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PROBLEMS OF SCIENCE

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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.

УДК 616-003.725:582.284.3

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):

% Inhibition =
$$[(AB-AA) \div AB] \times 100$$
 (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								
Species	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 $^{\bullet}$; 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 $^{\bullet}$; for ascorbic acid IC50 = 0.146 mg/mg DPPH $^{\bullet}$.

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)

	Phenolic acids content [μg/g DW]								
Species	Protocate chuc	4-OH- benzoic	Vanillic	Caffeic	Syringic	p- coumaric	Ferulic	Salicylic	Sum
S. hirsutu m	-	0.74 ± 0.02	-	_	-	0.2 ± 0.0	-	0.03 ± 0.0	0.9 7

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
	Gram-negative bacteria											
Species	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 \times 10⁻³ mg/ml and 0.25–1.0 \times 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.

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