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Evaluation of nutrients, trace metals and antioxidant activity in *Volvariella volvacea* (Bull. Ex. Fr.) Sing

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Abstract

Volvariella volvacea (Bull. Ex. Fr.) Sing, were collected from mushroom farms at Namakkal district, Tamil Nadu, South India. The different organic and inorganic additives were used in mushroom culture. Methanolic extracts were prepared from these mushrooms and their antioxidant properties, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, reducing power, and nutrient analysis were studied. Biomass productions, the additives Calcium carbonate and Sawdust were recorded better results. Calcium carbonate recorded maximum levels of enzymatic antioxidants that are catalase, superoxide dismutase, glutathione peroxidase, peroxidase, glutathione-s-transferase and Glutathione reductase, DPPH scavenging activities (31.71±0.05 – 92.05±0.06 mg/ ml), reducing power (0.29±0.05 – 0.45±0.04 mg/ ml), nutrients and trace metals were also significantly high in Calcium carbonate. Similar trends were recorded in nutrient analysis and mushroom yield potential (1356.98±1.1 g/ bed). The ongoing research will lead to a new generation of nutritional food because *V. volvacea* may have potential as natural antioxidants and the calcium carbonate could be used as a suitable additive to improve the production of antioxidative substances, nutritive value and yield in the mushroom food industry.

Key words: Antioxidant activity, Calcium carbonate, DPPH, Nutrients, Trace metals, *Volvariella volvacea*

Introduction

The genus *Volvariella* (paddy straw mushroom) comprises a group of several species, which can be found growing on a variety of substrates in tropical and subtropical regions. *V. volvacea* (Bull. Ex Fr.) Sing., is probably the best known species, as it has been traditionally cultivated in Southeast Asia since the eighteenth century (Chang, 1977). Currently, *V. volvacea* is the third most important cultivated mushroom reaching total world production of 287 metric tones (Chang and Miles, 1993). In India, Su and Seth (1940) first cultivated straw mushroom; however, scientific cultivation using spawn was successfully demonstrated by Thomas et al. (1943). Mushrooms are considered to be a good source of antioxidants, such as variegatic acid and diboviquinone, which have been found in mushrooms (Kasuga et al., 1995). Methanol and water extracts from *Volvariella volvacea* and *Lentinus edodes* were found to rich antioxidative activities (Cheung et al., 2003). In recent years, the use of some synthetic antioxidants has been restricted because of their possible toxic and carcinogenic effects (Gazzani et al., 1998). Foods rich in antioxidants have been shown to play an essential role in the prevention of cardiovascular diseases (Dragsted et al., 1993), neurodegenerative diseases, Parkinson's and Ahlzeimer's diseases (Joseph et al., 1999) inflammation, and problems caused by cell and cutaneous aging (Ames et al., 1993). Organism cells are prepared with many more protection systems against free radical damage, including oxidative enzymes, namely catalase and superoxide dismutase (Niki et al., 1994). Exogenous chemical and endogenous metabolic processes in the human body or in the food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of resulting in cell death and tissue damage. However, antioxidant supplements containing foods might be used to help human body cells to reduce oxidative scratch (Halliwell and Gutteridge, 2003). Thus the natural antioxidants

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present in foods and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic effects (Ames et al., 1993). In this study was discussed about, antioxidant properties, DPPH radical scavenging activity, reducing power, and nutrient analysis and trace metals were studied.

Materials and Methods

Isolation

Mushroom samples of *V. volvacea* (paddy straw mushroom) were collected from local mushroom form at Namakkal District, Tamil Nadu and South India. After collected mushrooms were immediately transferred to the laboratory. The sporophores of individual mushrooms were first swabbed with 80% of ethyl alcohol to remove external microbial contaminations. At the junction of pileus and strip, tissue bits were taken separately and using a sterile forceps, the tissue bits were surface disinfected with 10% of sodium hypochloride (commercial) for 5 min. and rinsed with three changes of sterile distilled water. The surface-sterilized tissue bits were placed on Potato Dextrose Agar (PDA) in petri dishes and incubated at laboratory temperature (30 ± 2°C) for 7 to 10 days. Six isolates were used for the present investigation.

Biomass production

For biomass production, 2% calcium carbonate was added separately in potato-dextrose broth. The 2% calcium carbonate was enhanced the mycelia growth, compared to other concentration (Kalaiselvan, 2007). Amended media (50 ml) were dispensed with additives in 100 ml Erlenmeyer flask and sterilized at 15 lbs pressure for 1 h. After cooling, the flask was inoculated with a disc (9 mm dia) taken from the peripheral growth of 7-day-old PDA culture of *V. volvacea*. For each treatment ten replications were maintained. The broth without amendment served as control. They were incubated at laboratory conditions (25± 2ºC) for complete coverage of mycelia. After extracting the supernatant from the individual treatment flask, the mycelial growth retained in weighed Whatman No. 1 filter paper was recorded. The mycelial was taken from individual flasks and oven dried separately, at 80ºC for 6 h and the mycelial dry weight (g/ lit) was determined.

Preparation of mushroom bed

The mushroom spawn and bed was prepared according to the method of Sudha et al. (2008) with some modifications. In mushroom bed preparation, organic and inorganic additives were used. Their six different additives were consisting of six beds; each bed was three replications were maintained. The harvested mushrooms were used for further study.

Nutrients and trace metals

Standard procedures (Jackson, 1958) were used to determine the proximate composition of dried samples of paddy straw mushrooms. Nutrients and trace metals such as Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Copper (Cu), Zinc (Zn), Iron (Fe), and Manganese (Mn) were analyzed.

Samples Preparation and estimation

The different harvested mushroom samples were dried in an oven at 60ºC for 30 min. The samples were powdered using pestle and mortal for the estimation of nutrients and trace metals. Oven-dried sample (0.5g) was digested over a hot plate with 50 ml of triple acid mixture (nitric acid: sulphuric acid: perchloric acids in the ratio of 9: 2: 1 v/ v). The silica that settled down was filtered through Whatman No. 42 filter paper (2.5 µm). Total nitrogen content of sample was estimated by Kjeldhal method (Peper, 1966). Phosphorus content was analyzed by Vandomolybdate method (Jackson, 1958). Flame photometer (Jackson, 1958) was used for estimation of potassium. The calcium and magnesium was determined by colorimetric method (Versenate method). The content of manganese, zinc, iron, and copper were determined in an Atomic Absorbance Spectrometer (AAS) using appropriate Cathode tubes and wave lengths (Cu = 3247.5 A°, Zn = 2319 A°, Fe = 2483.3 A°, Mn = 2794 A°).

Enzymatic antioxidant activity

Sample preparation

A fine dried mycelial mat (biomass filtrate paper) and harvested mushroom powder (20 meshes) samples (5g) were continuously extracted with methanol in a Soxhlet apparatus for 24h. The methanolic extract was evaporated to dryness at 40°C and redissolved in methanol at a concentration of 5 mg/ ml, and stored at 4°C prior to further use (Barros et al., 2007).

Catalase (CAT)

The assay mixture contained 0.5 ml of H_2O_2, 1.0 ml of 0.01 M Phosphate buffer (pH 7.0) and 0.4 ml water. To initiate the reaction, 0.2 ml of the enzyme was added. Dichromate/acetic acid reagent (2 ml) was added after 0, 30, 60, and 90 seconds of incubation. To the control tube, the enzyme was added after the addition of acid reagent. The tubes were then heated for 10 min and the colour developed

was read at 610 nm (Sinha 1972). The activity of catalase is expressed as μM of H_2O_2 decomposed /min/mg protein.

Superoxide dismutase (SOD)

An aliquot of 1.4 ml reaction mixture was pipetted out in a test tube. Enzyme extract (100 μl) was added followed by a pre-incubation at 37ºC for 5 min. Riboflavin (80 μl) was added and the tubes were exposed for 10 min to 200 W Philips fluorescent lamps. The control tube contained equal amount of buffer instead of sample. The sample and its respective control were run together. At the end of the exposure time, 1.0 ml of Greiss reagent was added to each tube and the absorbance of the colour formed was measured at 543 nm (Chao, 2001). The enzyme activity is expressed as inhibition of 50 per cent nitrite formation/min/mg protein.

Glutathione peroxidase (GPX)

An aliquot of 0.4 ml of 0.4 M sodium phosphate buffer (PH 7.0) was pipetted out along with 0.1 ml of sodium azide, 0.2 ml of reduced glutathione, 0.1 ml of H_2O_2, 0.2 ml of enzyme extract and 1.0 ml of water to a final incubation volume of 2.0 ml. The tubes were incubated for 0, 30, 60, and 90 seconds. The reaction was then terminated by the addition of 0.5 ml trichloroacetic acid. To determine the glutathione content, 2.0 ml of the supernatant removed by centrifugation was added to 3.0 ml disodium hydrogen phosphate solution and 1.0 ml of DTNB (2, 2, Dithiobis 5 nitro pyridine) reagent. The colour developed was read at 412 nm. Standards in the range of 200–1000 μg were taken and treated in the similar manner (Rotruck et al., 1973). The activity was expressed in terms of μg of glutathione utilized/min/mg protein.

Peroxidase (POX)

A reaction mixture was prepared in a test tube by adding 3.0 ml of 0.05 M pyrogallol solution and 0.1 ml of enzyme extract. The spectrometer was adjusted from 0–430 nm. Then 0.5 ml of 1 per cent H_2O_2 was added in the test cuvette mixed and the change was recorded in absorbance for every 30 sec up to 3 min. The difference in OD change per minute with and without enzyme addition was a measure of peroxidase activity (Sadasivam and Manickam, 1992). The activity is expressed in terms of μ moles of pyrogallol oxidized/min/mg protein.

Glutathione-S-transferase (GST)

A reaction mixture was prepared in a test tube by adding 1.0 ml of 0.5 M Phosphate buffer, 0.1 ml of enzyme extract, 1.7 ml of water and 0.1 ml of CDNB (1-chloro 2,4 dinitrobenzene) was incubated at 37 ºC for 5 min. After incubation, 0.1 ml of reduced glutathione was added. The increase in optical density of the enzyme was measured against the blank at 340 nm (Lin, 1999). The enzyme activity was calculated in terms of μM of CDNB conjugate formed/min/mg protein.

Glutathione reductase (GTR)

A mixture consisting 0.2 ml of enzyme extract, 1.5 ml of 0.3 M Phosphate buffer, and 0.5 ml of EDTA (Ethelene diamine tetra acidic acid) 0.2 ml of GSSG (Oxidised glutathione) and 0.1 ml NADPH (Nicotinanide adedine nucleotide disphosphate) were prepared in a test tube. The decrease in optical density of the enzyme was measured against the blank at 340 nm (Lin, 1999). The enzyme activity was calculated in terms of μM of glutathione utilized/min/mg protein.

DPPH radical scavenging activity

The scavenging activity of the free and bound extracts on 1, 1-diphenyl 2-picrylhydrazyl (DPPH) radical was measured according to the method of Cheung et al. (2003) with some modifications. Aliquots (various concentration of methanol extract) of 0.8 ml of 0.2 mM DPPH methanolic solution was mixed with 0.2 ml of the extracts. The mixture was vigorously shaken and left to stand for 10 min. under subdued light. The absorbance was measured at 520 nm. The DPPH radical scavenging activity (%) was calculated by the following equation:

Radical scavenging activity (%) = $(1 - A_{sample}/ A_{control}) \times 100$

Where A_{sample} is the absorbance in the presence of sample and $A_{control}$ is the absorbance in the absence of sample, respectively. All extracts were analyzed in triplicate.

Reducing power

The reducing power was determined according to the method of Oyaizu (1986) with some modifications. Various concentrations of methanolic extracts from mushrooms (2.5 ml) were mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6, Wako Pure Chemical Co., Osaka, Japan) and 2.5 ml of 1% potassium ferricyanide (Sigma), and the mixture was incubated at 50°C for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v, Wako) were added, the mixture was centrifuged at 650 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionised water and 1 ml of 0.1% ferric chloride (Wako), and the absorbance was measured at 700 nm in a 2020 Double Beem Spectrophotometer. A higher absorbance indicates a higher reducing power.

Statistical Analysis

Statistical analysis was performed on the data by Dunccan's Multiple Range Test (DMRT) with means followed by a common letter are not significantly different at the 5% level by DMRT. For graphical displays, Box plots and whisker plots were used for treating the results by ORIGIN PRO 6.0.

Results and Discussion

The successful commercial cultivation of paddy straw mushroom depends to a large extent on quality and purity of spawn (Kalaiselvan, 2007). In this *in vitro* study, it was seen that calcium carbonate significantly enhanced the biomass production up to 2.95±0.05 g/ l for the additives Calcium carbonate followed by Sawdust (Figure 1). The beneficial effect of calcium carbonate on fungal growth might be due to the creation of neutral pH in the medium that enhanced the fungal growth, chlamydospore formation and density, and biomass production (Kalaiselvan, 2007). Sangeetha (2004) reported that the media amended with gram powders enhanced the fungal growth and chlamydospores density of *V. volvacea* due to increase in protein content of the substrate as stated by Kattam et al. (1991). The great potentiality of *V. volvacea* by virtue of its fast mycelial growth and greater biomass production due to calcium carbonate was evident from this study.

Yield evaluations for selecting a better performing strain have also been performed earlier by several researchers (Ahlawat et al., 2008). However, some of them have correlated morphological and biochemical characteristics of a strain with yield potential. They considered these parameters for the selection of promising strains for commercial cultivation. This study showed that isolate Calcium carbonate recorded highest yield of 1356.98±1.1 g/ bed and was followed by Sawdust (1305± 0.9 g/ bed). The other additives were recorded fewer yields compared to Calcium carbonate and Sawdust (Figure 2). A variation in nutrients and trace metal levels of 26 strains of *V. volvacea* was also reported by Ahlawat et al. (2008). In this study, fabulous yielding isolates Calcium carbonate and Sawdust recorded high levels of macro and micronutrients, this results; N: 1.47±0.22; 1.45±0.37%, P: 1.22±0.14; 0.92±0.06%, K: 879.77±9.50; 841.41±9.2 ppm/g, Ca: 11.45±0.96; 10.73±1.09 ppm/g; Mg: 2.13±0.03; 1.95±0.05 ppm/g, Cu: 0.33±0.06 – 0.29±0.02 ppm/g, Zn 1.38±0.98 – 1.12±0.02 ppm/g, Fe: 0.41±0.1 – 0.35±0.05 ppm/g, Mn: 7.86±0.04; 3.45±0.05 ppm/g (Table 1).

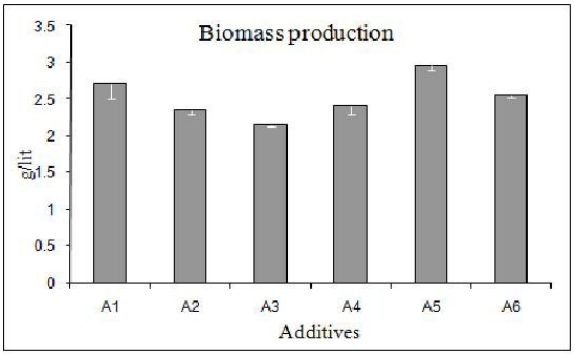

Figure 1. Biomass production of *Volvariella volvacea*.
A1- Sawdust, A2- Gypsum, A3- Calcium carbonate + gypsum, A4- Sawdust + rice bran, A5- Calcium carbonate, A6- Rice bran.
Each value is expressed as mean ± standard deviation.

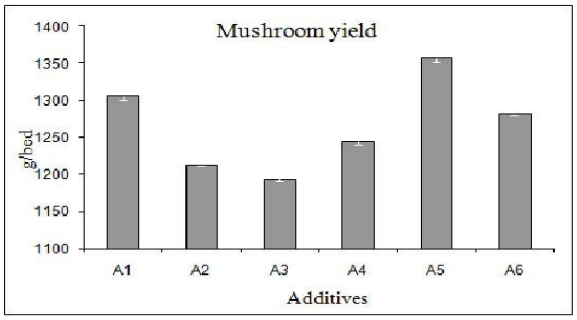

Figure 2. Yield performances in *Volvariella volvacea*.
A1- Sawdust, A2- Gypsum, A3- Calcium carbonate + gypsum, A4- Sawdust + rice bran, A5- Calcium carbonate, A6- Rice bran.
Each value is expressed as mean ± standard deviation

Table 1. Nutrient and trace metal levels in *Volvariella volvacea.*

Additive	N	P	K	Ca	Mg	Cu	Zn	Fe	Mn
A1	1.45±0.37^{a}	0.92±0.06^{b}	841.41±9.2^{b}	10.73±1.09^{b}	1.95±0.05^{b}	0.29±0.02^{b}	1.12±0.02^{b}	0.35±0.05^{b}	3.45±0.05^{b}
A2	0.97±0.17^{c}	0.72±0.07^{c}	666.60±4.07^{d}	7.66±0.90^{c}	0.79±0.09^{d}	0.16±0.04^{d}	0.93±0.21^{c}	0.27±0.07^{c}	2.93±0.03^{d}
A3	0.44±0.13^{d}	0.53±0.06^{d}	439.11±2.83^{e}	6.64±0.94^{d}	0.43±0.03^{e}	0.11±0.06^{e}	0.77±0.22^{d}	0.19±0.09^{d}	2.11±0.1^{e}
A4	0.92±0.09^{c}	0.72±0.07^{c}	680.74±9.51^{c}	7.68±1.00^{c}	0.89±0.04^{c}	0.18±0.03^{c}	0.93±0.05^{c}	0.31±0.05^{b}	3.03±0.03^{d}
A5	1.47±0.22^{a}	1.22±0.14^{a}	879.77±9.50^{a}	11.45±0.96^{a}	2.13±0.03^{a}	0.33±0.06^{a}	1.38±0.98^{a}	0.41±0.1^{a}	7.86±0.04^{a}
A6	1.14±0.20^{b}	1.01±0.12^{b}	841.41±5.61^{b}	10.63±1.16^{b}	0.94±0.05^{c}	0.19±0.05^{c}	0.96±0.04^{c}	0.33±0.02^{b}	3.25±0.05^{c}

A1- Sawdust, A2- Gypsum, A3- Calcium carbonate + gypsum, A4- Sawdust + rice bran, A5- Calcium carbonate, A6- Rice bran. Mean of three replicated. Means followed by a common letter are not significantly different at the 5% level by DMRT. Units: Nitrogen, phosphorus- %, Potassium, calcium, magnesium, copper, zinc, iron, and manganese- ppm/ g.

Food rich in antioxidative substances have been shown to play an essential role in the prevention of cardiovascular disease and cancers (Dragsted et al., 1993), neurogenerative diseases and inflammation (Joseph et al., 1999). The use of synthetic antioxidants has been restricted because of their possible toxic and carcinogenic effects (Gazzani et al., 1998). The present study clearly showed that the isolate Calcium carbonate (mycelial mat; Dried mushroom) recorded highest levels of antioxidants; catalase (90.15±0.15; 37.37±0.06 µmol), superoxide dismutase (22.69±0.09; 29.21±0.04 µmol), glutathione peroxidase (17.88±0.03; 48.88±0.07 µmol), peroxidase (5.11±0.03; 6.35±0.05 µmol), glutathione-S-transferase (30.52±0.06; 12.93±0.07 µmol), and glutathione reductase 8.15±0.05; 27.44±0.03 µmol), in mycelial mats and dried mushroom (Table 2, 3). This was followed by Sawdust and Sawdust + rice bran. Recently, Kalaiselvan (2007) reported high levels of antioxidative substances due to calcium carbonate activity in *P. euos* and *V. volvacea*. Murcia et al. (2002) reported that all truffles (*Terfezia* and *Piscoa* spp.) and five mushrooms *(Leptista nuda, Lentinus edodes, Agrocybe cylindracea, Cantharellus lutescens, and Hydrum sepundum*) exhibited higher percentage of oxidative inhibition based on lipid peroxidation, deoxyribose, and peroxidase.

Table 2. Antioxidant activity of the methanol extracts in mushroom mycelial mat.

Additives	CAT	SOD	GPX	POX	GST	GTR
Sawdust	88.21±0.20^{b}	20.11±0.04^{b}	16.74±0.06^{b}	4.23±0.09^{b}	29.85±0.06^{b}	8.03±0.06^{b}
Gypsum	80.65±0.05^{e}	18.13±0.1^{e}	14.15±0.06^{e}	3.48±0.07^{e}	27.95±0.05^{e}	7.59±0.05^{d}
Calcium carbonate + gypsum	79.39±0.09^{f}	15.19±0.09^{f}	13.15±0.04^{f}	3.04±0.06^{f}	27.05±0.06^{f}	7.04±0.07^{e}
Sawdust + rice bran	84.43±0.10^{d}	18.63±0.03^{d}	14.39±0.1^{d}	3.62±0.02^{d}	28.16±0.05^{d}	7.76±0.06^{c}
Calcium carbonate	90.15±0.15^{a}	22.69±0.09^{a}	17.88±0.03^{a}	5.11±0.03^{a}	30.52±0.06^{a}	8.15±0.05^{a}
Rice bran	86.03±0.63^{c}	18.82±0.08^{c}	16.63±1.72^{c}	3.88±0.07^{c}	28.73±0.04^{c}	7.84±0.08^{c}

Inhibition of 50 per cent nitrite formation / min / mg protein, GPX- 1 µ mole of glutathione utilized / min / mg protein, POX- 1 µ mole of pyrogallol oxidized / min / mg protein, GST - 1 µ mole of CDNB conjugate formed / min / mg protein, GTR - 1 µ mole of glutathione utilized / min /mg protein.

Table 3. Antioxidant activity of the methanol extracts in dried mushroom.

Isolate	CAT	SOD	GPX	POX	GST	GTR
Sawdust	35.05±0.06^{b}	28.13±0.06^{b}	47.29±0.06^{b}	5.9±0.4^{b}	12.05±0.05^{b}	26.48±0.24^{b}
Gypsum	32.42±0.04^{e}	25.15±0.05^{e}	45.28±0.08^{d}	4.95±0.06^{e}	10.75±0.06^{d}	23.69±0.11^{e}
Calcium carbonate + gypsum	29.05±0.08^{f}	23.92±0.07^{f}	41.12±0.03^{e}	4.09±0.03^{f}	10.18±0.36^{e}	22.18±0.05^{f}
Sawdust + rice bran	32.95±0.05^{d}	26.01±0.08^{d}	46.19±0.04^{c}	5.01±0.04^{d}	10.71±0.09^{d}	23.96±0.06^{d}
Calcium carbonate	37.37±0.06^{a}	29.21±0.04^{a}	48.88±0.07^{a}	6.35±0.05^{a}	12.93±0.07^{a}	27.44±0.03^{a}
Rice bran	33.93±0.61^{c}	27.16±0.06^{c}	47.02±0.08^{b}	5.16±0.03^{c}	11.09±0.07^{c}	24.14±0.06^{c}

Mean of three replications Means followed by a common letter are not significantly different at the 5% level by DMRT. Units: CAT - 1 µ mole of H_2O_2 decomposed / min / mg protein, SOD - µ mole of Inhibition of 50 per cent nitrite formation / min / mg protein, GPX- 1 µ mole of glutathione utilized / min / mg protein, POX- 1 µ mole of pyrogallol oxidized / min / mg protein, GST - 1 µ mole of CDNB conjugate formed / min / mg protein, GTR - 1 µ mole of glutathione utilized / min /mg protein.

Methanol and water crude extracts from shiitake and paddy straw mushroom were investigated for their antioxidant capacity in three different assays, the β-carotene and linoleic acid system, DPPH, radical scavenging activity, and inhibition of haemolysis of rat erythrocyte induced by peroxyl radicals (Cheung et al., 2003). In this study, DPPH scavenging activities (31.71±0.05 – 92.05±0.06 mg/ ml) (Figure 3) and reducing power (0.29±0.05 – 0.45±0.04 mg/ ml) (Figure 4) in dried mushroom were also higher in calcium carbonate isolate; this was followed by the isolate sawdust. Methanol extract of *P. florida* have potent hydroxyl radical scavenging and lipid peroxidation inhibition (antioxidant) activities. The antioxidant activities in *Ganoderma lucidum, P. florida*, and *P. sajor-caju*, as scavenging activity were demonstrated by Lakshmi et al. (2004). Cheung et al. (2003) reported that the four mushroom extracts from *L. edodes* showed the most potent radical scavenging activity in each assay, showing 75.9% (at 20 mg/ ml) in the β-carotene bleaching method, 55.4% in the DPPH radical scavenging method (at 6 mg/ ml), and 94.0% of inhibition of erythrocyte hemolysis (at 5 mg/ ml). Total phenolics in the water extracts were higher antioxidant activity compared to methanol extracts. The occurrence and distribution of different macro and micro elements in fruiting bodies of certain mushrooms is not only an important problem for physiology and ecology

of fungi, but also has practical environmental and toxicological aspects. As evident in this and several other studies (Maria Rudawska et al., 2005).

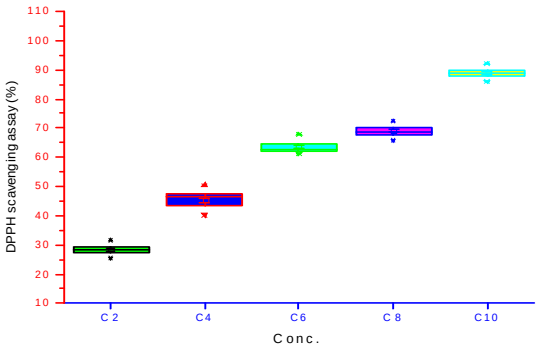

Figure 3. Scavenging effect of methanolic extracts from *Volvariella volvacea* on 1, 1-diphenyl-2-picrylhydrazyl radical.
Concentration: C2- 2mg/ ml, C4- 4mg/ ml, C6- 6 mg/ ml, C8- 8 mg/ ml and C10- 10 mg/ ml
Each value is expressed as mean± standard deviation.

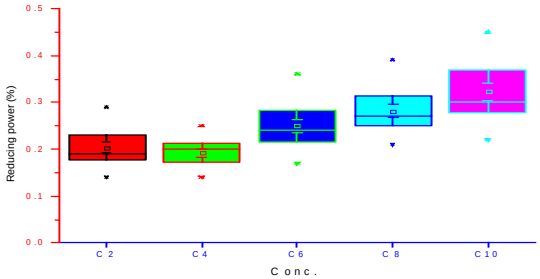

Figure 4. Reducing power of different concentrations of methanol extract of dried mushrooms.
Concentration: C2- 2mg/ ml, C4- 4mg/ ml, C6- 6 mg/ ml, C8- 8 mg/ ml and C10- 10 mg/ ml
Values are expressed as mean± standard deviation.

Conclusion

According to the results of this study, it is clearly indicated that the methanolic extract of mushroom species has significant antioxidant activity against various antioxidant systems. From the above assays, the possible mechanisms of the antioxidant activity of the mushroom extracts include scavenging of free radicals also get good responsible. Moreover, the mushroom species can be used as an easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry.

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