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Review Article

ANTIOXIDANT MUSHROOMS: A REVIEW

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ABSTRACT
The antioxidant properties of wild mushrooms have been extensively studied and many antioxidant compounds such as phenolic compounds, tocopherols, ascorbic acid, and carotenoids identified. The various antioxidant mechanisms of the mushroom species extracts may be attributed to strong hydrogen-donating ability, metal-chelating ability, and their effectiveness as good scavengers of superoxide and free radicals. This indicates the potential of mushrooms as panacea for many diseases and also reveals a novel potential to fight against tumors in man.

Keywords: Mushrooms, Antioxidants, Free radicals, Phenolic compounds, Tocopherols, Carotenoids.

INTRODUCTION

Mushrooms have continued to generate a lot of interest particularly in its consumption as food[1], in the cure of diseases[2], in bioremediation and as important items of commerce all over the world that stems from their nutritional, antioxidant and therapeutic values[3-6]. Mushrooms may prove to be one of the useful candidates in the search for bioactive compounds with radical scavenging activity as the fruiting body can be produced in much less time, the mycelium may also be rapidly produced in liquid culture and the culture medium can be manipulated to produce optimal quantities of active products[7-10]. The antioxidant properties of wild mushrooms have been extensively studied and many antioxidant compounds extracted from these sources were identified such as phenolic compounds, tocopherols, ascorbic acid, and carotenoids.

Naturally **phenolic compounds** are produced as accumulated end products from the shikimate and acetate pathways and can range from relatively simple molecules (phenolic acids, phenylpropanoids, flavonoids) to highly polymerised compounds (lignins, melanins, tannins), with flavonoids representing the most common and widely distributed sub-group, main phenolic compounds found in mushrooms were phenolic acids. Total phenols inhibit occurrence of atherosclerosis and cancer[11,12].

Vitamin C, a necessary nutrient, is thought to exert a protective role against various oxidative stress-related diseases such as heart disease, stroke, cancer, several neurodegenerative diseases and cataractogenesis[13]. Among simplest vitamins, it is one found in mushrooms that has been determined using HPLC coupled to UV or fluorescence detector, or following the spectrophotometer procedure based on the reaction with 2,6-dichlorophenolindophenol[14].

Carotenoids, exhibiting provitamin and antioxidant roles, particularly β- carotene were found in several mushroom species. As they can undergo isomerization, they are found in trans and cis isomers in which trans isomers are more common in food and are stable. Carotenoids reactivity depends on the length of the chain of conjugated double bonds and the characteristics of the end groups[15-17].

L-ergothioneine is a biologically active and stable antioxidant produced by certain fungal species and mycobacterium, not in plants. The precursors to the synthesis of L-ergothioneine are the amino acids- histidine, cysteine, and methionine. Supplementation with L-ergothioneine has been shown to have a protective effect on the organs of rats against lipid peroxidation and to conserve the consumption of endogenous glutathione and α-tocopherol. The ergothioneine content of mushrooms has been reported to be in the range of 0.4-2.0mg/g (dry weight)[18].

Methods for estimating Antioxidant activity

There are several methods to measure the efficiency of dietary antioxidants either as pure compounds or in food matrices. These methods focus on the different mechanisms of the antioxidant defense system, such as: the scavenging of oxygen and hydroxyl radicals, the reduction of lipid peroxyl radicals, the inhibition of lipid peroxidation, or the chelation of metal ions. The interaction between free radicals (such as superoxide and hydroxyl radicals) and antioxidants can show direct evidence for antioxidants to scavenge free radicals and has been widely used to evaluate the radical scavenging ability of antioxidants[19]. These include: Superoxide anion radical scavenging activity; Nitric oxide scavenging activity; Thiobarbituric reactive substances (TBARS assay); 1, 3-diethyl-2-thiobarbituric acid (DETBA) method; Reducing power; 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging activity; Cupric ion reducing antioxidant capacity (CUPRAC); Hydroxyl radicals scavenging activity; Chelating effects on ferrous ions; Linoleic acid assay; β-carotene bleaching inhibition; Hemolysis inhibition; SOD activity; Xanthine oxidase inhibition; Chorioallantoic membrane (CAM) assay ; Conjugated diene method; 2, 7-dichloro fluorescin diacetate (DCF) / 2, 2'-azobis(2-amidinopropane) dihydrochloride (AAPH) assay; 2, 2'-azinobis-(3-ethylbenzthiazoline-6-sulphoronic acid (ABTS) radical cation scavenging activity; Hydrogen peroxide scavenging activity; Thiocyanate method; Chelating effects on cupric ions; Lipid Peroxidation method; ferric reducing

antioxidant power assay(FRAP); Trolox equivalent antioxidant capacity(TEAC)[20,21].

Various studies done to determine Antioxidant potential of different species of mushrooms

Determination of antioxidant activity of ten species of wild Nigerian mushrooms including *Cantharelle cibarius, Laccaria amethysta, Clitocybe odora, Lepista nuda, Macrolepiotata procera, Lepista saeva, Lactarius deliciousus, Laccaria laccata, Pleurotus ostreatus* and *Hericium erinaceus* by studying inhibition of lipid peroxidation (antioxidant activity) revealed that all ten species of wild Nigerian mushrooms have antioxidant activity as they were able to inhibit peroxidation induced in liver homogenate. *C. cibarius, L. amethysta, C.odora* and *L. nuda* species elicited higher antioxidant activity compared to the standard ascorbic acid while the remaining species inhibited peroxidation to a lower extent[22,23].

Recent investigations carried out showed that medicinal mushrooms occurring in South India namely *Ganoderma lucidum, Phellinus rimosus, Pleurotus florida* and *Pleurotus pulmonaris* possessed profound antioxidant and antitumor activities and also showed significant antimutagenic and anticarcinogenic activities. The ethyl acetate, methanol and aqueous extracts were effective to scavenge O^{2-} generated from the photoillumination of riboflavin, $^{\cdot}OH$ generated from Fenton's reaction, nitric oxide radical released from aqueous solution of sodium nitroprusside in a dose dependent manner. Methanol extract effectively reduced ferric ion in FRAP assay and scavenged DPPH radicals[24,25].

The potential antioxidant and reducing properties and content of antioxidant compounds, phenolics and flavonoids, of three edible mushroom species *Lentinula edodes, Hericium erinaceous and Agrocybe aegerita* from Istra region and scavenging capacity on DPPH radicals were determined. The highest extraction yield was achieved in L. edodes extraction and highest total phenolics and total flavonoids content, as well as TF/TP ratio were determined for *A. aegerita* dry extract and all mushroom dry extracts possessed reductive capabilities. The results indicated that the antioxidant activity of mushroom extracts highly depends on extract concentration, i.e. concentration of active compounds[26,27].

Antioxidative potency of commercially available mushrooms in Taiwan was studied using the DPPH method and oil-in-water emulsion oxidation. The antioxidative activities of ethanol extracts of various mushrooms in an emulsified corn oil (o/w) system at 60° C were compared. The addition of test compounds in corn oil emulsions significantly extended the induction period of lipid oxidation. Mushroom *(Agaricus bisporus)* contained significant amounts of phenolic amino acids (tyrosine, L-glutaminyl-4-hydroxybenzene, 3, 4-dihydroxyphenylalanine and L-glutaminyl-3, 4-dihydroxybenzene) and small amounts of vitamin C which may be responsible for the relatively high antioxidative activity. The effect of mushroom extracts on retarding emulsion oxidation was *Agaricus bisporus >Hypsizigus marmoreus >Volvariella volvacea >Flammulina velutipes >Pleurotus eryngii >Pleurotus ostreatus >Hericium erinaceus >Lentinula edodes.* These results indicated that the ethanol extracts of *Agaricus bisporus* and *Hypsizigus marmoreus* are highly effective in retarding the oxidation of corn oil emulsion during storage[28,29].

Both the sulfation of acid *Auricularia auricular* polysaccharides (SAAAP) and the sulfation of neutral *Auricularia auricular* polysaccharides (SNAAP) derivatives possessed more powerful antioxidant competence than that of the native non-sulfated polysaccharides (AAAP and NAAP) while AAAP and NAAP exhibited stronger activity on scavenging both the hydroxyl radical and lipid peroxidation[30,31].

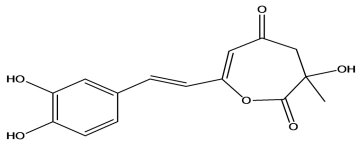
The antimicrobial activity of 90% ethyl acetate and antioxidant activity of fruiting bodies of mushrooms of four edible mushrooms *Agaricus bisporus, Pleurotus ostreatus, Volvariella volvace* and *Pleurotus sajor-caju was* investigated. The antioxidant activity, peroxidase, number of ascorbate oxidase units and catalase activity were found to be more significant with *Agaricus bisporus* than the other edible mushrooms used in the study and no peroxidase activity was observed in *P.ostreatus*[32].

The antioxidant activity of *Agaricus bisporus* was analysed by DPPH and hydroxyl ($^{\cdot}OH$) radical scavenging assays that showed more scavenging activity on DPPH than $^{\cdot}OH$. The phenolics composition of *A. bisporus* methanolic extracts was analysed by HPLC and found to contain rutin, gallic acid, caffeic acid and catechin which contributed to the antimicrobial and antioxidant activity. Total phenol and ascorbic acid content contributed to its antioxidant activity. This study revealed that *A.bisporus* is a natural source of antioxidant and antimicrobial agents against the tested organisms and had a potential as anticancer[33].

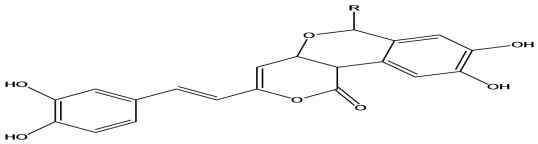
Ethanolic extracts of edible mushroom *Agaricus bisporus* before and after boiling for antioxidant, anticancer and antimicrobial activities were investigated and found that ABTS and DPPH free radical scavenging activities were similar in both the extracts, the extracts inhibited cell proliferation of HL-60 leukemia by the induction of apoptosis and antibacterial activity against both gram positive and gram negative bacteria, as well as anticandidal activity against *Candida albicans*[34].

The antioxidant activity of *Agaricus brasiliensis* strains extracted with methanol as solvent on different basidiocarp maturation phases was evaluated and also the best harvest period to obtain highest antioxidant activity was determined. Strains with closed basidiocarp had higher antioxidant activity than with opened basidiocarps[35].

Two natural antioxidants, named inonotusin A (1) and B (2), were isolated from the methanolic extract of the fruit bodies of *Inonotus hispidus*, together with (*E*)-4-(3,4-dihydroxyphenyl)but-3-en-2-one (3), hispidin (4) and 3,4-dihydroxybenzaldehyde (5). Their structures were identified by means of extensive NMR and MS data analysis. Compounds 1, 2 and 4 exhibited significant scavenging activity against the ABTS radical cation[36].

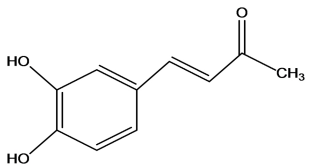


1



2 R = CH_2COCH_3

6 R = OCH_3



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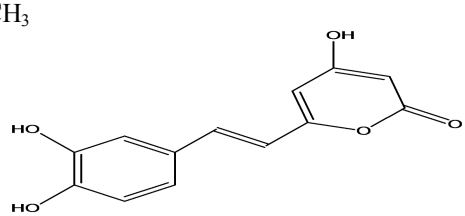

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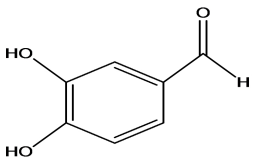

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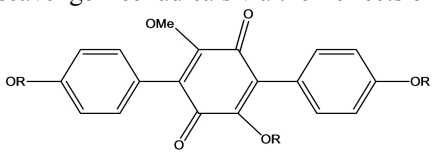
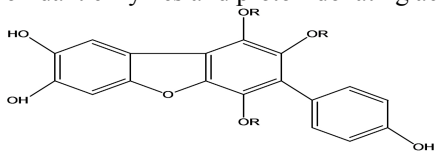
Three *p*-terphenyls isolated from the edible mushroom *Thelephora ganbajun* possessed potent lipid peroxidation inhibitory activity, superoxide dismutase (SOD) activity in rat liver homogenate, and DPPH radical scavenging activity which meant that compounds 1 to 3 scavenge free radicals via their effects on the antioxidant enzymes and proton-donating action[37].



1 R = H



3 R = $CH_2COC_6H_5$

2 R = $CH_2COC_6H_5$

Six major polyphenol antioxidants with free radical scavenging activities were found in the fruiting body of *Inonotus obliquus* namely inonoblins A, B, and C along with the known compounds, phelligridins D, E, and G and structures were established by extensive spectroscopic analysis. The compounds exhibited significant scavenging activity against the ABTS radical cation and DPPH radical, and showed moderate activity against the superoxide radical anion[38].

The bioactive compounds produced from the alcoholic extract of mushroom *Agaricus subrufescens* presented immunomodulatory, antioxidant and antitumor properties evaluated by cytotoxicity assays using primary cultures of granulocytes and macrophages colony forming cells (CFU-GM) and established V79 cell line. The extract exhibited an antioxidant activity on mitochondrial membranes and had low cytotoxicity potential[39].

The antioxidant and antimicrobial properties inherent in leaves and bulbs of geophytic, endemic species, *Ornithogalum alpigenum* Stapf were examined. The acetone bulb extract found to be most inhibitory to the growth of *Candida albicans* ATCC 10239. Total antioxidant activity of the extract from *O. alpigenum* Stapf. was determined using β–carotene linoleic acid model system and was found the highest in methanol bulb extracts. Free radical scavenging activity of the extracts using DPPH was also determined and found to be very close to those of Butylated Hydroxy Toluene (BHT). The leaf extracts were more potent with respect to free radical scavenging activity[40].

The antioxidant capacity and antimicrobial activities of ethanol extracts of *Laetiporus sulphureus* (Bull.) Murril were examined. The antioxidant activity (DPPH free radical-scavenging, β-carotene/linoleic acid systems), total phenolic content and total flavonoid concentration of *L. sulphureus* were determined. Inhibition values of *L. sulphureus* (LS) extract was found to be low than BHA and α-tocopherol standards which increased with the increase in concentration. DPPH free radical-scavenging activity increased with the increase in concentration. Positive correlations were found between total phenolic content in the mushroom extracts and their antioxidant activities[41].

Antioxidant capacity and antimicrobial activities of *Russula delica Fr.* (RD) extracts obtained with ethanol were studied using four complementary test systems, namely DPPH free radical scavenging, β-carotene/linoleic acid systems, total phenolic compounds and total flavonoid concentration. It was observed that inhibition values of both RD ethanolic extract and the standards (BHA and α-tocopherol) increased in parallel with the elevation of concentration in linoleic acid system[42].

The effects of an aqueous suspension of a commercial preparation of the mushroom *Coprinus comatus* on oxidative stress induced in rats by alloxane and carbon tetrachloride were studied by estimating changes in the biochemical parameters (xanthine oxidase, glutathione peroxidase and catalase activity, reduced glutathione content, and extent of lipid peroxidation) of liver homogenate as well as histological changes in the liver of the rats. Two screening doses of alloxane sufficient to induce diabetes in rats did not have any significant effect on the examined biochemical parameters of liver homogenate or on the cytoarchitectonics of liver cross-sections. Treatment with carbon tetrachloride resulted in a significant increase in the intensity of lipid peroxidation and peroxydasis activity, as well as decrease in catalase activity. Certain changes in liver cross sections were detected, such as lymphocyte infiltration of dilated sinusoid capillaries. Administration of *Coprinus comatus* suspension thus showed antioxidative potential, evidenced by an increase of antioxidative status of liver homogenate and prevention of histological changes in liver cross sections[43].

In vitro antioxidant activities of *Armillaria mellea Quel* were studied which showed significant inhibition of lipid peroxidation, potent hydroxyl and DPPH radical scavenging activity when compared with standard drug. IC_{50} values

followed the order crude> boiled > ethanolic extracts in hydroxyl radical scavenging activity, crude > ethanolic>boiled in DPPH radical scavenging activity, and boiled>crude>ethanolic in case of inhibition of lipid peroxidation respectively. Crude, boiled and ethanolic extracts also increased significantly nitric oxide production over control. The ethanolic extract was the most effective in relation to antioxidant activity and NOS activation property[44].

Antioxidant properties of hot water and ethanolic extracts prepared from cap and stipe of *C. comatus* fruit bodies were studied. Ethanolic extract from stipe showed high antioxidant activity while extracts from cap showed better scavenging ability on DPPH than stipe ones. Ethanolic extracts were more effective in scavenging ability on hydroxyl radicals but moderate on superoxide radicals than hot water extracts. Naturally occurring antioxidant components including total phenols, tocopherols, flavonoids and polysaccharides were found in the extracts. Overall, extracts from cap were more effective for the antioxidant properties assayed[45].

The in-vitro antioxidant and ACE inhibitory activities of selected culinary-medicinal mushrooms extracted by boiling in water for 30 mins were studied. Antioxidant capacity was measured using the following assays – DPPH free radical scavenging activity, β-carotene bleaching, inhibition of lipid peroxidation, reducing power ability and CUPRAC. Antioxidant potential of each mushroom species was calculated based on the average percentages relative to quercetin. *Ganoderma lucidum* (30.1%), *Schizophyllum commune* (27.6%) and *Hericium erinaceus* (17.7%) showed relatively high Antioxidant index (AI)[46,47].

For the first time it was reported that *Gastrodia elata* accumulated ergothioneine(ERG) whose levels were correlated with the concentrations of ERG in *Armillaria mellea,* one of the symbiotic fungi on which the lifecycle of *Gastrodia elata*, an achlorophyllous orchid plant, is completely dependent. The contents of ERG in *G. elata* were significantly higher in actively developing tissues, such as seed capsules and newly growing corms, than in mature rhizomes. The ERG levels in rhizomes were significantly correlated with antioxidant capacities[48].

Antioxidant properties and antioxidant compounds of various extracts from the Edible Basidiomycete *Grifola Frondosa* (Maitake) currently available in Taiwan were evaluated. Ethanolic, cold-water and hot-water extracts were prepared and their antioxidant properties were investigated. Cold-water extracts showed high reducing power and chelating abilities on ferrous ions than ethanolic and hot-water extracts. For the scavenging ability on DPPH radical, *G. frondosa* extracts were effective in the following order: ethanolic > hot-water > cold-water while the hot-water extract showed high scavenging ability on superoxide anions. Total phenols, flavonoids, ascorbic acid and α-tocopherol are the major antioxidant components found due to which they display potent antioxidative properties[49].

Antioxidant activity of the ethyl acetate soluble (PdEs) and insoluble (PdEi) fractions of methanol extract of plant-parasitic macrofungus *Phellinus durissimus* (Lloyd) Roy was investigated by in vitro chemical assays and established that P. durissimus has significant antioxidant potential which is comparable to or better than other species of Hymenochaetaceae[50].

The methanolic extracts of dried *Agaricus bisporus, Polyporus squamosus, Pleurotus ostreatus, Lepista nuda, Russula delica, Boletus badius,* and *Verpa conica* were investigated for antioxidant activity in different systems including reducing power, free radical scavenging, superoxide anion radical scavenging, total antioxidant activity, and metal chelating activities and compared to standard antioxidants BHA, BHT, and α-tocopherol. *Russula delica* showed highest percentage inhibition on peroxidation in linoleic acid system, reducing power and O^{2-} scavenging .Total phenolic compounds, α-tocopherol, and β-carotene were also determined[51].

Methanol and water crude extracts from Shiitake mushroom (*Lentinus edodes*) and straw mushroom (*Volvariella volvacea*) were investigated for their antioxidant capacity in three different assays, namely, the β-carotene and linoleic acid system, DPPH radical scavenging activity, and inhibition of hemolysis of rat erythrocyte induced by peroxyl radicals. The water extract from *L. edodes* showed the most potent radical scavenging activity. Total phenolics were higher in the water extracts[52].

ROS-generating activity in human cells and DPPH-TEAC antioxidant activity in hot water extracts of 2 groups of medicinal mushrooms from the genera *Agaricus, Antrodia, Auricularia, Coprinus, Cordyceps, Hericium, Grifola, Ganoderma, Lentinus, Phellinus,* and *Trametes* was determined[53].

The detailed mechanism whereby *G. lucidum* stimulates the catalase activity and expression was elucidated and found that methyl linoleate that is produced by *G. lucidum* stimulates the catalase expression at the transcription level[54].

An ergothioneine derivative, β-hydroxyergothioneine has been isolated from the mushroom *Lyophyllum connatum*. Ergothioneine, N-hydroxy-N', N'-dimethylurea, and connatin (N-hydroxy- N', N'-dimethylcitrulline) were also isolated. All the compounds displayed the ability to scavenge free radicals, based on DPPH radical scavenging assay. The radical scavenging activity of β-hydroxyergothioneine was very similar to that of ergothioneine. β- Hydroxyergothioneine showed the greatest protective activity against carbon tetrachloride-induced injury in primary culture hepatocytes[55].

The antioxidant capacity and total phenolic content of *Agaricus brasiliensis* in two stages of maturity, young (YB) and mature (MB), have been evaluated with minor differences in the composition of phenolic compounds being detected, but with similar antioxidant activities, except for the chelating ability for ferrous ions, which was higher in MB than in YB[56].

Antioxidant properties of methanolic extracts of three species of medicinal mushrooms in Taiwan- *Ganoderma lucidum* (Ling-chih), *Ganoderma tsugae* (Sung-shan-ling-chih), and *Coriolus versicolor* (Yun-chih) were studied. Results showed that *G. lucidum* and *G. tsugae* were higher in antioxidant activity, reducing power, scavenging and chelating abilities, which was attributed to their total phenolic content[57].

Membrane ultrafilteration and subfractionation of methanol and water extracts, of two edible mushrooms *Lentinus edodes* and *Volvariella volvacea* showed that the dichloromethane subfraction of the methanol extract of *V. volvacea* and the low molecular weight (LMW) subfraction of the water extract of *L. edodes* had the highest antioxidant activity against lipid peroxidation of rat brain homogenate correlated with the phenolic content in different subfractions[58].

A new potent antioxidant vialinin A together with a known compound ganbajunin B and a mixture of ganbajunins D and

E were isolated from dry fruiting bodies of Thelephora vial is and structure was elucidated by spectroscopic and chemical methods. This compound had strong DPPH free radical scavenging activity nearly equal to that of BHT[59].

Analysis for proximate composition , total phenols and antioxidant activity of methanolic extracts of three wild edible mushrooms (*Agaricus sp., Boletus sp., Macrolepiota sp.*) from North of Mexico and two commercial sp. (*Agaricus bisporus* white strain and brown strain) showed that wild mushrooms had higher phenolic content and antioxidant capacity then the commercial species[60].

CONCLUSION

Mushrooms can serve as a dietary supplement for proteins, vitamins, minerals as well a cheap and easily accessible source for natural antioxidants for both man and/or livestock. The various antioxidant mechanisms of the mushroom species extract may be attributed to strong hydrogen-donating ability, a metal-chelating ability, and their effectiveness as good scavengers of superoxide and free radicals. This indicates the potential of mushrooms as panacea for many diseases and also reveals a novel potential to fight against tumors in man. However, more intensive and extensive investigations are needed to exploit their valuable therapeutic potential.

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