Indian Journal of Natural Products and Resources
Vol. 5(1), March 2014, pp. 56-61

Study on *in vitro* antioxidant potential of some cultivated *Pleurotus* species (Oyster mushroom)

Gajendra S Jeena[1], H Punetha[1]*, Om Prakash[2], Mahesh Chandra[2] and K P S Kushwaha[3]

[1]Department of Biochemistry, [2]Department of Chemistry, College of Basic Sciences & Humanities, [3]M RTC, GBPUA &T, Pantnagar-263145, Uttarakhand, India

Received 2 July 2012; Accepted 4 November 2013

The antioxidant potential and quantitative phenolic from the methanolic extract of *Pleurotus sajor-caju* (Fr.) Singer, *P. ostreatus* (Jacq. ex Fr.) P. Kumm. and *P. sapidus* (Schulzer & Kalchbr.) Sacc. at mature fruiting stage and mycelium stage were investigated. Fruiting bodies of *Pleurotus* species exhibited the most potent free radical scavenging activity (*P. sajor-caju* 88.9 % at 10 mg/mL) and reducing power (1.98 at 10 mg/mL) while mycelia exhibiting higher chelating activity at 10 mg/mL (*P. sapidus* 99 %). Gallic acid, BHT and Catechin used as a standard were highly effective in inhibiting hydroxyl and superoxide radicals, showing IC_{50} values below 1 mg/mL. *Pleurotus sajor-caju* exhibited maximum antioxidant potential as compared to other two mushrooms. Analysis of total phenols and flavonoids in *Pleurotus* species has been carried out *in vitro. Pleurotus sajor-caju* fruiting contains highest phenol content (1.53 mg/g) and flavonoids (1.88 mg/g). These results suggest that these mushrooms may be used as a potential source of natural antioxidants for food supplements as well as in the development of nutraceuticals.

Keywords: *Pleurotus* species, Oyster, Mushrooms, Antioxidant activity, Phenols, Flavonoids,

IPC code; Int. cl. (2013.01)–A61K 36/00

Introduction

Oyster mushroom is an edible white-rot fungus and is classified into the genus *Pleurotus* (Jacq. ex Fr.) Kumm. comprises about 40 species[1]. Mushrooms have a great nutritional value since they are quite rich in protein, with an important content of essential amino acids and fibre and poor in fat. Edible mushrooms also provide a nutritionally significant content of vitamins (B1, B2, B12, C, D, and E) [2]. Edible mushrooms could be a source of many different nutraceuticals such as unsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid and carotenoids. Thus, they might be used directly in diet to promote health, taking advantage of the additive and synergistic effects of all the bioactive compounds present[3-5]. Oxidation is essential to many living organisms for the production of energy to fuel in biological processes. Free radicals are produced in normal and or pathological cell metabolism[6]. However, the uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with ageing. Antioxidant properties of phenolic compounds may help the endogenous defence system, assuming a major importance as possible protector agents reducing oxidative damage[4]. The edible mushrooms analysed could be directly used in the human diet to combat oxidative stress, while inedible species could represent a source of extractable phenolic compounds to be used as additives in the food industry or as components in pharmaceutical and cosmetic formulations, due to their well-known antioxidant properties[7]. Till now, research has tended to focus on the dietary value and cultivation of edible mushrooms; but, there is limited data in the literature concerning the antioxidant properties of the edible wild mushrooms and comparison between fruiting stage and mycelia stage. Hence, the present study focussed to determine antioxidant potential and *in vitro* phenolic at fruiting and mycelia stage of some cultivated *Pleurotus* species.

Materials and Methods

Preparation of methanolic extract

Three kinds of oyster mushroom *Pleurotus sajor-caju* (Fr.) Singer, *P. ostreatus* (Jacq ex Fr.)

*Correspondent author:
Phone: 09411300297
E-mail: punetha_hp@rediffmail.com,oporgchem@gmail.com

P. Kumm. and *P. sapidus* (Schulzer & Kalchbr.) Sacc (Plate 1) were harvested during early mature fruiting stage at Mushroom Research Training Centre, Pantnagar. To obtain the fungal mycelia of these oyster mushrooms, inoculums were grown in potato dextrose broth at 120 rpm, 25°C for 10-12 days. The fruit bodies were cleaned to remove any residue and mycelia were extracted through filtration. The freeze dried mushrooms were ground using a blender and then stored at -80°C along with mycelia for further analysis. A 20 g dried mushroom and mycelia sample was extracted using 100 mL of methanol overnight at room temperature, and the extract was filtered using a Whatman No.2 filter paper. The residue was then extracted with two additional portion of methanol under same condition. The methanolic extracts of mushrooms were combined and evaporated using a rotary evaporator at 40°C for dryness. For analysing samples, the dried extract was resolubilized in methanol and then five different concentrations of mushroom methanolic extracts (2, 4, 6, 8, 10 mg/mL) were prepared.

Scavenging effect on 1, 1-Diphenyl-2-picrylhydrazyl radical

The scavenging activity was determined by DPPH method developed earlier[8]. 1 mL of methanolic extract was mixed with 5 mL of 0.004% methanolic solution of DPPH. Each mixture was placed in dark for 30 min. The absorbance was measured at 517 nm using a spectrophotometer.

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

Reducing power

Reducing power was determined by the method developed earlier[8]. Each concentration of methanolic extracts (1 mL) was mixed with 2.5 mL phosphate buffer (200mM, pH6.6) and 2.5 mL of 1% potassium ferricyanide followed by incubate at 50°C for 20min. After incubation, a 2.5 mL of 10% trichloroacetic acid was added to the mixture and then centrifuged at 650 rpm for 10min. The supernatant (5 mL) was mixed with 5 mL distilled water and 1 mL of 1% ferric chloride, and then its absorbance was measured at 700 nm.

Chelating effects on ferrous ions

Chelating ability was determined according to the standard method[9]. Each extract (1 mL) was mixed with 0.1 mL of 2 mM $FeCl_2$. The reaction was initiated by the addition of 5 mM ferrozine and make up volume to 5 mL by adding methanol. After 10 min at room temperature, the absorbance of the mixture was determined at 562 nm % chelating activity = $(1 - A_{\text{sample}} / A_{\text{control}}) \times 100$

Total phenolic contents (TPC)

Total phenolic content of the methanolic extract of mushroom were measured according to the standard method developed earlier[10]. Each extract (1 mL) was mixed with 1 mL of saturated sodium carbonate solution and 0.4 mL Folin-Ciocalteau reagent. Made the volume up to 10 mL by adding distilled water. Incubate at 27° C for 1h and absorbance was recorded at 765 nm. TPC were expressed as Gallic acid equivalents.

Ortho-Dihydroxy phenols

Ortho-Dihydroxy phenols in the mushroom extract were determined by standard method[11]. Each extract (1 mL) was mixed with 1mL of 0.5N HCL and Arnow's reagent. To the reaction mixture add 2 mL of 1N aqueous sodium hydroxide and then absorbance was measured at 515 nm.

Total flavonoid contents (TFC)

Flavonoid content in the methanolic extract of oyster mushroom was determined by the method

Plate 1—Fruiting body of *P. sajor-caju* (a), *P. sapidus* (b), *P. ostreatus* (c)

developed earlier[12] and results were expressed as mg (+) catechins equivalents per gm of mushrooms. Standard solution or mushroom extract (1 mL) was mixed with 1.25 mL of distilled water and 75 µL of 5% $NaNO_2$ solution. After incubation of 5 min, 150 µL of 10 % $AlCl_3.H_2O$ was added. After 6 min, 500 µL of 1M NaOH and 275 µL of distilled water were added to the mixture. The solution was mixed and intensity of pink colour was measured at 510 nm.

Statistical analysis

All the analyses were performed in triplicates and these results were reported as means ± standard deviation (SD). The significance of differences among treatment means were determined by one way analysis of variance (ANOVA) with a significant level of ($p < 0.05$).

Results and Discussion

DPPH radical scavenging activity

The DPPH radical scavenging activity of the methanolic Oyster mushroom extracts increased with increase in concentrations. At 10 mg/mL the radical scavenging activities of *P. sajor-caju, P. ostreatus* and *P. sapidus* fruiting bodies (fb) were 88.9, 87.4 and 85 % which is much higher as compared to their mycelia (53.9, 44.1 and 75%). Fruiting bodies of *P. sajor-caju* and *P. ostreatus* showing free radical scavenging activity equivalent to that of standard antioxidants, BHT, gallic acid and catechin at 10 mg/mL. DPPH radical scavenging activities of *P. ostreatus* were 81.8% at 6.4 mg/mL and 68.4% at 5 mg/mL[13-14]. *P. eryngii* (DC. ex Fr.) Quel., *P. dijamor* (Fr.) Boedjin and *P. flabellatus* (Berk. & Br.) Sacc. have higher radical free scavenging activity than *P. sajor-caju* and *P. florida* at 100 % mycelia extract[15]. Our results showed that *P. sajor-caju* (fb) has the lowest IC_{50} value which is about 2 mg/mL. Among the mycelia, *P. sapidus* was found to be more efficient than *P. sajor-caju* and *P. ostreatus* with IC_{50} value about 3 mg/mL (Fig.1). Free radical scavenging activity has been known as an established phenomenon in inhibiting lipid oxidation, which otherwise can be deleterious to the cellular components and cellular function[16]. With the presence of radical scavenging activity, consumption of wild mushrooms might be beneficial to protect human body against oxidative damage, which can be further developed into health related degenerative illnesses.

Reducing power

Reducing power of a compound may serve as a significant indicator of its potential antioxidant activity[17]. The presence of reducers (antioxidants) causes the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form. In present study, *P. sajor-caju* fruiting had an excellent reducing power (0.759 at 2 mg/mL and 1.980 at 10 mg/mL), showing that its reducing ability was more effective than the fruiting of *P. sapidus* and *P. ostreatus* (1.97 and 1.78 at 10 mg/mL) (Fig. 2). Among the mycelia of these *Pleurotus* species, highest reducing power was shown by *P. sajor-caju* (1.672) followed by *P. ostreatus* (1.371) and *P. sapidus* (1.010) at 10 mg/mL (Fig 2). Ethanolic extract of the mushroom *Pleurotus citrinopileatus* Singer has been reported to exhibit the

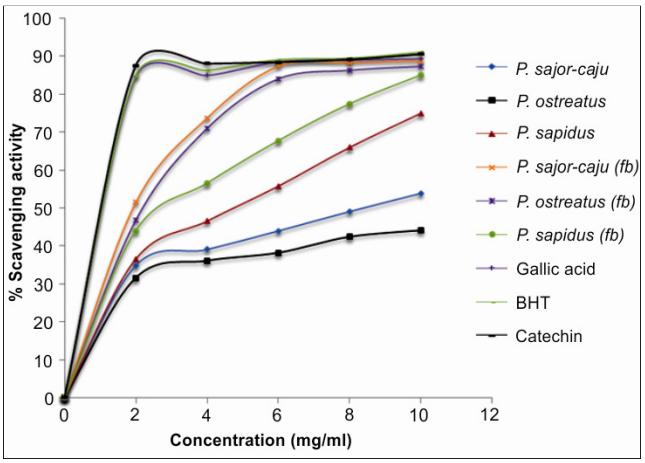

Fig.1—Scavenging effects of DPPH radical of the methanolic extracts of some *Pleurotus* species at different concentrations. Each value is expressed as mean ± SD (n=3).

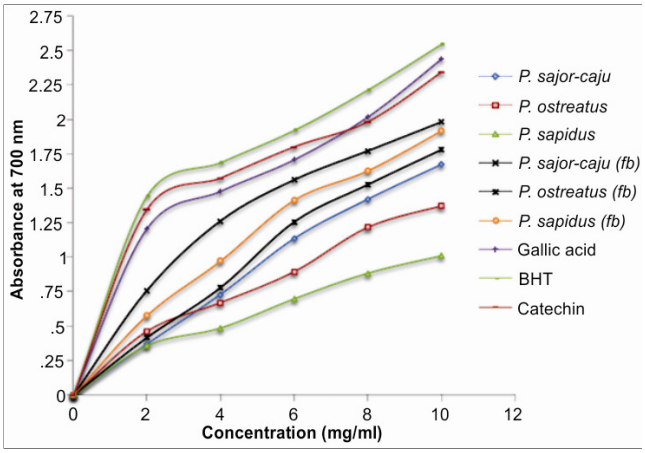

Fig. 2—Reducing power of methanolic extracts of some *Pleurotus* species at different concentrations. Each value is expressed as mean ± SD (n=3).

reducing power of 1.05 at 10 mg/mL[18]. The antioxidant activity has been reported to be concomitant with the development of reducing capacity[19]. Therefore, reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[20]. However, absorbance of BHT at 20 mg/mL was much higher (2.660) indicating synthetic antioxidant has better reducing ability compared to antioxidant from wild mushrooms. Similarly, the reducing power of the wild mushrooms was much lower than other edible mushrooms reported elsewhere[21]. However, the reducing power of the methanolic extract from *P. porrigens* (Pers. ex Fr.) Kumm. steadily increase to 0.2 at 20 mg/mL[22] which is found to be lower as compared to the edible mushrooms used in present study.

Chelating effect on ferrous ion

The range and the mean of Fe^{2+} chelating capacities varied significantly among different types of mushrooms. In this assay, extract of the mushroom species interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture ferrous ion before ferrozine. Chelating effect of methanolic extracts from different oyster mushrooms on ferrous ions increased as the concentration increases from 2 mg/mL to 10 mg/mL (Fig. 3). At 10 mg/mL, fruiting bodies of *P. sapidus* had an outstanding chelating ability (94 %) which is more than the synthetic metal chelators EDTA (92.3 %) and citric acid (87.6 %) while *P. ostreatus* and *P. sajor-caju* has 88.2 % and 69.7 % chelating ability. *Pleurotus* species at their mycelia stage have greater chelating

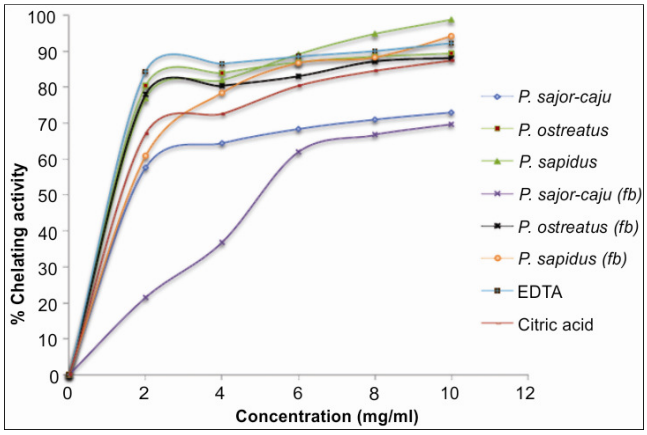

Fig. 3—Chelating effects on ferrous ion of methanolic extracts of some *Pleurotus* species at different concentrations. Each value is expressed as mean ± SD (n=3).

effect on ferrous ion as compared to their fruiting bodies. *P. sapidus* was found to be the lowest IC_{50} value below 1.5 mg/mL which shows that *P. sapidus* has highest ferrous ion chelating activity. The order of chelating effect on ferrous ion is *P. sapidus* (99 %) > *P. ostreatus* (89.4 %) > *P. sajor-caju* (73 %). Reverse case has been observed to that of DPPH radical scavenging activity and reducing power. Fig. 3 reveals that the methanolic extract of edible mushrooms in this study showed a marked capacity for iron binding, suggesting that their action as per oxidation protector may be related to its iron binding capacity. Fruit bodies of *P. citrinopileatus* have been reported to chelate ferrous ion by 82.1% at 5 mg/mL, showing that it is more effective than its mycelia[17]. Chelating abilities of *P. eryngii, P. ferulae* and *P. ostreatus* at 5 mg/mL were 41.4-64 %[14]. For commercial mushrooms including winter, oyster mushrooms and shiitake, their methanolic extracts chelated 45.6-81.6 % of ferrous ion at 1.6 mg/mL[13]. A heteroglycan isolated from the mycelia of *P. ostreatus* also have a marked capacity for iron binding ability of 54.82 % at a concentration of 1 mg/mL[23].

Total phenolic contents (TPC)

The measurement of the amount of total phenolic compounds as well as the identification of the main phenolics in mushrooms, have great importance in nutritional and functional characterization[24]. The TPC of oyster mushrooms fruiting body were in the order of *P. sajor-caju* (1.53 mg/g) > *P. ostreatus* (1.32 mg/g) > *P. sapidus* (1.10 mg/g) while the TPC of the mycelia were found to be *P. sajor-caju* (0.69 mg/g), *P. ostreatus* (0.68 mg/g) and *P. sapidus* (0.50 mg/g) (Table 1). These TPC results could show the significant correlation to the antioxidant activities of the three oyster mushrooms in terms of radical

Table 1—Total Phenol Content (TPC), o-Dihydroxyphenol content and Total Flavonoid Content (TFC) of methanolic extracts from Oyster mushrooms fruiting bodies (fb) and mycelia

Samples	TPC (mg/g)	o-Dihydroxy phenol (mg/g)	TFC (mg/g)
P. sajor-caju	0.69±0.10	0.17±0.20	1.78±0.08
P. ostreatus	0.68±0.10	0.16±0.10	1.56±0.06
P. sapidus	0.50±0.05	0.10±0.01	0.98±.02
P. sajor-caju (fb)	1.53±0.09	0.36±0.05	1.88±0.10
P. ostreatus (fb)	1.32±0.10	0.30±0.02	1.82±0.50
P. sapidus (fb)	1.10±0.05	0.27±0.08	1.39±0.03

Each value is expressed as mean ± SD (n=3). Mean with different letters within a row are significantly different ($p<0.05$).

scavenging, reducing power, and chelating effects. Phenolics are the major naturally occurring antioxidant components found in methanolic extract of several mushroom species[25]. The most abundant polyphenols reported in edible mushroom extracts are *p*-benzoic acid, *p*-phenyl acetic acid, *o*-coumaric acid, ferulic acid and chrysin[26]. Phenolic groups such as flavonoids, lignin, and phenolic acids were found in *Agaricus bisporus* (J.E. Lange) Emil J. Imbach. (Button mushroom), *P. ostreatus* (Oyster mushroom) and *Lentinus edodes* (Berk.) Pegler (Shiitake mushroom) which contribute to the high phenolics content[27]. Numerous studies have showed the consumption of foods high in phenolics can reduce the risk of heart disease by slowing the progression of atherosclerosis due to their antioxidative properties[28-29]. In addition, it was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid per oxidation[30].

Total flavonoid contents (TFC)

Flavonoids are usually glycosylated and can be classified as anthocyanidins, flavanols (catechins), flavones, flavanones and flavonols which are responsible for the orange, red and blue color in fruits and vegetables. Generally, deep-colored fruits, vegetables or foods are recognized as more healthy to human body[31]. There has been a growing interest in pigment components of natural food, which may promote human health or lower the risk for diseases. As shown in Table 1, total phenolic contents in all the three mushrooms were higher than corresponding mycelia. Similar case was observed in total flavonoid contents. *P. sajor-caju* fruiting has higher flavonoid content (1.88 mg/g) as compared to *P. ostreatus* (1.82 mg/g) and *P. sapidus* (1.39 mg/g). The concentration of flavonoids varies depending on the mushroom species and the total flavonoid content does not correlate with the phenolic concentration. Thus, *Lactarius deliciosus* (L. ex Fr.) S.F.Gray has the higher amount and *A. bisporous* and *P. ostreatus* present the lower flavonoid content. Total flavonoid content found in *P. ostreatus* is below 1 mg/g[32]. Total flavonoid content was estimated in oyster mushroom (*P. ostreatus*) dark grey, yellow and pink strains[33]. Reportedly dark grey strain has the highest flavonols (2.16 mg/g). Our results showed the significant increase in the flavonoid content at fruiting and mycelia stage.

Conclusion

This study showed that the methanolic extracts from different *Pleurotus* species had considerable antioxidant activities. Especially, *P. sajor-caju* fruiting exhibited the strongest antioxidant activities including scavenging ability and reducing power as compared to *P. ostreatus* and *P. sapidus*. Furthermore, antioxidant properties of three different oyster mushrooms were intimately linked with phenolic compounds content. *P. sajor-caju* fruiting body has highest phenol and flavonoid content. Therefore, these results indicate that matured fruiting of oyster mushroom could be very beneficial for defending radical mediated toxicity and used as a potential source of natural antioxidants for food supplements as well as in the development of nutraceuticals.

Acknowledgements

Authors are thankful to DES Pantnagar and Joint Director MRTC for providing necessary facilities.

References

1 Jose N and Janardhanan KK, Antioxidant and antitumor activity of *Pleurotus florida*, *Curr Sci,* 2000, **79**, 941-943.

2 Heleno SA, Barros L, Sousa MJ, Martins A and Ferreira ICFR, Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity, *Food Chem,* 2010, **119**, 1443-1450.

3 Barros L, Baptista P and Ferreira ICFR, Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays, *Food Chem. Toxicology,* 2007, **45**, 1731-1737.

4 Ferreira ICFR, Barros L and Abreu RMV, Antioxidants in wild mushrooms, *Curr Med Chem,* 2009, **16**, 1543-1560.

5 Pereira E, Barros L, Martins A and Ferreira ICF, Towards chemical and nutritional inventory of Portuguese wild edible mushrooms in different habitats, *Food Chem,* 2012, **130**, 394-403.

6 Elmastas M, Isildak O, Turkekul I and Temur N, Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms, *J Food Comp Anal,* 2007, **20**, 337-345.

7 Josiana A, Vaz Barros L, Martins A, Morais JS, Vasconcelos MH and Ferreira ICFR, Phenolic profile of seventeen Portuguese wild mushrooms, *Food Sci Technol,* 2011, **44**, 343-346.

8 Yen GC and Duh HY, Antioxidative properties methanolic extracts from peanut hulls, *J Amer Oil Chemist's Soc,* 1993, **70**, 383-386.

9 Hsu CL, Chen W, Weng YM and Tseng CY, Chemical composition, physical properties and antioxidant activities of yam flours as affected by different methods, *Food Chem,* 2003, **83**, 85-92.

10 Singleton VL and Rossi JA, Colorimetry of total phenolics with phosphomolybdic and phosphotungtic acid reagents,

American Journal of Enology and Viticulture, 1965, **16**, 144-158.

11 Arnow LE, Colorimetric determination of the components of 2, 4-dihydroxyphenylalanine–tyrosine mixtures, *J Biol Chem,* 1937, **118**, 531-53.

12 Choi Y, Lee SM, Chun J, Lee HB and Lee J, Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shitake *(Lentinus edodes)* mushroom, *Food Chem,* 2006, **99**, 381-387.

13 Mau JL, Chao GR and Wu K, Antioxidant properties of methanolic extracts from several ear mushroom, *J Agric Food Chem*, 2001, **49**, 5461-5467.

14 Lo SH, Quality evaluation of *Agaricus bisporus*, *Pleurotus eryngii*, *Pleurotus ferulae*, *Pleurotus ostreatus* and their antioxidant properties during postharvest storage, Master's thesis, 2005, National Chung-Hsing University, Taichung, Taiwan.

15 Mishra KK, Pal RS, Arunkumar R, Chandrashekara C, Jain SK and Bhatt JC, Antioxidant properties of different edible mushroom species and increased bioconversion efficiency of *Pleurotus eryngii* using locally available causing materials, *Food Chem*, 2013, **138**, 1557-1563.

16 Puttaraju NG, Venkateshaiah SU, Dharmesh SM, Urs SMN and Somasundaram R, Antioxidant activity of indigenous edible mushrooms, *J Agric Food Chem,* 2006, **54**, 9764-9772.

17 Oyaizu M, Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine, *Jap J Nutr*, 1986, **44**, 307-315.

18 Lee YL, Huang GW, Liang ZC and Mau JL, Antioxidant properties of three extracts from *Pleurotus citrinopileatus, Food Sci Technol,* 2007, **40**, 823-833.

19 Tanaka M, Kuie CW, Nagashima Y and Taguchi T, Application of antioxidative Maillard reaction products from histidine and glucosa to sadine products, *Nippon Suisan Gakkaishi,* 1988, **54**, 1409-1414.

20 Meir S, Kanner J, Akiri B and Hadas SP, Determination and involvement of aqueous reducing compounds in oxidative defence systems of various senescing leaves, *J Agric Food Chem,* 1995, **43**, 1813-1815.

21 Lee YL, Jian SY, Lian PY and Mau JL, Antioxidant properties of extract from a white mutant of the mushroom *Hypsizigis marmoreus*, *J Food Comp Anal,* 2008, **21**, 116-124.

22 Wong JY and Chye FY, Antioxidant properties of selected tropical wild edible mushrooms, *J Food Comp Anal,* 2009, **22**, 269-277.

23 Patra S, Patra P, Maity KK, Mandal S, Bhunia SK, Dey B, Devi KSP, Khatua S, Acharya K, Maiti TK and Islam SS, A heteroglycan from the mycelia of *P. ostreatus*: structure determination and study of antioxidant properties, *Carbohydrate Res*, 2013, **368**, 16-21.

24 Carvajal AESS, Koehnlein EA, Soares AA, Eler GJ, Nakashima ATA and Bracht A, Bioactives of fruiting bodies and submerged culture mycelia of *Agaricus brasiliensis* (*A. blazei*) and their antioxidant properties, *LWT- Food Sci Technol*, 2012, **46**, 493-499.

25 Tsai SY, Huang SJ and Mau J, Antioxidant properties of hot water extracts from *Agrocybe cylindracea*, *Food Chem,* 2006, **98**, 670-677.

26 Kalogeropoulos N, Yanni AE, Koutrotsios G and Aloupi M, Bioactive microconstituents and antioxidant properties of wild edible mushrooms from the island of Lesvos, Greece, *Food Chem Toxicol*, 2013, **55**, 378-385.

27 Mattila P, Konko K, and Eurola M, Contents of vitamins, minerals elements, and some phenolic compounds in cultivated mushrooms, *J Agric Food Chem,* 2001, **49**, 2343-2348.

28 Visioli F, Borsani L and Galli C, Diet and prevention of coronary heart disease: the potential role of phytochemicals, *Cardiovascular Res,* 2000, **47**, 419-425.

29 Meng CQ, Somers PK, Rachita CL, Holt LA, Zheng XS, Simpson JE, Hill RR, Olliff LK, Kunsch C, Sundell CL, Parthasarathy S, Saxena U, Sikorshi JA, Wasserman MA and Hoong LK, Novel phenolics antioxidants as multifunctional inhibitors of inducible VCAM-1 expression for use in atherosclerosis, *Bioorg Med Chem Letters*, 2002, **12**, 2545-2548.

30 Yen GC and Wu JY, Antioxidant and radical scavenging properties of extracts from *Ganoderma tsugae*, *Food Chem,* 1993, **65**, 375-379.

31 Lin JY and Tang CY, Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation, *Food Chem*, 2007, **101**, 140-147.

32 Palacios I, Lozano M, Moro C, Arrigo MD, Rostagno MA, Martinez JA, Garcia-lafuente A, Guillamon E and Villares A, Antioxidant properties of phenolic compounds occurring in edible mushrooms, *Food Chem,* 2011, **128** , 674-678.

33 Kim JH, Kim SJ, Park HR, Choi JI, Ju YC, Nam KC, and Lee SC, The different antioxidant and anticancer activities depending on color of Oyster mushrooms, *J Med Plants Res,* 2009, **3**, 1016-1020.