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Therapeutic Importance of *Lentinus edodes*: A Review of Its Bioactive Constituents

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ABSTRACT

Lentinus edodes also known as shiitake are considered as second most widely cultivated mushroom in the world. In India, its cultivation was carried on different substrates in the form of wood logs, artificial medium, saw dust, wheat straw. Different bioactive components (lentinan, eritadenine, shiitake mushroom mycelium, and culture media extracts) are present in the mycelium and fruiting body with proven pharmacological properties. Submerged cultivation of this mushroom has received considerable attention in terms of reproducible and efficient mycelial and metabolite production in a shorter time with chance of contamination in comparison to solid state fermentation. Lentinan is recognized as biological response modifier against cancerous cells by activating cytotoxic macrophages, helper T-cells, NK cells and T cell differentiation. The medicinal properties of different bioactive components of this mushroom would make an efficient contribution in the field of pharmaceutical industries and medical word as a potent anti-cancerous drug, high blood pressure, diabetes, cholesterol-lowering, antimicrobial activity with potential value for healthy food and therapeutics. Still its mechanism is unclear because of the complex structure of lentinan which hinders its application in pharmaceutical industry.

Keywords: Bioactive components, Lentinus edodes, Pharmaceutical and Submerged.

INTRODUCTION

Lentinus edodes (Berk.) Pegler, commonly known as shiitake mushroom, is one of the most widely grown species of mushrooms which ranks second in world mushroom production after the button mushroom (Chang 2005). These mushrooms are either sold as fresh or dried and are recognized as one of the most important functional food ingredients of cuisines of countries such as China, Japan, Taiwan and Korea. Pegler (1983) included shiitake to the genus *Lentinula* in 1980s. It grows in nature on the wood of broad leaf trees, mainly oak, willow, poplar and

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chestnut. However, in India, its cultivation started on wood logs, artificial medium, saw dust and wheat straw supplemented with wheat and rice bran (Thakur and Sharma 1992).

Lentinus edodes is the source of several well-documented proven pharmacological properties, especially in the form of polysaccharides viz. lentinan, eritadenine, shiitake mushroom mycelium, and culture media extracts. Different compounds with antibiotic, anticarcinogenic, antidiabetic, antiobesity and antiviral properties have been isolated from fruiting body, mycelium (intracellularly) and from culture media (extracellularly); (Friedman 2016). This mushroom is having numerous medicinal applications that are used for diseases involving depressed immune function (including AIDS), cancer, environmental allergies, fungal infection, frequent flu and colds, bronchial inflammation, heart disease, hyperlipidemia (including high blood cholesterol), hypertension, infectious disease, diabetes, hepatitis and regulating urinary inconsistencies. Recently, its submerged cultivation has received a considerable attention in terms of reproducible and efficient mycelial and metabolite production in a shorter time with very less chance of contamination (Elisashvili 2012). The purpose of this review is to focus on the pharmacological importance of *Lentinus edodes* along with its bioactive molecules which could make an efficient contribution in the development of mushroom nutraceuticals.

Habitat and Morphology of *Lentinus* species

Lentinus edodes has been classified in the genus *Lentinula,* family Tricholomataceae, order Agaricales, and subphylum Basidiomycotina based on the macro- and micro-morphological characteristics of mushroom. Its name *Lentinus edodes* is derived from Latin word (*Lent* means "supple," *inus* means "resembling," and *edodes* means "edible"). This mushroom has been known by different names in different countries such as Black forest mushroom (United States), Xiang-gu-the fragrant mushroom, Dong-gu-the winter mushroom, and Hua-gu-the flower mushroom or variegated mushroom in China (Chen 2001). It occurs naturally throughout Southeast Asia and has been reported from China, Japan, Korea, Vietnam, Thailand, Burma, North Borneo, the Philippines, Taiwan, and Papua New Guinea (Wasser and Weis 1997, Stamets 2000). It is often seen on fallen wood of a wide variety of deciduous trees, especially shii, oak, chestnut, beech, maple, sweet gum, poplar, alder, hornbeam, ironwood, mulberry in the forest

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from May-September (Dulay *et al* 2017).

Lentinus edodes is an edible wood-rotting fungus with an aromatic, fleshy, golden or dark brown to blackish cap along with stipe, gills, and veil. The fruit body development begins with the primordial formation at the tip of the bump followed by development of dark brown young pinheads which later on develops into mature fruiting body with lighter brown in color. These raw mushrooms are of high nutritional value with 88–92 per cent water, protein, lipids, carbohydrates, vitamins and minerals in comparison to commonly consumed vegetables. The nutritional composition of these mushrooms includes 58–60 per cent carbohydrates, 20–23 per cent protein (digestibility of 80–87%), 9–10 per cent protein fiber, 3–4 per cent protein lipids, and 4–5 per cent protein ash (Wasser 2005). It is a good source of vitamins especially provitamin D_2 (ergosterol), which under ultraviolet (UV) light and heat yields calciferol (vitamin D_2) along with vitamin B-complex (B_1, B_2, B_5 and B_{12}; Hobbs 2000) and minerals (Chen, J. et al., 2024).

Bioactive Molecules of *Lentinus edodes*

The edible mushrooms are considered as a nutritionally functional food with physiologically bioactive compounds. Among them, mushroom polysaccharides represent a diverse class of macromolecules which exist as a structural component of cell wall with chitin and β-glucan as two main polymers. These polymers are interlinked with hydrogen bridges to form a network matrix by forming covalent bond (Xiaofei *et al* 2014). These polysaccharides are formed by repetitive units of monosaccharides joined by glycosidic linkages. One way interconnections occur among nucleotides in nucleic acids and the amino acids in proteins while in polysaccharides, the monosaccharide units are interconnected at several points to form a wide variety of branched or linear structures (Ooi and Liu 1999). It also forms secondary structures on the basis of conformation of sugar residues, molecular mass, inter- and intra-chain bonding. It possesses the highest capacity to carry biological information due to its greater structural variability. This potential variability in its structure provides the required flexibility for carrying the precise regulatory mechanisms of different cell-cell interactions in higher organisms. These days much attention has been paid to extracellular polysaccharide (EPS) of microbial origin in comparison to polysaccharides of plant origin. These microbial originated polysaccharides are independent of various factors such as environmental variations, can be easily recovered and

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purified, highly stable at broad temperature, pH and high ionic concentrations and giving high quality final product. These microbial EPS possess numerous applications in the field of cosmetic, pharmaceutical, food industries, enhanced oil recovery and medical applications etc. (Moscovici 2015). The main polysaccharides responsible for the immuno-modulator effects of mushrooms are basically β-D-glucans which are either water-soluble β-D-glucans, β-D-glucans with heterosaccharide chains of xylose, mannose, galactose and uronic acid or β-D-glucan-protein complexes-proteoglycans (Wasser 2002). The immunomodulator effect of β-D-glucans depends on water solvency, degree of branching, polymer lengths and tertiary structure (Brown and Gordon 2003). The β-D-glucans of *L. edodes* have been used as therapeutic adjuvant for treating gastric cancer, colon carcinoma and pancreatic cancer (Batbayar *et al* 2015). It has been reported that soluble β-glucans from *L. edodes* increased the number of B cells without changing the number of NK cells in healthy elderly people during oral intake for 6 weeks (Gaullier *et al* 2011).

Extraction, Fractionation and Purification of Lentinan

Submerged culture of mushrooms is one of the best alternatives for the production of high mycelial biomass in shorter time with compact space along with less chances of contamination (Puchkova *et al* 2010). In addition, exopolysaccharides which have a synergistic effect with mycelium on biological activities can be simultaneously produced in submerged culture (Kim *et al* 2002). The cultivation of mushrooms requires several months for the development of the fruiting body which is more time consuming and also delays the production and quantity of required bioactive components (2.5%-3.0%) (Wang, Y. et al., 2024). For its extraction, different methods have been developed for polysaccharide extraction from mushroom fruiting bodies, mycelium and liquid media. However, these methods are having broad similarity with one another (Mizuno 1999). Most of the methods involve the extraction of dried mushroom powder or mycelium with ethanol, 1 per cent ammonium oxalate and 5 per cent sodium hydroxide. Its further purification involves the combination of techniques including ethanol concentration, fractional precipitation, and acidic precipitation with acetic acid, ion-exchange chromatography, gel filtration and affinity chromatography.

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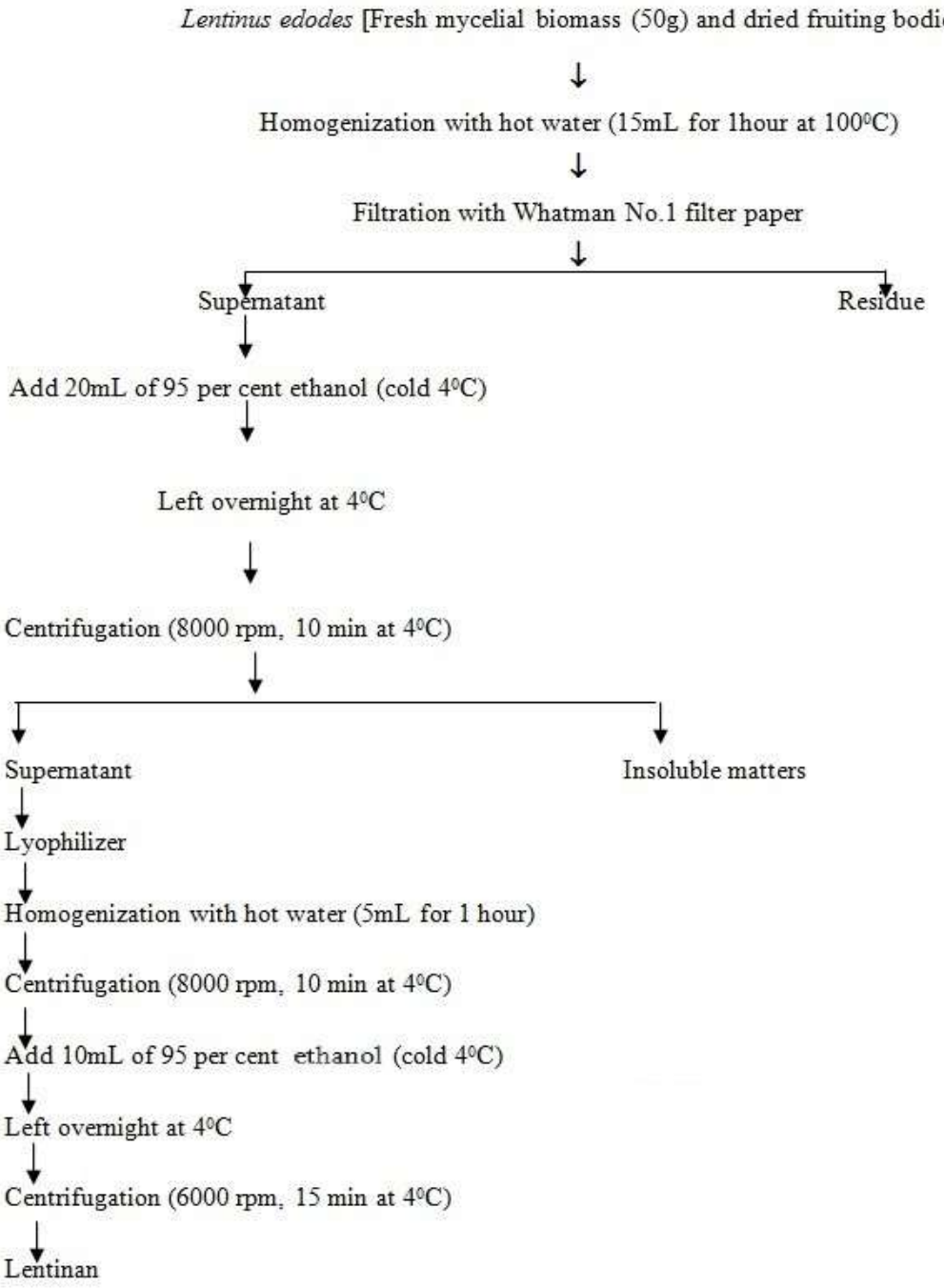


Figure 1. Method for extraction of Lentinan from *L. edodes* strains (Shivani 2012).

There would be a growing interest in increasing the activity of medicinal mushroom polysaccharides by various chemical modifications and perhaps creating a range of sem-isynthetic compounds. Such chemical modifications are required for improving the anticancer

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and antiviral activities of bioactive components present in mushrooms (Li, X. *et al*., 2023). These modifications can be achieved by oxido-reducto hydrolysis (Smith degradation) and also by formolysis. These modifications lead to some positive improvements in the extraction process (Mizuno 1999). A more efficient procedure has been established by Yap and Ng (2001) for the extraction of ß -D-glucans from *L. edodes.* The ß -D-glucan was isolated through ethanol precipitation and freeze-drying in liquid nitrogen. This method was less time-consuming, more efficient and of relatively low cost on commercial aspect in comparison to Mizuno's method.

An efficient protocol for the extraction of polysaccharides has been standardized by Shivani (2012) that involves homogenization of fresh mycelial biomass and dried powder of *L. edodes* with hot water, filtration, ethanol precipitation, centrifugation and lyophilization (Fig. 1). High-pressure cooking treatment has been used in order to improve the extraction efficiency of polysaccharides from *Lentinus edodes* (Li *et al* 2018).

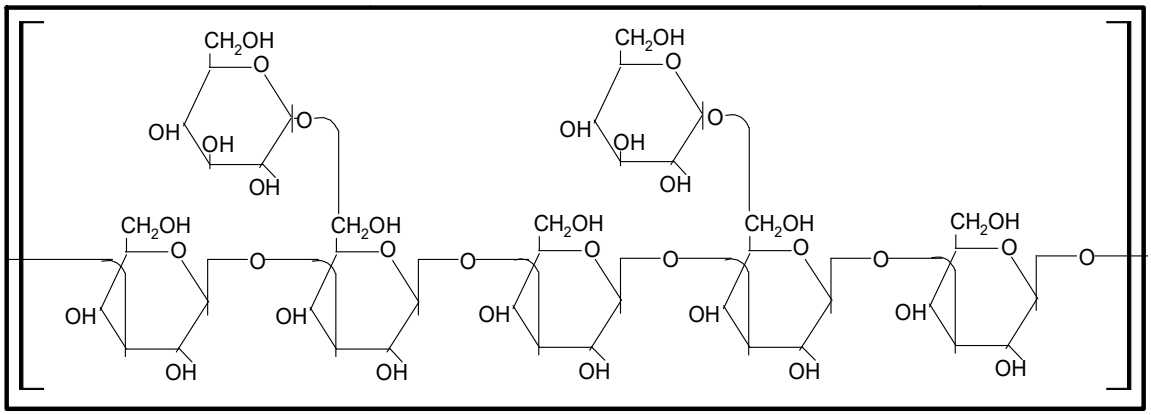


Figure 2. Structure of Lentinan (Bluhm and Sarco 1977).

Lentinan is one of the major cell wall constituent extracted from the fruiting bodies or mycelium of *L. edodes* with its molecular formula ($C_6H_{10}O_5$) and the mean molecular weight is about one million (~5 x 10^5 Da). It is a water soluble, heat stable, alkali labile, and protein free that is completely devoid of any nitrogen, phosphorus, sulphur or any other atoms of carbon, oxygen and hydrogen (Hobbs 2000). It is a water-soluble complex of β -1,3 glucan polysaccharide in a triple helix structure (Fig 2), containing only glucose molecules with mostly (1,3)- β -D glucan linkages in the regularly branched main chain with two β -(1,6)-D-

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glucopyranoside branchings for every five β-(1,3)-glucopyranoside linear linkages (Bluhm and Sarco 1977).

Lentinan exists in aqueous solution as a triple helix while in DMSO it exists in the form of a random coil. Intra-molecular hydrogen bonding acts as a major strength for maintaining the helical structure of lentinan in water while inter-molecular hydrogen bonding is required for triple-strand formation. Any disordering in the intra- and inter-molecular hydrogen bonding leads to transition in its structure and forms a helix-coil conformation (Zhang 2011).

Different techniques such as ^{13}C NMR, size-exclusion chromatography in combination with multi-angle laser light scattering (SEC-MALLS), dynamic light scattering (DLS), and optical rotation confirmed the triple-helical conformation of lentinan in water solution at a particular temperature of $25^{O}\pm1$ C, while at high temperature, the conformational changes takes place from triple helix to single flexible chains. *Lentinus edodes* extracts of water-soluble crude polysaccharides were isolated from the sporophore and broth by hot-water extraction, precipitated by ethanol, fractionated by column chromatography that results in the separation of six polysaccharide fractions, CPFN-G-I, CPFN-G-II, CPFN-G-III, CPFA-G, CPBN-G, and CPBA-G. Out of these, three fractions namely CPFN-GI, CPBN-G, and CPBA-G possess anticancer properties by stimulating the activation of macrophages which in turn enhance the cytokine expression and phagocytosis and also enhance the host immune response against cancerous cells invasion (Lee *et al* 2009).

Medicinal Importance of Lentinan

Lentinan possess various pharmacological properties in terms of anticancer, anti-diabetic, anti-viral, antibiotic properties while its medicinal effect is known to be varying and inconsistent. Therefore, its medicinal effect depends on branching pattern, the length of the polymer and its tertiary structure (Ina *et al* 2013). These polysaccharides are used against various forms of cancer by increasing the host resistance and possess an ability to retain the immune function of cancer patients (Chihara *et al* 1989, Chihara 1992). This immunomodulatory activity of lentinan is basically linked to modulation of hormonal factors that plays a potential role in tumor growth (Chihara 1992). Lentinan and its derivatives such as lentinan sulphate possessed pharmacological

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effects in terms of anti-tumoral cell activity (Zhang *et al* 2002). These polysaccharides have no direct cytotoxic effects against tumor but it may retain and enhance the immunological property in host cells. In terms of pharmacology, lentinan is considered as one of the major anti-cancer drug developed using mushrooms and recognized as biological response modifier against cancerous cells (Ina *et al* 2016). Its mode of action against cancerous cells influence the number of peripheral blood mononuclear cells of patients which results in the formation of certain factors such as Interleukin-1α (IL-1α), IL-1β and tumor necrosis factor-α (TNF- α). It also increases the potential of cancer patients in response to lymphocytokines or other intrinsic bioactive factors and thus protects patients against metastasis (Chihara 1992). It maintains balance, stimulates interferon production and enhances the disease resistance by improving the physiological conditions of host defense mechanism. One of the researchers reported the action of lentinan by activating cytotoxic macrophages, helper T-cells, NK cells and T cell differentiation (Bohn and BeMiller 1995). Its main effect is related with particular types of tumor not with all kinds especially used for gastric and colorectal carcinomas (Taguchi *et al* 1985).

The biological activities of lentinan involves an increase in stimulation of non specific inflammatory response by protein production, activation of helper and cytotoxic T cells, activation of macrophages by secreting mediators, activation of MAPK signaling pathways (Suga *et al* 1986; Chihara 1992; Lee *et al* 2008; Xu *et al* 2011). Intake of lentinan along with cancer therapies would increase the survival time and betterment of life. Its antioxidant activity has been studied by Thetsrimuang *et al* (2011) by evaluating crude polysaccharides of mature fresh and dried fruiting bodies of *Lentinus* spp. Different parameters such as proteins, reducing sugars and phenolic compounds indicated its natural antioxidant potential. Moreover, the yield of total polysaccharides of *Lentinus* sp. (47.7% and 42.5% for FB and DB, respectively) was quite high in comparison to *L. edodes* (3.5-10.0 %) reported by Surenjav *et al* (2006).

Other Bioactive Molecules

Not only lentinan is present in *L. edodes* but also various other bioactive molecules are present in the mushroom with pharmacological properties. Its water extract increase the number of colon inhabiting beneficial microorganisms (*Lactobacillus brevis* and *Bifidobacterium breve*) by disaccharide sugar, trehalose and thus prevents colon cancer by reducing the harmful effects of

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β-glucosidase, β-glucuronidase and tryptophanase and possesses anticoagulant properties (Bae *et al* 1997). The extracts prepared from mycelium and culture media (LEM and LAP extracts) are basically glycoproteins with sugars such as glucose, galactose, xylose, arabinose, mannose and fructose. They possess strong anti-tumor activities. For the preparation of LEM, hot water extract of powdered mycelia was used, incubated for 50-60 h at 40-50^{O}C, partially hydrolysed by enzymes, centrifuged and the filtrate was freeze dried which later on turned into light brown powder. The extract LAP is obtained from filtrate of LEM by adding four volumes of ethanol. Another immunoactive fraction EP3 has been obtained by fractionation of LEM. This fraction is made up of water soluble lignin with carboxyl groups (Susuki *et al* 1990).

Other bioactive substances of *L. edodes* are eritadenine, lectin, antimicrobials and lentin which possess pharmaceutical properties. LC 11 (β-1, 6-D-glucan with ramifications 1-4) and KS-2 (peptidomannan) are the bioactive fractions of *L. edodes* which also posses immune regulatory activities. Eritadenine is a bioactive molecule used against cholesterol which was correlated with hepatic metabolism of lipids (Sugiyama and Yamakawa 1995). Lectin is a group of proteins linked with carbohydrates which possess *in vitro* anti-fungal activity and inhibitory activity to HIV-1 virus and leukemia cells (Ngai and Ng 2003). The extraction of nucleic acid from *L. edodes* showed significant antithrombotic activity (Hokama and Hokama 1981). Another key component, lenthionine inhibits platelet aggregation that serves as a crucial medicine for treating thrombosis (Shimada *et al* 2004).

Antimicrobial Activity

The inappropriate and indiscriminate use of antibiotics which results in bacterial resistance towards antibiotics is becoming a major issue for today's era. Therefore, scientists are looking for an alternate option and extraction of antimicrobial compounds from mushroom species would be one of the best options that show antibacterial, antifungal, antiviral, antitumor, hypoglycemic, antiallergic, immunomodulating, anti-inflammatory, hypolipidemic, and hepatoprotective activity (Lindequist *et al* 2005). Many studies were carried out using different mushroom extracts with different types of microorganisms. However, the response of microorganisms against mushroom extracts depends on the growth conditions in which it has been growing (Iwalokum *et al* 2007).

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One of the studies on dried shiitake mushrooms carried by Hirasawa *et al* (1999) in which antibacterial extracts were prepared by chloroform, ethyl acetate or water which possess efficient antibacterial activities against *Streptococcus* spp., *Actinomycetes* spp., *Lactobacillus* spp., *Prevotella* spp. and *Porphyromonas* spp. It was also found by Ishikawa and Kasuya (2001) that mycelia free culture of *L. edodes* showed greater antimicrobial effect against gram-positive bacteria in comparison to gram-negative bacteria. Four solvent systems (Hexane, Dichloromethane, Chloroform and Ethyl acetate) were used for testing culture filtrate of *Lentinus tuberregium* for antimicrobial activity and ethyl acetate extract showed more effective antimicrobial activity against pathogenic bacteria and yeast (Manjunathan and Kaviyarasan 2011). The bioactive component lentinan has been found to be active against *Mycobacterium tuberculosis, B. subtilis, S. aureus, Micrococcus luteus, Candida albicans* and *S. cerevisiae* (Wasser and Weis 1999). Its derivatives are effective against bacterial, fungal, viral and parasitic infections (Wasser and Weis 1997, Hobbs 2000, Smith *et al* 2002). The effectiveness of azidothymidine (AZT) was found to more in conjunction with lentinan from *L. edodes* in suppressing the surface expression of HIV or T cells in comparison to AZT when used alone. Lentinan and its derivative lentinan sulphate inhibit the viral replication and cell fusion that showed its anti-HIV activity. Its lignin fractions EP3 and EPS4 possess antiviral and immune-modulatory effects (Hanafusa 1990). The protein fraction of shiitake prevents the infection of plants with tobacco mosaic virus (Wasser and Weis 1997). The clinical trials for low molecular weight shiitake extracts proved that such extracts also possess anti-caries properties and also reduces the metabolic activity of dental biofilms (Lingstrom *et al* 2012).

The antibacterial activity of four types of extracts (crude filtrate, alcoholic, aqueous and crude lentinan extracts) of seven different strains (LeC, LeS, LeI, OE-38, OE-142, OE-329 and OE-388) of *Lentinus edodes* mycelium and three extracts (alcoholic, aqueous and crude lentinan) of fruiting bodies of five strains (LeS, OE-38, OE-142, OE-329 and OE-388) on *Acinetobacter* sp., *E. coli, Klebsiella pneumoniae*, *Salmonella typhii* and *Vibrio cholera* were studied by Sharma *et al* (2014). Different extracts showed different antimicrobial potential against various microbial cultures and crude lentinan extract showed maximum antimicrobial potential. Therefore, these bioactive components of *L. edodes* would serve as one of the alternate for

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antibiotic resistance and serve as a promising antimicrobial agent with potent antibacterial and antifungal activity.

Future Perspectives

Polysaccharides in comparison to protein and nucleic acid are considered as a renewable source in terms of pharmacologically active agent with immuno-stimulant, anti-tumor, anti-cancer and anti-viral activities. These polysaccharides possess numerous applications in the field of medicine and food industry.

Though β-glucans possess high molecular weight that plays a key role in activating immune response of patients either absorption via M cells of intestinal membrane or by macrophages. Thus the bioactive components from *Lentinus edodes* would serve as a promising nutraceutical used for treating various forms of cancer and several other diseases such as high blood pressure, diabetes, cholesterol-lowering with potential value for healthy food and therapeutics. Because of the complex structure of lentinan, its mechanism is still uncertain which hinders its application in the field of pharmaceutical industry.

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