ANTIOXIDANT ACTIVITY OF SUBMERGED MYCELIUM EXTRACTS FROM HIGHER BASIDIOMYCETES MUSHROOMS

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Abstract: *the alternative and promising approach is to search for new, safe natural antioxidants from mushroom submerged cultures. Surprisingly, very scarce information is available on the capability of fungi to produce antioxidants in submerged cultures. Therefore, the aim of this study was analysis of chemical composition and biological activity of wild growing mushroom species, including saprophytic.*
Keywords: *antioxidant activity, Basidiomycetes, submerged mycelium.*

АНТИОКСИДАНТНАЯ АКТИВНОСТЬ ЭКСТРАКТОВ ГЛУБИННОГО МИЦЕЛИЯ ИЗ ВЫСШИХ ГРИБОВ БАЗИДИОМИЦЕТОВ

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Аннотация: *альтернативным и перспективным подходом является поиск новых, безопасных природных антиоксидантов из глубинных культур грибов. Однако мало информации о способности грибов продуцировать антиоксиданты в погруженных культурах. Поэтому целью этого исследования был анализ химического состава и биологической активности дикорастущих видов грибов, в том числе сапрофитных.*
Ключевые слова: *антиоксидантная активность, базидиомицеты, глубинный мицелий.*

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Oxygen-centered free radicals and other reactive oxygen species are continuously produced in vivo. Although almost all organisms are well-protected against free-radical damage by enzymes such as superoxide dismutase and catalase or by compounds such as ascorbic acid, glutathione and tocopherols, these systems are insufficient to prevent damage entirely [1].

Among many sources of natural bioactive substances, mushrooms constitute a huge and almost unexplored group. The number of mushroom species all over the world is estimated at about 150 000. However only 22 000 species are known to science and only approximately 5% of them has been studied [2]. Since ancient times, mushrooms have been valued as both food and medicines. Mushrooms *P. ostreatus* are widely recognized as a functional food and as a source of various physiologically active compounds [3-7]. Fungal compounds have been repeatedly reported to exert biological effects which have prompted their use in pharmaceutical and cosmetic industry. Moreover, bioactive compounds from mushrooms have been reported to exert immunomodulating, antiviral, antitumor, antioxidant, radical scavenging and antibacterial effects [3, 4, 8-21].

Antiradical activity of extracts is calculated by the following formula (1):

$$\% \text{ Inhibition} = [(AB - AA) \div AB] \times 100 \qquad (1)$$

Where: AB – absorption of a blank sample (DPPH$^{\bullet}$ solution and methanol instead of the test extract), AA – absorption of a tested sample with DPPH$^{\bullet}$ reagent.

Recently, certain mushrooms have been found to possess antioxidant activity. It can be related to the presence of other groups of compounds with antioxidant activity in mushrooms, e.g. tocopherols, ascorbic acid and carotenoids [8-10]. Numerous wild growing mushrooms possess significant antioxidant potential which is often related to the phenolic compound content [8] (table 1-2).

Table 1. Total phenolic content (TPC) and antiradical activity of mushroom extracts

Species	Mean values of three replicate assays with standard deviations				
	TPC	**IC50**	**AE**	**TE**	**VCE**
S. hirsutum	8.70±0.37	18.84±0.34	0.05	140.60	129.04

TPC expressed as mg of gallic acid equivalents per gram of dried extract; IC50 expressed as mg of dry extract per mg DPPH•; AE – antiradical efficiency (1/IC50); TE – Trolox equivalent; VCE – ascorbic acid equivalent. Equivalents were calculated by dividing extract mean IC50 by standard mean IC50. For Trolox IC50 = 0.134 mg/mg DPPH•; for ascorbic acid IC50 = 0.146 mg/mg DPPH•.

Table 2. Phenolic acid contents in mushroomextracts expressed in μg per g of dry weight of mushrooms (mean values of three replicate assays with standard deviation)

Species	Phenolic acids content [μg/g DW]								
	Protocatechuc	**4-OH-benzoic**	**Vanillic**	**Caffeic**	**Syringic**	**p-coumaric**	**Ferulic**	**Salicylic**	**Sum**
S. hirsutum	–	0.74 ± 0.02	–	–	–	0.2 ± 0.0	–	0.03 ± 0.0	0.97

Abbreviations: «-» not detected; Trace – trace amounts.

There is also a growing interest in investigations of natural chemopreventive agents, which can act as blockers and or suppressors by inhibiting carcinogenesis at the initiation, post-initiation as well as promotion stages. Submerged mycelium extracts has been previously isolated from cultures of *S. hirsutum*, this metabolite was reported for its in vitro antitumour activity [11-17].

Numerous diseases, even those that were once easily healed, are becoming a huge problem. *S. aureus, E. coli or K. pneumoniae* are among the most resistant strains posing a real risk to the society. Antibacterial activity of submerged mycelium extracts *S. hirsutum* [17-21] against Gram-positive (*B. subtilis, S. aureus, S. epidermidis, M. luteus*) and Gram-negative (*E. coli, K. pneumonia, P. aeruginosa, P. mirabilis)*, microbial strains was analyzed [8] (table 3).

Table 3. Antimicrobial activity of mushroomextracts expressed inmg of dry extract per ml (mean values of three replicate assays)

Species	Reference microbial strains											
	Gram-positive bacteria											
	S. epidermidis			*S. aureus*			*B. subtilis*			*M. luteus*		
	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R
S. hirsutum	2.5	2.5	1	2.5	2.5	1	2.5	5	2	2.5	2.5	1
Species	Gram-negative bacteria											
	E. coli			*K. pneumoniae*			*P. areuginosa*			*P. mirabilis*		
	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R
S. hirsutum	2.5	2.5	1	2.5	2.5	1	2.5	2.5	1	5	5	1

Abbreviations: R–MBC/MIC ratio; «-» not determined; MICs of gentamicin ranged from 0.03–0.12 × 10^{-3} mg/ml and 0.25–1.0 × 10^{-3} mg/ml for Gram-positive and Gram-negative bacterial strains, respectively.

For every extract examined, determined the MBC – minimal bactericidal concentration, which was subsequently compared with the corresponding MIC value. The MBC to MIC ratio indicates bactericidal properties when it ranges from 1 to 4.

This study indicates that the submerged mycelium of most investigated mushroom species possess high radical scavenging potential and might serve as a good source of safe natural antioxidants. Further studies are needed to determine the morphological mechanisms regulating antioxidant accumulation, to isolate active components, and to establish the pharmacological efficacy of promising mushroom extracts.

Conclusion for the majority of selected species of *S. hirsutum, P. ostreatus*, which could be obtained in industrial amounts constituting interesting material for preparation of pharmaceutical and cosmetic products.

References / Список литературы

1. *Asatiani M.D., Elisashvili V.I., Wasser S.P., Reznick A.Z., Nevo E.* Free-radical scavenging activity of submerged mycelium extracts from higher basidiomycetes mushrooms. Bioscience, Biotechnology, and Biochemistry, 2007, Vol. 71, No. 12, pp. 3090–3092.
2. *Lindequist U., Niedermeyer T.H., Jülich W.D.* The pharmacological potential of mushrooms. Evid Based Complement Alternat Med, 2005, Vol 2, No 3, pp. 285–299.

3. *Oyetaya V.O., Ariyo O.O.* Antimicrobial and antioxidant properties of *Pleurotus ostreatus* (Jacq.: Fries) cultivated on different tropical woody substrates. Journal of Waste Conversion, Bioproducts and Biotechnology, 2013, Vol. 1, No 2, pp. 28–32.
4. *Palmieri G., Bianco C., Cennamo G., Giardina P., Marino G., Monti M., Sannia G.* Purification, characterization and functional role of novel extracellular protease from *Pleurotus ostreatus.* Applied and Environmental Microbiology, 2001, Vol. 67, no. 6, pp. 2754–2759.
5. *Gregori A., Svagelj M., Pohleven J.* Cultivation techniques and medicinal properties of *Pleurotus* spp. Food Technology Biotechnology, 2007, Vol. 45, No. 3, pp. 238–249.
6. *Dohmae N., Hayashi K., Miki K., Tsumuraya Y., Hashimoto Y.* Purification and characterization of intracellular proteinases in *Pleurotus ostreatus* fruiting bodies. Bioscience, Biotechnology, and Biochemistry, 1995, Vol. 59, no. 11, pp. 2074–2080.
7. *Deepalakshmi K., Mirunalini S. Pleurotus ostreatus*: an oyster mushroom with nutritional and me-dicinal properties. Journal of Biochemical Technology, 2014, Vol. 5, No.2, pp. 718–726.
8. *Nowacka N., Nowak R., Drozd M., Olech M., Los R., Malm A.* Antibacterial, Antiradical Potential and Phenolic Compounds of Thirty-One Polish Mushrooms. PLoS ONE, 2015, Vol. 10, No. 10, pp. 1–13.
9. *Lee J. [et al.]* The antioxidant properties of solid-culture extracts of basidiomycetous fungi. J. Gen. Appl. Microbiol, 2013, Vol. 59, No. 4. pp. 279–285.
10. *Yun B.S. [et al.]*, Sterins A and B new antioxidative compounds from *Stereum hirsutum*. J. Antibiot, 2002, Vol. 55. pp. 208–210.
11. *Nukamura H., Iitaka, Y., Kurazawa S., Takeuchi T., Umazawa H*. The crystal and molecular structure of a dibromo-derivative of MS-3: a glyoxalase I inhibitor produced by a mushroom, *Stereum hirsutum*. Agricultural and Biological Chemistry, 1976, Vol. 40, pp. 1781–1784.
12. *Kurazaw, S., Naganawa H., Takeuchi T., Umezawa H.* The structure of MS-3: a glyoxalase I inhibitor produced by a mushroom. Agricultural and Biological Chemistry, 1975, Vol. 39, No. 10, pp. 2009–2014.
13. *Kurazawa S., Takeuchi T., Umezawa H.* Studies on glyoxalase inhibitor of a new active agent, MS-3, from a mushroom culture. Agricultural and Biological Chemistry, 1975, Vol. 39, No. 10, pp. 2003–2008.
14. *Bu M., Yang B.B., Hu L.* Natural bioactive sterol 5a, 8a-endoperoxides as drug lead compounds. Med. Chem., 2014, Vol. 4, pp. 709–716.
15. *Poyraz B. [et al.]* Antibacterial and antitumor activity of crude methanolic extracts from various macrofungi species. BİBAD, 2015, Vol. 8, No. 1, pp. 5–10.
16. *Schwartz B., Hadar Y., Sliva D.* The use of edible mushroom water-soluble polysassharides in the treatment and prevention of chronic diseases: a mechanistic approach. Antitumor potential and other emerging medicinal properties of natural compounds, Dordrecht et al.: Springer, 2013, pp. 263–283.
17. *Qin H. [et al.]* Cell factories of higher fungi for useful metabolite production Adv. Biochem. Eng. Biotechnol, 2016, Vol. 155, pp. 199–235.
18. *Kleinwächter P., Dahse H.M., Luhmann U., Schlegel B., Dornberger K.* Epicorazine C, an antimicrobial metabolite from *Stereum hirsutum* HKI 0195. J. Antibiot, Tokyo, 2001, Vol. 54, No. 6, pp. 521–525.
19. *Rai M.* Mycotechnology : present status and future prospects. New Delhi: I.K. International, 2007, 306 pp.
20. *Ma K. L., Bao J., Han T., Jin X., Yang F., Zhao S., Li F., Song M., Liu H.* New benzoate derivatives and hirsutane type sesquiterpenoids with antimicrobial activity and cytotoxicity from the solid-state fermented rice by the medicinal mushroom *Stereum hirsutum*. Food Chemistry, 2014, Vol. 143, pp. 239–245.
21. *Dubin G.-M., Fkyerat A., Tabacchi R.* Acetylenic aromatic compounds from *Stereum hirsutum*. Phytochemistry, 2000, Vol. 53, pp. 571–574.