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Laetiporus sulphureus – CHEMICAL COMPOSITION AND MEDICINAL VALUE

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ABSTRACT

The higher fungi are a rich source of chemical compounds with multi-directional therapeutic and pro-health effects. This review summarizes the results of the most important chemical and biological studies of the fruiting bodies and the mycelial cultures of *Laetiporus sulphureus*. Numerous studies have demonstrated the antimicrobial, anticancer, cytotoxic, hypoglycemic anti-inflammatory and antioxidant activity of the extracts. Currently, only a few wood-decay fungi have practical use in medicine. Therefore it seems important to continue research on the effectiveness and safety of extracts and compounds of natural origin, including fungi, whose potential is not still used.

Key words: *Laetiporus sulphureus*, medicinal mushrooms, biological activity

TAXONOMIC POSITION, PREVALENCE, AND ETYMOLOGY

Laetiporus name is a combination of two words "*laeti*" (Lat.) and "*por*" (Lat.) – and refers to a hymenial layer and the size of the specifically shaped fruiting bodies [Plezia 2007]. The adjective "*sulphureus*" (Lat.) originates from the characteristic color of fruiting bodies. *L. sulphureus* is a cosmopolitan species, it is present on all continents, except Antarctica. It is widely distributed in Europe and North America. It attacks and colonizes both living tree trunks and dead wood of deciduous species, such as *Aesculus* sp., *Populus* sp., *Quercus* sp., *Robinia* sp., and more rarely coniferous species, for example, *Larix* sp., *Taxus* sp. Fruiting bodies consist of fleshy, semicircular hats with a characteristic sulfuric-yellow color, intensively grow in May, but may be produced until the autumn. *L. sulphureus* belongs to conditionally edible mushrooms because only young fruiting bodies may be eaten while raw fruiting bodies may be toxic [Gumińska and Wojewoda 1985]. The case of severe poisoning with fruiting bodies in a 6-year old girl, which resulted in symptoms of ataxia and hallucinations, was described in 1988 [Appleton et al. 1988]. The English names "chicken mushroom", "chicken of the woods" or "chicken fungus" suggest that properly prepared fruiting bodies may resemble the taste of chicken meat.

This paper presents mycochemical and biological characteristics of the individual, isolated compounds, and extracts from fruiting bodies and mycelial cultures of *L. sulphureus.*

CHEMICAL COMPOSITION

Due to the fact that the young fruiting bodies of *L. sulphureus* are edible, they were examined in terms of basic nutrients content. Total carbohydrates content was – 64.90; fats – 5.85, and proteins – 11.90 g 100 g^{-1} DM (dry mass) of the fruiting body.

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In addition, the content of organic acids such as malic (3.68 g kg^{-1} DM), citric (3.13 g kg^{-1} DM) and ascorbic acid (0.06 g kg^{-1} DM) was analyzed. The energy content of 100 g of fresh fruiting bodies determined according to Ayaz [2011] is 360 kcal.

One of the best-recognized group of metabolites in fruiting bodies are polysaccharides. The following exopolysaccharides as linear water-insoluble β-1,3-glucan – laminaran, and fuco-galactomannan, heteropolysaccharide consisting of the main chain and α-D-galactopyranosyl residues attached to it with 1→6 bonds, were identified in the fruiting bodies of that species. These residues are substituted with 3-O-D-L-mannopyranosyl-L-fucopyranosyl, α-D-mannopyranosyl and α-L-fucopyranosyl moieties at positions 2 and 3 [Alquini et al. 2004].

Water-soluble endopolysaccharides were isolated in subsequent studies. They constituted 3.67% of the fruiting body, a dominant structure was laetiporan – β-1,3-glucan, with a mass of 56 kDa, where sugar molecule (mannose, galactose, fucose, xylose or rhamnose) can be attached at position C6 [Olennikov et al. 2009a]. Another fraction isolated from fruiting bodies were polysaccharides soluble in the alkaline solution. The main component of this fraction was latiglucan I, a linear β-1,3-glucan with a mass of 180 kDa, moreover, the structure latiglucan II and latiglucan III were determined [Olennikov et al. 2009b]. Linear polysaccharides with the structure similar to latiglucan I are not typical for *Basidiomycota*, but they are more common in bacteria and algae. According to Olennikov, latiglucan I is a valuable source of substrates for potential anticancer agents production [Olennikov et al. 2009b].

The fraction of polysaccharides insoluble in water and soluble in an alkaline environment was also examined at Maria Curie-Skłodowska University in Lublin and the result of this study was the isolation of α-(1→3)-D-glucans from that fraction [Wiater et al. 2011].

The study carried out in the 90s of the twentieth century allowed to obtain Laetiporus sulphureus Lectin (LSL-) specific to N-acetyllactosamine from the fruiting bodies. LSL is a heterotetrameric protein with a mass of 190 kDa, composed of subunits with masses of 36 and 60 kDa [Końska et al. 1994]. Subsequent studies proved that lectin molecule has two modules i.e. N-terminal module, responsible for hemagglutination induction, and C-terminal module, structurally similar to blowing bacterial toxins exhibiting hemolytic activity [Tateno and Goldstein 2003].

A number of volatile compounds such as (Z)-3-methylcinnamic aldehyde (27.5%) and 2-phenylethanol (6.4%) were identified in the fruiting bodies, and they are responsible i.a. for characteristic odor. Also, methyl orselinate, the compound which was described so far only in lichens and mosses, was identified. The studies on volatile fraction confirmed that its composition varies depending on the age of the fruiting body, tree species colonized by the fungus, and the place of collection. The young fruiting bodies contained oct-1-en-3-one, oct-1-en-3-ol, methylbutanoic acid, phenylethanol and phenylacetic acid. In turn, 2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid and phenylacetic acid were predominant in older specimens [Rapior et al. 2000, Wu et al. 2005].

L. sulphureus contains mainly long chain fatty acids (C16-C20), and ethyl esters of fatty acids with 16 to 24 carbons, as well as 28-, 29-, 30-carbon sterols. Fatty acids and their esters isolated from fruiting bodies, except margaric acid and arachidonic acid, are characteristic of *Fungi* kingdom. Also sterols, derivatives of ergostane (ergosta-7,22-dien-3β-ol, ergosterol, ergosta-7-en-3β-ol and 24 ethylcholestan-3β-ol), are commonly found in fungi. In turn, sterols being the derivatives of lanostane, 24- and 25-methylene-lanostane, are characteristic for the fungi of *Polyporaceae* family [Ericsson and Ivonne 2009].

Analysis of lipid fraction allowed to define the structure of cyclodepsypeptide referred to as beauvericin – mycotoxin produced by certain species of *Ascomycota* division (fig. 1). Acidic hydrolysis of that compound allowed obtaining N-methyl-phenylalanine and α-hydroxyisovaleric acid [Deol et al. 1978].

The structure of masutakeside I (fig. 2) and masutakic acid (fig. 3) were isolated and determined in the fruiting bodies of *L. sulphureus* var. *miniatus*. Already known compounds: egonol (fig. 4), demethoxyegonol, egonol glucoside and egonol gentiobioside as well as 2-(3,4-dihy-droxyphenyl)-2,3-dihydro-7-hydroxy-3--hydroxymethyl-5-benzofuranpropanol were concur-

rently observed in the same experiment [Yoshikawa et al. 2001].

The compound named (±)-laetirobin of the chemical formula $C_{44}H_{32}O_{12}$ (fig. 5) was obtained from the fruiting bodies parasitic on *Robinia pseudoacacia* species. Its structure differs from the previously known benzofuran derivatives present in *L. sulphureus*, and also in other species of fungi or plants [Lear et al. 2009].

Triterpene compounds present in fruiting bodies of *L. sulphureus* are lanostane derivatives: 3-oxo-sulfurenic acid (fig. 6) and eburicoic acid (fig. 7), as well as 15α-hydroxy-trametenolic acid (fig. 8) and sulfurenic acid (fig. 9) were determined among the triterpene acids [Léon et al. 2004]. Another triterpene derivative noted in fruiting bodies was dehydrotrametenolic acid, commonly found in the wood-decay fungi, including *Fomitopsis officinalis*.

Phenolic compounds which total content amounted to about 7.25 and 0.33 mg × g − 1 DM, respectively, were determined in methanolic and chloromethane extracts. Gallic acid in an amount of 2.06 mg g^{-1} and protocatechuic acid in an amount of 1.21 mg g^{-1} were quantitatively dominant among the determined phenolic acids [Karaman et al. 2010]. The total content of flavonoids and phenolic compounds, which amounted to 14.20 ±0.12 µg mg^{-1} (as quercetin concentration), and 63.80 ±0.25 µg mg^{-1} (as pyrocatechol concentration), respectively, was determined in the fruiting bodies in a further experiment with ethanol extract [Turkoglu et al. 2006]. Quantitative content of phenolic compounds: quercetin 11.37 mg g^{-1}, kaempferol 5.01 mg g^{-1}, (+)-catechin 14.04 mg g^{-1}, gallic acid 28.57 mg g^{-1}, chlorogenic acid 22.61 mg g^{-1}, caffeic acid 20.07 mg g^{-1}, and *p*-coumaric 18.84 mg g^{-1}, was determined in the extract obtained using ethyl acetate [Olennikov et al. 2011a]. Analysis of selected wood-decaying fungi from Poland confirmed the presence of protocatechuic acid in the fruiting bodies [Sułkowska-Ziaja et al. 2012].

Analysis of sulfuric-yellow dye structure of the fruiting bodies showed that this is laetiporic acid A (fig. 10), a polyene compound built from non-isoprenoid structures. This dye appeared to be very durable and can be used as a natural food dye [Davoli et al. 2005]. This is a new structure, not described yet in fungi. Moreover, several other dyes were detected in the extract of *L. sulphureus* fruiting bodies, including 2-dehydro-3-deoxylaetiporic acid [Weber et al. 2004]. Two other polyene dyes, laetiporic acid B and laetiporic acid C, were also identified in mycelial cultures [Davoli et al. 2005].

The dyes present in the fruiting bodies include also melanins, high molecular heterogeneous complexes, dihydronaphthalene derivatives, which were isolated with a performance of 2.5% DM of the fruiting body. Elemental analysis demonstrated that they contain 49.0% of carbon, 6.3% of hydrogen, 2.3% of nitrogen and 42.4% of oxygen [Olennikov et al. 2011b].

The content of macro- and microelements was examined in the fruiting bodies of *L. sulphureus* collected in Turkey. The estimated macroelements included potassium (18 500 mg kg^{-1} DM), calcium (4 200 mg kg^{-1} DM), magnesium (2 100 mg kg^{-1} DM) and sodium (285.0 mg kg^{-1} DM) [Ayaz et al. 2011]. In turn, the contents of microelements were as follows: chromium (58.3 mg kg^{-1} DM), manganese (30.7 mg kg^{-1} DM), lead (24.5 mg kg^{-1} DM), copper (22.7 mg kg^{-1} DM), nickel (22.7 mg kg^{-1} DM), cadmium (0.68 mg kg^{-1} DM) and silver (0.26 mg kg^{-1} DM) [Doğan et al. 2006]. Other studies confirmed the presence of aluminum (53.9 mg kg^{-1} DM), boron (16.4 mg kg^{-1} DM), cobalt (1.2 mg kg^{-1} DM) and tin (4.5 mg kg^{-1} DM) [Durkan et al. 2011].

It was proved already in the 60s of the last century that submerged and superficial cultures of *L. sulphureus* constitute a valuable source of compounds with therapeutic properties. Special attention should be paid to the presence of compounds of triterpene structure, which content reaches up to 30% of the dry biomass, including up to 75% of eburic acid. In 2015, He et al. isolated seven new sesquiterpenoids of drimane type, called sulphureuines B-H, from mycelial cultures.

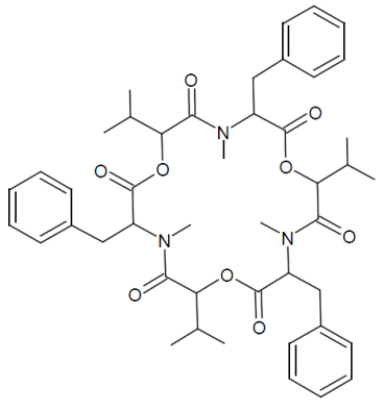

Fig.1. Beauvericin

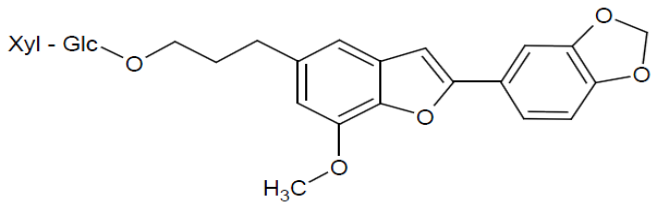

Fig. 2. Masutakeside I

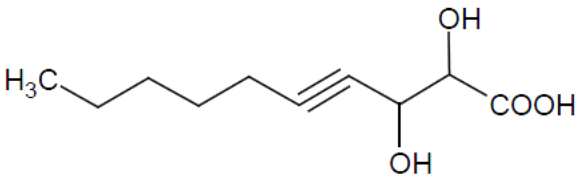

Fig. 3. Masutakic acid

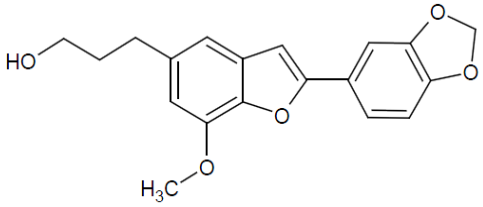

Fig. 4. Egonol

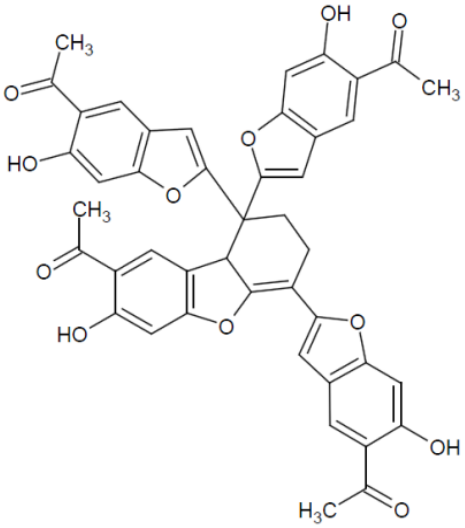

Fig. 5. (±)-Laetirobin

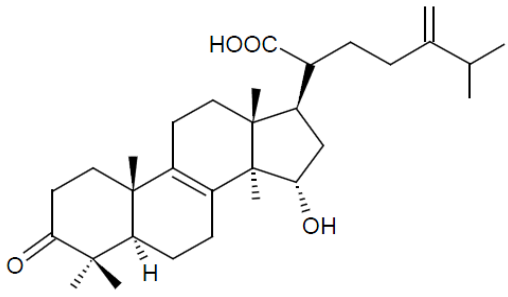

Fig. 6. 3-oxosulphurenic acid

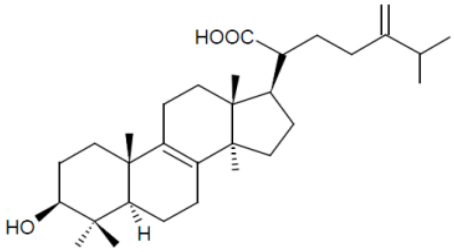

Fig. 7. Eburicoic acid

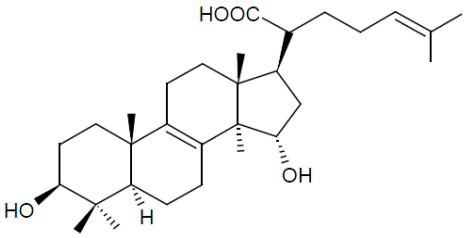

Fig. 8. 15α-hydroxytrametenolic acid

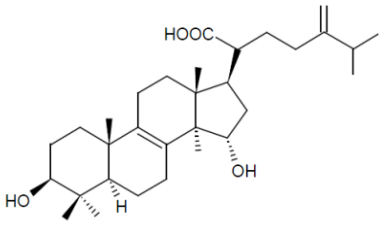

Fig. 9. Sulphurenic acid (15α-hydroxyeburicoic acid)

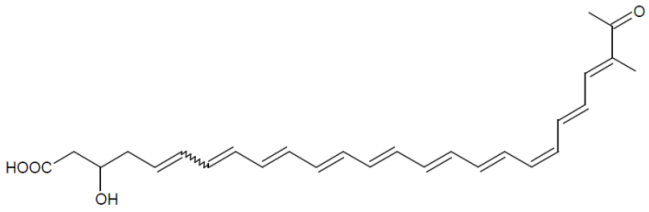

Fig. 10. Laetiporic acid A

PHARMACOLOGICAL PROPERTIES

L. sulphureus has been used for centuries in traditional medicine in many European countries, where it was valued for its antipyretic, antitussive and antirheumatic activity.

Extensive examinations of both extracts and individual compounds confirm the known, traditional uses, and also demonstrate the new profiles of biological activity.

Antimicrobial activity

Cultured strains of *L. sulphureus* exhibit antimicrobial activity against gram-negative and gram-positive bacteria, including methicillin-resistant *S. aureus* strains, and *Leuconostoc mesenteroides* strains resistant to glycopeptides [Ershova et al. 2003].

Fruiting bodies extracts demonstrated an action against the following strains: *Bacillus cereus*, *Enterobacter cloacae*, *Escherichia coli*, *Listeria monocytogenes*, *Micrococcus flavus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus* [Šiljegović et al. 2011], *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus luteus*, *Micrococcus flavus* [Turkoglu et al. 2006], *Enterococcus faecium*, *Proteus vulgaris* [Demir et al. 2008].

Ethanol extracts from fruiting bodies, as a few of fungi species already examined in this direction, exhibit strong antifungal activity against *Candida albicans* [Turkoglu et al. 2006].

In turn, water-ethanol extract showed an antifungal activity against the following strains: *Aspergillus niger, Botrytis cinerea, Fusarium oxysporum* f. sp. *tulipae*, *Penicillium gladioli* and *Sclerotinia sclerotiorum.* The minimum concentration inhibiting the growth of these microorganisms was comparable to that of the known antifungal fluconazole [Pârvu et al. 2010].

It was proved that the extracts from mycelium of *L. sulphureus* affect an inhibition of the growth of strains pathogenic to humans and animals as *Alternaria alternata*, *Aspergillus wentii*, *Fusarium tricinctum*, *Penicillium griseofulvum* and *Microsporum gypseum* [Sakeyan 2006].

Mlinarić et al. [2005] also investigated an antiviral activity of 63 species of fungi from *Basidiomy-*

cota division. The strongest inhibiting activity of the reverse transcriptase of HIV-1 virus (90.1%), was demonstrated for methanol-water extracts of *L. sulphureus*. The presence of acidic compounds with amino groups was noted in the most active fraction.

Antioxidant activity

Ethanolic extracts of fruiting bodies demonstrate antioxidant activity confirmed by several studies, including DPPH radical scavenging assay, linolenic acid emulsion stability test, and also based on the measurement of the total content of flavonoids and phenolic compounds. 320 µg of the fungus fruiting bodies ethanol extract showed an antioxidant effect in DPPH test corresponding to 40 µg of α-tocopherol. Antioxidant activity is proportional to the applied concentration of the extract and phenolic compounds content.

Laetiporan A, isolated from fruiting bodies demonstrated strong antioxidant activity *in vitro*, preventing an occurrence of hepatitis in test animals treated with carbon tetrachloride [Olennikov et al. 2011a]. This high antioxidant potential of extracts from fruiting bodies of *L. sulphureus* is probably caused by oxalic acid. Among 7 species of polyporoid fungi, *L. sulphureus* showed the highest ability to scavenge hydroxyl radicals, while only *G. lucidum* demonstrated stronger antioxidant potential in the DPPH test. Methanol extracts of *L. sulphureus* demonstrated about 40% inhibition of lipid peroxidation process *in vitro* [Karaman et al. 2010].

Anti-inflammatory activity

Exopolysaccharide (EPS) isolated from this species demonstrated an anti-inflammatory activity. In BV2 microglial cells, it significantly inhibited the production of inflammatory mediators induced by LPS, such as NO, prostaglandin E2 and TNF-α, without significant cytotoxicity. This is a very important discovery, since uncontrolled or abnormal activation of microglial cells in the brain can cause serious damage to neurons, and may consequently lead to Alzheimer's and Parkinson's diseases, septic shock, atherosclerosis or multiple sclerosis [Jayasooriya et al. 2011].

Hypoglycemic effect

EPS also demonstrated the hypoglycemic effect *in vivo*. Administered orally to rats 48 h after streptozotocin injection, it decreased mean plasma glucose concentration to 43.5% compared to the control group, decreased cholesterol and triglyceride levels to near normal. EPS caused an increased proliferation and regeneration of pancreatic islet β cells and also increased an activity of antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase [Hwang et al. 2008].

Dehydrotrametenolic acid isolated from fruiting bodies exhibits potential anti-diabetic properties. This triterpene compound demonstrates biological activity similar to the effects induced by PPAR-γ receptor agonists – thiazolidinediones. It induces the differentiation of adipocytes *in vitro,* and reduces hyperglycemia in mice with induced non-insulin-dependent diabetes mellitus. Dehydrotrametenolic acid is therefore, the compound of potential hypoglycemic properties, which mechanism involves tissues sensitizing to insulin [Sato et al. 2002].

Cytotoxic activity

Triterpenes, derivatives of lanostan isolated from the fruiting bodies, as well as their semi-synthetic derivatives, demonstrate cytotoxic activity. The strongest activity was noted for acetyl derivative of the eburic acid, which has apoptosis inducing properties by an activation of caspase-3 and degradation of poly(ADP-ribose) polymerase (PARP), one of the enzymes repairing DNA damages. Acetyl-eburic acid is a valuable source of structures that may lead to the discovery of new anticancer drugs [Léon et al. 2004].

Furthermore, egonol, demethoxyegonol and egonol glucoside isolated from *L. sulphureus var. miniatur* showed cytotoxic activity *in vitro* against human gastric cancer cell line KATO III [Yoshikawa et al. 2001].

Also cytotoxic activity of (±)-laetirobin was revealed, which mechanism of action differs from the previously known mechanism of anti-mitotic drugs. In *in vitro* tests, it quickly penetrated to cancer cells, inhibited their proliferation in late mitosis and induced apoptosis [Lear et al. 2009]. Likewise, poly-

saccharides of *L. sulphureus* have potential anticancer activity. Carboxymethyl derivatives of α-(1→3)--D-glucans isolated from fruiting bodies of *L. sulphureus* have a significant activity to inhibit tumor cell lines metabolism, and they do not inhibit significantly normal cells metabolism [Wiater et al. 2011].

Anticancer diagnostics

Laetiporus sulphureus Lectin (LSL) can be used to detect cancer antigens belonging to TACA group, which includes, i.a. free N-acetyllactosamine chains (antigen Ii (Galβ 1-4 GlcNAc)n) specific to thyroid, lung and breast cancer cells. *L. sulphureus* lectins may also be used for therapeutic purposes because they exhibit hemolytic and hemagglutination features, and an activity close to the toxic lectins belonging to the group of RIP proteins, i.e. ricin, abrin, modeccin, having enzymatic properties (RNA--glycosidase) and causing an inhibition of protein synthesis by ribosomes inactivation [Końska et al. 2008a, 2008b].

Other pharmacological effects

Water extracts of fruiting bodies display lipid-lowering effect, which was confirmed in animal studies and in clinical trials. The hypolipemic effect in rats after 4 weeks of treatment with *L. sulphureus* extract was similar to the effect induced by lovastatin administration at a dose of 1.8 mg. and showed no toxic effects. A statistically significant decrease in cholesterol level was observed in clinical studies. The mechanism of *L. sulphureus* extracts activity is associated with an inhibition of cholesterol synthesis, antilipemic activity of the extract is probably caused by lovastatin – a compound lowering lipids concentration, and demonstrating pleiotropic effect on cardiovascular system affecting endothelial function, stabilization of atherosclerotic plaques, inhibition of coagulation system, stimulation of fibrinolytic system, inhibition of inflammatory and immunomodulatory effect. The study conducted by [Aryantha et al. 2010] points to the possibility of water extracts application as the hypolipidemic agent.

Table 1. Biological activity of selected compounds isolated from *Laetiporus sulphureus*

Biological activity	Active compounds	References
Anticoagulant	Extracts from fruiting bodies	Okamura et al. 2000
Hypoglycemic	Dehydrotrametenolic acid EPS – exopolysaccharides	Sato et al. 2002 Hwang et al. 2008
Hipolipemic	Water extracts from fruiting bodies	Aryantha et al. 2010
Anticancer diagnosis	Lectins	Końska et al. 2008a Końska et al. 2008b
Cytotoxic	(±)-Laetirobin CM-α-(1→3)-D-glucans Egonol Demethoxyegonol and egonol glycoside Lanostane triterpenoids derivatives Sesquiterpene drimane type	Lear et al. 2009 Wiater et al. 2010 Yoshikawa et al. 2001 Léon et al. 2004 He et. al. 2015
Anti-inflammatory	EPS–egzopolysaccharides	Jayasooriya et al. 2011
Acetylcholinesterase inhibiting	Ethanol extract	Orhan et al. 2011
Antioxidants	Laetiporan A Phenolic compounds Oxalic acid	Olennikov et al. 2009a Turkoglu et al. 2007 Karaman et al. 2010
Reverse transcriptase of HIV-virus inhibitors	Methanol-water extracts from fruiting bodies	Mlinaric et al. 2005
Antifungal *Candida albicans* *Aspergillus, Penicilium, Fusarium*	 Ethanol extract Water extract	 Turkoglu et al. 2007 Parvu et al. 2010

It was demonstrated that the extracts of *L. sulphureus* fruiting bodies acted *in vitro* as a potent inhibitor of pancreatic lipase, a key enzyme involved in consumed fat metabolism. Dichloromethane extract inhibited the enzyme at a level of 83%, while methanol one at 41% [Slanc et al. 2004].

Extracts from the fruiting bodies exhibit anticoagulant activity, which was determined based on an extension of some parameters connected with parameters of blood coagulation (thrombin time, prothrombin time, and kaolin-kefalin time). Thrombin time (TT) was more over 44-fold longer than in the control group (TT exceeded 600 seconds). They may be a component of the diet in heart disease prevention [Okamura et al. 2000].

Ethanol extract of fruiting bodies showed high acetylcholinesterase inhibiting activity *in vitro* and it raises the possibility to use the fruiting bodies of in Alzheimer's disease treatment [Orhan et al. 2011].

Table 1 shows biological activity of extracts and isolated compounds from *L. sulphureus.*

CULTIVATION OF *Laetiporus sulphureus* FRUITING BODIES

In 2013, Polish research team under the leadership of Pleszczyńska described the results of the experiment involving the production on the large-scale of *L. sulphureus* fruiting bodies on an artificial medium. A few strains of *L. sulphureus* isolated from the natural state were cultured on the various variants of media enriched with organic and inorganic components. In the case of two strains, the primordia were obtained after 5-6 days after initiation, and after further 2 days the fruiting bodies started to develop. The results of this experiment open the way for the commercial preparation of these valuable fruiting bodies [Pleszczyńska et al. 2013]. A great success of the team Szczodrak J., Siwulski M., Biernacki J. K., Wiater A., Pleszczyńska M. was the Patent No. P.397668, 15.01.2015 aimed at developing a simple and high-yielding method of growing the fruiting bodies of *Laetiporus sulphureus* for use on an industrial scale. In the past, numerous attempts were made to cultivate *Laetiporus sulphureus* under different conditions. The fruiting bodies obtained in these ways in most cases did not reach the appropriate size, satisfying for the industry. The manner of breeding fruiting bodies according to this invention was characterized by the fact that the substrate is placed in a special, hermetically sealed container. Such cultivated fruiting bodies gaining weight from 250 to 300 g within 6–12 days, which is an excellent mass to obtain a lot of valuable metabolites.

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