Research Article

Antioxidant Activity in Terms of Total Phenol and Reducing Power of Two Strains of *Hypsizygus ulmarius* CO2 and IIHR Hu1

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ABSTRACT

In the present study the antioxidant potential of two strains of *Hypsizygus ulmarius* CO2 and IIHR Hu1 (Blue oyster mushroom) were analyzed by means of different assays namely Total phenol content and Reducing power. The total phenol content in two strains of *Hypsizygus ulmarius* CO2 and IIHR Hu1 ranged from 8.569 mg/g and 6.120 mg/g respectively. The reducing power of mushroom methanolic extracts increased with increasing concentration. Among the two strains of *Hypsizygus, H. ulmarius* CO2 strain shows higher reducing power (0.89/2.5 mg/ml). Therefore, these two strains of *Hypsizygus ulmarius* CO2 and IIHR Hu1 edible mushroom may have potential as natural antioxidants.

Keywords: Antioxidant activity, Edible mushroom, Reducing Power, Total Phenol.

INTRODUCTION

Mushrooms are known for their rich source of biologically active compound which offer protection to the human body in a natural way. Moreover, they contain proteins and nutritive fibers, as well as other vitamins necessary for the normal functioning of the human body. Mushrooms are rich in dietary fiber, minerals, vitamins and low in fat.[1] Mushrooms are widely consumed as an edible and medicinal resource. Studies have found that some species of mushrooms are having therapeutic properties such as antioxidant, anticancer, antimicrobial, cholesterol lowering and immune stimulatory effect.[2, 3]

Oxidation is essential for the production of energy to fuel biological processes in many living organisms. The recent changes in lifestyle or environmental factors such as pollution, radiation, cigarette smoke and herbicides can generate free radicals among people worldwide. Free radicals produced by radiation, chemical reactions and several redox reactions of various compounds may contribute to protein oxidation, DNA damage, lipid peroxidation in living tissue and cells.[4] This oxidative stress may be related to many disorders, such as cancer, atherosclerosis, diabetes and liver cirrhosis.[5] Recent epidemiological studies have indicated that increased consumption of certain foods, such as fruits and vegetables, as associated with reduces risks of chronic diseases.[6] This association may be attributed from the antioxidants in the foods including vitamins C, vitamin E, carotenoids, Polyphenolic compounds and flavonoids, which prevent free radical damage.[7]

Antioxidants obtained through diet are taking on major significance as possible protector agents to diminish oxidative damage and edible mushrooms might be used as neutraceuticals or directly eaten in the diet to maintain good health. However antioxidant supplements or antioxidant containing foods may be used to help the human body to reduce oxidative damage or to protect food quality by preventing oxidative deterioration. Hence, the objective of this study was aimed to determine the antioxidant properties of methanolic extract from dried mushrooms.

MATERIALS AND METHODS

Preparation of Mushroom extract

The fruiting bodies of mushroom were cleaned and washed by using water. These samples were cut into pieces and stored at 80°C. Then, they were lyophilized using freeze-dryer to remove the moisture content. After freeze-dryer, the dried samples were ground into fine powder by using micro-grinder and kept in airtight container for further analysis.

Five grams of each powdered samples were extracted with 100ml of methanol. The mixture was placed in a conical flask and agitated at 150 rpm with orbital shaker for 24 hours at 25°C. The extract was then separated by filtration through Whatman No.4 filter paper. Then methanol extracts was removed under reduced pressure at 40°C using a rotary evaporator to dryness. For aqueous extraction, the aqueous extract was lyophilized by using a freeze dryer. This dried extract was used directly for further analysis.

Determination of Total Phenolic Content

Total phenols were determined by Folin ciocalteau's reagent method.[8] A known quantity of the sample was ground in pestle and mortar in 80% ethanol. Centrifuge the homogenate at 10,000rpm for 20 minutes. Save the supernatant and it was evaporated to dryness. Residue was dissolved in distilled water. Pipette out different aliquots (0.2-2ml) into test tubes and make up the volume in each tube with 3mL water and further add 0.5 mL of Folin ciocalteau's phenol reagent. After 3 minutes, add 2

mL of 20% sodium carbonate solution to each tube and mixed thoroughly. Place the tubes in a boiling water for exactly one minute, cool and measure the absorbance at 650 nm against a reagent blank and prepare a standard curve using different concentration of catechol. The total phenol content in the test samples was calculated from the standard curve and expressed as mg catechol equivalent of phenol/ g sample.

Determination of Reducing Power

The reducing power was determined according to the method of Oyaizu.[9] Various concentrations (0.5 – 2.5mg/ml) of mushroom methanolic extracts were mixed with 2.5 ml of 200 mmol/1 sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 650 rpm for 10 minutes. The upper layer (5 ml) was mixed with 5ml deionized water and 1 ml of 0.1% of ferric chloride, and the absorbance was measured at 700nm. Higher absorbance indicates higher reducing power. Ascorbic acid was used as standard.

RESULTS AND DISCUSSION

The two strains of edible mushroom species (*Hypsizygus ulmarius* CO2 and *Hypsizygus ulmarius* IIHR Hu1) were evaluated for their antioxidant ability in terms of total phenol and reducing power capacity. Total phenol content was determined using Folin ciocalteau's phenol reagent. The reducing power was evaluated measuring absorbance at 700nm after mixing the sample with ferric compounds. Higher absorbance indicates higher reducing power.[10, 11]

Phenols are important constituent because of their scavenging ability due to their hydroxyl groups.[12] In addition, it was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation.[13] Phenolic compounds have been reported to be the major naturally occurring antioxidant compounds found in the extracts of edible mushrooms, whereas other potential antioxidants such as ascorbic acid, β – carotene, lycopene and γ – tocopherol have only been found in very small amount.[14, 15]

In the present study the total phenolic content in mushroom methanolic extract of *Hypsizygus ulmarius* CO2 was 8.569 mg/g and in *Hypsizygus ulmarius* IIHR Hu1 was 6.120 mg/g (Table 1) respectively. Phenol concentration of five species of *Agaricus* mushroom extract showed that phenols were the major antioxidant compounds found in the extracts of *Agaricus silvaticus* revealed a higher content (8.95 ± 0.30 mg/g) of phenol compounds.[16] In *Lactarius deliciosus* the total phenols content in methanolic extracts from 17.25 ± 0.65 mg/g while in *Tricholoma portentosum* extracts contained only 10.80 ± 0.47 mg/g.[17] A direct correlation between mushrooms antioxidant activity and total phenolic content, although the antioxidant action is raised by phenols.[10] The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals.[18]

Table 1: Total Phenol Content of fruit bodies of mushroom *Hypsizygus ulmarius* CO2 & IIHR Hu1

Name of the species	Total Phenol Content (mg/g)
Hypsizygus ulmarius CO2	8.569
Hypsizygus ulmarius IIHR Hu1	6.120

Table 2: Reducing power of methanolic extracts from fruit bodies of mushroom *Hypsizygus ulmarius* CO2 & IIHR Hu1

Name of the Species	Concentration	OD value (700nm)
Ascorbic acid	0.5	0.35
	1.0	0.68
	1.5	0.77
	2.0	0.90
	2.5	0.98
Hypsizygus ulmarius CO2	0.5	0.36
	1.0	0.60
	1.5	0.73
	2.0	0.82
	2.5	0.89
Hypsizygus ulmarius IIHR Hu1	0.5	0.24
	1.0	0.32
	1.5	0.39
	2.0	0.45
	2.5	0.64

The reducing power of a compound is related to its electron transfer ability and may serve as a significant indicator of its potential antioxidant activity. Usually the ability of mushroom to donate electron and reducing Fe^3 to Fe^2 is assessed. The reducing power was measured as the absorbance. In the present study, reducing power of two strains of *Hypsizygus ulmarius* were assessed (Figure 1; Table 2) Results indicate the reducing power of mushroom methanolic extract increased with increasing concentration. Among the two strains of *Hypsizygus, H. ulmarius* CO2 strain shows higher reducing power (0.89/2.5 mg/ml). It was reported that the reducing power of mushrooms might be due to their hydrogen – donating ability.[19] The methanolic extract from *Antrodia camphorate* showed an excellent reducing power of 0.96-0.97 at 10 mg/mL.[20] Reducing powers of methanolic extracts from *Grifola frondosa*, *Hericium erinaceus* and *tricholoma magnivelare* increased along with the increased concentrations and were 1.18, 1.01 and 0.63 at 9 mg/ml, and 2.50, 1.78 and 1.11 at 24 mg/ml[16] respectively. Reducing powers of methanolic extracts from two strains of *Flammulina velutipes* mushrooms were 0.52 and 0.65 at 10 mg/mL.[21] Accordingly, *Hypsizygus ulmarius* CO2 might contain higher amounts

of reductone, which could react with free radicals to stabilize and block radical chain reactions.

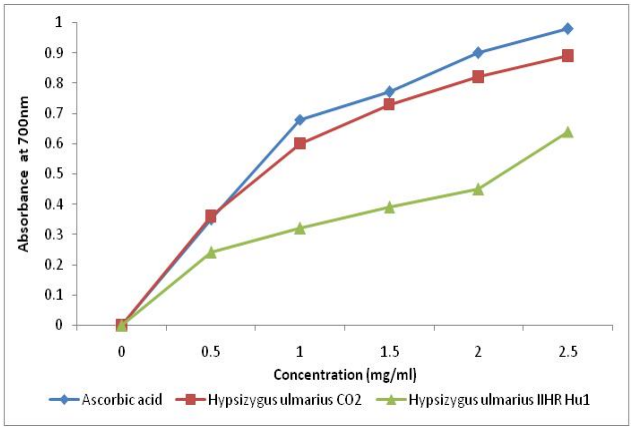

Figure 1: Reducing power of methanolic extracts from fruit bodies of mushroom *Hypsizygus ulmarius* CO2 & IIHR Hu1

CONCLUSION

In conclusion searching new sources may bring natural products in to the food industry with safer and better antioxidants that provide good protection against the oxidative damage, which occur both in the body and our daily foods. This study suggests that high antioxidant activity in methanolic extracts of mushrooms can potentially be used as a source of natural antioxidant due to the presence of radical scavenging property and total phenol content since mushroom are easily available and acceptable to the public. Over all, both strains proved to have antioxidant property in terms of reducing power and total phenol content. Thus the present study revealed that two strains of edible mushroom *Hypsizygus ulmarius* CO2 and IIHR Hu1 can be used in food as natural antioxidant.

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