Review Article

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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF MUSHROOM- *LENTINUS* SP.: A SHORT REVIEW

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ABSTRACT

In search of new therapeutic alternatives, scientific community had studied many kinds of mushrooms and have been shown to contain valuable sources of bioactive agents that result in beneficial health effects such as antitumor, anticancer, antiviral, anti inflammatory and hypolipidemic activity. The most significant medicinal effects of mushrooms derived compounds, both cellular components and secondary metabolites, have been shown to stimulate the immune system and could be used to treat variety of diseases, including Cancers. So these medicinal mushrooms can be called as "Immunomodulators" or "Biological Response Modifiers (BRMS)", "Immunopotentiators" and "Immunostimulant".

Keywords: Shiitake mushroom, *Lentinus* sp., antitumor, anticancer, antiviral

INTRODUCTION

The importance of the Shiitake mushroom (*Lentinus* sp), the second most popular edible mushroom in the global market, is attributed not only to its nutritional value, but also to possible medicinal and food industrial applications[1]. *Lentinu* sp (Berk Sing) also known as Shiitake, Black Forest or Chinese mushroom is most popular edible mushroom in world reaching a production of 7.5 million ton in 2000[2] and this represents 14 % of the world's total production of edible mushrooms[3]. Shiitake mushroom contains several therapeutic actions such as antioxidant and antimicrobial properties, carried by the diversity of its components[4]. It is traditionally cultivated on logs of broad-leaved trees in Japan, Taiwan, China and Indonesia.

In search of new therapeutic alternatives, Scientific community had studied many kinds of mushrooms and have been shown to contain valuable sources of bioactive agents that result in beneficial health effects such as antitumor, anticancer, antiviral, anti-inflammatory and hypolipidemic activity[5,6]. The most significant medicinal effects of mushrooms derived compounds, both cellular components and secondary metabolites, have been shown to stimulate the immune system and could be used to treat variety of diseases, including Cancers[7,8].

Reactive Oxygen Species (ROS)

Free radicals are defined as any atom or molecule capable of independent existence that contains one or more unpaired electrons. An unpaired electron is one that occupies an atomic or molecular orbit by itself, which include H_2O_2, singlet oxygen and ozone, lack unpaired electrons, can participate in cellular redox reaction these together with the oxygen containing radical are collectively called as "Reactive Oxygen" or "Active Oxygen Species"(ROS or AOS)[9].

The most important ROS are the superoxide anion (O_2^-), hydroxyl radical (OH), nitric Oxide (NO) and hydrogen peroxide (H_2O_2). The primary ROS formed *in vivo* are superoxide and H_2O_2[10]. H_2O_2 is generated through non enzymatic or enzymatic dismutation of superoxide[10]. ROS are also regarded as essential participants in cell signaling and gene regulation. They play an important role in host defense, since activated phagocytes generate ROS to fight foreign organisms especially through membrane bound NADPH Oxidase, a situation which is often referred as the respiratory burst[11].

Oxidants and Human Diseases

Oxidant species such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH.) and lipid peroxidation are becoming increasingly implicated in human disease, including rheumatoid arthritis, reperfusion injury, immune injury to lung and kidney and cerebral trauma ischemia and some new concepts were developed to understand the primary mechanism of oxidant toxicity to cell and the antioxidant protection were reviewed[12].

Antioxidant

Antioxidants are the substances that when present at low concentration compared to those of oxidisable substrate significantly delays or inhibit oxidation of that substrate by

1. Preventing the production of free radicals
2. Scavenging the unpaired electron
3. Quenching the energy of executed molecule
4. Terminating chain reaction[9].

Antioxidants are our first-time defense against free radical damage and are critical for maintaining optimum health. The need for antioxidants becomes with increased exposure to free radicals.

Pollution, Cigarette smoke, drug illness, stress can increase free radical exposure. Thus, for the part of healthy lifestyle and well balanced, wholesome diet, antioxidant supplement is now being recognized as an important means of improving free radical protection[13].

Sources of Antioxidants

Plant cells normally contain a suite antioxidant localized in different compartments. The antioxidant complement often changes as organs grow and develop and is markedly affected by environmental stresses. Antioxidants are broadly categorized into two groups. They are

a) The Enzymatic Antioxidants
b) Non-Enzymatic Antioxidants (low molecular weight).

The enzymatic antioxidants are catalases, superoxide dismutase peroxidases. Non-Enzymatic low molecular weight antioxidants include vitamins, glutathione and several Plant pigments[9].Occurring naturally in foods and beverages from plant sources such as fruits, vegetables, berries, tea and wine. Flavonoids are phenolic compounds that provide an important dietary source of antioxidants[14,15]. Flavonoids have the chemical structure C6-C3-C6, which includes two benzene rings linked by three carbons[16]. Flavonoids can be classified according to the variations in the C3- ring as

- Flavonols
- Flavones
- Flavanols (catechins)
- Flavanones
- Anthocyanins
- Isoflavonoids.

More than 6000 flavonoids have been identified, having properties related to their chemical structures[16]. Many Indian Medicinal Plants are considered potential sources of antioxidant compounds. In some cases, their active constituents are known. *Terminalia chebula*, *Terminalia bellerica*, *Terminalia muelleri* and *Phyllanthus emblica* all of which have antioxidant activity, showed high content of phenolics like Gallic Acid. Specifically, in Bangladesh about 250 species are used as medicinal plants[17]. It has now been established that the plants which naturally synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils and contain minerals and vitamins possesses medicinal properties[18].

Phytochemical analysis of the dried aerial part of *Senna tora* (Family – Fabaceae) indicated the presence of reducing sugars, tannins, steroids, saponins and gums. The pharmacological interest of these compounds coupled with the use of this plant in traditional medicine prompted for its probable antibacterial and analgesic activities[19]. Several medicinal plants (Rasayana) have also been extensively used in the Indian traditional (Ayurveda) system of Medicine for the treatment of number of diseases. Some of these plants have shown potent antioxidant activity. The plants namely *Acorus calamus, Hemidesmus indicus, Holarrhena antidysenterica and plumbago zeylanica* were used to determine their antioxidant and free radical scavenging properties[20].

Tea is rich in Catechins and contains the Flavonols quercetin, kaempferol and myricetin[16]. Catechins are oxidized during the processing of black tea, which involves fermentation of tea leaves and forms fermentation products such as the arubigins and theaflavins. Blue berries have attracted interest because of their high anthocyanin content[21], which is reflected in their high antioxidant capacity. Lowbush "wild" blueberries, bilberries (*Vaccinium myrtillus*) have been reported to have a higher anthocyanin content and antioxidant capacity than cultivated high bush blueberry. Epidemiological studies show that consumption of fruits and vegetables with high phenolic content correlate with reduced cardio and cerebrovascular diseases and cancer mortality[14].

Antioxidant activity of Mushroom

Mushroom fruiting bodies were investigated *in vitro* for antioxidant activity. Mushroom extracts of *Armillariella mellea, Formitella farinea* and *Pleurotus cornuspirae* markedly exhibited inhibition on lipid peroxidation of rat liver microsome. A. melea were separated using silica gel column chromatography and recrystallization. The structure of the compound was determined by NMR GC / MS and X-ray crystallography[22]. Total antioxidant property of *Morchella conicapers* using ethanol and methanol was carried out and compared with appropriate standards like butylated hydroxyanisol and α- tocopherol. The inhibitory effect was found to be more in ethanol extract of *Morchella conicapers*. Presence of total phenol and total flavonoid content were correlated with the antioxidant property[23]. Ethanol extract of *Ramaria flava* were also found to have potent antioxidant property when total tested with appropriate standards[24].

In vitro evaluation of the antioxidant property of *Cordyceps militaris* were studied using methanol such as DPPH free radical scavenging, hydroxyl radical eliminating, iron chelating, lipid peroxidation and ferrous reducing power. 80 % scavenging effect were seen in method DPPH, eliminating hydroxyl radicals, chelating ferrous ions and also positive results of lipid peroxidation and reducing power were observed[25]. Total antioxidant property methanol and ethyl acetate extracts of *Phellinus rimosus, Pleurotus florida, Pleurotus sojar-caju* and *Ganoderma lucidum* using different methods like 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) Ferric Reducing Antioxidant Power Assay (FRAP), lipid peroxidation and Oxygen Radical Absorbance Capacity (ORAC). Among these methanol extract of *Pleurotus florida* and *Phellinus rimosus* showed significant antioxidant property of around 70 %[26,27].

Methanolic and water crude extracts of *Lentinus edodes* and *Volvariella volvacea* tested for their antioxidant activity and total phenolic content using three different methods. Among these extracts, water extract from *Lentinus edodes* showed potent radical scavenging activity in all the methods. Total phenolics in the water extracts were higher than that of the methanol ones[28].

In vitro evaluation of antioxidant activity of *Auricularia auricula* showed significant inhibition of lipid peroxidation a potent hydroxyl radical scavenging when compared with standard drug catechin having potential therapeutic use[29]. Fruiting body of *Phellinus linteus* extracted with 7 % ethanol showed strong anti angiogenic activity detected using chick embryo Chorio Allantoic Membrane assay (CAM). The *in vitro* antioxidant activities of *Phellinus linteus* evaluated using two different bio assay DPPH and lipid peroxidation concentration dependent method with standard Vitamin – C[30].

The Methanolic extracts of macro fungus *Pleurotus rimosus* possessed significant *in vitro* superoxide anion hydroxyl radical and nitric oxide scavenging and lipid peroxidation inhibiting activities. The extract showed remarkable anti-inflammatory activity when compared with the standard reference drug diclofenae. The results obtained showed anti inflammatory activity of *Pleurotus rimosus* is attributed to its free radical

scavenging activity property[31]. Twenty-two patients with Rheumatoid Arthritis (RA) and twenty healthy volunteers were taken to examine the oxidant and antioxidant systems. Levels of Malondialdehyde (MDA) and antioxidant vitamins (A, E, C) in serum samples were determined by High Performance Liquid Chromatography (HPLC). Spectrophotometric methods were used to determine activity levels of antioxidant enzymes, Superoxide dismutase (SOD) and Glutathione Peroxidase (GSH – Px), in erythrocytes. Thus, the results indicate that there was an increased oxidative stress and low antioxidant status in patients with Rheumatoid Arthritis. These changes are probably due to efforts for reducing lipid peroxidation and hence lower tissue damage[32].

Measurement of Antioxidant Capacity

Several methods have been developed during last decade to measure the antioxidant capacity of plasma, serum and of other biological samples and in foods. Most of these methods measure the extent to which free radical generation is inhibited by antioxidants present in the sample. The methods are based on different technologies using different free radical generators, target molecules and end points. Recently, it was proposed that the procedures and applications of the ORAC assay, and possibly those of the TEAC assay, should be standardized[33].

The Trolox Equivalent Antioxidant Capacity (TEAC) Assay

The TEAC assay uses only the degree of free radical inhibition at a fixed time to determine antioxidant capacity and does not take the duration of inhibition into account, which may result in under estimation of antioxidant capacity. This assay has been criticized because a non physiological radical is used and because of dilution effects[34]. However, commercial the Trolox equivalent antioxidant capacity (TEAC) assay kits are available and the assay is relatively fast. Several other assays such as the earlier mentioned Telomeric Repeat Amplification Protocol (TRAP), the Ferric Reducing ability (FRAP) Assay and the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay have been used for the determination of antioxidant capacity[33].

Antimicrobial activity in Mushroom Extracts

There appear to be an increasing number of reports on Gram positive bacteria developing resistance to virtually every clinically available drug[34], and Basidiomycetous mushrooms have been shown to possess antibacterial activity against this group of bacteria. The mycelium of *Flammulina velutipes* was shown to have bacterial activity against the Gram-positive bacteria *Bacillus subtilis* and *staphylococcus aureus*[35]. In addition to these two bacteria, the fruiting body extract from *Armillariella mellea* also exhibited activity against *Bacillus cereus,* under *in vitro* condition[36].

Early studies proposed that most antibacterial components from Basidiomycetous fungi were potent against Gram Positive bacteria only but more recent work explained that extracts were also active against Gram Negative organisms like *Proteus vulgaris* and *Escherichia coli, in vitro*[37]. *Agaricus campestis, Agaricus bisporus* and *Agaricus arvensis* have been shown to produce compounds active against both types of bacteria[38] and extracts from *Lentinus edodes* have also been shown to be active against both types[39].

CONCLUSION

Two edible Macrofungi by *Lycoperdon* and *Lycoperdon giganteum* were studied *in vitro* for their antimicrobial activities using water methanol and ethanol as extraction solvents. Ethanolic extract of *Lycoperdon giganteum* showed more actively followed by methanol and water[40]. *Pholiotaadiposa* a macro fungus rich in mucous matter a polysaccharide covered the fruit body surface was evaluated for antimicrobial activity and showed active against both bacteria and yeast[41]. Ethanol extract of *Morchella conicapers* were tested against six species of gram positive bacteria, 7 species of gram negative bacteria and one species of yeast and showed to be *Micrococcus flavus* was most susceptible and did not exhibit anticandidal activity against *Candida albicans*[23].

Ethanolic extracts of *Ramaria flava* were tested for antimicrobial activity and found that the growth of gram positive bacteria inhibited better than gram negative bacteria and yeast[24].

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