Study on Extraction Technology of Antioxidant Components from Lentinus Edodes

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Abstract: Natural antioxidants have broad application prospects in medicine, food and other fields. Lentinus edodes is a well-known edible and medicinal fungus. The research on extracting antioxidants from Lentinus edodes has certain theoretical and practical value. In this paper, the isolation and basic properties of antioxidants from dried Lentinus edodes were studied. A component with high antioxidant activity was obtained by boiling water extraction, ethanol precipitation, Sephadex G-75 molecular sieve chromatography, DEAE-52 ion exchange chromatography and Sephadex G-75 molecular sieve chromatography.

1. Introduction

Excessive free radical production or excessive elimination will cause the accumulation of free radicals in the body. It attacks the macromolecular substances and various organelles, causing various damages at the molecular level, cell level and tissue and organ level [1-3]. The body's aging process and induce various diseases. In addition, oxidative modification of lipids is closely related to free radicals. Because of the potential toxicity and side effects of traditional synthetic antioxidants, the development of natural antioxidants has become one of the hot topics in the field of medical and food science [4-6]. There are many methods to determine and screen antioxidants. DPPH method is widely used for its direct, sensitive, fast and simple advantages. DPPH method can be used to detect the scavenging ability of this stable free radical, and express the activity of scavenging reactive oxygen species (ROS) in vivo. Some foreign scholars define EC50 as the amount needed to remove 50% DPPH, which is used as an index of antioxidant capacity [7-9]. Lentinus edodes not only tastes delicious and nutritious, but also has antimicrobial, anti-aging, anti-cancer, hypoglycemic and lipid-lowering effects. In this paper, the antioxidant components of Lentinus edodes were separated by DPPH method and some common column separation methods in laboratory, so as to further study the components.

2. Antioxidant Activity of Lentinus edodes

Oxygenation is essential for energy production, but most of these physiological processes are accompanied by the production of oxygen free radicals or other reactive oxygen free radicals, which can lead to diseases, including atherosclerosis, diabetes, cancer and cirrhosis of the liver [10]. Although most organisms have their own antioxidant defense systems, the ability of these systems is limited, so it is necessary to study the antioxidant components in natural plants.

The methanol and water extracts of Lentinus edodes were scavenged with beta-carotene and 1,1-diphenyl-2-trinitrophenylhydrazine (DPPH) to determine their antioxidant capacity and inhibition on erythrocyte hemolysis induced by peroxide free radicals in rats. The water extract of Lentinus edodes had the strongest scavenging rate of free radicals [11-12]. The scavenging rate of beta-carotene was 75.9% at 20 mg/ml, and that of DPPH was 55.4% at 6 mg/ml. The inhibition rate

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of erythrocyte hemolysis at 5 mg/ml was 75.9%. The total phenolic acid content in water extract was higher than that in methanol extract. Lentinus edodes is expected to become a natural antioxidant.

Sasidharan et al. showed that the methanol extract of Lentinus edodes (Lentinus edodes Soxhlet extractor plus 95% methanol (w:v=1:5) for 48 h extract) had antioxidant activity, and had a high concentration dependence. The half inhibition concentration was 4.4 mg/ml. Kitzberger et al. compared the DPPH scavenging capacity of extracts assisted by organic reagents such as n-hexane, ethyl acetate, dichloromethane extracts, high pressure carbon dioxide, carbon dioxide and organic reagents. It was concluded that supercritical fluid extraction could improve the antioxidant activity of Lentinus edodes extract. When ethanol was used as the assistant solvent for carbon dioxide extraction, the activity of Lentinus edodes extract was most affected. The optimum concentration was 15%(w/w).

Kang et al. studied the antioxidant activity of ethanol extracts from Lentinus edodes and Coprinus comatus. The reduction ability of ferricyanide, nitrite scavenging ability, DPPH scavenging ability, superoxide scavenging ability, hydroxyl radical scavenging ability, chelating ability with ferrous ions and inhibition of linoleic acid peroxidation were determined. The results showed that they all had good concentration dependence, but the antioxidant activity of Lentinus edodes was better than that of Coprinus comatus.

3. Isolation of Antioxidant Components from Lentinus edodes

3.1 A scheme for separating antioxidant components

(1) Hot water extraction

Weighing 100 g dried mushroom powder, Soxhlet extraction and degreasing, dissolving in 500 mL pure water, boiling water bath extraction for 4 hours, centrifugation extract supernatant, obtain crude extract.

(2) Ethanol precipitation treatment of crude extract

Absolute ethanol was gradually added to the supernatant, and the alcohol concentration was from 0 to 80% (V/V) at intervals of 10%. The alcohol precipitation sections were collected and resolved with Tris-HCl buffer of 0.05 mol/L and pH 8.0 and dialyzed. The scavenging rate of DPPH by alcohol precipitation concentration was determined, and the components with high scavenging activity were selected to continue to be used in molecular sieve chromatography.

(3) Sephadex G-75 zeolite chromatography

Samples separated from the previous step were further applied to Sephadex G-75 zeolite chromatography. The self-made Sephadex G-75 column was 1.1 x 60 cm, the injection volume was 1 mL, and the flow rate was 0.3 mL/min. The removal rate of DPPH by the peaks was determined, and the high removal activity was retained. It was continued to be applied in ion exchange chromatography.

(4) Separation of DEAE-52 ion exchange chromatography

Samples were dissolved in Tris-HCl buffer of 0.05 mol/L and pH 8.0, and gradient elution was carried out. The eluent was sodium phosphate buffer with pH 6.0 and 0.02 mol/L. The NaCl gradient was 0.1 mol/L and the concentration ranged from 0 to 0.9 mol/L. The peaks with high clearance were collected and enriched, and the dialysis freeze-drying was continued to be applied to molecular sieve chromatography.

(5) Sephadex G-75 zeolite chromatographic separation

The components with high clearance obtained from ion exchange chromatography were applied to Sephadex G-75 molecular sieve chromatography again. The samples were dissolved in Tris-HCl buffer with 0.05 mol/L and pH 8.0, and eluted with the same buffer. The elution conditions are the same as above.

3.2 Screening and determination of antioxidant components activity

DPPH method was used. DPPH is a relatively stable free radical synthesized artificially. Its

ethanol solution is purple and its maximum absorption peak is 517 nm in the visible region. When antioxidants were added to DPPH solution, the color of DPPH solution became lighter and the absorbance at 517 nm decreased, and the degree of absorbance decreased linearly with the degree of scavenging. Therefore, the antioxidant capacity of a substance can be evaluated by its scavenging effect. The higher the scavenging rate is, the stronger the antioxidant capacity of the substance is. The clearance of each component was determined by the same mass volume concentration (W/V). METHODS: 0.01% PPH ethanol solution 2 mL and 2 mL sample solution were added into the test tube, shake well and keep the temperature at 37 C for 30 min. The control was treated in the same way, and then the absorbance of the solution was determined at 517 nm. The clearance rates of substances were calculated according to the following formulas:

$$\text{Clearance rate}=\left[\left(A_0 - A_s\right)/A_0\right]\times 100\% \quad (1)$$

In formula A_0 is blank absorbance, A_s is sample absorbance.

The determination of polysaccharide content in samples by phenol-sulfuric acid method is shown in Figure 1.

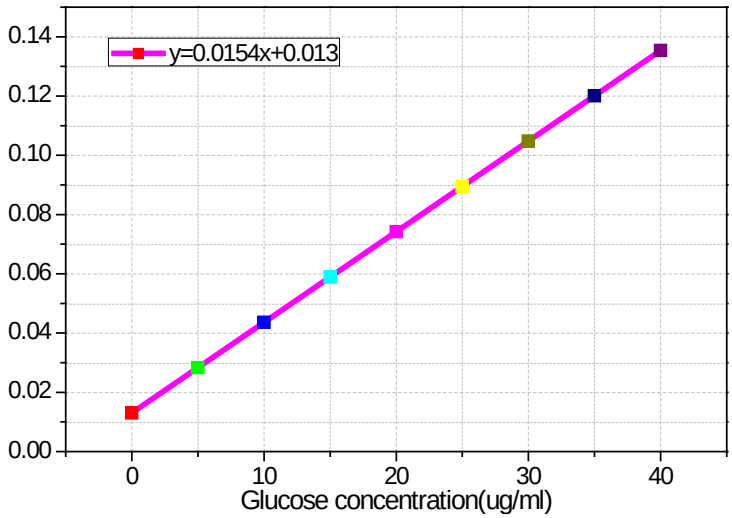

Figure 1. Standard curve of glucose concentration

The determination of protein content in samples by LOWRY method is shown in Figure 2.

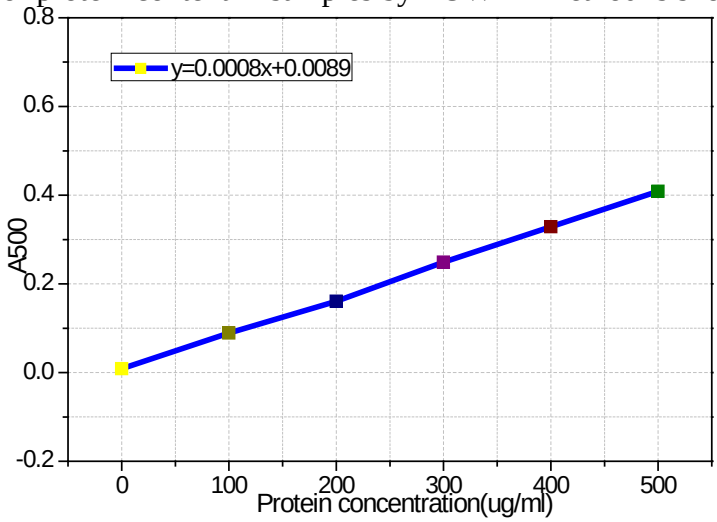

Figure 2. Standard curve of protein concentration

4. Results and discussion

The dry powder of the mushroom was Soxhlet extracted and degreased, and the hot water was extracted for 4 h. The supernatant was centrifuged, and absolute ethanol was gradually added to the

supernatant to carry out segmental alcohol precipitation, and the respective alcohol precipitation sections were separately reconstituted with pure water. If there is a poorly soluble insoluble matter, it is removed by centrifugation. The DPPH clearance rate of each part is as follows [no precipitation when the ethanol concentration is 0-20%, and the same mass (each is wet weight) volume concentration of each alcohol precipitation component]:

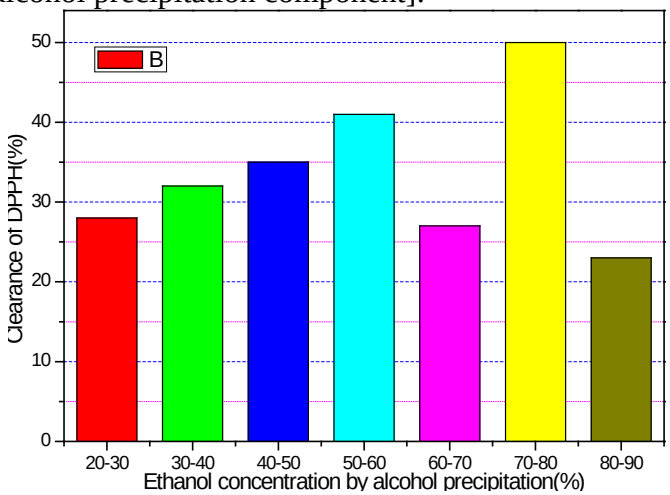

Figure 3. Removal of DPPH by alcoholic precipitation components

As shown in Figure 3 above, the DPPH clearance rate of the alcohol precipitation saturation 70-80% is obviously higher. Meanwhile, the polysaccharides of the above components are measured. The relative protein content is as follows Table 1.

Table 1. Contents of polysaccharides and proteins in alcohol precipitation components

Alcoholic precipitation (%)	Polysaccharide content (mg/mL)	Protein content (mg/mL)
20-30	**0.043**	**0.101**
30-40	**0.163**	**0.044**
40-50	**0.151**	**0.168**
50-60	**0.123**	**2.162**
60-70	**0.067**	**1.115**
70-80	**1.379**	**2.663**
80-90	**0.164**	**0.239**

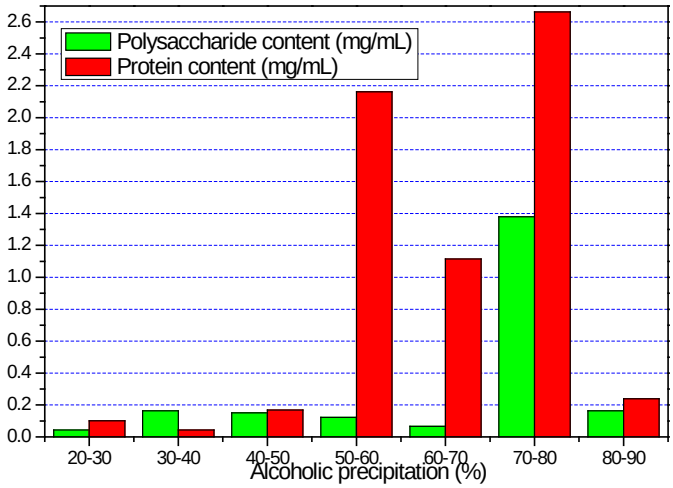

Figure 4. Comparison of polysaccharide and protein contents

From Figure 4, we can see that the relative contents of polysaccharides and proteins in the fractions with higher scavenging rates are higher. From the analysis of the relative contents of polysaccharides and proteins in each fraction, the relative contents of polysaccharides or proteins are related to the free radical scavenging rates of the corresponding fractions.

5. Conclusion

In this paper, the isolation and basic properties of antioxidants from dried Lentinus edodes were studied. The scavenging rates of DPPH were measured at different concentrations, and it was found that the antioxidant activity was related to the polysaccharides and proteins in the components. The protein composition of Le-II is much higher than that of polysaccharide in Le-2. It can be inferred that both proteins and polysaccharides play a certain role in the antioxidant activity of Lentinus edodes, but they are not simply superimposed, which may be related to polysaccharides, protein content, types and their synergies.

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