Chou, S.-T.; Chao, W.-W. Studies on the antioxidant activities of Hsian-tsao (*Mesona procumbens* Hemsl) leaf gum. *J. Agric. Food Chem.* **2001**, *49*, 963−968.

(26) Ng, T. B.; Liu, F.; Wang, Z. T. Antioxidative activity of natural products from plants. *Life Sci.* **2000**, *66*, 709−723.

(27) Wulf, L.; Nagel, C. W. Analysis of phenolic acids and flavanoids by HPLC. *J. Chromatogr.* **1976**, *116*, 271−279.

(28) Valentão, P.; Andrade, P. B.; Rangel, J.; Ribeiro, B.; Silva, B. M.; Baptista, P.; Seabra, R. M. Effect of the conservation procedure on the contents of phenolic compounds and organic acids chanterelle (*Cantharellus cibarius*) mushroom. *J. Agric. Food Chem.* **2005**, *53*, 4925−4931.

(29) Vaskovsky, V.; Khotimcheko, S.; Eugenya, M. Distribution of diacyl glycerotrimethylhomoserine and phosphotidylcholine in mushrooms. *Phytochemistry* **1998**, *47*, 755−760.

(30) Tsai, S. Y.; Huang, S. J.; Mau, J. L. Antioxidant properties of hot water extracts from *Agrocybe cylindracea. Food Chem.* **2006**, *98*, 670−677.

(31) Mau, J. L; Lin, H. S.; Song, S. F. Antioxidant properties of several specialty mushrooms. *Food Res. Int.* **2002**, *35*, 519−526.

(32) Yang, J. H.; Lin, H. C.; Mau, J. L. Antioxidant properties of several commertial mushrooms. *Food Chem.* **2002**, *77*, 229−235.

(33) Mau, J. L.; Tsai, S. Y.; Tseng, Y. H.; Huang, S. J. Antioxidant properties of hot water extracts from *Ganoderma tsugae* Murrill. *LWT−Food Sci. Technol.* **2005**, *38*, 589−597.

(34) Mau, J. L.; Tsai, S. Y.; Tseng, Y. H.; Huang, S. J. Antioxidant properties of methanolic extracts from *Ganoderma tsugae. Food Chem.* **2005**, *93*, 641−649.

(35) Mau, J. L.; Chang, C. N.; Huang, S.; Chen, C. C. Antioxidant properties of methanolic extracts from *Grifola frondose*, *Morchella esculenta* and *Termitomyces albuminosus. Food Chem.* **2004**, *87*, 111−118.

(36) Mau, J. L.; Lin, H. C.; Chen, C. C. Antioxidant properties of several medicinal mushrooms. *J. Agric. Food Chem.* **2002**, *50*, 6072−6077.

(37) Cheung, L. M.; Peter, C. K. Mushroom extracts with antioxidant activity against lipid peroxidation. *Food Chem.* **2005**, *89*, 403−409.

(38) Cheung, L. M.; Cheung, P. C. K.; Ooi, V. E. C. Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chem.* **2003**, *81*, 249−255.

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