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EVALUATION OF IN-VITRO FREE RADICAL SCAVENGING ACTIVITY OF AGARICUS BISPORUS EXTRACT

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ABSTRACT
Medicinal plants and natural products have attracted global attention due to their safety as well as their considerable antioxidant content that helps to prevent or ameliorate various disorders including memory impairments. The phenolic compound in fruits, vegetables, herbs and spices possess potent antioxidant, anti-inflammatory, antigenotoxic and anticancer activities. Mushrooms are functional foods largely consumed in Asia and Europe. The consumption of mushroom in Brazil is very low, in part due to the lack of information about the nutritional value of the edible mushrooms cultivated in the country. The dried shoots of *Agaricus bisporus* was extracted with methanol using a Soxhlet extractor. The total phenolics content of leaf as determined by Fenton reaction and was found to be good antioxidant activity as different dose concentrations. The antioxidant activity of plant extract was carried out with ascorbic acid as a standard reducing agent. The present results were made with the use of UV-Visible Spectrophotometer. In this plant *Agaricus bisporus* Extract there was a remarkable concentration dependent free radical scavenging and reducing power was exhibited. In conclusion the present study indicates that *Agaricus bisporus* extract may be a potential source of natural antioxidant. A. bisporus, appears as an excellent option to enrich the diet with antioxidant phenolic compounds.

KEYWORDS: Antioxidant activity, Fenton Reaction, Hydroxyl radical, ascorbic acid, *Agaricus bisporus*, TBARS.

1. INTRODUCTION
Mushrooms have been recognized as important food items since the ancient times because of their nutritional values and therapeutic properties. In ancient China, people believed that the mushroom establishes human body and health, preserves the youth for as long as possible, it was using as food and medicine (Safwat and Al Kholi, 2006 The most widely consumed farmed edible fungus worldwide is the button mushroom, Agaricusbisporus (J.E. Lange) Imbach. The current version has a white form with a white cap, stalk, and meat with dark brown gills, but the original wild form had a brownish cap and dark brown gills (Loganathan et al., 2009). Fruit bodies of A. bisporus are typically grown in polythene bags filled with sterilised sawdust. The bags are opened when the vegetative mycelia growth is finished, and the colonised substrate is then exposed to environmental factors that are known to promote fruiting. Depending on where and how the mushrooms are grown, environmental pressures may cause changes in the nutritional value, including antioxidants.The amount of antioxiodants in mushrooms may also be influenced by the cultivation environment, diet, and substrate type (Román et al., 2006). The complete cluster or several clusters are put into each overwrapped bundle with the mushroom. A. bisporus is typically viewed as having less value than other cultivated mushrooms that are mostly grown in Asia in terms of nutrition and medicine (Aisya et al., 2010). According to recent research, A. bisporus also includes significant amounts of compounds that may be useful medically, including tyrosinase, aromatase inhibitors, and immunomodulating and antitumor polysaccharides (Aisya et al., 2010).A. bisporus extracts in cold water have the power to counteract the genotoxic effects of reactive oxygen species. The enzyme tyrosinase was connected to this genoprotective effect. Recent investigations show that A. bisporus extract has anticancer properties both in vivo and in vitro, and that its primary fatty acid contents, which inhibit aromatase activity and oestrogen production, may have chemopreventive effects on breast cancer (Savoie et al., 2008). Other intriguing elements for the development of A. bisporus as a nutraceutical include ergosterol, vitamin D2 concentration, and antioxidant activity.A. bisporus, like other mushrooms, is regarded as a valuable part of the human diet because of its nutritional value, with a low calorie, purine, carbohydrate, and salt content as well as a high

concentration of numerous vitamins, potassium, phosphorus, and other trace minerals (Savoie et al., 2008). This implies that further research is necessary to fully understand the usefulness of A. bisporus as a functional food (Savoie et al., 2008). Our goal was to assess the antioxidant qualities of ethanolic and hot water extracts from the fruit bodies and mycelia of A. bisporus, including their reducing power and radical-scavenging capacities against hydroxyl, DPPH, and superoxide anion radicals. Therefore, we have planned out the antioxidant activity of the *Agaricus bisporus* Mycelia and fruit bodies extract using Fenton reaction.

2. MATERIALS AND METHODS

Plant material – *Agaricus bisporus* Mycelia and fruit bodies extract was collected from Local Market, Raipur (Chhattisgarh), India.

Chemicals and Reagent samples – The reagents used were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany) and other. Sample absorbances were read using a Lambda 532 nm, UV Spectrometer made by Varian.

Preparation of extract - Dried powdered of *Agaricus bisporus* Mycelia and fruit bodies extract (15 g) were extracted by continuous mixing in 500 ml 50% methanol and water, 24 h at room temperature. After the filtration process, methanol was evaporated until only water remained through evaporation on water bath at 60-70 0c temperature. The final extract was kept in air tied box.

Deoxyribose assay to assess OH -radical scavenging activity

The OH- radical scavenging activity of *Agaricus bisporus* Mycelia and fruit bodies extract extract (10–100 ug/ml) was determined according to the deoxyribose method reported of Halliwell, *et al*., (1987). In the protocol the presence of 100 lM EDTA. $FeCl_3$, H_2O and ascorbic acid were prepared in degassed H_2O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 lM EDTA, 1 mM H2O2, 100 lM L- ascorbic acid, 100 lM $FeCl_3$, H_2O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Samples was kept in incubation at 38º C, 1 hrs, 1.0 ml 1.0% TBA in 0.05 M NaOH and 1.0 ml 10% TCA were added to the reaction mixture after that samples was heated in a boiling water bath for 15 min. After the samples were cooled, the absorbances were read at 532 nm. The IC50 value of the plant extract was compared with that of ascorbic acid, which was used as the standard. The result of lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percentage of inhibition of hydroxyl radical was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Abs: 532 nm Control Abs. - 532 nm sample Abs.} \times 100}{\text{532 nm Control Abs}}$$

Antioxidant capacity of test compounds was expressed as IC_{50}, the concentration necessary for 50% inhibition concentration of TBARS.

3. RESULT

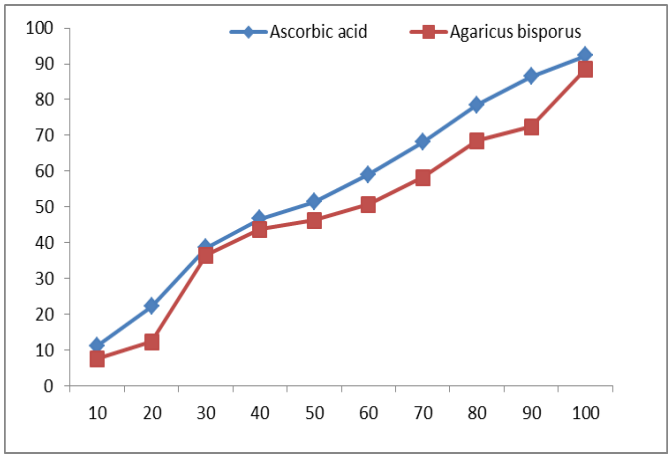
The results of the effects of the examined *Agaricus bisporus* Mycelia with fruit bodies extract extract as well as control solutions on OH- radical production. They show that all extract of *Agaricus bisporus* Mycelia with fruit bodies extract *extract* and control solutions as a ascorbic acid inhibited the production of OH- radicals. The % of free racial scavenging activity of methanolic extract of *Agaricus bisporus* Mycelia with fruit bodies extract presented in Table 1 have reducing power, the free radial OH- scavenging activity of the extract increases with increasing the concentration.

Table 1: Antioxidant activities of *agaricus bisporus* mycelia with fruit bodies extract extract using Fenton reaction.

Concentration (in µl)	Ascorbic acid (Mean + SE)	Agaricus bisporus Mycelia with fruit bodies extract(Mean + SE)
10	11.12 ± 1.90	7.50 ± 0.17
20	22.20 ± 3.01	12.27 ± 0.96
30	38.57 ± 1.51	36.53 ± 0.33
40	46.72 ± 1.15	43.69 ± 0.99
50	51.33 ± 1.25	46.17 ± 1.40
60	59.03 ± 1.00	50.68 ± 1.10
70	68.03 ± 0.91	58.24 ± 0.10
80	78.59 ± 1.01	68.51 ± 0.65
90	86.44. ± 1.41	72.38 ± 0.34
100	92.34 ± 0.87	88.55 ± 0.33

Table 2: Inhibitory concentration 50% values (IC_{50}) of Ascorbic Acid and Agaricus bisporus Mycelia with fruit bodies extract using fenton reaction.

Sl	Group	IC_{50} Value
1.	Ascorbic acid	49.0 (µg/ml)
2.	Agaricus bisporus Mycelia with fruit bodies extract	60.0 (µg/ml)



Graph 1: Antioxidant activities of *Agaricus bisporus* Mycelia with fruit bodies extract extract using Fenton reaction.

4. DISCUSSION AND CONCLUSION

Mushrooms are considered as potential source of many essential nutrients and therapeutic bioactive compounds. Agaricus bisporus belongs to Basidiomycetes family and the most important commercially cultivated mushroom in the world. The rich nutrients like carbohydrates, proteins, lipids, fibers, minerals, and vitamins present this mushroom as famous healthy food. Moreover, because of the presence of some active ingredients, such as polysaccharides, lipopolysaccharides, essential amino acids, peptides, glycoproteins, nucleosides, triterpenoids, lectins, fatty acids and their derivatives, these mushrooms have been reported to have antimicrobial, anticancer, antidiabetic, antihypercholesterolemic, antihypertensive, hepatoprotective and antioxidant activities. This study is focused on reviewing the recent studies published in the medical and nutritional properties of Agaricus bisporus. Investigations on the mushroom have accelerated during the last ten years so that only reports published after 2006 have been considered. The secondary plant metabolites total phenols, flavonoids, ascorbic acid, -carotene, and lycopene were shown to be abundant in A. bisporus extracts. Usually found in estuary marshes, A. bisporus has special adaptations to deal with environmental stresses such high salinity, high temperature, inadequate nutrients, and intense radiation. Production of ROS is a natural byproduct of this activity, and as a result, the expression of these antioxidant genes was altered, upregulating the antioxidant enzymes (Jitesh et al., 2006Phenolics are regarded as traditional herbivore defence chemicals that shield plants from predators. Since it was postulated that secondary metabolites in plants evolved for such reason. Contrary to these theories, it has been proposed that many plant phenolics' primary function may be to protect leaves from photodamage rather than herbivores. They can do this by acting as antioxidants, and their levels may change depending on the environment in order to prevent this potential photodamage (Banerjee et al., 2008).It has been demonstrated that phenolics, in particular flavonoids, shield mushrooms from UV light (Agati et al., 2007). Given that they were cultivated and survived in stressful conditions, the various mushroom extracts had significant phenolic content and showed higher synthesis. The effective antioxidant effects of the mushroom extracts (MHWE-50.34 and 30.77 mg/g for FBHWE) were in part due to their high total phenol content. In fact, it had been noted that the amount of phenolic chemicals in plant materials is highly connected with their antioxidant activity (Ferreira et al., 2007).The amount of phenolics found in the mushroom samples that were studied (50.34 to 5.62) was higher than the total phenolics found in other edible wild mushrooms, such as Lactariusdeliciosus (pine mushroom), which were found to range from 17.25 to 0.65 mg/g, according to Ferreira et al (2007). A. bisporus is therefore a potentially better natural supply of phenols, which are well-known to be powerful antioxidants. But it was clear from the solvents employed that water extracts had higher TPC than ethanol extracts. This might be because the extract contains more phenols that are water soluble. With higher extract concentrations, greater levels of phenolic chemicals were produced, which was consistent with reports (Chirinang and Intarapichet, 2009). A. bisporus' high phenolic content in this study is comparable to that of widely consumed vegetables including lettuce, celery, and cucumber (Chu et al., 2002). According to Gursoy et al., A. bisporus (also known as the button mushroom) contains phenolic groups such as flavonoids, carotenoids, anthocyanins, phenolic acids, tannins, and lignans (2010).There is a correlation between antioxidant components and reducing power, hydroxyl radical scavenging ability, super-oxide anion scavenging ability, and DPPH scavenging ability, suggesting that the mechanisms of action of the extracts for the antioxidant activity may be the same and related to total phenol content. When compared to water extracts, ethanolic extracts (FBEE and MEE) were found to have a greater RSA (p 0.05). (FBHWE and MHWE). For the majority of mushrooms previously described, ethanolic extracts were generally superior than hot water extracts in terms of their scavenging activities (Savoie et al., 2008).The ability of all hot water extracts from Ganoderma tsugae to scavenge hydroxyl radicals increased with concentration, according to Oyetayo et al. (2009),

however the ability of the methanolic extract from G. tsugae did not increase with concentration. As a result, the sample from A. bisporus possesses a sizable hydroxyl radical scavenging capacity. Both the ethanolic and aqueous mushroom extracts had a peak superoxide anion scavenging action at the thirty minute mark, and then the effects started to decline. This is most likely a result of radical scavengers becoming worn out (Kim et al., 2009).This confirms earlier findings by Oyetayo et al. (2009) that hot water extract from Cordyceps sinensis natural and cultured mycelium had a greater superoxide scavenging effect than ethanolic extract, confirming the importance of extraction solvent polarity in superoxide anion scavenging ability. In general, the samples have some notable impact on scavenging superoxide free radicals.

In conclusion, fruit bodies and mycelia's ethanolic and hot water extracts had potent antioxidant qualities, making them useful as prophylactic agents against a variety of diseases caused by oxidative stress. Samples from A. bisporus that underwent phytochemical analysis revealed the presence of chemicals with antioxidant and antibacterial properties. Total phenols and total tocopherols were the two main anti-oxidant substances present in hot water and ethanolic extracts, respectively. Therefore, A. bisporus is a more affordable and organic source of antioxidants important for the health of a living body system. The discovered data could provide a solid foundation for choosing which species of mushrooms to study further in the hopes of discovering novel, important bioactive chemicals.

Disputed interests

There were no declared conflicts of interest by the authors.

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