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Antioxidant Activity of three Species of Wild Mushroom Genus *Cantharellus* Collected from North-Western Himalaya, India

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ABSTRACT

The efficiency of antioxidant activity from *Cantharellus* species, namely *C. friessi, C. subcibarius* and *C. cinerius* mushrooms collected from North-Western Himalayan region of India was compared with *Pleurotus florida*. The total phenol contents of each species were analyzed in addition to some bioactive compounds. The analysis revealed that the total phenol contents showed major antioxidants components ranged from 9.55 to 16.8 mg/g in different mushrooms. The antioxidant activity by *C. friessi* was significantly higher than other mushrooms. The mushrooms investigated in the present study could represent easily accessible sources of natural antioxidant. The present study showed significant interest for the wild edible mushrooms due to the presence of natural antioxidants in them. © 2011 Friends Science Publishers

Key Words: Antioxidant; Nutraceutical; Total phenolics; Ascorbic acid; β-carotene

INTRODUCTION

Mushrooms are a well balanced food stuff that provide definite nutrition and health benefits for human. Mushrooms are known to produce many kind of bioactive compounds, generally linked with mycelial cell wall, that help in enhancing the immune capacity to fight against carcinogens (Ramesh & Pattar, 2010). Traditionally, wild edible mushrooms are used by most of the Asian and other countries worldwide as food and medicinal sources (Manzi *et al*., 1999; Sanmee *et al*., 2003) and also are important diet food based on their nutritive characteristics, as well as a good source of antioxidants. The world consumption of *Cantharellus* (also known as chanterelle) mushroom has been estimated at 150-200 km t/yr (Watling, 1997).

Various antioxidant compounds are used widely in different food products that help in providing protection to oxidative damaged by free-radical molecules. Natural products that contain antioxidant property help to protect the endogenous system. Such properties lead to growing interest of using mushrooms in various nutraceutic products (Yaltirak *et al*., 2009). Barros *et al*. (2007) reported the presence of antioxidant compounds in foods diets, which act as agents to reduce oxidative damage in human body. There are various edible mushroom species, which are sources of physiological agents for medicinal applications, antiviral, possessing antitumour, cardiovascular and antibacterial (Chang, 1996; Halpern & Miller, 2002; Wasser, 2002). Still there are several varieties of wild mushrooms whose nutritive and medicinal profiles have not been described well. The members of *Cantharellus* species are of one of such edible mushrooms that are needed to study.

North-Western Himalayan region of India has been known to place containing rich varieties of wild edible mushrooms including *Cantharellus* (Kumar *et al*., 1990; Upadhyay *et al*., 2008). During our survey in the forests of North-Western Himalayas, India the local people informed about the edibility and habitat of various species of *Cantharellus*. However, this precious knowledge is limited to the old aged villagers only and common people still do not know about the edibility of these mushrooms. The identity of these mushrooms is done based on their color and sweet smell but they have no idea about their nutritional values and uses. Local people collect such types of mushrooms from this region for their own food and also to sale in local market. They recognize the benefit of the additional foods, added flavor and the income from local sales and from export to other states or countries.

The members of *Cantharellus* species are well known edible mushrooms (Pilz *et al*., 2003). Some studies have confirmed that chanterelles are nutritious and rich sources of fat, carbohydrates, fiber and energy (Caglarirmak *et al*., 2002; Colak *et al*., 2007) but antioxidant properties of such wild mushrooms have not been reported. In the present scenario the phenolic compounds are frequently used for their advantageous biological property and play an important role in the improvement of overall health of human being (Merkl *et al*., 2010).

Keeping interest in the antioxidant properties of *Cantharellus* species, in the present study, we collected

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three different varieties of these mushrooms from North-Western Himalayan region of India. To date there is no report available on detailed antioxidant properties of *Cantharellus* collected from Himalayan region in literature. The efficiency of antioxidant activity of these mushrooms was also compared with a known edible mushroom, *Pleurotus florida*. The aim of current research work was to screen the antioxidant properties along with total phenolics, flavonoids, ascorbic acid and β-carotene contents of different varieties of mushrooms.

MATERIALS AND METHODS

Collection of mushrooms: Fruiting bodies of *Cantharellus* species were collected from the Dhalli reserve forest, Karol and Karsog forests of North Western Himalayas, India. Microscopic examinations (spore, hyphae, basidia & cystidia) were performed using Leica DM LS2 microscope. The identification of the species was made according to above systematical criteria obtained from macroscopic, microscopic and molecular examinations (Kumari *et al*., 2010). Mushroom samples were carried into the laboratory in an ice bath and stored in deep-frozen at –42°C. The antioxidant activities from these mushrooms were assayed in a week. The specimens collected in the present study, were deposited at the herbarium of Punjabi University, Patiala, India and also at the herbarium of Directorate of Mushroom Research (DMR), Solan, India. *Pleurotus florida* was procured from herbarium of DMR, Solan, India.

Preparation of extracts for antioxidant assay: The methanolic extracts of all the four mushrooms species were done according to Barros *et al*. (2007) with little modifications. The fruit bodies were dried and powdered. Five gram sample was used for extraction with 10 mL of methanol at room temperature and centrifuged followed by filtration. The resultant solution was extracted twice with same volume of methanol. The methanol was evaporated by keeping the extracts open in 100 mL beakers at 37ºC for 2-3 days in an incubator. The yield was calculated after evaporation and the remaining residue from the extracts was re-suspended in methanol and water to make final concentration, 10 mg/mL. The extracts were stored in the dark at 4°C.

Total antioxidant activity: The antioxidant property from the extracts of mushroom was calculated as per β-carotene bleaching method according to Velioglu *et al*. (1998). The reagent mixture consisted of β-carotene (1 mL, 0.2 mg/mL in chloroform), linoleic acid (20 µL) and Tween 80 (200 µL). The chemical reagents used were of Sigma, USA. The contents of mixture were evaporated under nitrogen stream. A 50 mL of oxygenated water was later added to reaction mixture. Antioxidant activity was assayed in 4.8 mL of reagent mixture added with 200 µL of methanolic extracts of mushrooms with concentrations ranging from 2-10 mg/mL. Methanol and water served as control and blank respectively and reaction mixture consisted of all chemicals except β-carotene, incubated for 2 h at 50°C to form liposome solutions. The absorbance of an aliquot was measured at 470 nm at 20 min interval. Butylated hydroxyanisole (Sigma) was used as the standard. The bleaching rate (R) of β-carotene was determined as per equation (1):

$$R = \ln (a/b)/t \qquad (1).$$

Where a is absorbance at time 0, b is absorbance at time t, and t is incubation interval 20, 40, 60, 80, 100 or 120 min.

Further, antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control, according to equation (2):

$$AA = [(R_{control} – R_{sample})/R_{control}] \times 100 \qquad (2).$$

Determination of Bioactive Compounds

Phenolic compound estimation: The presence of phenolic compounds was estimated in the methanolic extracts by a colorimetric assay as per Barros *et al*. (2007). Briefly, in 1 mL of sample was added in one ml of Folin and Ciocalteu's phenol reagent. The mixture was incubated at room temperature for 3 min followed by addition of 1 mL of saturated Na_2CO_3 solution. The final volume was made up to 10 mL with sterilized water. The reaction mixture was incubated for 90 min in dark condition. The absorbance was measured at 765 nm using Shimadzu UV-1601 spectrophotometer. Gallic acid at concentrations of 0.01-0.4 mM was used to make standard curve. The mean values were expressed as mg of gallic acid equivalents (GAEs) per gram of extract.

Flavonoid contents: Flavonoid contents from the fruit body were estimated from methanolic extract. One mL of extract was diluted using 80% aqueous ethanol. 100 µL 10% aluminum nitrate and 100 µL, 1 M aqueous potassium acetate were mixed into the solution and incubated at room temperature for 40 min. The intensity of the pink color solution was recorded at 415 nm using Shimadzu UV-1601 spectrophotometer. Quercetin was used as standard to calculate the concentration of total flavonoids [0.002108 µg quercetin-0.01089 (R^2=0.9999)].

Ascorbic acid determination: The ascorbic acid content was determined from dried methanolic extract. A 100 mg of the extract was mixed with 1% metaphosphoric acid (10 mL) and incubated at room temperature for 45 min and filtered. One mL of filtrate was mixed with 9 mL of 2, 6-dichloroindophenol and absorbance was recorded at 515 nm in 30 min against a blank. The ascorbic acid content was calculated using calibration curve of L-ascorbic acid (0.020–0.12 mg/mL; Y = 3.4127X-0.0072; R^2 = 0.9905). The results were expressed in terms of mg of ascorbic acid/g of extract.

β-carotene estimation: β-carotene was determined from the dried methanolic extract. 100 mg of extract was mixed with 10 ml of acetone–hexane mixture (4:6) for 1 min and filtered. The absorbance was recorded at three different

wavelengths (453, 505 & 663 nm). The β-carotene content was calculated by: β-carotene (mg/100 mL) = 0.216×A663-0.304×A505+0.452×A453. The results were presented as μg of carotenoid/g of extract.

Statistical analysis: All the experimental analysis was carried out in triplicates. Further the results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test using SAS v. 9.1.3 program.

RESULTS AND DISCUSSION

Antioxidant activity: Polyphenols are considered to be major contributors to the antioxidant property of fruits, vegetables and mushrooms (Ferreira *et al*., 2007). To consider the importance of polyphenolic compounds and its presence in many varieties of mushrooms, the total antioxidant activity of three different edible wild *Cantharellus* mushrooms were determined in the present study. There was an increased in the total antioxidant activities in linoleic acid emulsion for all the *Cantharellus* species, but at the second hours of activity increased significantly as compared to first and third hours. The alcoholic extracts were most effective and maximum activity was observed in fruit bodies of *C. friessi*. The percent inhibition values increased from 33.5 to 55.8% after 2 h of incubation, while decreased to 24.4% after 3 h of incubation. The minimum inhibition was observed in the aqueous extract of *P. florida* (7.5%) after one hour of incubation. The inhibition values were increased up to 8.7% after 2 h, while decreased to 6.3% after 3 h, whereas the antioxidant activities of water extract from the *Cantharellus* species gradually decreased (Table I). Supposedly, this water extract from *Cantharellus* species contained compounds that inhibit the antioxidant activity.

Bioactive components: The present studies also concentrate on four different bioactive components; like phenol, flavonoids, ascorbic acid and β-carotene (Table II). As discussed earlier, phenolic compounds are known to be major bioactive component present in fruit bodies expressed as mg per gram of fruit body, which ranged from 9.55 to 16.80 mg/g. Average concentration of flavonoid ranged from 1.23 to 1.92 mg/g, ascorbic acid (range, 0.35 to 1.1 mg/g) followed by very small concentration of β-carotene (9.88 to 13.7 μg/g) among all mentioned isolates. The amount of calculated bioactive compound was found to be quite higher in *C. friessi* (Table II) but the concentration of β-carotene was found maximum in *C. subcibarius* (13.7 μg/g) and minimum in *C. cinerius* (9.88 μg/g). It seems that with regard to bioactive components, *P. florida* was comparably less effective than *Cantharellus* species. Bioactive components of these *Cantharellus* mushrooms were significantly higher than some widely used edible mushrooms such as species of *Lycoperdon, Clavaria, Ramaria, Marasmius, Pleurotus, Russula* (Turkoglu *et al*., 2007; Yaltirak *et al*., 2009; Ramesh & Pattar, 2010). Furthermore, we analyzed that the *C. friessi* contained various useful neutraceuticals such as phenolics, carotenoids and ascorbic acid. These extracted bioactive compounds could be used as functional ingredients mainly against microbial infections. The comparison of *Cantharellus* species to *P. florida* show the all the three species of *Cantharellus* had significantly higher antioxidant potential in both the extract. It has been found out that many pharmacological effects of phenolics and flavonoids are linked together and act as strong antioxidants as well as free radical scavengers that help in chelation of heavy metals along with interaction with enzymes, adenosine receptors, and biomembranes (Saija *et al.,* 1995). Phenols are one of the major components of plants and mushrooms (Barros *et al*., 2007). These are known to eliminate (free) radicals due to the presence of hydroxyl groups (Hatano *et al*., 1989), and also associated with antioxidant effect of whole system (Duh *et al*., 1999). There are also some reports that suggest the role of polyphenolic compounds in stabilizing lipid oxidation (Wagner *et al*., 1992). Further, it can be suggested that such mushrooms diet containing high polyphenolic compounds might show inhibitory effects on various diseases arising from mutagenesis and carcinogenesis in humans. Therefore, it is very important to set up a nutraceutical database of *Cantharellus* mushrooms to retain

Table I: Total antioxidant activities of different *Cantharellus* species and comparison with *Pleurotus florida*

Mushrooms	Inhibition (%) of linoleic acid peroxidation					
	Alcoholic Extract			Aqueous Extract		
	1 h	2 h	3 h	1 h	2 h	3 h
C. friessi	33.5 ± 0.33^{b}	55.8 ± 0.32^{a}	24.4 ± 0.33^{ab}	8.4 ± 0.33^{c}	18.7 ± 0.24^{a}	5.4 ± 0.37^{d}
C. subcibarius	36.6 ± 0.34^{ab}	48.4 ± 0.32^{b}	25.8 ± 0.24^{a}	10.2 ± 0.46^{b}	13.2 ± 0.43^{b}	7.3 ± 0.45^{b}
C. cinerius	37.2 ± 0.40^{a}	45.1 ± 0.24^{c}	20.5 ± 0.32^{b}	14.2 ± 0.46^{a}	9.6 ± 0.45^{c}	9.2 ± 0.46^{a}
P. florida	26.2 ± 0.36^{c}	37.8 ± 0.31^{d}	15.8 ± 0.38^{c}	7.5 ± 0.30^{d}	8.7 ± 0.32^{d}	6.3 ± 0.41^{c}

Table II: Bioactive components present in different *Cantharellus* species and *Pleurotus florida*

Mushrooms	Phenols (mg/g)	Flavonoids (mg/g)	Ascorbic acid (mg/g)	β-carotene (μg/g)
C. friessi	16.80 ± 1.05^{a}	1.92 ± 0.12^{a}	1.10 ± 0.10^{a}	12.66 ± 0.11^{ab}
C. subcibarius	12.97 ± 1.77^{b}	1.78 ± 0.13^{b}	0.67 ± 0.03^{b}	13.70 ± 0.07^{a}
C. cinerius	11.40 ± 0.31^{c}	1.34 ± 0.17^{c}	0.48 ± 0.10^{c}	9.88 ± 0.53^{c}
P. florida	9.55 ± 0.21^{d}	1.23 ± 0.11^{d}	0.35 ± 0.10^{d}	10.50 ± 0.44^{b}

All values are Mean ± SD (n = 3). Values bearing different letters in the same column are significant at $P<0.05$

the information of these unique and indigenous species. Such nutraceutical database based on nutrition and antioxidative properties may be helpful in conserving important natural resources.

CONCLUSION

All the three different *Cantharellus* species in the present study were found to contain significant amount of antioxidant activity along with higher amount of total phenolics, flavonoids, β-carotene and ascorbic acid. As a result of regular global demand of food industry, such edible mushrooms can be important nutritional sources. In addition, the mushrooms provide source of additive and synergistic effects based on presence of all the bioactive compounds. This is for the first time, wild edible *Cantharellus* mushrooms collected from North-Western Himalayas were submitted to these studies.

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