

Full length Research Paper

Antioxidant properties of ethanolic and hot water extracts from *A. bisporus* fruit bodies and mycelia

Tegla Musyoka Wainaina

Department of Biochemistry and Biotechnology, Kenyatta University, P.O Box 43844, 00200, Nairobi, Kenya.

Accepted 27 October, 2016

6

Antioxidant activities and phytochemical compounds of ethanol and hot water extracts of *Agaricus bisporus* species fruiting body and mycelia cultivated in Kenya were spectrophotometrically determined and evaluated. The total antioxidant activity was analysed using 1,1-diphenyl-2-picrylhydrazil, hydroxyl, superoxide radical scavenging and reducing power assays, while phytochemicals were assayed through calorimetric assays. Total phenolic, β -carotene, lycopene, flavanoid and ascorbic acid composition of *A. bisporus* extracts was analysed by calorimetric assays and found to contain 40.26 to 4.61 mg/g, 48.99 to 2.86 mg/g, 67.82 to 11.87 mg/g, 93.8 to 17.2 mg/g and 11.62 to 10.22 mg/g respectively. The mineral elemental analysis done using energy dispense x-ray fluorescence (EDXRF) analytical method revealed that the samples contain zinc (42.9 mg/l), iron (33 to 48.5 mg/l), copper (18 to 24 mg/l) and manganese (7.5 to 9 mg/l). Generally, the mycelium extracts were more effective radical scavengers than the fruiting bodies. Due to the above characteristics, *A. bisporus* mushroom could be considered a food complement with antioxidative activity in the diet for the health benefits they present. Their effectiveness was also evaluated by their EC₅₀ values through interpolation from linear regression analysis of their respective data.

Key words: Button mushroom (*Agaricus bisporus*), edible mushroom, antioxidant, reducing power, scavenging ability, phytochemicals.

INTRODUCTION

The button mushroom, *Agaricus bisporus* (J.E. Lange) Imbach, is the premier cultivated edible mushroom and is consumed throughout the world. The original wild form

bears a brownish cap and dark brown gills but more familiar is the current variant with a white form, having white cap, stalk and flesh and brown gills (Loganathan et

*Corresponding author: E-mail: tegmusyoka05@yahoo.com

Author(s) agreed that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

al., 2009). In cultivation of *A. bisporus*, fruit bodies are usually produced in polythene bags containing sterilized sawdust. After the completion of vegetative mycelia growth, the bags are opened and the colonized substrate subjected to environmental conditions known to stimulate fruiting. Environmental stresses depending on where/how the mushrooms are cultured may lead to variations in terms of nutritional value including antioxidants. Culturing conditions/feed/substrate type) may also influence the antioxidants levels in mushrooms (Román et al., 2006). The mushroom is packed by placing the entire cluster or several clusters in each overwrapped package. *A. bisporus* is usually considered to be of lesser value nutritionally and medicinally compared with other cultivated mushrooms that are predominantly grown in Asia (Aisya et al., 2010). Recent evidence suggests that *A. bisporus* also contains high levels of substances of possible medicinal importance, such as tyrosinase, aromatase inhibitors and immunomodulating and antitumour poly-saccharides (Aisya et al., 2010). Cold water extracts of *A. bisporus* have the ability to neutralise genotoxic effects of reactive oxygen species. This genoprotective effect was associated with tyrosinase.

Recent studies illustrate the anticancer activity *in vitro* and *in vivo* of *A. bisporus* extract, and its major fatty acid constituents that suppress aromatase activity and oestrogen biosynthesis are responsible for the potential breast cancer chemopreventive effect (Savoie et al., 2008). Ergosterol, vitamin D₂ content and antioxidant activity are also proposed as interesting components for the development of *A. bisporus* as a nutraceutical. Owing to its nutritional value, with a low calorie, purine, carbohydrate and sodium content as well as a high content of several vitamins, potassium, phosphorus and some trace elements, *A. bisporus*, like other mushrooms, is considered to be a valuable component of the human diet, especially by health-conscious people (Savoie et al., 2008). This suggests that the value of *A. bisporus* as a functional food warrants more detailed study (Savoie et al., 2008). Our objective was to evaluate the antioxidant properties of ethanolic and hot water extracts from *A. bisporus* fruit bodies and mycelia including reducing power, scavenging abilities on hydroxyl, DPPH and superoxide anion radicals. The contents of potential antioxidant components were also determined as well as the trace element contents.

MATERIALS AND METHODS

Mushroom fruit bodies and mycelia

Fresh fruit bodies and mycelia of *A. bisporus* were obtained from 6 bags of substrates for growing the mushrooms. For each *A. bisporus* growing bag, 4 kg wheat straw were used as growing substrate. The wheat straws were watered for 4 days after which a heap was raised and watered overnight. After a day's rest, 2 kg chicken manure, 0.5 kg urea, and 0.5 kg molasses were added to the wheat straw and watered for a day. After a days' rest, the pile

was mixed while sprinkling with a little water. This was repeated six times. The substrate was then filled in a tunnel for pasteurization by allowing the temperature to increase upto about 60°C and allowing to stay for 8 to 10 h then cooled to 48°C in a long stack step for a four day conditioning. It was finally allowed 68 to 72% moisture content at 25°C temperature. *A. bisporus* spawns (0.35 kg) was seeded followed by packing into polythene bags 36 x 24 ft with 7 kg substrate each. They were then left to incubate for 14 days until colonization was complete, the polythene bags were then folded and casing done using sterile loam soil, slight watering was done to maintain moisture after which pinning was observed then harvested after three days by gently holding the mushroom body and twisting. The mycelia and fruiting bodies of the harvested mature mushrooms were separated, placed in freezer bag containers and stored in deep freezer at 4°C.

Extraction of the phytochemicals

Ethanolic extraction was done according to Bo et al. (2010). A sub sample (20 g) was extracted by stirring with ethanol (200 mL, 95% pure) at 25°C for 24 h and filtering through Whatman No.1 filter paper. The residue was then extracted with two additional 200 mL portions of ethanol as described above. The combined ethanolic extracts were then rotary evaporated at 40°C to dryness. Hot water extractions was done according to Bo et al. (2010), a subsample (20 g) was heated with deionized water (200 mL) at reflux for 1 h, centrifuged at 4,000 rpm for 15 min, followed by filtering through Whatman No.1 filter paper. The residue was then extracted with two additional 200 mL portions of boiling water as described above. The combined hot water extracts were freeze-dried. The dried extracts were used directly for analyses of antioxidant components or re-dissolved in water or ethanol to a concentration of 50 mg/mL and stored at 4°C for further uses.

Determination of antioxidants component

Phenols, polyphenols, ascorbic acid, flavanoid, β-carotene and Lycopene were determined in both ethanolic and aqueous mushrooms extracts by colorimetric assays, based on previously described procedures except for minor modifications (Barros et al., 2008).

Determination of total phenolic content

A sample of the extract (1 ml) was mixed with 1 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm. Gallic acid was used to calculate the standard curve (0.01 to 0.4 mM). Estimation of the phenolic compounds was carried out in triplicate. The results were in mean values ± standard deviations and expressed as mg of gallic acid equivalents (GAEs) per g of each extract.

Determination of total flavonoid concentration

Mushroom extracts solution (1 ml) were diluted with 4.3 ml of 80% aqueous ethanol and to the test tubes 0.1 ml of 10% aluminium nitrate was added followed by 0.1 ml of 1 M aqueous potassium acetate. After 40 min at room temperature, the absorbance was determined spectrophotometrically at 415 nm. Total flavonoid concentration was calculated using quercetin as standard (Park et al., 1997). Absorbance = 0.002108 µg quercetin – 0.01089 (R²: 0.9999).

Ascorbic acid determination

The dried extract (100 mg) was re-extracted with metaphosphoric acid (1%, 10 ml) for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The filtrate (1 ml) was mixed with 2, 6-dichloro-phenolindophenol (9 ml) and the absorbance was measured within 30 min at 515 nm against a blank. Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (0.020 to 0.12 mg/ml; $Y = 3.4127X - 0.0072$; $R^2 = 0.9905$) and the results were expressed as mg of ascorbic acid/g of extract.

β -Carotene and lycopene determination

The dried extract (100 mg) was vigorously shaken with acetone-hexane mixture (4:6, 10 ml) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at $\lambda = 453, 505$ and 663 nm. Contents of β -carotene and lycopene were calculated according to the following equations: lycopene (mg/100 ml) = $-0.0458 A_{663} + 0.372 A_{505} - 0.0806 A_{453}$; β -carotene (mg/100 ml) = $0.216 A_{663} - 0.304 A_{505} + 0.452 A_{453}$. The result was expressed as μ g of carotenoid/g of extract.

Determination of antioxidant activities

Determining of DPPH radical scavenging activity

This was carried out according to the DPPH spectrophotometric method of Mensor et al. (2001). A range of concentrations (1 to 20 mg/ml) of extract and standards (gallic acid and ascorbic acid) were used. 1 ml of a 0.3 mM DPPH methanol solution was added to 2.5 ml solution of the extract or standards and allowed to react at room temperature for 30 min. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA%) using the formula: $AA\% = 100 - [(Abs \text{ sample}/Abs \text{ control}) \times 100]$. Methanol (1.0 ml) plus extract solution (2.5 ml) was used as a blank. 1 ml of 0.3 mM DPPH plus methanol (2.5 ml) was used as a negative control while ascorbic acid and gallic acid solutions were used as positive control.

Determination of hydroxyl radical scavenging activity

The effect of hydroxyl radical was assayed by using the 2-deoxyribose oxidation method (Chung et al., 1997). Hydroxyl radicals (OH \cdot) are generated from Fe $^{2+}$ -ascorbate-EDTA-H $_2$ O $_2$ system (Fenton's reaction) which attack the deoxyribose and set off a series of reactions that eventually result in the formation of malondialdehyde (MDA), measured as a pink MDA-TBA chromogen at 535 nm. Reaction mixture (1 ml) contained deoxyribose (2.8 mM), KH $_2$ PO $_4$ KOH (20 mM; pH 7.4), FeCl $_3$ (100 μ M), EDTA (104 μ M), H $_2$ O $_2$ (1 mM) and ascorbate (100 μ M). Reaction mixture was incubated at 37°C for 1 h and a colour developed as described above. Catechin was used as positive control.

Determination of superoxide anion radical scavenging activity

Superoxide radical was generated from auto oxidation of hematoxilin and was detected by an increase in absorbance at 560 nm, in a spectrophotometer (Martin, 1987). The reaction mixture contained 0.1 M of phosphate buffer (pH 7.4), EDTA (0.1 M), hematoxilin (50 μ M) and incubated at 25°C for different time periods. Inhibition of auto oxidation of hematoxilin by water and ethanolic extracts over the control (gallic acid) was measured.

Reducing power assay

The reducing power was determined according to the method of Oyaizu (Oyaizu, 1986), which measure the power of extracts to reduce ferricyanide to ferrocyanide. Each extract (1 to 20 mg/mL) in water or ethanol (2.5 mL) was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 10 mg/mL potassium ferricyanide (2.5 mL), and the mixture was incubated at 50°C for 20 min. After trichloroacetic acid (2.5 mL, 100 mg/mL) was added, the mixture was centrifuged at 3,000 rpm for 10 min. The upper layer (5 mL) was mixed with deionized water (5 mL) and 1 mg/mL ferric chloride (1 mL), and the absorbance was measured at 700 nm against a blank. A higher absorbance will indicate a higher reducing power. EC $_{50}$ value (mg extract/ml) was the effective concentration at which the antioxidant activity was inhibited by 50%; absorbance was obtained by interpolation from linear regression analysis. Ascorbic acid was used for comparison.

Mineral element analysis

The elemental analysis was done using the Energy Dispenser X-ray Fluorescence analytical method (EDXRF) of Shahidi et al. (1992) for the detection of both detrimental heavy and trace metals in the hot water extracts of the mushrooms. Each mushroom sample was air-dried at 105°C overnight, and crushed using a mortar and pestle. Digestion of the samples was performed using a mixture of HNO $_3$: H $_2$ SO $_4$: H $_2$ O $_2$ (10:1:1, 12 ml/g-1 of sample) and heated at 100°C for about 10 to 15 min. After cooling, 50 mL of distilled water added and the mixture filtered. The solution was then used for elemental analysis using EDXRF and a concentration of each element was calculated on percentage of dry matter. The mineral elements analyzed included potassium, calcium, chromium, manganese, iron, copper, zinc and lead.

Data analysis

For each of the hot water and ethanolic extractions from fruit body and mycelia, three samples were prepared for every assay of every antioxidant attribute and component. Each value was expressed as mean \pm SD (n=3). The antioxidant activity was expressed as percentages. The experimental data was also subjected to Analysis of variance (ANOVA) to compare the values of the mycelium and fruiting bodies and test the significance levels at $p \leq 0.05$. Student's t-test was used to compare the values of the mycelium and fruiting bodies. The efficient concentration of antioxidant required to induce a 50% effect. Also, the data was evaluated by using one-way analysis of variance.

RESULTS

Phytonutrients present in fruiting body and mycelium ethanolic and hot water extracts

The various phytochemical present in mycelium ethanolic extract (MEE) and fruiting body ethanolic Extract (FBEE) of the mushrooms extract as detailed in Table 1. From the analysis, all the mushroom extracts had β -carotene with the content decreasing in the order FBEE > FBHWE > MEE > MHWE. There were significant differences ($p < 0.05$) in β -carotene amount between ethanol mushroom extracts and the water mushroom extracts. Similarly,

Table 1. Phytochemical levels of fruiting body and mycelium hot water and ethanoic extracts of *Agaricus bisporus*.

Phytochemicals	Concentration of phytochemicals (mg/mL)				
	FBHWE	FBEE	MHWE	MEE	Control
Phenols	31.87±3.89 ^a	16.77±4.69 ^c	40.26±0.55	4.61±0.31 ^c	36.20±1.86
β-Carotene	44.57±1.93 ^a	48.99±1.51	2.86±1.15 ^c	19.88±0.17 ^b	50.86±2.25
Lycopene	67.82±0.39	11.87±0.43 ^c	16.80±0.62 ^b	42.94±1.08 ^a	68.14±1.60
Flavonoids	85.36±1.01 ^a	21.87±0.09 ^b	93.80±0.36	17.20±0.09 ^c	85.10±0.20 ^a
Ascorbic acid	10.51±0.18	10.22±0.24	10.39±0.11	11.62±0.09	11.10±1.58

Results are expressed as Mean ± Standard Deviation (SD) of the three independent determinations. Means within respective rows followed by similar lower case letters are not significantly different at $p \leq 0.05$ by ANOVA and Tukey B test. FBHWE, fruiting body hot water extract; FBEE, fruiting body ethanoic extract, MHWE, mycelium hot water extract; MEE, mycelium ethanoic extract.

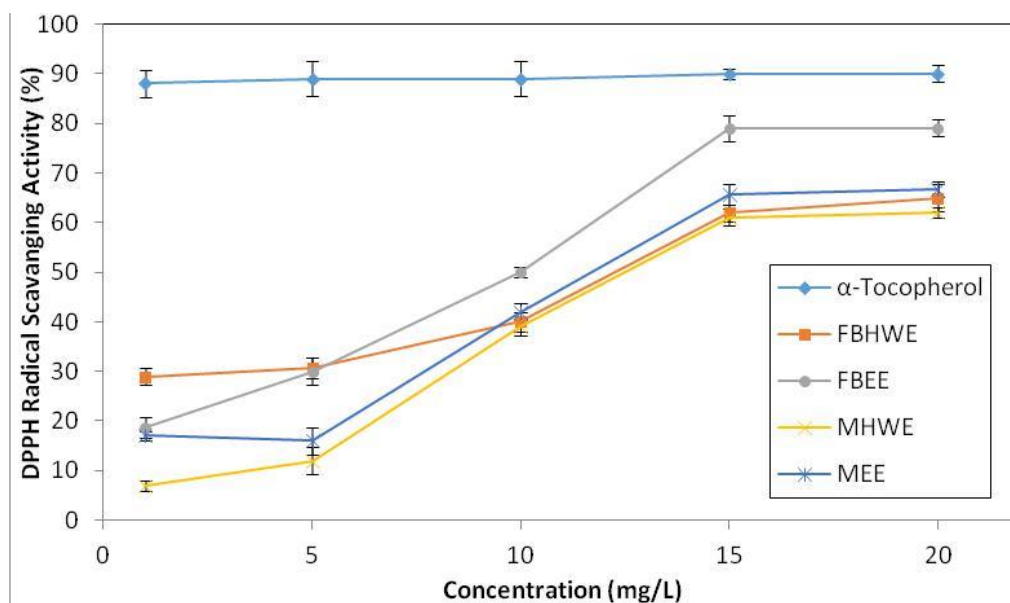


Figure 1. DPPH Radical scavenging activities (%). Results are expressed as Mean ± Standard Deviation (SD) of the three independent determinations. FBHWE, fruiting body hot water extract; FBEE, fruiting body ethanoic extract, MHWE, mycelium hot water extract; MEE, mycelium ethanoic extract.

there were significant differences ($p < 0.05$) in β-carotene amount between the fruiting body extracts and mycelium extracts. However, there was no significant difference ($p < 0.05$) in β-carotene amount among the ethanoic extracts (Table 1). The water extracts of β-carotene amount also differed significantly ($p < 0.05$). All the extracts had high lycopene content. FBHWE had the highest amount of lycopene at 67.82 μg/g. The lycopene amount decreased in the order FBHWE > MEE > MHWE > FBEE. There was a significant difference ($p < 0.05$) in lycopene content between FBHWE and FBEE, MHWE and MEE. The lycopene content differed significantly ($p < 0.05$) between MEE, MHWE and FBEE. Ethanol extracts had a significantly higher ($p < 0.05$) total flavanoid content than the water extracts. Among the ethanol

extracts, MEE had higher total flavanoid content than FBEE. The difference was not significant at $p < 0.05$. Among the water extract, FBWE had a higher, though not significant different ($p < 0.05$) total flavanoid content than MHWE. All the extracts had substantial amount of ascorbic acid content. MEE had the highest amount of ascorbic acid at 11.62±0.13 mg/g. There was no significant difference ($p < 0.05$) in the ascorbic acid content among the mushroom extracts (Table 1).

Determination of antioxidant activities

Figure 1 shows that there was significant DPPH radical scavenging activity in the fruiting body and mycelium hot

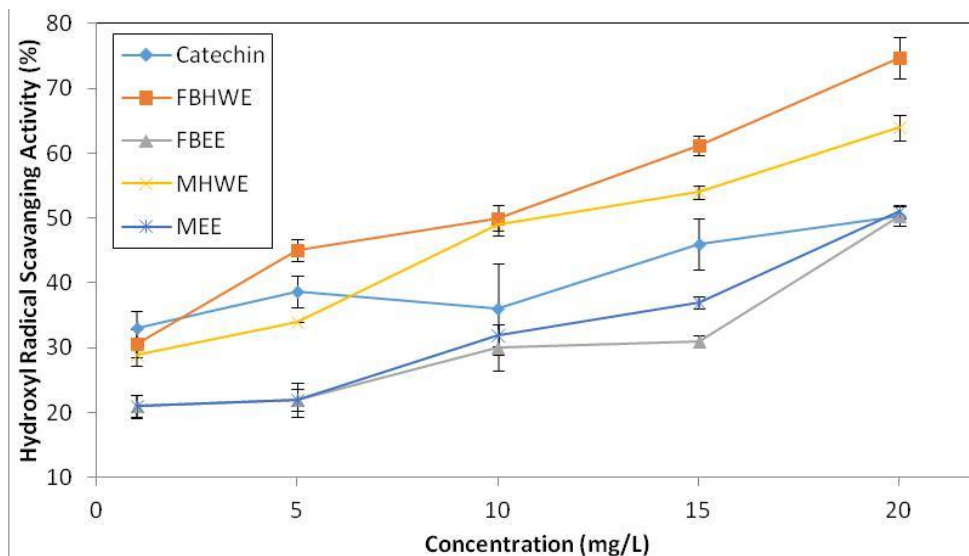


Figure 2. Hydroxyl Radical Scavenging Activity. Results are expressed as Mean \pm Standard Deviation (SD) of the three independent determinations. FBHWE, fruiting body hot water extract; FBEE, fruiting body ethanolic extract, MHWE, mycelium hot water extract; MEE, mycelium ethanolic extract.

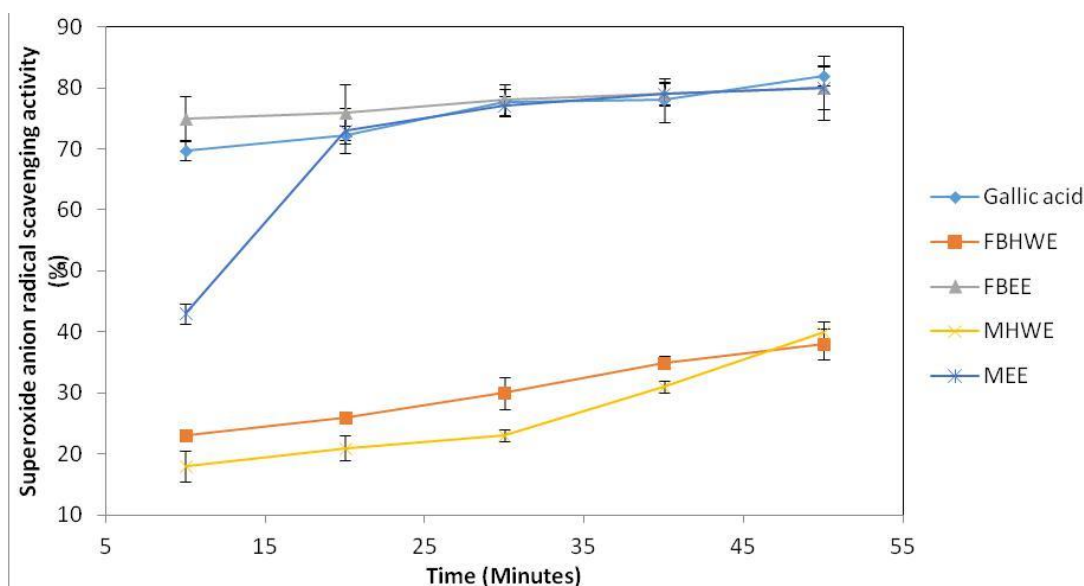


Figure 3. Superoxide Anion Radical scavenging Activity. Results are expressed as Mean \pm Standard Deviation (SD) of the three independent determinations. Gallic acid was used as the control. FBHWE, fruiting body hot water extract; FBEE, fruiting body ethanolic extract, MHWE, mycelium hot water extract; MEE, mycelium ethanolic extract.

water and ethanolic extracts of *A. bisporus*. The ethanolic extracts of both the mycelium and the fruiting body have a greater DPPH scavenging activity than the water extracts. Result also shows the hydroxyl radical scavenging activity of the fruiting body and mycelium hot water and ethanolic extracts of *A. bisporus* (Figure 2). The hydroxyl radical scavenging activity increased with the

increasing concentration of the extracts. The fruiting body hot water extracts had a better hydroxyl radical scavenging activity. The order of hydroxyl radical scavenging activity was fruiting body hot water extract followed by mycelium hot water extract followed by fruiting body ethanolic extract. Figure 3 shows the superoxide anion radical scavenging activity of the fruiting

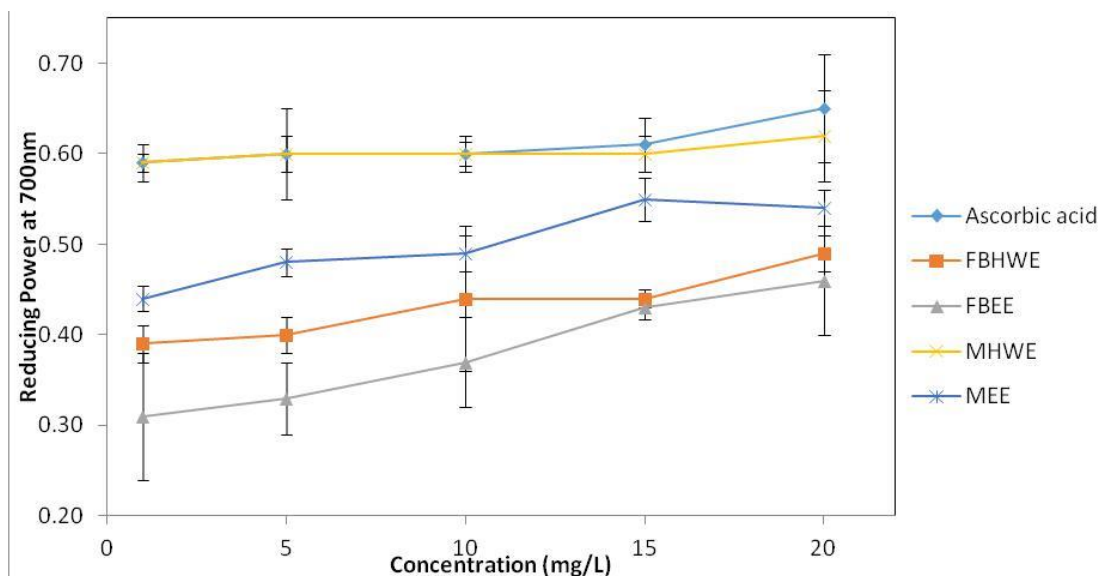


Figure 4. Reducing power of *Agaricus bisporus*. Results are expressed as Mean \pm Standard Deviation (SD) of the three independent determinations. FBHWE, fruiting body hot water extract; FBEE, fruiting body ethanolic extract, MHWE, mycelium hot water extract; MEE, mycelium ethanolic extract.

body and mycelium hot water and ethanolic extracts of *A. bisporus*. Results show that generally the superoxide anion radical scavenging activity was similar in fruiting body ethanolic and mycelium ethanolic extracts which were then followed by similar lower superoxide anion scavenging activity of the fruiting body and mycelium hot water extracts. An increasing superoxide anion radical scavenging activity was noted with increasing incubation time for fruiting body and mycelium hot water extracts.

Figure 4 shows the reducing power of the fruiting body and mycelium hot water and ethanolic extracts of *A. bisporus*. Results show that the reducing power of both ascorbic acid and mycelium hot water extracts were similar for concentrations ranging from 1 to 15 mg/L but this was significantly increased when the concentrations of the two was raised to 20 mg/L (Figure 3); for the fruiting body hot water extracts, fruiting body ethanolic extracts, and mycelium ethanolic extracts, increasing the concentrations increased the reducing power. In general, the order of reducing power decreased from ascorbic acid and mycelium hot water extracts followed by mycelium ethanolic extracts followed by fruiting body hot water extracts. EC₅₀ values showed that antioxidant activity was inhibited by 50% at 0.5 absorbance for reducing power. DPPH and Hydroxyl radicals were also scavenged by 50%

Mineral element analysis

Some antioxidant enzymes such as cytoplasmic and mitochondrial superoxide dismutases require micro-

Table 2. Mineral element composition of the fruiting body and mycelium of *Agaricus bisporus*.

Mineral	Mineral concentration (mg/kg)	
	Fruiting body	Mycelium
Copper	24.0 \pm 2.7	18.0 \pm 2.7
Zinc	46.7 \pm 3.6	42.0 \pm 1.0
Manganese	9.0 \pm 3.0	7.5 \pm 0.1
Iron	33.0 \pm 2.7	48.5 \pm 1.0

Results are expressed as Mean \pm Standard Deviation (SD) of the three independent determinations.

elements for their activities. All the samples from *A. bisporus* analysed recorded substantial amounts of the elements tested (Table 2). The Fruiting body sample contained high amounts of zinc (46.7 \pm 3.6 mg/kg) while mycelium recorded 42.0 \pm 1.0 mg/kg zinc. The amount of iron was 33.0 \pm 2.7 mg/kg in fruiting body and 48.5 \pm 1.0 mg/kg in mycelium. The amount of copper recorded was 24.0 \pm 2.7mg/kg in fruiting body and 18.0 \pm 2.7mg/kg in mycelium. Fruiting body had the highest amount of Zinc while mycelium had the highest amount of iron.

DISCUSSION

A. bisporus extracts were found to be rich in secondary plant metabolites like total phenols, flavonoids, ascorbic acid, β -carotene and lycopene. *A. bisporus* usually grow

in estuarine swamps; have unique adaptations to combat environmental stress conditions for example high salinity, high temperature, low nutrient and excessive radiation. An inevitable consequence of this process results in the production of ROS and accordingly the antioxidant enzymes were upregulated due to altered expression of these antioxidant genes (Jitesh et al., 2006). Phenolics have been considered classic defence compounds for protecting plants from herbivores. Ever since plant secondary metabolites were suggested to have evolved for that reason. In contrast to these concepts, it has been suggested that the main role of many plant phenolics may be to protect leaves from photo damage, not herbivores; they can achieve this by acting as antioxidants; and their levels may vary with environmental conditions in order to counteract this potential photo damage (Banerjee et al., 2008). The phenolics especially flavonoids were shown to protect mushrooms from UV radiation (Agati et al., 2007). The different extracts from mushroom were high in phenolic content and reflected greater synthesis since these were grown and survived in stress condition. The high content of total phenols in the mushroom extracts was partially responsible for their effective antioxidant properties (MHWE-40.26±0.9 and 31.87±0.42 mg/g for FBHWE). In fact, it had been reported that the antioxidant activity of plant materials is well correlated with the content of phenolic compounds (Ferreira et al., 2007). The level of phenolics in mushroom samples analysed (40.26 to 4.61 mg/g) was higher than the content of the total phenolics obtained from other edible wild mushroom like *Lactarius deliciosus* (pine mushroom), which was 17.25 to 0.65 mg/g as reported by Ferreira et al. (2007). As a result *A. bisporus* is a potential superior natural source of phenols which are known to be effective antioxidants.

However, with regard to solvents used, it was obvious that water extracts contained higher TPC than ethanol extracts. This could be due to the availability of more water soluble phenols in the extract. Higher amounts of phenolic compounds were obtained with increasing the concentrations of extracts, which was in agreement with reports (Chirinang and Intarapichet, 2009). The high phenolic contents of *A. bisporus* in this study are comparable to those in commonly consumed vegetables such as lettuce, celery and cucumber (Chu et al., 2002). It has been reported that phenolic groups such as flavonoids, carotenoids, anthocyanins, phenolic acids, tannins, lignans, and phenolic acids were found in *A. bisporus* (Button mushroom) Gursoy et al. (2010). A relationship between the reducing power, scavenging ability on hydroxyl radicals, scavenging ability on superoxide anion radical and scavenging ability on DPPH and antioxidant components was found, indicating that the mechanisms of action of the extracts for the antioxidant activity may be identical, being related to the content of total phenols. The RSA of ethanolic extracts (FBEE and MEE) were found to be higher ($p < 0.05$) than those of

water extracts (FBHWE and MHWE). Generally, ethanolic extracts were more effective in scavenging abilities than hot water extracts for most mushrooms reported previously (Savoie et al., 2008). Similar observation was found in our experimental data. From the analysis of Figure 1, we can conclude that the scavenging effects of mushrooms ethanolic extracts on DPPH radicals increased with the concentration increase. The higher scavenging abilities in ethanolic extracts might be attributed to the high level of antioxidant components in extracts, which could react rapidly with DPPH radicals and reduce most DPPH radical molecules. These results indicated that extracts were free radical scavengers, acting possibly as primary antioxidants (Savoie et al., 2008).

The results of hydroxyl radical scavenging powers of the mushroom extracts showed that MHWE exhibited the highest hydroxyl radical scavenging activity (70%) at the highest dose of 20 mg/ml concentration. Oyetayo et al. (2009) had earlier reported a concentration dependent increase in the scavenging abilities of all hot water extracts from *Ganoderma tsugae* on hydroxyl radicals while methanolic extract from *G. tsugae* showed a non-concentration dependent hydroxyl radical scavenging ability. Therefore, the sample from *A. bisporus* has an appreciable scavenging power on hydroxyl radicals. The superoxide anion scavenging effect of both ethanolic and water mushroom extract peaked at the 30th min, after which the superoxide anion radical scavenging effects decreased. This is probably due to exhaustion of radical-scavengers (Kim et al., 2009). This supports previous observations by Oyetayo et al. (2009) that hot water extract from natural and cultured mycelium of *Cordyceps sinensis* had a better superoxide scavenging effect than ethanolic extract affirming the role of extraction solvent polarity in superoxide anion scavenging ability. Generally, the samples have some appreciable effect on superoxide free radical scavenging. The result also showed that all the 4 extracts exhibited good antioxidant activity as evidence by their lower EC₅₀ values. With regard to the scavenging activity on hydroxyl radicals, various extracts were effective in order of their EC₅₀ values: Hot water extracts of mycelium was the most effective with EC₅₀ value of 9.8± 0.5 against fruiting body hot water extract (10.2± 0.7). The ethanoic extracts with EC₅₀ values of 15.3±0.5 and 15.1±0.5 of fruiting body and mycelium, respectively, were also effective. Therefore, the hydroxyl ion scavenging ability exhibited suggests that extracts have potentials of being used as alternative to synthetic antioxidants in arresting oxidative activity of hydroxyl ions (Oyetayo et al., 2009). There is an excellent correlation between contents of total phenols and EC₅₀ values of antioxidant activity (Savoie et al., 2008). The presence of zinc and iron in high levels also explains the effectiveness of the samples in scavenging superoxide anion radicals. Cytoplasmic and mitochondrial superoxide dismutase enzyme requires Cu, Zn and Mn to catalyse

the removal of superoxide radicals. Furthermore H₂O₂ in the cell is removed by catalase (C 1.16.1.16) which require Fe (Duthie, 1993). Iron is also required for transport of oxygen and oxidative metabolism (Bothwell et al., 1979). *A. bisporus* could therefore be considered as a complement in the human diet for the health benefits they present.

In conclusion, the ethanolic and hot water extracts from fruit bodies and mycelia were effective in antioxidant properties which represent antioxidant agents to provide prophylaxis against various diseases related to oxidative stress. Phytochemical screening of samples from *A. bisporus* indicated presence of compounds responsible for antioxidant and antibacterial activity. The major antioxidant components found in hot water extracts were total phenols and in ethanolic extracts were total toco-pherols. *A. bisporus* is therefore a cheaper and more natural source of antioxidants useful for the wellbeing of a living body system. The obtained results could form a good basis for selection of mushroom species for further investigation in the potential discovery of new valuable bioactive compounds.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES

- Agati G, Matteini P, Goti A, Tattini M(2007). Chloroplast located flavonoids can scavenge singlet oxygen. *New Phytol.* 174:77-89.
- Aisya B, Naveen T, Pravalika K, Harith G, Anusha K (2010). Treatment of cancer by medicinal mushroom. *Int. J. Pharm. Technol.* 2(2):102-117.
- Banerjee D, Chakrabarti S, Hazra AK, Banerjee S, Ray J, Mukherjee B(2008). Antioxidant activity and total phenolics of some mangroves in Sundarbans. *Afr. J. Biotechnol.* 7(6):805-810.
- Barros L, Falcão S, Baptista P, Freire C, Vilas-Boas M, Ferreira I(2008). Antioxidant activity of *Agaricus sp.* mushrooms by chemical, biochemical and electrochemical assays. *Food Chem.* 111:61-66.
- Bo L, Fei L, Xigomin S, Haijuan N, Ban L (2010). Antioxidant properties of cap and stipe from *Coprinus comatus*. *Molecules* 15: 1473- 1486.
- Bothwell TH, Charlton RW, Cook JD and Finch C A (1979). Iron metabolism in man. Blackwell Scientific, Oxford, England. p. 7.
- Chirinang P, Intarapichet K(2009). Amino acids and antioxidant properties of the oyster mushrooms, *Pleurotus ostreatus* and *Pleurotus sajor-caju*. *Sci. Asia* 35:326-331.
- Chu YH, Sun J, Wu XZ, Liu RH (2002). Antioxidant and antiproliferative activities of common vegetables. *J. Agric. Food Chem.* 50:6910-6916.
- Chung K, Haddad L, Ramakrishna J, Riely F (1997). Identifying the Food Insecure: The Application of Mixed-Method Approaches in India. International Food Policy Research Institute, Washington, DC.
- Duthie G (1993). Lipid peroxidation. *Eur. J. Clin. Nutr.* 47:759-764.
- Ferreira I, Baptista P, Vilas-Boas M, Barros L(2007). Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. *Food Chem.* 100:1511-1516.
- Gursoy N, Sarikurkcü C, Tepe B, Solak MH (2010). Evaluation of Antioxidant Activities of 3 Edible Mushrooms. *Ramariaflava* (Schaeff.: Fr.) Quél, *Rhizopogonroseolus* (Corda) TM, *Fries* and *Russuladelica* Fr. *Food Sci. Biotechnol.* 19(3):691-696
- Jitesh M, Prasanth SR, Sivaprakash KR, Parida A (2006). Monitoring expression profiles of antioxidant genes to salinity, iron, oxidative light and hyperosmotic stresses in the highly salt tolerant gray mangrove *Avicennia marina* (Forsk)Vierh by mRNA analysis. *Plant Cell Rep.* 25:865-876.
- Kim JH, Kim SJ, Park HR, Choi Jj, Ju YC, Nam KC, Kim SJ, Lee SC (2009). The different antioxidant and anticancer activities depending on the color of oyster mushrooms. *J. Med. Plants Res.* 3(12):1016-1020.
- Loganathan K, Jagadish V, Krishnan V, Shenbhagaraman R, Kaviyarasan V (2009). Comparative study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* (J.E Lange) Imbach before and after boiling. *Afr. J. Biotechnol.* 8(4):654-661.
- Mensor L, Menezes FS, Leitao GG, Reis AS, Dos Santos TC, Coube CS, Leitao SG (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother. Res.* 15:127-130.
- Oyazuru M (1986). Studies on products of browning reactions: antioxidative activities of browning reactions prepared from glucosamine. *Jpn. J. Nutr.* 103:413-419.
- Oyetayo VO, Dong CH, Yao YJ (2009). Antioxidant and Antimicrobial Properties of Aqueous Extract from *Dictyophora indusiata*. *Open Mycol. J.* 3:20-26.
- Park W, Weyand CM, Schmidt D, Coronzy JJ(1997). Co-stimulating pathways controlling activation and peripheral tolerance of human CO₄ + CD28- T cells. *Eur. J. Immunol.* 27(5):102-1090.
- Román M, Boa E, Woodward S (2006). Wild-gathered fungi for health and rural livelihoods. *Proc. Nutr. Soc.* 65:190-197.
- Savoie JM, Minvielle N, Largeteau ML (2008). Radical-scavenging properties of extracts from the white button mushroom, *Agaricus bisporus*. *J. Sci. Food Agric.* 88:970-975.
- Shahidi F, Janitha PK, Wanasundara PD (1992). Phenolic antioxidant. *Crit. Rev. Food Sci. Nutr.* 32(1):67-103.

