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Research Article

Antioxidant activity study of pleurotus eryngii polysaccharide

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Abstract: The antioxidant ability of pleurotus eryngii polysaccharide was investigated in the experiment and was compared with vitamin C. The results showed as followed. In terms of reducing power and hydroxyl radical scavenging ability, the antioxidant activity of pleurotus eryngii polysaccharide from 0.5 to 8 μg/mL was higher than that of vitamin C. Compared from superoxide anion and nitroso scavenging ability, the antioxidant activity of pleurotus eryngii polysaccharide between 0.5 and 8 μg/mL was lower than that of vitamin C. As for DPPH scavenging ability, in low concentrations (about below 3μg/mL), the antioxidant activity of pleurotus eryngii polysaccharide was higher than that of vitamin C; at high concentrations, it was lower than that of vitamin C.
Keywords: pleurotus eryngii, polysaccharide, antioxidant activity.

INTRODUCTION

Pleurotus eryngii is a new rare edible fungus variety, successfully developed in recent years. It might be used as food, medicant or food therapy. Pleurotus eryngii possesses snow-white and fleshy sporophore, crisp and tender texture, and abundant nutrition [1,2]. Pleurotus eryngii contains rich polysaccharide that is benefit of the growth and reproduction of probiotics such as Bacillus bifidus. Probiotics might improve gastrointestinal function. Meanwhile, pleurotus eryngii polysaccharide might lower blood sugar, fat and cholesterin, prevent oxidation and aging, and enhance the immunity of human body [3-5].

In this experiment, the antioxidant activity of pleurotus eryngii polysaccharide was investigated. The methods including reducing power and the radical scavenging ability of DPPH (diphenyl-picryl hydrazide) radical, superoxide anion, hydroxyl radical and nitroso radical, were used to evaluate antioxidant activities of pleurotus eryngii polysaccharide. In addition, the antioxidant activities of pleurotus eryngii polysaccharide were compared with vitamin C. This experiment might provide certain reference for the deep exploitation and utilization of pleurotus eryngii.

MATERIALS AND METHODS

Materials and Reagents

Pleurotus eryngii was originated of Linfen, China. Methanol, vitamin C, diphenyl-picryl hydrazyl, ferric chloride and salicylic acid (analytical grade) were purchased from Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Anthrone, thiourea, glucose, anilinparasulfonic acid and α-naphthylamine (analytical grade) were purchased from Xiya Reagent Co., Ltd. (Chengdu, China). Alfa Aesar Company (Tianjin, China) supplied other reagents.

Equipments and instruments

GZX-9246 MBE Digital blast drying box, Shanghai Boxun Industrial Co., Ltd. medical equipment factory, Shanghai, China; RJ-TDL-40C Centrifuge, Ruijiang Analysis Instrument Co., Ltd., Wuxi, China; UV-1100 spectrophotometer, Shanghai Meipuda Instrument Co., Ltd., Shanghai, China; MJ-25BM04B Mill, Guangdong Midea premium appliances manufacturing Co., Ltd., Guangzhou, China; DZF-6020 Vacuum drying oven, Shanghai Suoyu Experiment Equipment Co., Ltd., Shanghai, China.

Preparation of pleurotus eryngii polysaccharide

Fresh pleurotus eryngii was cleaned and torn into strips; dried to constant weight at 55 ℃ for 10 h. afterward, dried fresh pleurotus eryngii was smashed and sieved with 40 mesh. 5g of pleurotus eryngii powder was added into a 250-mL Erlenmeyer flask, and then 100 ml of 0.03% cellulase solution was added into the flask. The pH of suspension was adjusted to 4.5. Subsequently, the suspension was shaken to extract polysaccharide at 55℃ for 40 min. Finally, the suspension was centrifuged at 3000 r/min and the supernatant was concentrated by rotary evaporator. The concentrated liquid was dried at 40℃ under vacuum. The polysaccharide content was determined using phenol sulphate colorimetry [6].

Determination of antioxidant activity

Determination of DPPH free radical scavenging activity

DPPH radical scavenging capacity was assayed as followed [7]. Briefly, 2 mL of pleurotus eryngii polysaccharide or vitamin C with different concentration was respectively added to 10 mL of DPPH (65 μmol·L^{-1}) in methanol. After the reaction mixtures were incubated for 30 minutes under ambient temperature in the dark, its absorbance at 517 nm was determined. The scavenging activity of DPPH radicals was calculated as followed. Scavenging activity (%) = (A_c - A_s) / A_c × 100, where A_c is the absorbance of the control solution, and A_s is the absorbance of the pleurotus eryngii polysaccharide or vitamin C in DPPH solution.

Determination of reducing power

The reducing power of pleurotus eryngii polysaccharide was determined as followed [8]. 1 mL aliquot of pleurotus eryngii polysaccharide or vitamin C with different concentration was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide in 10 mL test tubes. The mixtures were incubated for 20 min at 50 °C. After incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixtures, followed by centrifugation at 3000 *g* for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL of distilled water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm. The increase in absorbance of the reaction mixture indicated the reducing power of the samples.

Determination of scavenging activities of superoxide anion and hydroxyl radical

For superoxide anion radical assay, the superoxide anion radicals were generated by a pyrogallol autoxidation system [9]. 4.5 mL of Tris-HCl buffer solution (50 mmol·L^{-1}, pH 8.2) and 0.5 mL of deionized water were added into a test tube, and the test tube was incubated in a water bath at 25℃for 20 min. A volume of 0.4 mL of pyrogallol solution (25 mmol·L^{-1} of pyrogallol in 10 mmol·L^{-1} of HCl), which was also pre-incubated at 25℃, was injected to the above test tube with a microliter syringe and mixed. The mixture was incubated at 25 °C for 3 min and then 1 mL of 10 mol·L^{-1} HCl was dripped into the mixture promptly to terminate the reaction. The absorbance at 420 nm marked as A_0 was measured 5 min later, and this A_0 denotes the speed of pyrogallol autoxidation. The A_1 autoxidation speed was obtained applying the above method and with the addition 1 ml of pleurotus eryngii polysaccharide or vitamin C solution substituting for deionized water into the Tris-HCl buffer solution. Simultaneously, a blank control of pleurotus eryngii polysaccharide or vitamin C with different concentration was obtained as A_2. The scavenging percentage was calculated according to the following formula: Superoxide radical scavenging activity (%) = [A_0– (A_1– A_2)] /A_0 × 100.

Determination of superoxide anion scavenging activity

The hydroxyl radicals were generated in an H_2O_2–$FeSO_4$ system by oxidation of $FeSO_4$ and were assayed by the color change of salicylic acid [10]. The hydroxyl radicals were generated in 7.0 mL of reactive solution containing 2.0 mL of 6mmol·L^{-1} $FeSO_4$, 2.0mL of 6mmol·L^{-1} salicylic acid, 2.0mL of 6mmol·L^{-1} H_2O_2 and 1.0 ml of pleurotus eryngii polysaccharide or vitamin C with different concentration. The mixture was incubated at 37℃ for 1 h. The change in absorbance caused by the color change of salicylic acid was measured at 510 nm. The scavenging activity of hydroxyl radical was calculated as follows: Scavenging activity (%) = [1-(A_1-A_2)/A_0] × 100, where A_0 was the absorbance of control sample, A_1 was the absorbance of mixture containing pleurotus eryngii polysaccharide or vitamin C and A_2 was the solution absorbance of pleurotus eryngii polysaccharide or vitamin C.

Determination of nitroso scavenging activity

5 mL of sodium citrate-muriatic acid buffer solution (0.5mol·L^{-1}, pH 3) was added into a 25mL colorimetric cylinder containing 1 mL of 0.01% sodium nitrite [11]. 10 ml of pleurotus eryngii polysaccharide or vitamin C with different concentration was added the mixture. The mixture was diluted to 25 mL and incubated in a water bath at 37℃for 1 h. Afterward, 1 mL of mixture was added into 50 mL-volumetric flask, and 2 mL of 0.4% anilinparasulfonic acid and 1 mL of 0.2% α-naphthylamine hydrochloride were added into the volumetric flask. After 15 minutes, the absorbance was determined at 540 nm and nitroso scavenging activity was calculated as followed.Nitroso scavenging activity (%) = (A_0-A_x) / A_0 × 100,where A_0 was the absorbance of control sample and A_x was the absorbance of mixture containing pleurotus eryngii polysaccharide or vitamin C with different concentration.

RESULTS AND ANALYSIS

DPPH free radical scavenging activity

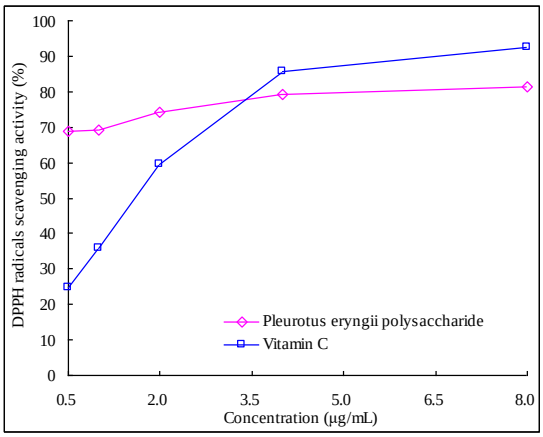

Fig-1: The DPPH radical scavenging activity of pleurotus eryngii polysaccharide

DPPH radical scavenging activity indicates the ability of providing hydrogen atom [12]. As shown in Figure 1, the DPPH radical scavenging activity of pleurotus eryngii polysaccharide had little variation with concentration change, and vitamin C showed an increasing trend to scavenge DPPH radical with concentration enhancement. In low concentrations (about below 3μg/g), the antioxidant activity of pleurotus eryngii polysaccharide was higher than that of vitamin C, but at high concentrations, it was lower than that of vitamin C.

Reducing power

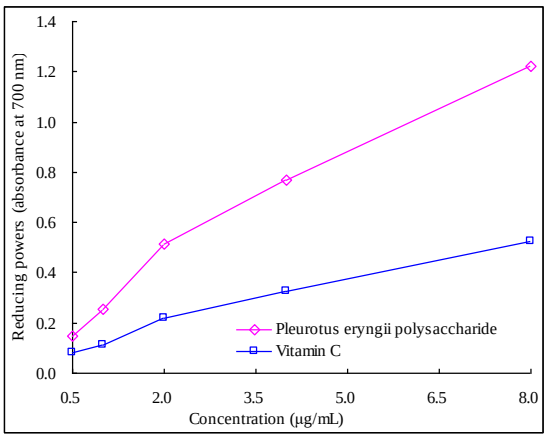

Fig-2: The reducing power of pleurotus eryngii polysaccharide

Reducing power characterizes the ability of providing electrons, usually closely associated with the antioxidant activity of substance [13]. As described in Figure 2, with the increase of pleurotus eryngii polysaccharide concentration, reducing power gradually strengthened. Moreover, the reducing power of pleurotus eryngii polysaccharide is higher than that of vitamin C. At 8 μg/mL, it was as about 3 times as vitamin C.

Superoxide anion scavenging activity

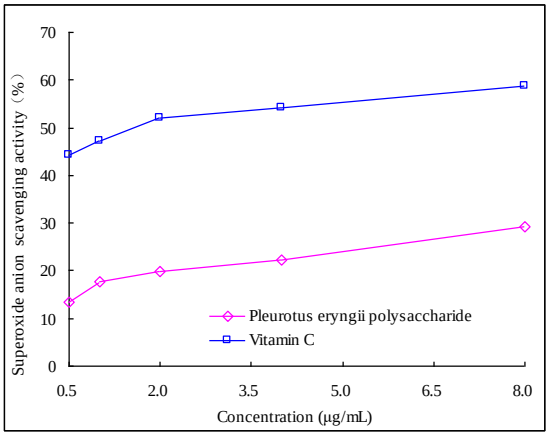

Fig-3: The superoxide anion scavenging activity of pleurotus eryngii polysaccharide

As shown in Figure 3, the ability to scavenge superoxide anion of pleurotus eryngii polysaccharide was weaker, mainly concentrating in between 10% ~ 30%. Moreover, there was little change with polysaccharide concentration variation. The superoxide anion scavenging activity of vitamin C was obviously higher than that of pleurotus eryngii polysaccharide. And at 8 μg/mL, the superoxide anion scavenging activity of pleurotus eryngii polysaccharide was 29.1%, which was only about one half of vitamin C.

Hydroxyl radical scavenging activity

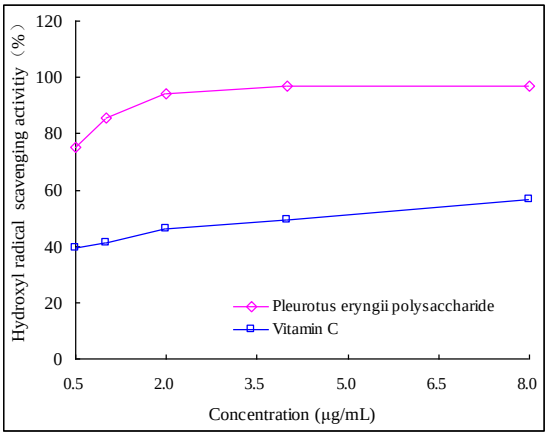

Fig-4: The hydroxyl radical scavenging activity of pleurotus eryngii polysaccharide

As described in Figure 4, from 0.5 to 2 μg/mL, the hydroxyl radical scavenging activity gradually increased with the concentration enhancement of pleurotus eryngii polysaccharide, but there was little change between 2 and 8 μg/mL. On the whole, the hydroxyl radical scavenging ability of pleurotus eryngii polysaccharide was higher than that of vitamin C. At 8μg/mL, the hydroxyl radical scavenging ability of pleurotus eryngii polysaccharide was 97.1%, which was about as two times as vitamin C.

Nitroso scavenging activity

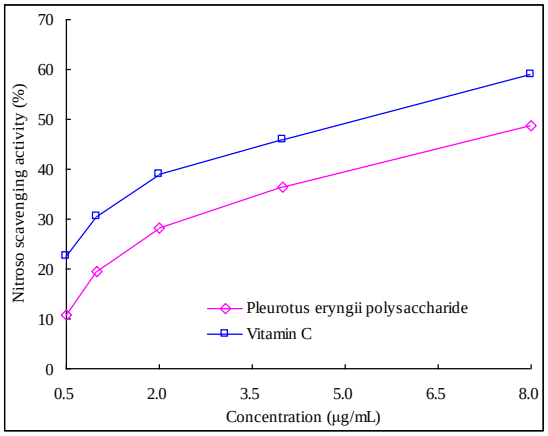

Fig-5: The nitroso scavenging activity of pleurotus eryngii polysaccharide

The nitroso scavenging activity increased with the concentration enhancement of pleurotus eryngii polysaccharides (Figure 5). At 8 μg/mL, it reached to 48.8%, suggesting that pleurotus eryngii polysaccharide had certain ability to scavenge nitroso. However, at the same concentration, the nitroso scavenging activity of vitamin C was higher than that of pleurotus eryngii polysaccharide.

CONCLUSION

In terms of reducing power and hydroxyl radical scavenging ability, the antioxidant activity of pleurotus eryngii polysaccharide from 0.5 to 8 μg/mL was higher than that of vitamin C. Compared from superoxide anion and nitroso scavenging ability, the antioxidant activity of pleurotus eryngii polysaccharide between 0.5 and 8μg/mL was lower than that of vitamin C. As for DPPH scavenging ability, in low concentrations (about below 3 μg/mL), the antioxidant activity of pleurotus eryngii polysaccharide was higher than that of vitamin C; at high concentrations, it was lower than that of vitamin C.

Acknowledgments

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