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Evaluation of Antioxidant Properties in Extracts of Strains of the Medicinal Fungus Schizophyllum Commune

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Abstract The purpose of this article is to study the antioxidant properties of extracts of medicinal *S.commune* strains (JE4, JE5, JE6) grown in liquid nutrient media. In the study, technologies were used to measure the formation of DPPH radicals in the extracts of strains of the *S.commune* fungus grown in Suslo (brewer's yeast), yeast and potato-glucose liquid nutrient media, antioxidant properties, scavenging activity against free radicals. Among the extracts of S. commune fungus, it was found that the optimal range for the formation of DPPH radicals corresponds to 21 days, and Suslo (brewer's yeast) nutrient medium is the most optimal nutrient. Antioxidant properties of *S.commune* in strain JE6 were recorded as 98.63 ± 3.02 in Suslo (brewer's yeast) nutrient medium. The fact that the formation of DPPH radicals in the extract is recorded in a high range makes it possible to use them in the fields of biotechnology and pharmaceuticals.

Keywords Fungus, Mycelium, Strain, Fruit body, Free radical, Extract, Antioxidant

1. Introduction

Fungi are a major source of non-toxic drug substitutes for the food and pharmaceutical industries. Fungi contain various free radical scavenging molecules, such as polysaccharides, polyphenols, and antioxidants (vitamin C, E, and carotenoids), which are used in the fight against diseases [1]. So far, phenolic compounds are considered one of the most important sources for the inhibition of free radicals in the prevention of vascular diseases, some forms of cancer and oxidative stress [2].

Many physiological processes in fungi additionally generate oxygen-based free radicals and other reactive oxygen species (ROS). The most common types of free radicals are superoxide radicals, peroxide radicals, reactive nitrogen radicals, and nitric oxide [3]. Uncontrolled production of free radicals and ROS can lead to cell death and tissue damage [4]. Oxidative damage caused by free radicals contributes to various pathological conditions such as atherosclerosis, respiratory diseases, neurodegenerative diseases, inflammatory diseases, cancer, hypertension, and aging [5].

Liquid extracts of the fungus are in a concentrated form and have a beneficial effect when used in the pharmaceutical industry [6]. For the first time, selection of nutrient media for growing the medicinal fungus *S. commune* in liquid nutrient media and planting mycelia in liquid nutrient media were carried out by scientists Hu et al. in 2003 and Survase in 2006 [7], [8]. Yifeng Zhang found that the medicinal fungus *S. commune* synthesizes schizophyllan polysaccharide when grown in a liquid nutrient medium [9].

Vitamins B, D, K, A and C were isolated for the first time from S. commune isolates grown in liquid nutrient media (Alam et al., 2007) [10]. Proteins, vitamins, lipids and U P, Mg, K mineral elements in the fungus (Adejoye et al., 2007) [11] were completely separated.

The extract of the fungus *S.commune* has been found to be used as an oxidative stabilizer and scavenger of free radicals to extend the shelf life of lipid foods [12]. Flavonoid, beta-carotene, and lycopene of the antioxidant compounds of the S. commune fungus have been used for a long time in Japan as medicinally active compounds for the treatment of cancer [13].

Reactive oxygen species (ROS) represent the most powerful free radicals, as they can have destructive effects on various cells and cause oxidative stress. An imbalance between the production and elimination of free radicals can lead to many diseases, including neurodegenerative diseases such as Alzheimer's (AD) [14].

Scientists J.P. Sherkulova and E.Y. Eshonkulov [15], [16] have conducted scientific research on molecular analysis of strains of *S.commune* fungus obtained from the territory of Uzbekistan and cultivation in liquid nutrient media.

This article provides information on the methods and results of studying the antioxidant properties of the extracts of medicinal *S.commune* strains (JE4, JE5, JE6) grown in liquid nutrient media.

2. Materials and Methods

Strains JE4, JE5 and JE6 of *S.commune* fungus were

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isolated in the microbiology and virology laboratories of Karshi State University and are currently stored in the collection of the department under oily oil. The strains were submitted to the collection of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan "Collection of microorganisms important for industry" and were listed under the collection number SKB-592 based on the given reference.

A microbiological shaker (muli-functional orbital shaker PSU-20i) was used to obtain mycelial biomass of *S.commune*. In this case, thick mycelial films are formed on the surface and in the depth of the layer of liquid medium. Erlenmeyer flasks with a capacity of 250 ml are used for surface sowing. For this, the mycelium mixture was placed in a liquid nutrient medium and grown in oscillators for 30 days at an average vibration speed of 180 and 250. Our next experiments were conducted in a laboratory fermenter with a capacity of 6 l. The experiments were carried out 3 times in 500 cm3 Erlenmeyer flasks. 200 ml of prepared nutrient media were poured into each flask and sterilized at one atmosphere pressure. For inoculation, seven-day-old mycelia of macromycetes grown in a shaker were planted. To grow *S.commune* fungus in a liquid nutrient medium, the following nutrient mediums were selected:

1) Potatoes - liquid nutrient medium with glucose: (potatoes 400, glucose 100, agar 2 g);
2) Liquid nutrient medium with yeast: (g / l): 7-10 g of dried yeast per 1 liter of distilled water is boiled for 20-30 minutes; It is left in the refrigerator for 12 hours, filtered, filled with one liter of water; It is boiled for 30 minutes and 10% sucrose, 2 g agar and 0.5% NaCl are added to the medium;
3) Wort (beer yeast): water up to 70C according to the Balling hydrometer is added to the beer wort. 2 g of agar is added to the resulting wort and heated.

The antioxidant property of *S.commune* fungus strains was measured by the method of scavenging activity against DPPH radical and free radical [17].

The extract-free mixture was used as a negative standard and butyl hydroxytoluene (BHT) as a positive standard. Making the free radical DPPH is calculated according to the following formula [18]:

$$\text{Cleaning activity: } (\%) = (A_1 - A_2/A_0) \times 100\ (\%) = (A_1 - A_2/A_0) \times 100$$

where A_0 is absorbance of DPPH without sample and A_1 is absorbance of sample with DPPH, A_2 is absorbance of blank only.

The performed experiments were carried out in 3 replicates and statistical analysis Microsoft Excel 2010 was used to calculate the average and standard for all multiple measurements. Antioxidant activity results (EC50) were performed using analysis of variance to test the mean. Analysis of mean comparisons was performed using IBM SPSS Statistics version 22.0 for Windows. $p < 0.05$ was taken as an average result.

3. Results and Discussion

S.commune fungal strains were incubated in an optimal liquid nutrient medium in a thermostat at a temperature of 370C for 27-30 days. The culture fluid of the strains was 5000 rpm for 20 minutes. was centrifuged at high speed. The antioxidant activity of the supernatant and sedimented cells from the centrifuge was determined. Intact sedimented cells were washed twice in isotonic solution and resuspended in 0.2 M (pH=7.4) phosphate buffer, and the cell titer was brought to turbidity of 108 HHB/ml. A 0.05mM ethanolic solution of diphenylpicrylhydrazine (DFPG) was used as a free oxygen radical donor.

The experiment was carried out in triplicate. Vitamin C was taken as a control.

Two common solvents were used for antioxidant, ethanol and aqueous extract as standard.

Table 1. Results of generation of DPPH radicals among the extracts of *S.commune* fungal strains

Liquid nutrient		Suslo (brewer's yeast),		yeast		potato-glucose	
Extracts		PSH	EtOH	PSH	EtOH	PSH	EtOH
strains	days	16.03 ± 4.30	72.65 ± 9.15	6.64 ± 0.66	5.17 ± 0.03	65.11 ± 6.27	42.58 ± 3.47
JE4	7 day						
	14 day	18.92 ± 6.12	377.71 ± 14.85	7.78 ± 0.41	6.01 ± 0.07	20.89 ± 1.67	11.44 ± 0.84
	21 day	71.55 ± 3.43	**96.28 ± 2.52**	3.17 ± 0.44	3.02 ± 0.10	9.55 ± 0.51	5.99 ± 0.20
	28 day	69.13 ± 4.24	74.65 ± 1.74	2.30 ± 0.31	5.43 ± 0.53	5.61 ± 1.83	10.77 ± 1.05
JE5	14 day	15.76 ± 0.63	70.86 ± 2.52	2.60 ± 0.14	3.80 ± 0.08	0.28 ± 0.09	61.81 ± 4.78
	21 day	32.42 ± 3.09	53.61 ± 2.32	7.40 ± 0.19	3.26 ± 0.96	9.69 ± 3.67	89.97 ± 2.81
	28 day	30.18 ± 2.25	55.96 ± 1.31	7.62 ± 0.62	4.65 ± 0.31	57.10 ± 2.35	**91.86 ± 2.8**
	14 day	69.69 ± 3.9	67.31 ± 1.03	3.25 ± 1.98	3.40 ± 0.09	4.36 ± 1.36	80.28 ± 3.21
JE6	14 day	14.89 ± 0.41	12.69 ± 0.93	5.16 ± 1.63	5.29 ± 0.05	4.03 ±0.41	6.39 ± 3.84
	21 day	14.45 ± 6.83	14.34 ± 0.65	1.63 ± 1.71	5.20 ± 0.08	6.49 ± 7.40	7.30 ± 1.98
	28 day	85.39 ± 1.61	**98.63 ± 3.02**	7.49 ± 0.67	5.84 ± 0.06	79.12 ± 10.77	74.22 ± 1.32
	14 day	78.48 ± 0.01	78.39 ± 1.66	55.12 ± 0.39	44.85 ± 0.19	33.67 ± 5.35	32.46 ± 1.27

Among the extracts of *S.commune* strains, DPPH radicals were determined on days 7-14-21-28 and their average results were calculated. The antioxidant activity analyzed among the extracts had variations, but nevertheless, on the 28th day, they proved to have the best properties for free radical neutralization (Table 1).

The average results of the generation of DPPH radicals among the extracts of the S.commune fungus strains were determined by the scientists of the University of Malaysia and the Borneo Marine Research Institute [19] when determining the antioxidant properties and antimicrobial activity of the extracts of *Pleurotus sajor caju* and *Schizophyllum commune* fungi extract was found to range in concentration from 54.11% to 97.19%. According to our results, the highest range was 98.63 ± 3.02, which showed that the antioxidant property of the fungus is very high.

4. Conclusions

Based on the results of the conducted scientific research, formation of DPPH radicals among the extracts of medicinal *S.commune* strains grown in Suslo (brewer's yeast), yeast and potato-glucose liquid nutrient mediums in Suslo (brewer's yeast) nutrient medium strain showed the highest result of 96.28 ± 2.52 in 21 days. The JE5 strain showed a result of 91.86 ± 2.8 in the potato-glucose medium in 21 days, while the JE6 strain showed a result of 98.63 ± 3.02 in the Suslo (brewer's yeast) nutrient medium in 21 days. Among the extracts of *S.commune* fungus, the most optimal range for the formation of DPPH radicals corresponded to 21 days. Suslo (brewer's yeast) nutrient medium can be indicated as the most optimal nutrient. As a result of investigating the antioxidant properties of the medicinal S.commune strains in the extract of fungi, the formation of DPPH radicals in the extract showed a high range, which makes it possible to use them as a promising medicinal species for the fields of biotechnology and pharmaceuticals.

REFERENCES

[1] Dulay R. Antioxidant activity and total phenolic content of Volvariellavolvacea and *Schizophyllum commune* mycelia cultured in indigenous liquid media. *Mycosphere.* 2016; 7: 131–138.

[2] Veurink G., Perry G., Singh S.K. Role of antioxidants and a nutrient rich diet in Alzheimer`s disease: AD and Nutraceuticals. *Open Biol.* 2020; 10. doi: 10.1098/rsob.200084.

[3] Moneim A.E.A. Oxidant/Antioxidant Imbalance and the Risk of Alzheimer`s Disease. *Curr. Alzheimer Res.* 2015; 12: 335–349. doi: 10.2174/1567205012666150325182702.

[4] Nunomura A., Perry G., Aliev G., Hirai K., Takeda A., Balraj E.K., Jones P.K., Ghanbari H., Wataya T., Shimohama S., et al. Oxidative Damage Is the Earliest Event in Alzheimer Disease. *J. Neuropathol. Exp. Neurol.* 2001; 60: 759–767. doi: 10.1093/jnen/60.8.759.

[5] Jomova K., Vondrakova D., Lawson M., Valko M. Metals, oxidative stress and neurodegenerative disorders. *Mol. Cell. Biochem.* 2010; 345: 91–104. doi: 10.1007/s11010-010-0563-x.

[6] Chopra K., Misra S., Kuhad A. Current perspectives on pharmacotherapy of Alzheimer`s disease. *Expert Opin. Pharmacother.* 2011; 12: 335–350. doi: 10.1517/14656566.2011.520702.

[7] Xu, C.P., Kim, S.W., Hwang, H.J., Choi, J.W., Yun, J.W., 2003. Optimization of submerged culture conditions for mycelial growth and exobiopolymer production by *S. commune* C240. Process Biochem. 38, 1025–1030.

[8] Survase, S.A., Saudagar, P.S., Singhal, R.S., 2006. Production of scleroglucan from *S. commune* MTCC 2156. Bioresour. Technol. 97 (8), 989–993.

[9] Yifeng Zhang, Huiling Kong, Yapeng Fang, Katsuyoshi Nishinari, Glyn O. Phillips. "Schizophyllan: A review on its structure, properties, bioactivities and recent developments". Bioactive Carbohydrates and Dietary Fibre. Elsevier, January 2013.

[10] Alam, N., Khan, A., Hossian M.S., Amin, S.M.R., Khan, L.A. 2007 Nutritional analysis of dietary mushroom Pleurotus florida Egger and Schizophyllum commune (Fr) Singer. Bangladesh J Mushroom 1(2): 1-7).

[11] Adejoye, O.D., Adebayo-Tayo, B.C., Ogumjobi, A.A., Afolabi, O.O. 2007. Phytochemical studies on Schizophyllum commune (Fries) a Nigerian fungus. World ApplSci J. 2(1), 73-76.

[12] Pinho B.R., Ferreres F., Valentão P., Andrade P.B. Nature as a source of metabolites with cholinesterase-inhibitory activity: An approach to Alzheimer`s disease treatment. *J. Pharm. Pharmacol.* 2013; 65: 1681–1700. doi: 10.1111/jphp.12081.

[13] Moniruzzaman A., Hossain S., Sarker J., Rahman S.M.A., Rashid M., Rahman M. In vitro antioxidant and cholinesterase inhibitory activities of methanolic fruit extract of *Phyllanthus acidus. BMC Complement. Altern. Med.* 2015; 15: 403. doi: 10.1186/s12906-015-0930-y.

[14] Basso V, Schiavenin C, Mendonça S, Gonçalves de Siqueira F, Salvador M, Camassola M (2020) Chemical features and antioxidant profile by *Schizophyllum commune* produced on different agroindustrial wastes and byproducts of biodiesel production. Food Chem 329: 127089.

[15] Eshonkulov E., Sherkulova J. Molecular analysis of medicinal Schizophyllum commune fungi // Universum: химия и биология: электрон. научн. журн. 2023. 11(113).

[16] Eshonkulov E., Sherkulova J. Technology of deep cultivation of medicinal fungus schizophyllum commune // Universum: химия и биология : электрон. научн. журн. 2023. 12(114).

[17] Moniruzzaman A., Hossain S., Sarker J., Rahman S.M.A., Rashid M., Rahman M. In vitro antioxidant and cholinesterase inhibitory activities of methanolic fruit extract of *Phyllanthus acidus. BMC Complement. Altern. Med.* 2015; 15: 403. doi: 10.1186/s12906-015-0930-y.

[18] Vougiouklaki D., Loka K., Tsakni A., Houhoula D. Characterization of Metabolites Production by Lactobacillus gasseri ATCC 33323 and Antioxidant Activity. *Nutr. Food Sci. Int. J.* -2022. -№11. –P.1–8.

[19] Sujjat Al-Azad, Vivian Chong Ai Ping "Antioxidant Properties and Antimicrobial Activity in the Extracts of Two Edible Mushroom, Pleurotus sajor caju and Schizophyllum commune" published by Advances in Bioscience and Biotechnology, Vol.13 No.9, 2022.