

Full Length Research Paper

Comparative study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* (J. E. Lange) Imbach before and after boiling

Loganathan K. Jagadish^{1*}, V. Venkata krishnan², R. Shenbhagaraman¹, and V. Kaviyaranan¹

¹University of Madras, Center for Advanced Studies in Botany, Chennai, India.

²Anna University, Center for Biotechnology, Chennai, India.

Accepted 5 January, 2009

In this study, ethanol extracts of an edible mushroom *Agaricus bisporus*, before and after boiling were investigated for antioxidant, anticancer and antimicrobial activities. To confirm the total antioxidant activity, ABTS, DPPH free radical-scavenging assay was carried, along with total phenolic and flavonoid concentration. In measuring ABTS and DPPH free radical scavenging activities, scavenging activity was found to be similar in both the extracts. Total flavonoids were 16.4 ± 0.5 and 15.2 ± 0.2 mg/g (Quercetin equivalent), the phenolics were 90.2 ± 0.6 and 70.6 ± 0.1 mg/g (Catechin equivalent) in raw and boiled extract, respectively. *A. bisporus* extracts inhibited cell proliferation of HL-60 leukemia by the induction of apoptosis. In addition, *A. bisporus* extracts exhibited antibacterial activity against both gram positive and gram negative bacteria, as well as anticandidal activity against *Candida albicans*. Therefore, *A. bisporus* could be considered as a functional food with antimicrobial, anti-oxidative and antiproliferative activity.

Key words: *Agaricus bisporus*, anticancer, antimicrobial, ABTS, DPPH, HL-60.

INTRODUCTION

The arising awareness of the relationship between diet and diseases has evolved the concept of "functional foods" and the development of a new scientific discipline, Functional Food Science (Sadler and Saltmarsh, 1998). A food may be considered to be functional if it contains a food component (whether a nutrient or not) which affects one or more identified functions in the body in a positive manner, which are in different name forms, e.g. dietary supplements, nutraceuticals, medicinal foods, vita foods, pharma foods, phytochemicals, mycochemicals and foods for specific health uses (Hasler, 1996).

Mushrooms, because of their special fragrance and texture, have been used for many years in oriental culture as tea and nutritional food (Manzi et al., 1999). The scientific community, in searching for new therapeutic

alternatives, has studied many kinds of mushrooms and has found variable therapeutic activities such as anti-carcinogenic, anti-inflammatory, immuno-suppressor and antibiotic among others (Asfors and Ley, 1993). It has been known for many years that selected mushrooms of higher Basidiomycetes origin are effective against certain cancer types, and this have stirred a growing interest in such mushrooms from industry, the media and the scientific community (Wasser, 2002).

Agaricus bisporus, known as table mushroom, cultivated mushroom or button mushroom, is an edible basidiomycete fungus which naturally occurs in grasslands, fields and meadows across Europe and North America. It has spread much more widely and is one of the most widely cultivated mushrooms in 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; it can be found boiled on pizzas and casseroles, stuffed mushrooms, raw on salads, and in various forms in a variety of dishes.

*Corresponding author. E-mail: jk_l_phd@yahoo.co.in, manikavi53@gmail.com. Tel: + 91 9884410966/ Fax: 044-22352494.

Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, and atherosclerosis as well as in degenerative processes associated with aging (Halliwell and Gutteridge, 1984). Organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols and glutathione (Mau et al., 2002). As an integral part of the human diet, vegetables, fruits, seeds, tea, wines and juices have received much attention since many epidemiological studies suggest that consumption of poly phenol-rich foods and beverages is associated with a reduced risk of cardiovascular diseases, stroke and certain types of cancer. These protective effects have partly been ascribed to the antioxidant properties. Among flavanone aglycons, naringenin, hesperetin, eriodictyol and isosakuranetin are the most common, but they are present in much smaller quantities than glycosides. Citrus flavonoids, especially hesperidin, have shown a wide range of therapeutic properties such as anti-inflammatory, antihypertensive, diuretic, analgesic and hypolipidemic activities (Inga et al., 2007).

Researchers showed antimicrobial activity of several mushrooms (Gezer et al., 2006; Mercan et al., 2006 and Turkoglu et al., 2007). In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists for searching new antimicrobial substances from various sources which are the good sources of novel antimicrobial chemotherapeutic agents (Karaman et al., 2003). Extracts from fruiting bodies and the mycelia of various mushrooms have been reported for antimicrobial activity against wide range of infectious bacteria (Hirasawa et al., 1999; Dulger et al., 2002).

There is no doubt that edible mushrooms are nutritionally sound tasteful food source for most people and can be a significant dietary component for vegetarians. Nevertheless, the edible mushrooms consumed as raw food in most of the western countries, whereas in countries like India most of the food items were boiled, cooked and consumed. In that case whether it retains its biological activity, can they be considered as a functional food? Based on this problem, the present work is carried out to evaluate the antioxidant, anticancer and antimicrobial activities of *A. bisporus* before and after boiling. One part of the tissue was prepared by boiling and extracted with ethanol for further analysis.

MATERIALS AND METHODS

Mushroom

The Button mushroom *A. bisporus* was cultivated in our laboratory.

It was divided into two, one part was shade dried at room temperature and the other part of the tissue was boiled for one h at 100°C. The tissues collected were shade dried at room temperature. Dried mushroom sample (50 g) was extracted by stirring with 500 mL of ethanol at 30°C at 150 rpm for 24 h and filtered through Whatman No. 4 filter paper. The residue was then extracted with two additional 500 mL of ethanol as described above. The combined ethanol extract was then rotary evaporated at 40°C to dryness, re-dissolved in ethanol to a concentration of 10 mg/mL and stored at 4°C for further use.

Chemicals

2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) 1,1-Diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and α -tocopherol, Trolox (6-hydroxy-2-5-8 tetra methyl chroman-2-carboxylic acid) were purchased from Sigma (Sigma, Aldrich GmbH, Sternheim, GERMANY). Catechin, Folin-ciocalteu's phenol reagent (FCR), sodium carbonate, ethanol, RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin (100 IU/ml), and streptomycin (100 μ g/ml), MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, propidium iodide. and the other chemicals and reagents were purchased from Merck (Darmstadt, GERMANY). All other unlabelled chemicals and reagents were analytical graded.

ABTS radical scavenging activity

The Trolox equivalent antioxidant capacity (TEAC) was estimated using the Feryl Myoglobin/ ABTS method for total antioxidant activity (Pellegrini et al., 1999). In this assay, ABTS is oxidized by peroxy radicals or other oxidants to its radical cation, ABTS⁺, which is intensely colored, and AOC (antioxidant capacity) is measured as the ability of test compounds to decrease the color reacting directly with the ABTS⁺ radical. Results of test compounds are expressed relative to Trolox. The stock solution included 7.4 mM ABTS solution and 140 mM potassium per-sulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in dark. The solution was then diluted by mixing 1 ml ABTS solution with ethanol to obtain an absorbance of 0.7 \pm 0.02 units at 734 nm using UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan). The antioxidant property was determined by reduction in the O.D. compared with the standard Trolox. Inhibition of free radical by ABTS⁺ in percent (%) was calculated in following way:

$$I (\%) = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. The values of inhibition were calculated for the various concentrations of ethanol extracts. Tests were carried out in triplicates.

DPPH assay

The hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-colored methanol solution of 2, 2 diphenylpicrylhydrazyl (DPPH). This spectrophotometer assay uses the stable radical DPPH as a reagent. One thousand micro liters of various concentrations of the extracts in ethanol were added to 4 mL of 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against

a blank at 517 nm. Inhibition of free radical by DPPH in percent (I %) was calculated in following way:

$$I (\%) = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition per-centage against extract concentration. Tests were carried out in triplicate.

Determination of total phenolic compounds

Total phenolic compounds in the ethanol extracts were determined using Folin–Ciocalteu method, (Ragazzi and Veronese, 1973). One mL of the extract was added to 10.0mL distilled water and 2.0 mL of Folin-Ciocalteu phenol reagent. The mixture was allowed to stand at room temperature for 5 min and then 2.0 mL of 20% sodium carbonate was added to the mixture. The resulting blue complex was then measured at 680 nm. Catechin was used as a standard for the calibration curve. The phenol compound was calibrated using the linear equation based on the calibration curve. The contents of the phenolic compound were expressed as mg catechin equivalent/g dry weight.

Determination of total flavonoid concentration

The $AlCl_3$ method (Lamaison and Carnet, 1990) was used for determination of the total flavonoid content of the sample extracts. Aliquots of 1.5 ml of extracts were added to equal volumes of a solution of 2% $AlCl_3$ (2 g in 100 mL methanol). The mixture was vigorously shaken, and absorbance at 367 nm was read after 10 min of incubation. Quercetin was used as a standard for the calibration curve. The flavonoid compound was calibrated using the linear equation based on the calibration curve. The contents of the flavonoid compound were expressed as mg Quercetin equivalent/g dry weight.

Antimicrobial activity

The following strains of bacteria were used: *Pseudomonas aeruginosa* ATCC 2036, *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 27736, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 and *Candida albicans* (ATCC 90028). They were collected from National cell culture collection (NCCS) Pune, India.

Antimicrobial activity of ethanol extract of *A. bisporus* was determined by the agar-well diffusion method. All the microorganisms mentioned above were incubated at $37 \pm 0.1^\circ\text{C}$, ($30 \pm 0.1^\circ\text{C}$). *C. albicans* was incubated in YEPD broth at $28 \pm 0.1^\circ\text{C}$ for 48 h. The culture suspensions were prepared and adjusted by comparing against 0.4-0.5 McFarland turbidity standard tubes. Nutrient Agar and YEPD Agar (20 ml) were poured into each sterilized Petri dish (10 X 100 mm diameter) after injecting cultures (100 μl) of bacteria and yeast and the medium was distributed in the Petri dishes homogeneously. For the investigation of the antibacterial and anticandidal activity, the dried mushroom extracts were dissolved in dimethylsulfoxide (DMSO) to a final concentration of 20% and sterilized by filtration through a 0.22 μm membrane filter (Tepe et al., 2005). Each sample (100 μl) was filled into the wells of agar plates directly. Plates injected with the yeast cultures were incubated at 28°C for 48 h. At the end of the incubation period, inhibition zones formed on the medium were evaluated in mm. Studies were performed in duplicates and the inhibition zones were

compared with those of reference discs. Inhibitory activity of DMSO was also tested. Reference discs used for reference value and as a control were as follows: nystatin (100 U), ketoconazole (50 μg), tetracycline (30 μg), ampicillin (10 μg), penicillin (10 U), oxacillin (1 μg), tetracycline (30 μg) and gentamycin (10 μg). All determinations were done in duplicates.

MTT assays

Human promyelotic leukemia HL-60 cells purchased from NCCS (Pune, India) were grown in a humidifier atmosphere containing 5% CO_2 in RPMI-1640 medium supplemented with 10% FBS and penicillin (100 IU/ml), and streptomycin (100 $\mu\text{g}/\text{ml}$), HL-60 cells were seeded at a density of 1×10^5 cells/ml and treated with the extract after 24 h.

Cell suspensions were prepared at a concentration of 2×10^4 cells/ml were seeded in 96-wells micro-culture plates with or without extract treatment in a volume of 100 μl . After 72 h incubation at 37°C , 20 μl of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well, and the samples were incubated for further 2 h at 37°C . Plates were analyzed on a micro-titer plate reader at 492 nm. The inhibition ratio (I) was calculated according to the following equation:

$$I (\%) = [(A_{\text{control group}} - A_{\text{AB treated group}})/A_{\text{control group}}] \times 100$$

Assessment of apoptosis

Apoptotic cell death was measured as the percent of cells with hypodiploid DNA. HL-60 Cells cultured with or without the samples (100 $\mu\text{g}/\text{ml}$) at 37°C for 48 h were harvested, washed with PBS, and fixed with 75% ethanol at 4°C for 2 h. Cells were then treated with RNase A (0.25 mg/ml) at 37°C for 1 h. After washing, the cells were stained with 50 mg/ml propidium iodide at room temperature for 10 min. Cell cycle analysis was performed on FACS calibur flow cytometer (Becton-Dickinson, San Jose, CA).

RESULTS

Antioxidant activity of ethanolic extracts of *A. bisporus*

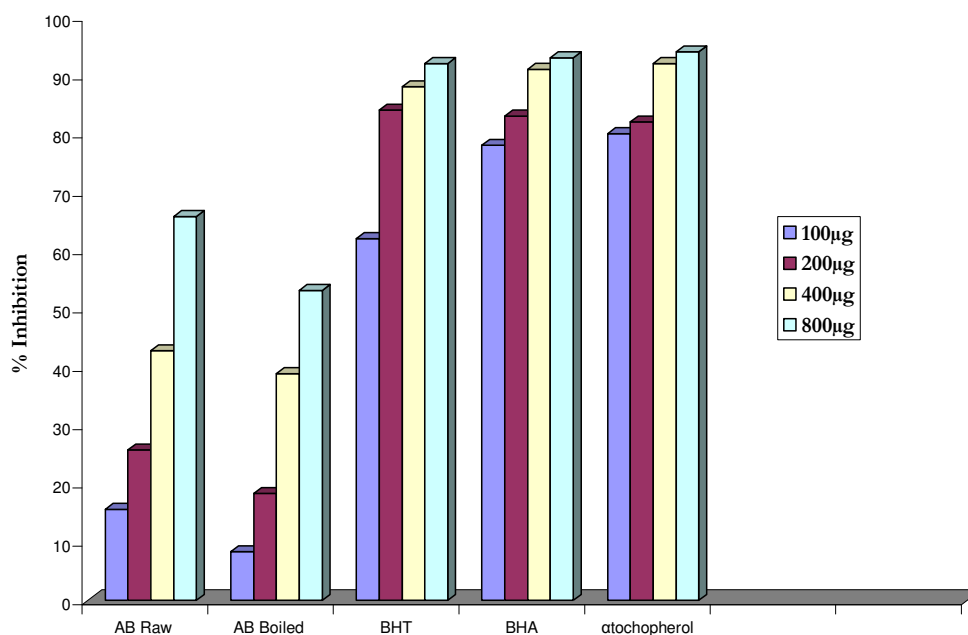
To find out the antioxidant activity of the ethanolic extracts of *A. bisporus*, four complementary test systems, namely ABTS free radical-scavenging, DPPH free radical scavenging systems, total phenolic and total flavonoid concentration were used for the analysis. ABTS, a stable free radical with a characteristic absorption at 734 nm, was used to study the radical-scavenging effects of different organic extracts because the TEAC (Trolox equivalent antioxidant capacity) is operationally simple. TEAC values of many compounds and food samples have been reported (Proteggente et al., 2002).

It was found that inhibition values of ethanol extracts of *A. bisporus* both boiled, raw and the standards increased with increase in concentration. Inhibition values were found to be 15.77, 39.7, 78.23, and 85.44% in raw extract, and 17.32, 42.95, 78.32, and 82.44% in boiled extracts at a concentration of 100, 200, 400 and 800 $\mu\text{g}/\text{ml}$, respectively (Table 1). In both extracts, the inhibi-

Table 1. *In vitro* antioxidant activity of ethanol extracts of *Agaricus bisporus* using ABTS.

ABTS assay	Raw (μg)				Boiled (μg)			
	100	200	400	800	100	200	400	800
Inhibition (%)	15.77 ^a	39.7	78.23	85.44	17.32	42.95	78.32	82.44
Trolox equivalent antioxidant activity (mmol/kg)	7	32	84	92	5	27	54	88

^aMeans \pm SD from triplicate determination.

**Figure 1.** Free radical scavenging capacity of the extract measured in DPPH assay.

tory concentration (IC_{50}) was found to be $\geq 400 \mu\text{g/ml}$. This suggests that antioxidant activity was retained even after boiling or cooking and the antioxidant compounds were found to be thermostable.

DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of extracts. As antioxidants donate protons to these radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging. Free radical scavenging capacities of the extracts, measured by DPPH assay, shown in Figure 1, revealed that with the increase in the concentration of *A. bisporus* ethanol extract, an increase in DPPH free radical-scavenging was observed in both cases. Inhibition values of 15.59, 25.78, 42.77 and 65.76% in raw extract, and 8.30, 18.30, 38.87 and 53.09% in boiled extracts were observed at a concentration of 100, 200, 400 and 800 $\mu\text{g/ml}$, respectively. In both the extracts the inhibitory concentration (IC_{50}) was $\geq 800 \mu\text{g/ml}$. The values obtained here shows that raw ethanol extract of *A. bisporus* showed more activity when compared with boiled one but the difference was found to be around 8%.

Amount of total phenol and flavonoids

Amounts of total flavonoid components of ethanolic extracts of raw and boiled *A. bisporus* were found to be $16.4 \pm 0.5 \text{ mg}^{-1}$ and 15.2 ± 0.2 (quercetin equivalent) while the phenolics were $90.2 \pm 0.6 \mu\text{g mg}^{-1}$ and 70.6 ± 0.1 (pyrocatechol equivalent) respectively (Table 2). The amount of antioxidant compounds, found to be very similar in both boiled and raw extract, is the major compound responsible for antioxidant activity.

Antimicrobial activity of extract

The antimicrobial effect of ethanol extracts of *A. bisporus* was tested against two species of Gram-positive bacteria, three species of Gram-negative bacteria and one species of yeast. As summarized in Table 3, *A. bisporus* extract had a narrow antibacterial spectrum against Gram-negative bacteria and strongly inhibited the growth of the Gram-positive bacteria tested, including *B. subtilis*. The maximal zones of inhibition ranged from 12 to 22 mm. The most susceptible bacterium was *S. aureus* for both

Table 2. Amounts of total flavonoid and total phenolic compounds in *Agaricus bisporus* ethanolic extract.

<i>Agaricus bisporus</i>	Total phenolic compounds [catechin equivalents (m /g)]		Total flavonoid content [quercetin equivalents (mg/g)]	
	Raw	Boiled	Raw	Boiled
Ethanolic extract	90.02±0.6 ^a	70.67 ± 0.1	16.4±0.5	15.2±0.2

^aMeans ± SD from triplicate determination.

Table 3. Antimicrobial activities of ethanol extract *Agaricus bisporus* (AB) and antibiotic sensitivity of microorganisms (zone size, mm).

Test bacteria/fungus	<i>A. bisporus</i>		N	A	P	G	O	T
	Raw	Boiled						
<i>Staphylococcus aureus</i> ATCC 25923	22±4	18±1	NT	NT	31	16	21	20
<i>Bacillus subtilis</i> ATCC6633	12±1	12±5	NT	NT	12	NT	8	17
<i>Escherichia coli</i> ATCC 35218	-	-	NT	10	11	NT	NT	8
<i>Klebsiella pneumonia</i> ATCC 27736	15	-	NT	-	18	NT	NT	5
<i>Pseudomonas aeruginosa</i> ATCC 2036	16±0.2	12±0.3	NT	NT	NT	16	NT	8
<i>Candida albicans</i> ATCC 90028	14±0.5	12±0.4	19	NT	NT	NT	NT	NT

N, Nystatin (100 U); A, ampicillin (10µg); P, penicillin (10 U); G, gentamycin (10 µg); O, oxacillin (1µg); T, tetracycline (30 µg); (NT) not tested; (-) No inhibition.

Table 4. The inhibitory effect of *Agaricus bisporus* (AB) extracts on HL-60 leukemia cells using cell proliferation or MTT assay.

Cell proliferation or MTT Assay	Raw (mg)			Boiled (mg)		
	0.125	0.25	0.5	0.125	0.25	0.5
Inhibition (%)	40.2 ^a	54.8	66.4	48.6	60.25	71.31

^aMeans ± SD from triplicate determination.

boiled and raw extract with a diameter of 18 ± 1 and 22 ± 4 mm, respectively. The ethanol extract of *A. bisporus* boiled showed no antibacterial activity against *K. pneumoniae*, but in raw extract clear zone was observed of 15 ± 1 mm diameter. Against *P. aeruginosa*, both the extracts of *A. bisporus* showed antibacterial activity but the activity range was less in boiled extract (12 ± 0.3) when compared with raw mushroom extract (16 ± 0.2). This might be due to the influence of temperature that disturbed the compound which is responsible for the activity. In the present study, the ethanol extracts of *A. bisporus* both boiled and raw exhibited anti-candidal activity against *C. albicans*.

MTT assay

To examine the effect of *A. bisporus* extracts on HL-60 leukemia cell proliferations, the cells were treated with increasing concentration of 0 – 0.5 mg/ml and the cell growth was determined. As shown in Table 4, both the extracts of *A. bisporus* inhibited the cell proliferation in dose dependent manner, because after 72 h of treatment

with 0.125, 0.25 and 0.5 mg/ml of *A. bisporus* extracts, raw and boiled inhibited proliferation of HL-60 cells by 40.2, 54.8, 66.4 and 48.6, 60.25, 71.3, respectively. The activity was very similar in both the extracts; the percentage of inhibition was high in boiled ethanolic extract when compared with the raw ethanolic extract of *A. bisporus*.

Assessment of apoptosis

To evaluate whether the cytotoxic effect observed upon treatment of HL-60 cells with both the extracts was due to the induction of apoptosis, cells were treated with the extracts at 0.5 mg/ml for 24-48 h and the cell cycle distribution was determined by flow-cytometry after 48 h as shown in Figure 2. Thus, the treatment of HL-60 leukemia significantly changes the amount of cells at M1 phase of around 65.41% in *A. bisporus* raw extract and 51.88 % in boiled extract. These data suggest that *A. bisporus* extract inhibited the growth of HL-60 cells by cell cycle arrest, that is, by the induction of apoptosis.

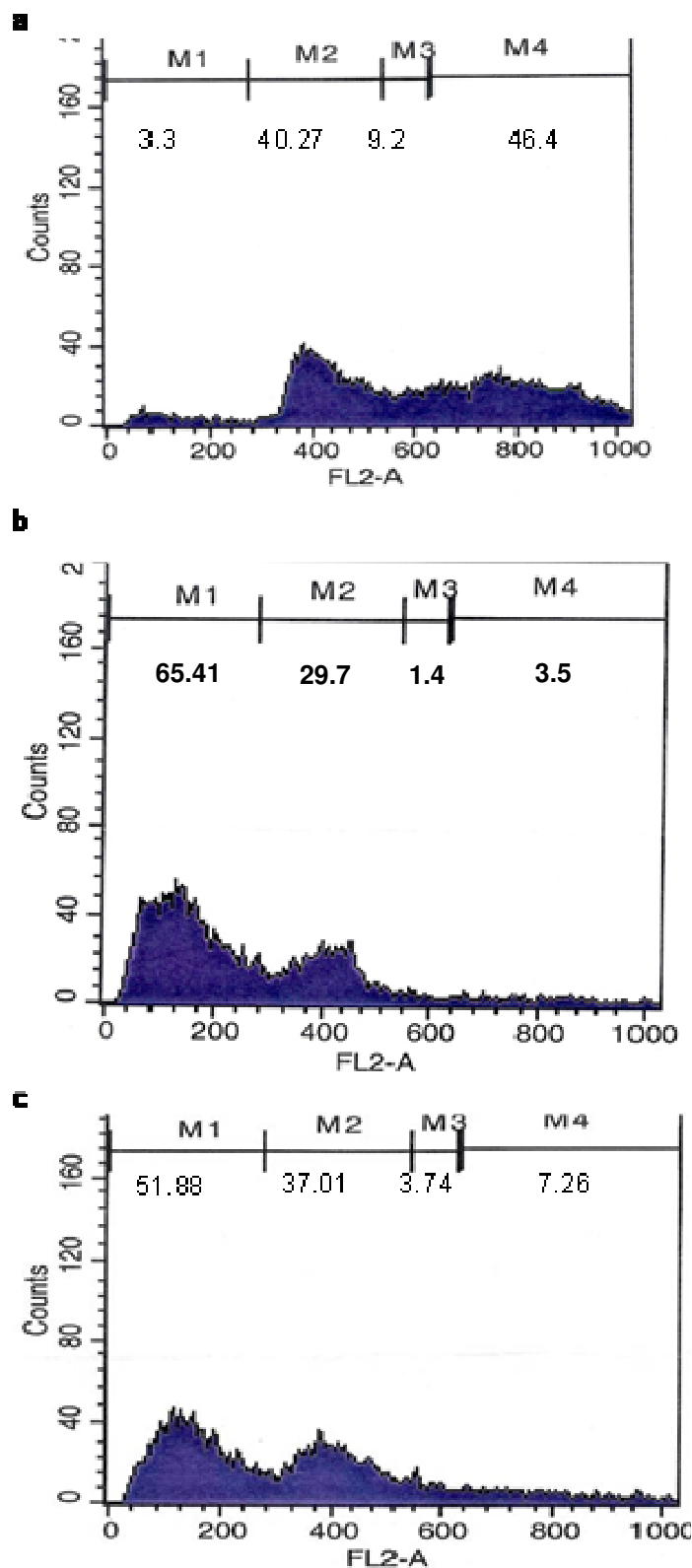


Figure 2. Induction of apoptosis by *Agaricus bisporus* extracts in HL-60 cells. Cells were treated with extract (0.5 mg/ml) for 48 h (a) Control (b) cells treated with raw ethanol extract (C) cells treated with boiled ethanol extract. The percentage of apoptotic cells was assessed by flow cytometry.

DISCUSSION

ABTS⁺ reacts rapidly with antioxidants, and it can be used over a wide pH range to study the effects of pH on antioxidant mechanisms (Lemanska et al., 2001). Also, ABTS⁺ is soluble in both aqueous and organic solvents and is not affected by ionic strength, and thus can be used in multiple media to determine both hydrophilic and lipophilic antioxidant capacities of extracts and body fluids (Awika et al., 2003). But the compounds involved in biological relevance were found to reduce DPPH less when compared to the synthetic antioxidant compounds like BHT and BHA (Prior et al., 2005). One possible reason for lesser amount of inhibition found in DPPH assay when compared to ABTS antioxidant assay would be because of the chemical nature of DPPH.

The role of phenolic compounds as scavengers of free radicals is widely reported; phenolic compound plays a major role in antioxidant activity of many vegetables (Komali et al., 1999; Moller et al., 1999). It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ingested daily from a diet rich in fruits and vegetables (Tanaka et al., 1998). Polyphenolic compounds seem to have important role in stabilizing lipid oxidation and they are associated with antioxidant activity (Gulcin et al., 2003). Many studies had attributed antioxidant properties to the presence of phenols and flavonoids (Gezer et al., 2006; Turkoglu et al., 2007). Previously, in a study performed with *Hypericum hyssopifolium*, it was found that antioxidant activity was based on flavonoid-type compounds (Cakir et al., 2003).

In this study, performed with *A. bisporus*, it is thought that the high free radical-scavenging activity and total antioxidant activity may result from the existence of phenolic and flavonoid type compounds.

The antimicrobial activity of the extract *A. bisporus* showed prominent result against gram positive organisms when compared with gram negative organisms. Similar observation was made in ethanolic extract of a polyporus mushroom *Laetiporus sulphureus* (Bull. Murill (Turkoglu et al., 2007). The antimicrobial properties of the *A. bisporus* confirm previous studies that mushrooms possess antimicrobial effects (Hur et al., 2004; Sheena et al., 2003).

The activity against HL60 cells shows the anti-proliferative property. Though there have been number of reports that various basidiomycetes have antitumor activity by direct or indirect mechanisms including antioxidant defense or host immune system potentiation (Zaidman et al., 2005). Though many reports indicated that most of the polysaccharide or polysaccharide protein complexes from mushroom or natural sources cannot exert direct cytotoxicity on tumor cells, but predominantly by host mediated immune response, this type of inhibitory action may be interwoven and the mechanism of action is varied from person to person and the type of cancer. Therefore, the mechanism of action is urgently needed

(Daba and Ezeronye, 2003). Extraction of small molecule exerting direct cytotoxicity in relation with antioxidant compounds like phenol and flavonoids have demonstrated that chemotherapy induced apoptosis and subsequent phagocytosis of cancer cells depend on the redox status and the intracellular balance between pro- and antioxidants (Shacter et al., 2000). Ergosterol, a phenolic compound extracted from white button mushroom showed inhibitory effect on breast cancer cell line *in vitro* by aromatase inhibition without side effect (Baiba et al., 2001).

In conclusion, the edible mushroom *A. bisporus* consumed has essential medicinal properties. Boiling or cooking did not dilute or reduce the medicinal properties. Hence it is necessary to identify the biological and pharmacological potential of mushrooms especially edible mushrooms which are collected indigenously and cultivated locally or sold in local and international market. In 2003-2004, 857 million pounds of mushrooms were grown in the United States alone with a value of almost 1 billion dollars (USDA, 2004). The production and marketing of mushrooms and their products is vital for an economic importance. Therefore, it is also necessary to intensify research in identifying and isolating different varieties of mushrooms having nutraceutical and medicinal properties and to commercialize their production and marketing, which will boost the food industry and create employment especially in villages.

ACKNOWLEDGEMENT

The authors thank the Director, CAS in Botany, University of Madras for the laboratory facility.

REFERENCES

- Asfors KE, Ley K (1993). Sulfated polysaccharides in inflammation. *J. Lab. Clin. Med.* 121: 201-202.
- Awika JM, Rooney LW, Wu X, Prior RL, Cisneros-Zevallos L (2003). Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. *J. Agric. Food Chem.* 51: 6657-6662.
- Baiba JG, Elizabeth TE, Yeh-Chih K, Annette K, Shiuan C (2001). White button mushroom phytochemicals inhibit Aromatase activity and Breast cancer cell proliferation. *J. Nutr.* 131: 3288-3293.
- Cakir A, Mavi A, Yildirim A, Duru ME, Harmandar M, Kazaz C (2003). Isolation and characterization of antioxidant phenolic compounds from the aerial parts of *Hypericum hyssopifolium* L. by activity-guided fractionation. *J. Ethnopharmacol.* 87: 73-83.
- Daba AS, Ezeronye OU (2003). Anticancer effect of polysaccharide isolated from higher basidiomycetes mushrooms. *Afr. J. Biotechnol.* 2(12): 672-678.
- Dulger B, Ergul CC, Gucin F (2002). Antimicrobial activity of the macrofungus *Lepista nuda*. *Fitoterapia*, 73: 695-697.
- Gezer K, Duru E, Kivrak, Turkoglu A, Mercan N, Turkoglu H, Gukan S (2006). Free radical Scavenging capacity and antimicrobial activity of Wild –Edible mushroom from Turkey. *Afr. J. Biotechnol.* 5(20): 1924-1928.
- Gulcin Y, Buyukokuroglu ME, Oktay M, Kufruevioglu OY (2003). Antioxidant and analgesic activities of turpentine of *Pinus nigra* Arn. subsp. *palliana* (Lamb.) Holmboe. *J. Ethnopharmacol.* 86: 51-58.
- Halliwell B, Gutteridge JMC (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.* 219: 1-4.
- Hasler CM (1996). Functional food: the western perspective, *Nutr. Rev.* 54: 506-510.
- Hirasawa M, Shouji N, Neta T, Fukushima K, Takada K (1999). Three kinds of antibacterial substances from *Lentinus edodes* (Berk.) Sing. (Shiitake, an edible mushroom). *Intl. J. Antimicrobial Agents.* 11: 151-157.
- Hur JM, Yang CH, Han SH, Lee SH, You YO, Park JC (2004). Antibacterial effect of *Phellinus linteus* against methicillin-resistant *Staphylococcus aureus*. *Fitoterapia.* 73: 603-605.
- Inga K, Maria M, Miroslawa S, Anna G (2007). Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. *J. Food Compost. Anal.* 20: 313-322.
- Karaman Y, Bin SA, Gu F, Ilu` ce M, g`u` tc, u` O`, S` engu` HIM, Adigu` ZA (2003). Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. *J. Ethnopharmacol.* 85: 213-235.
- Komali AS, Zheng Z, Shetty K (1999). A mathematical model for the growth kinetics and synthesis of phenolics in oregano (*Origanum vulgare*) shoot cultures inoculated with *Pseudomonas species*. *Proc. Biochem.* 35: 227-235.
- Lamaison JLC, Carnet A (1990). Teneurs en principaux flavonoids des fleurs de *Crataegus monogyna* Jacq et de *Crataegus laevigata* (Poiret D. C) en fonction de la vegetation. *Pharm. Acta. Helv.* 65: 315-320.
- Lemanska K, Szymusiak H, Tyrakowska B, Zielinski R, Soffer AEMF, Rietjens IMCM (2001). The influence of pH on the antioxidant properties and the mechanisms of antioxidant action of hydroxyflavones. *Free Rad. Biol. Med.* 31: 869-881.
- Manzi P, Gambelli L, Marconi S, Vivanti V, Pizzoferrato L (1999). Nutrients in edible mushroom: An inter-species comparative study. *Food Chem.* 65: 477-482.
- Mau JL, Lin HC, Song SF (2002). Antioxidant properties of several specialty mushrooms. *Food Res. Intl.* 35: 519-526.
- Mercan N, Duru ME, Turkolu A, Gezer K, Kivrak, Turkolu H (2006). Antioxidant And Antimicrobial Properties Of Ethanolic Extract From *Lepista nuda* (Bull.) Cooke., *Ann. Microbiol.* 56(4): 339-344.
- Moller JKS, Madsen HL, Altonen T, Skibsted LH (1999). Dittany (*Origanum dictamnus*) as a source of water-extractable antioxidants. *Food Chem.* 64: 215-219.
- Pellegrini N, Roberta RE, Yang M, Evans C (1999). Screening of Dietary Carotenoids and Carotenoid-Rich Fruit Extracts for Antioxidant Activities applying 2, 2'-Azinobis (3-ethylene benzothiazoline) 6-sulphonic acid and radical cation decolourisation assay. *Methods Enzymol.* 299: 379-390.
- Proteggente AR, Pannala AS, Paganga G, Van Buren L, Wagner E, Wiseman S, Van De Put F, Dacombe C, Rice-Evans CA (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Rad. Res.* 36: 217-233.
- Ragazzi E, Veronese G (1973). Quantitative analysis of phenolics compounds after thin layer chromatographic separation. *J. Chromotogr.* 77: 369-375.
- Shacter E, Williams JA, Hinson RM, Senturker S, Lee YJ (2000). Oxidative stress interferes with cancer chemotherapy: inhibition of lymphoma cell apoptosis and phagocytosis. *Blood.* 96: 307-313.
- Sadler M, Saltmarsh M (1998). Functional foods: The consumer, the products and the evidence, (Royal society of chemistry, Cambridge.
- Sheena N, Ajith A, Mathew TA, Janardhanan KK (2003). Antibacterial activity of three Macrofungi *Ganoderma lucidum*, *Navesporus floccose* and *Phellinus rimosus* occurring in South India. *Pharm. Biol.* 41: 564-567.
- Tanaka M, Kuei CW, Nagashima Y, Taguchi T (1998). Application of antioxidative maillard reaction products from histidine and glucose to sardine products. *Nippon Suisan Gakkaishil.* 54: 1409-1414.
- Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M (2005). Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salviatomentosa* Miller (Lamiaceae). *Food Chem.* 90: 333-340.
- Turkoglu A, Duru ME, Mercan N, Kivrak I, Gezer K (2007). Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Muril. *Food Chem.* 101: 267-273.

- Wasser SP (2002). Review of medicinal mushroom advances Good news from old allies. *Herbal Gram* 56: 2833.
- Zaidman BZ, Yassin M, Mahajna J, Wasser SP (2005). Medicinal mushroom modulators of molecular targets as cancer therapeutics. *Appl. Microbiol. Biotechnol.* 67: 453-468.