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THE STUDY ON THE ANTIOXIDANT ACTIVITIES OF POLYSACCHARIDES ISOLATED FROM CULTURED MYCELIUM OF THE *CORDYCEPS MILITARIS*

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Keywords: *Cordyceps* fungus, *Cordyceps militaris*, Polysaccharides, Antioxidant activity

Abstract. The article describes the antioxidant activities of polysaccharides isolated from cultivated mycelium of the fungus *Cordyceps militaris*. Polysaccharides of the fungus *Cordyceps militaris* showed high antioxidant activity in vitro and in vivo.

ИССЛЕДОВАНИЕ АНТИОКСИДАНТНЫХ АКТИВНОСТЕЙ ПОЛИСАХАРИДОВ, ВЫДЕЛЕННЫХ ИЗ КУЛЬТИВИРОВАННЫХ МИЦЕЛИЕВ КОРДИЦЕПСА ВОЕННОГО *CORDYCEPS MILITARIS*

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Ключевые слова: кордицепс военный, *Cordyceps militaris*, Полисахариды, антиоксидантная активность.

Аннотация. В статье описаны антиоксидантные активности полисахаридов, выделенных из культивированных мицелиев гриба *Cordyceps militaris*. Полисахариды гриба *Cordyceps militaris* показали высокие антиоксидантные активности in vitro и in vivo.

Introduction

Mushrooms contain many proteins, polysaccharides, amino acids, vitamins and microelements, which are used as nutritious foods and spices to impart taste, as well as for the prevention of various diseases such as cancer and atherosclerosis.

In particular, *Cordyceps* mushrooms, “winter-worm summer-grass”, are used as a valuable medicinal material to increase longevity and physical strength, it attracts attention for the development of anticancer drugs during clinical studies, because it has a high immunostimulatory activity that inhibits the action of cancer cells.

Cordyceps sinensis and *Cordyceps militaris* are most often used as medicines, but they are depleted from natural sources, and artificial cultivation methods are not fully developed [5].

Therefore, studies of the technology of liquid cultivation of fungi, studied recently, are conducted to increase the production of mycelium as a substitute for a fruit body [4].

In particular, polysaccharides of ergot fungi possess high pharmacological activity, such as immunosuppressive and inhibitory effects on tumor cells, and are in demand in the development of new drugs and clinical study [3, 6].

The purpose of the course work is to study the antioxidant activities of polysaccharides from *Cordyceps militaris* of the genus *Cordyceps*.

Materials and Methods

The material of the study was polysaccharides isolated from cultivated mycelium of the *C. militaris* (L) Link strain.

DPPH reduction activity, hydroxyl radical reduction activity, Fe^{3+} inhibition activity, superoxide anion reduction activity (O_2^-), inhibiting activity of lipid peroxidation, effect on content of malonic dialdehyde (MDA) and effect on superoxide dismutase(SOD) measured using methods that described in differences [1,2].

Results and discussion

1) In vitro Antioxidant activity

(1) DPPH reduction activity analysis

This method is one of the most common for measuring in vitro antioxidant activity. As shown in Table 1, the reduce activity of DPPH of polysaccharide increased linearly with increasing concentration of the polysaccharide, and the concentration (IC_{50}) at which the recovery rate was 50% was 190.2 $\mu\text{g/ml}$.

Tab. 1. DPPH reduction activity of the polysaccharide

Concentration of polysaccharide ($\mu\text{g/ml}$)	OD_{517}	Reduce rate (%)
100	0.311 ± 0.013	40.8 ± 2.4
200	0.244 ± 0.012	50.9 ± 2.3
300	0.187 ± 0.016	61.2 ± 3.3
400	0.157 ± 0.007	69.2 ± 1.5
500	0.143 ± 0.009	72.2 ± 1.7

(2) Analysis of hydroxyl radical reduction activity ($\cdot\text{OH}$)

As shown in Table 2, the hydroxyl radical reduction activity ($\cdot\text{OH}$) of the polysaccharide increased linearly with increasing polysaccharide concentration, and the concentration (IC_{50}) at which the reduction rate was 50% was 855.9 $\mu\text{g/ml}$.

Tab. 2. Hydroxyl radical($\cdot\text{OH}$) reduction activity of the polysaccharide

Concentration of polysaccharides ($\mu\text{g/ml}$)	OD_{560}	Recovery rate (%)
200	0.542 ± 0.006	9.8 ± 1.1
400	0.451 ± 0.007	26.9 ± 1.1
600	0.355 ± 0.013	40.6 ± 2.2
800	0.259 ± 0.008	48.9 ± 1.4
1000	0.167 ± 0.005	53.6 ± 0.9

(3) Fe^{3+} inhibition activity of the polysaccharide

As shown in Table 3, Fe^{3+} inhibition activity of the polysaccharide increased linearly with increasing concentration of polysaccharides and the concentration (IC_{50}), at which the inhibition rate was 50%, was 641.7 $\mu\text{g/ml}$.

Tab. 3. Fe^{3+} inhibition activity of the polysaccharide

Concentration of polysaccharides ($\mu\text{g/ml}$)	OD_{510}	Recovery rate (%)
250	0.421 ± 0.008	30.7 ± 1.3
500	0.334 ± 0.007	43.9 ± 1.1
750	0.254 ± 0.008	57.8 ± 1.3
1000	0.194 ± 0.007	67.9 ± 1.2
1250	0.166 ± 0.006	72.8 ± 1.0

(4) Superoxide anion (O_2^-) reduction activity of the polysaccharide

As shown in Table 4, the recovery activity of superoxide anion (O_2^-) polysaccharide increased linearly with increasing concentration of polysaccharides, and the concentration (IC_{50}) at which the recovery rate was 50% was equal to $194.3 \mu\text{g/ml}$.

Tab. 4. Superoxide anion (O_2^-) reduction activity of the polysaccharide

Concentration of polysaccharides ($\mu\text{g/ml}$)	OD_{560}	Reduction rate (%)
50	0.161 ± 0.004	15.6 ± 2.3
100	0.142 ± 0.006	28.2 ± 2.8
150	0.113 ± 0.006	46.1 ± 2.6
200	0.087 ± 0.005	52.6 ± 2.4
250	0.059 ± 0.007	68.6 ± 3.9

(5) Inhibition activity of lipid peroxidation

Based on the data in Table 5, the inhibition activity of lipid peroxidation of the polysaccharide of ergot fungi of the pupa increased linearly with increasing polysaccharide concentration, and the IC_{50} , at which the degree of inhibition was 50%, was equal to $148.7 \mu\text{g/ml}$.

Tab. 5. Inhibiting activity of lipid peroxidation of the polysaccharide

Concentration of polysaccharides ($\mu\text{g/ml}$)	OD_{540}	Reduction rate (%)
50	0.347 ± 0.005	15.5 ± 1.7
100	0.326 ± 0.007	33.6 ± 2.1
150	0.305 ± 0.006	51.7 ± 1.5
200	0.291 ± 0.006	63.8 ± 2.2
250	0.286 ± 0.004	68.1 ± 2.4

2) in vivo antioxidant activity

(1) Effect on malonic dialdehyde (MDA)

As shown in Table 6, the level of MDA in the liver and serum in the experimental group obtained by injection of the polysaccharide under study was significantly lower than in the control group.

Tab. 6. The effect of polysaccharide on the content of MDA ($\bar{X} \pm SE$)

Animal group	Dose (mg/kg)	The content of MDA liver (nmol / g • fabric)	The MDA content of serum (nmol/ml)
Intact	-	143.6±21.2	17.3±1.12
Control	-	456.3±32.4	58.2±4.56
Experiment (Polysaccharide)	40	320.4±30.6*	28.8±3.54*
Experiment (Vitamine C)	30	303.5±28.8*	26.5±2.94*

*; $p < 0.05$ (comparison with intact), separately $n = 5$

(2) Effect on superoxide dismutase (SOD) activity

As shown in Table 7, SOD activity in the spinal fluid, muscle tissue, and red blood cells significantly increased in the experimental group obtained by injecting a polysaccharide under study than in the control group.

Tab. 7. The effect of polysaccharide on SOD activity ($\bar{X} \pm SE$)

Animal group	Dose (mg/kg)	Liver SOD activity (U / mg • protein)	Erythrocyte SOD activity (E / mg • GB)
Intact	-	2.38±0.22	3.45±0.23
Control	-	0.86±0.08	2.07±0.26
Experiment (Polysaccharide)	40	1.64±0.09*	3.26±0.20*
Experiment (Vitamine C)	30	1.68±0.09*	3.32±0.22*

*; $p < 0.05$ (comparison with intact), separately $n = 5$

Conclusion

Polysaccharides of the fungus *Cordyceps militaris* showed high antioxidant activity in vitro and in vivo. In vitro: IC₅₀ of DPPH reduction activity - 190.2 µg/ml, hydroxyl radical ($\bullet$ OH) reduction activity - 855.9 µg/ml, Fe³⁺ inhibition activity - 641.7 µg/ml, superoxide anion (O₂⁻) reduction activity - 194.3 µg/ml, and the degree of inhibition activity of lipid peroxidation is 650.9 µg/ml. In vivo: polysaccharides decreased the activity of lipid peroxidation and increased SOD activity after liver poisoning with CCl₄.

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