

## LENTINULA EDODES (SHIITAKE) – BIOLOGICAL ACTIVITY

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### Abstract:

*Lentinula edodes* (Berk.) Pegler (shiitake) is a species with medicinal properties that is used primarily in traditional medicine, but also in conventional oncology treatment. The first records of *Lentinula edodes* cultivation reach back to China under the Song dynasty (960-1127). The Japanese adopted the Chinese technique of shiitake cultivation, thus becoming its main producer. Currently, shiitake is used in the treatment of lifestyle diseases. Polysaccharides contained in this species strengthen the immune system, eliminate side effects of chemo and radiotherapy and have strong antitumor, antiviral and antibacterial properties. The year 1972 saw a discovery of substances with anti-atherosclerotic effects, with the most important being eritadenine (2 (R), 3 (R)-dihydroxy-4 – (9-adanyl) butyric acid) and statin – lovastatin. *L. edodes* is of interest to researchers due to its content of therapeutic compounds. These substances have antitumor, antifungal, antibacterial, anti-inflammatory, hypocholesterolemic, antihypertensive, hypoglycaemic and antioxidant effects.

**Keywords:** *Lentinula edodes*, shiitake, lentinan, eritadenine, immunostimulating effect

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*Lentinula edodes* (Berk.) Pegler – shiitake of the family *Omphalotaceae* (Basidiomycota) commonly called shiitake is an edible mushroom from East Asia which is cultivated and eaten in many Asian countries as well as in Europe. It is a species with medicinal properties, which is used primarily in traditional medicine, but also in conventional oncology treatments [1,2].

The Japanese name shiitake consists of *shii*, derived from the common name of the *Castanopsis cuspidata* tree, whose wood is used for the cultivation of this species, and *take* the Japanese term for “mushroom”. The generic name *edodes* comes from Latin, meaning “edible” [3].

*L. edodes* grows in groups on decaying deciduous trees, particularly on the *Castanopsis cuspidata* species and other species of the following genera: *Aesculus sp.*, *Quercus sp.*, *Acer sp.*, *Fagus sp.*, *Eucalyptus sp.*, *Populus sp.*, *Carpinus sp.*, *Morus sp.* It grows naturally in the warm and humid climates of Southeast Asia [4].

### Biologically active substances found in *Lentinula edodes* fruiting bodies

Fresh *Lentinula edodes* fruiting bodies contain 88-92% water. Their calorific value is 387-392 kcal per 100 g of dry matter. The content

of carbohydrates in the dry matter of *Lentinula edodes* fruiting bodies is 67.5-78.0%, of which simple sugars constitute 15.87%. Among carbohydrates, mono-, di-, tri- and polysaccharides can be distinguished. The simple sugars are as follows: glucose, mannose, galactose, xylose, ribose, fucose and rhamnose. The disaccharides include sucrose and maltose. Raffinose trisaccharide is also present. There is a high percentage of polysaccharides in the content, including (1→3)-β-D-glucans, which determine therapeutic properties of this species (antioxidant and protecting against UV). Polysaccharides, which are present both in fungal cell walls and intracellularly, are soluble (α- and β-glucans, galactates, manganates, xyloglucans) and insoluble in water (heteroglycans, polyuronids, β-glucans). Polysaccharides contained in this species strengthen the immune system, eliminate side effects of chemo and radiotherapy and have strong antitumor, antiviral and antibacterial properties. Lentinan is a branched β-D-glucan. Other polysaccharides responsible for immunomodulation and identified in edible shiitake extracts are: KS-2 (mannopeptide), LE (polysaccharide-protein complex), L-II (α-(1→3)-D-glucan) and JLS-18 (lignin) [1-6].

The fruiting bodies of this species contains all exogenous amino acids. The essential amino acid content is 39% of the total amount of all amino acids. Thanks to their presence, shiitake fruiting bodies has a nutritional value ranking after meat products and before dairy products [2].

As antioxidant properties of the shiitake extract have been shown to depend on extract concentration, i.e. the concentration of active ingredients, we have also determined the contents of phenolic compounds and flavonoids. Antioxidant properties have been found to be weaker than in the case of ascorbic acid. Polyphenols (gallic acid, protocatechuic acid, catechin), tocopherols,  $\beta$ -carotene, polysaccharide fractions are responsible for antioxidant properties [2,3]. In addition, in 1976, Ac2Ps with strong anti-viral properties were isolated from aqueous *L. edodes* dried fruits. Ac2P is a high molecular weight polysaccharide composed of pentose with the highest activity against smallpox virus [7-9].

In addition to polysaccharides, several antimicrobial substances have been identified, many of which have been patented. Edible shiitake is therefore a source of antibacterial and antifungal compounds. The isolated antibiotics include: lentin (protein), lenthionine (exobiopolymer containing sulphur), lentysine (purine compound), lentinamycin A and B (polyacetylene derivatives). Lentin and lenthionine also exhibit the antifungal activity. Th protein content in edible shiitake fruiting bodies varies from 13.4 to 17.5% of dry matter [8].

In 1972, the first anti-atherosclerotic substances such as lentysine were detected in fruiting bodies of *L. edodes*. Another one eritadenine (2 (R), 3 (R)-dihydroxy-4-(9-adanyl) butyric acid) is one of the most important compounds reducing lipid levels in the blood and derived from *L. edodes* fruiting bodies. It is an inhibitor of S-adenosyl-L-homocysteine hydrolase (SAHH). The amino acid called lentinacin shows similar activity [9].

The lipid content in the fruiting bodies is low and ranges from 4.8 to 8.0% of dry matter. Fats include fatty acids, mono-, di-, triglycerides, phospholipids and sterols. Fats with unsaturated fatty acids comprise a high percentage (78%). Linoleic acid has the highest proportion in the lipid content – about 68%. There is also oleic acid – 5.5%; among saturated acids – palmitic acid –16% of the total fat content. Among sterols, ergosterol is the highest percentage (83-89%). This compound is part of the cell membranes of mushrooms. It was

determined in the amount of 679 mg per 100 g of dry matter of the mushrooms. Ergosterol in the fungal cells is present in free form, as peroxides and esters with higher fatty acids, glycosides or complexes with polysaccharides. Apart from ergosterol, shiitake fruiting bodies also contains its derivatives: ergosta-7,22-dienol, ergosta-7,5-dienol and fungisterol.

Like other mushroom species, edible shiitake fruiting bodies are a good source of vitamins, especially from group B. The content of vitamin B<sub>1</sub> is comparable to that of cereal grains and larger than in eggs. Vitamin B<sub>2</sub> is present in higher amounts than in vegetables. It is important to have vitamin B<sub>12</sub>, especially for vegetarians. Like animals, mushrooms have the ability to biosynthesize vitamin D. In mushrooms, vitamin D<sub>2</sub> (ergocalciferol) is produced from ergosterol under the influence of ultraviolet radiation (sunlight). Studies in rats showed an increase in both serum 25-hydroxyvitamin D and bone mineral density following *Lentinula edodes* fruiting bodies-based diet irradiated with UV light. The results indicate that vitamin D<sub>2</sub> from UV-irradiated mushrooms was well absorbed and metabolized in this animal model [10, 11]. Nuclease enzyme with the antithrombotic activity was isolated from this species (eye pouch and anti-cellulite preparations).

Like other mushrooms, edible shiitake has a high potency to accumulate elements. The content of elements in the fruiting bodies depends on the composition of the cultivation medium. *Lentinula edodes* contains more calcium than other species of edible mushrooms. It also comprises significant amount of potassium, magnesium, sodium, zinc and phosphorus [12].

### ***Lentinula edodes* cultivation**

Currently cultivated Shiitake is used in the treatment of lifestyle diseases. The first records of *Lentinula edodes* cultivation reach back to China during the reign of the Song dynasty (960-1127). The Japanese adopted the Chinese technique of shiitake cultivation, and thus became its main producer. The first methods of shiitake cultivation consisted of the inoculation of logs of oak, chestnut and eucalyptus. Cultivation techniques were then developed using special plastic bags filled with sawdust. Sawdust is the most basic substrate for synthetic mixtures used to produce shiitake, but other substrates may include straw or corn cobs. Regardless of the main ingredient, starchy supplements such

as wheat bran, rice, soybean, millet, rye or corn may be added to the mixture. Cultivating in special plastic bags reduces production time, increases productivity and allows for production throughout the year. Shiitake cultivated on both wooden logs and on sawdust blends, produces enzymes, such as cellulases, hemicellulases, cell wall breakdown enzymes, acid phosphatases, acid proteins, laccases, manganese peroxidases and ligases that contribute to degradation and circulation of elements in nature [4].

### Healing properties of *Lentinula edodes*

*L. edodes* is of interest to researchers because of its content of therapeutic compounds. These substances have antitumor, antifungal, antibacterial, anti-inflammatory, hypocholesterolemic, antihypertensive, hypoglycaemic and antioxidant effects. Some of these compounds are well characterized, e.g. lentinan, KS-2, lentinacin or eritadenine. The shiitake antimicrobial activity has been confirmed against bacteria, filamentous fungi, yeasts and viruses [4]. The most studied active ingredient isolated from shiitake is pure  $\beta$ -(1,3)-D-glucan known as lentinan (Figure 1).

This compound has a triple helix structure, the main chain is  $\beta$ -(1,3)-glucan, to which  $\beta$ -(1,6)-glucanes are attached in the number of 2/5. Molecular weight is  $4-8 \times 10^5$ .

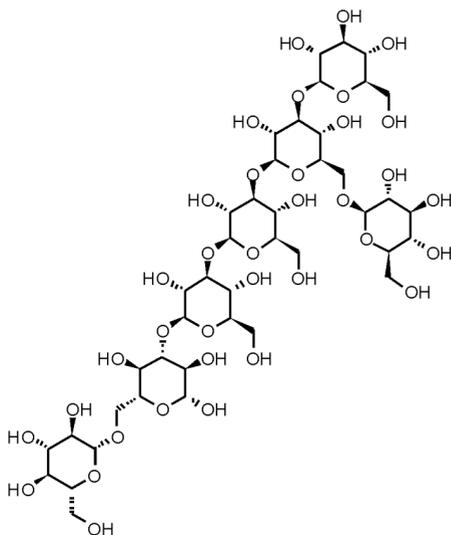


Fig. 1. Structural pattern of lentinan

### Antitumor properties

Research into the potential uses of antitumor substances found in mushrooms began in Japan in the 1960's. According to the British Imperial Cancer Research Fund, there is a 25% chance of developing cancer; therefore, the shiitake antitumor properties became of particular interest.

In the experiment conducted by Kurashige et al. (1997), the control group consisting of six-week old mice was treated with carcinogen N-butyl-N'-butanolonitrosoamine and fed with standard feed. The study group, which was subjected to the same carcinogen, was fed with diet containing 5% dried and powdered *L. edodes*. 100% of the mice in the control group developed bladder cancer, whereas the proportion of this malignancy in the study group was 53% [13]. A diet supplemented with shiitake increased the macrophage activity and lymphocyte response to concanavalin A to the normal level, which had been lowered by the use of N-butyl-N'-butanolonitrosoamine. This diet also increased the impaired NK (natural killer) cell activity. The resulting immunomodulating effects can explain the mechanism of shiitake antitumor activity. They coincide with antitumor properties of lentinan, which inhibits both induced carcinogenic and viral carcinogenesis. Another experiment demonstrated that intraperitoneal injections of aqueous shiitake extract containing lentinan significantly inhibited (81%) the growth of malignancies from sarcoma cells implanted into laboratory mice [5]. In addition, lentinan enhances the effects of other drugs, such as 5-fluorouracil (5-FU) [14] and cisplatin in the treatment of cancer [15,16].

Lentinan is a drug that has documented effects of prolonging the life of patients with neoplasms, especially with gastric and colorectal cancers. In patients with non-resectable gastric cancer, the administration of lentinan in combination with 2 other chemotherapeutics increased the annual survival compared to those who received only 2 chemotherapeutic agents. During the lentinan treatment, patients reported improvement in Quality of Life (QOL) [17].

*In vitro* and *in vivo* studies have demonstrated that shiitake and lentinan are effective anticancer agents, and therefore their formulations are used in oncology clinics.

### Immunomodulation effects

Ingestion of the aqueous *L. edodes* extract resulted in immunomodulatory effects such as

those described above, whereas drinking isolated lentinan was not effective. As  $\beta$ -glucans lose much of their antitumor activity with decreasing molecular weight, their digestion after the oral administration is connected with the reduced activity. Differences in therapeutic efficacy between shiitake extract and lentinan extract indicate that some other components of this species exhibit biological effects.

The mechanism of antitumor activity of lentinan is not fully understood, but it has been proven that lentinan is a modulator of the biological response. It is believed to enhance cellular immune responses *in vitro* and *in vivo*; this mechanism is also effective against cancer. The effect of lentinan on T cells has been studied. The intraperitoneal administration of lentinan resulted in complete tumour regression in 7 out of 8 BDF1 mice vaccinated with Di Guglielmo syndrome FBL-3 cells. When monoclonal antibodies against CD4 and CD8 were administered prior to the lentinan treatment, tumour growth inhibition was stopped (Suzuki et al., 1994). Further studies showed that patients with advanced malignancies exhibited an impaired balance between Th1 (Th – T helper) and Th2 responses, meaning that they had a defective cellular immune response. Flow cytometry of blood samples revealed that the intravenous administration of lentinan (2 mg, three times a week) resulted in restoration of the correct Th1 to Th2 ratio [18].

The vascular response to the intravenous administration of lentinan was investigated in experiments conducted in mice. This compound enhanced skin reactions following the bradykinin administration. In addition, it stimulated the body's response to induce acute phase proteins, while vasodilation caused haemorrhaging from the ears. This reaction occurred only in strains of lentinan-sensitive mice. They had a specific gene for this sensitivity.

Skin reactions were not observed in T-cell deficient mice. In addition, the administration of lipoxygenase inhibitors, thrombin and plasmin to mice with leukaemia resulted in decreased lentinan skin response and reduced tumour necrosis.

The results suggest that vascular responses may play a key role in the antitumor effect of lentinan and that the dermal response may be a useful tool for monitoring lentinan host sensitivity during antineoplastic therapy [19].

### Hypocholesterolemic properties

Cardiovascular diseases are the leading cause of death in the world. As high blood cholesterol is an important risk factor in the development of cardiovascular disease, cholesterol-lowering substances are crucial in its prevention. The ability of *L. edodes* to lower cholesterol was described for the first time in the 1960's by Kamyia et al. (1969) [9]. It was discovered that a diet containing dried soil siderophores lowered the average blood cholesterol levels in rats. Eritadenine (2 (R), 3 (R)-dihydroxy-4-(9-adanyl) butyric acid) and statin – lovastatin are the main active ingredients responsible for this effect (Figure 2).

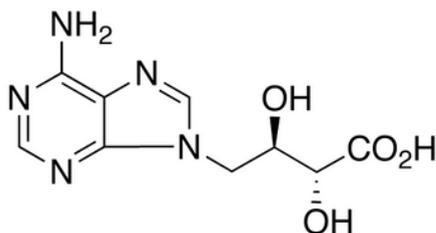


Fig. 2. Structural formula of eritadenine

Statins are inhibitors of 3-methyl-glutaryl co-enzyme reductase A. By acting on this enzyme, they inhibit the synthesis of endogenous cholesterol. They block the mevalonic acid pathway, which results in reduction of cholesterol levels in the body. Other proven biological effects of statins, *in vitro* and *in vivo*, are connected with the cytotoxic and cytostatic activity against various tumour cell lines. They result from proapoptotic, antimetastatic and antiangiogenic mechanisms of action [20]. Eritadenine is likely to lower all blood lipoprotein fractions in mammals. The oral administration of this substance has proven to be effective and having low level toxicity, although only 10% of this compound is absorbed from the gastrointestinal tract. The intravenous administration of eritadenine is ineffective because of the fact that the compound is rapidly removed from the bloodstream and excreted by the kidneys.

Fulushima et al. (2001) have denied the above thesis that all lipoprotein fractions are reduced by *L. edodes* [20]. Rats fed with diet containing this species of mushrooms in the amount of 50 g/kg body weight for 4 weeks showed reduced VLDL, IDL, and LDL levels compared to the control group receiving cellulose in the amount of 50 g/kg body weight. There were no significant

changes in the concentrations of HDL, hepatic cholesterol and the amount of mRNA encoding LDL receptors, although the concentration of excreted cholesterol was significantly increased in the study group. A decrease in plasma cholesterol levels in rats was evident [20]. Statins are inhibitors of 3-methyl-glutaryl coenzyme A reductase. Natural statins also include lovastatin, which is found in large amounts in *L. edodes* (Figure 3).

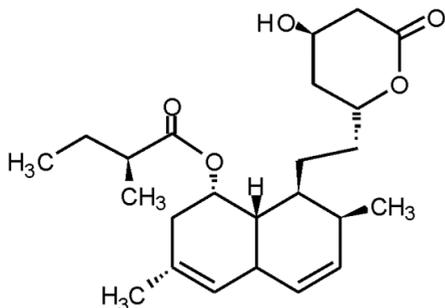


Fig. 3. Structural formula of lovastatin

### Anti-inflammatory activity

Ergosterol, which is present in the *L. edodes* fruiting bodies, has anti-inflammatory and anti-cancer effects. In mitogen-induced C57B1/6 mice (concanavalin A) with hepatitis (concanavalin A – agent immunomodulating the liver function), supplementation with *shiitake* extract enriched with vitamin D by UV-B, significantly reduced liver damage. The histopathological image of tissues has improved and the plasma level of aminotransferases and INF- $\gamma$  decreased. In addition, it was found that the anti-inflammatory effect of vitamin D and fungal extract was synergistic [6]. Typical  $\beta$ -glucans with anti-inflammatory activity and among the longest used in treatment is lentinan from *Leninus edodes*.  $\beta$ -glucans were also found to influence the production of both pro – and anti-inflammatory cytokines. One of the  $\beta$ -glucan mechanisms is binding immune system of cells with pattern recognition receptors (PRRs) as pathogen-associated molecular patterns [6]. Such receptors include, among others, dectins-1, complementary receptor 3 (CR3, CD11b/CD18) and toll-like receptors (TLRs). Thus,  $\beta$ -glucans activate proliferation and maturation of the immune system cells and stimulate the activation of macrophages and NK cells [1].

### Antibacterial activity

An interesting aspect of immunomodulating effects after the administration of *L. edodes* extract is the increased host resistance to bacterial and viral infections. Lenthionine is a cyclic compound containing sulphur in the ring ( $C_2H_4S_5$ ) and partly responsible for the particular taste of this species (Figure 4).

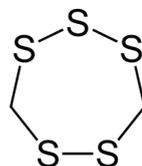


Fig. 4. Structural pattern of lenthionine

This compound has antimicrobial and antifungal properties, while bis [(methylsulfonyl) methyl] disulfide (lenthionine derivative) has strong antimicrobial properties against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* [12].

Chloroform and ethyl acetate extracts from dried mushrooms have bactericidal properties against *Streptococcus mutans* and *Prevotella intermedia* [21].

The postulated lentinian antibacterial mechanism may be activated by complement proteins C3 and C3b. In addition, many studies have reported non-specific modulation of the immune system and this may also be responsible for the antimicrobial effects of lentinan [22]. Other immunomodulatory effects of lentinan have also been noted, such as the increased IL-1 production [23], TNF $\alpha$  circulating inhibition [24,25] and elevated cytokine expression. The analysis of cytokine expression after the administration of lentinan showed increased amounts of IL-1 $\alpha$ , IL-1 $\beta$ , TNF- $\alpha$ , IFN- $\gamma$  and M-CSF mRNAs in the cells of peritoneal exudate and splenocytes [25].

Moreover, Braga et al. (2011) have shown that aqueous *L. edodes* extracts are bacteriostatic to Gram positive bacteria [4].

### Anti-viral activity

In several experiments, the HIV virus showed susceptibility to *L. edodes* extracts. Lentinan was used in combination with 3'-azido-3'-deoxythymidine (AZT – zidovudine) because it suppressed the surface expression of HIV antigens more strongly than AZT alone *in vitro*. It also amplified

the effect of AZT on HIV replication in various hematopoietic cell lines *in vitro* [17, 28]. In another experiment, aqueous *L. edodes* extract inhibited cytopathic HIV effects [12].

HIV infection leads to AIDS. CD4 particles present on T-helper cells serve as receptors for the HIV infection. Viral RNA is converted into DNA by reverse transcriptase and incorporated into cellular DNA, leading to cellular immunodeficiency. Immunomodulatory effects of lentinan may be useful in the treatment of AIDS patients, although this information is still fragmentary.

It is suggested that aqueous extract, ethanol extract and *L. edodes* polysaccharides act at initial replication stages of the polio virus infection (PV-1), bovine viral diarrhoea and bovine herpesvirus infection of the mucous membranes (BoHV-1). For this reason, these extracts and polysaccharide may be considered as potential sources of antiviral substances [28].

### Other biological properties of *Lentinula edodes*

It has been found that *L. edodes* may be enriched in trace elements such as selenium and thus serve as a potential nutraceutical [11].

It has been demonstrated that fungal polysaccharide fractions, which are isolated from *L. edodes* and enriched with selenium (Se), may have higher biological activity than untreated fractions currently used in cancer therapy [29,30]. In order to obtain selenium-enriched formulations, *L. edodes* was cultured in a medium supplemented with sodium selenite. The effect of selenium on the fungal hyphae composition was determined in Se media at concentrations ranging from 0 to 30 µg/mL. In each culture, the mycelium growth, total Se concentration and Se distribution were determined in different polarity mycelial fractions. The content of exopolysaccharide, polyphenols and ergosterol increased in proportion to the concentration of Se in the range 0-5 µg/mL. At higher concentrations, the content of chitin decreased. The data showed that in fungal media Se had a significant effect on the structure of the cell wall and semipermeable cell membrane as well as on the content of polyphenols involved in the detoxification processes.

### Conclusion

*Lentinula edodes* – shiitake is one of the longest-used species of edible therapeutic mushrooms. Proven healing properties of this species resulting from the rich content of biologically

active substances make it irreplaceable in the treatment of lifestyle diseases, especially cancer and cardiovascular diseases.

### Resumo

*Lentinula edodes* (Berk.) Pegler (Ŝiitako) estas specio de fungoj kun kuracaj ecoj, kiuj estas uzata ĉefe en la tradicia medicino, sed ankaŭ en onkologia kuracado. La unuaj informoj pri kulturado de *Lentinula edodes* atingis Ĉinion dum la regado de Song dinastio (960-1127). La japanoj adoptis la ĉinan teknikon de Ŝiitakoplantado kaj fariĝis ĝia ĉefa produktanto. Nuntempe, Ŝiitako estas uzata en la kuracado de civilizaciaj malsanoj. Polisakaridoj, kiuj estas en ĉi tiu specio, fortigas la imunan sistemon, eliminas kromefikojn de kemi – kaj radioterapio kaj havas fortan kontraŭtumoran, kontraŭvirusan kaj kontraŭbakteriajn ecojn. En la jaro 1972 oni malkovris substancojn, kiuj estas efikaj kontraŭ arteriosklerozo, ĉefe eritadeninon (2 (R), 3 (R) – dihidroxy-4 – (9-adanyl) butera acido) kaj statinon – lovastatinon. Pri *L. edodes* interesiĝas sciencistoj pro ĝia enhavo de kuracaj substancoj. Ĉi tiuj substancoj havas kontraŭtumoran, kontraŭfungan, kontraŭbakterian, kontraŭinflanman, antihipertensian, hipokolesteroleman, hipoglikemian kaj kontraŭoksidan efikon.

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