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几种银耳属真菌提取物的抗氧化活性研究

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摘　要:以四种银耳属真菌为研究对象,采用水提法和酸提法,从中获得多个粗提取物,并对其进行抗氧化活性研究。经过超氧自由基实验、羟自由基实验和 DPPH 实验后,发现来自血耳的两种提取物 TSPA 和 TSPW 展现出较高的羟自由基和超氧自由基清除活性,其活性甚至大于 Vc,TSPA 也表现出 DPPH 清除能力,可以作为潜在的抗氧化剂开发。

关键词:银耳属;提取;抗氧化活性

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Study on Antioxidant Activities of Several Extracts from *Tremella*

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Abstract: Several products were extracted from four fungi of *Tremella* by boiling-water extraction and acid isolation. On the basis of superoxide radical assay, hydroxyl radical assay and DPPH assay, their antioxidant activity were investigated. Two extracts TSPA and TSPW from *Tremella samguinea Peng* exhibit the higher scavenging activity of hydroxyl radical and superoxide radical than Vc, TSPA has a scavenging activity of DPPH, and they can be explored as potential antioxidants.

Key words: *Tremella*; isolation; antioxidant activities

Introduction

Many synthetic antioxidants are used as medical materials at the present time. However, the most commonly have been suspected of being responsible for liver damage and carcinogenesis [1]. Thus, it is essential to develop and utilize effective and natural antioxidants [2]. Published data indicates that fungi polysaccharides in general have strong antioxidant activities and can be explored as novel potential antioxidants [3,4]. The fungi of *Tremella* are well known as traditional edible fungi in oriental countries. It has been widely used as Chinese herbal medicine and health food for hundreds of years. In this study, we reported on the extraction of eight extracts from *Tremella*. In addition, antioxidant activities of the extracts were also identified.

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Experiments

Materials and Chemicals

Four dried samples (*Tremella fuciformis Berk.*, *Tremella aurantialba Bandoni et Zang*, *Tremella samguinea Peng and Tremella encephala Pers.*) were purchased from local stores. Nitro Blue Tetrazolium (NBT), Phenazine Methosulfate (PMS) and Nicotinamide Adenine Dinucleotide Hydrogen (NADH) were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other reagents used were of analytical grade.

Isolation of crude extracts

Isolation of crude extracts by boiling water

The sample (200 g) was extracted with 8000 mL of distilled water at 95 ℃ for 2.5 h. After each extraction, the soluble polymers were separated from residues by filtration, and extracts were combined and concentrated. The above extract was submitted to graded precipitation with four volumes of ethanol and the mixture was kept overnight at 4 ℃ to precipitate the polysaccharides. The precipitate was collected by centrifugation,

washed successively with ethanol and aether, and dried at reduced pressure, giving crude extracts (TFBW, TABW, TSPW and TEPW).

Isolation of crude extracts by acid

The sample (100 g) was mixed with 4000 mL of distilled water, adjusted pH = 2 by 1 mol/L HCl and extracted at 95 ℃ for 2.5 h. After each extraction, the soluble polymers were separated from residues by filtration or centrifugation, and extracts were concentrated. The above extract was submitted to graded precipitation with four volumes of ethanol and the mixture was kept overnight at 4 ℃ to precipitate the polysaccharides. The precipitate was collected by centrifugation, washed successively with ethanol and aether, and dried at reduced pressure, giving crude extracts (TFBA, TABA, TSPA and TEPA).

Antioxidant activities assay

Superoxide radical assay

The superoxide radical assay was measured by the method of Robak and Gryglewski with a minor modification [5]. Samples were dissolved in distilled water at 0(control), 0.5, 1, 5, 10, 20, 50, 60 or 80 mg/mL. A 0.1 mL aliquot of each sample solution was mixed with 1 mL of 16 mM Tris - HCl (pH 8.0) containing 557 um NADH, 1 mL of 16mM Tris - HCl (pH 8.0) containing 45 um PMS, and 1 mL of 16 mM Tris - HCl (pH 8.0) containing 108 um NBT. After 5 min of incubation at 25 ℃, the absorbance was measured at 560 nm. The superoxide radical effect was calculated as scavenging activity (%) = (1-absorbance of sample/absorbance of control) × 100%.

Hydroxyl radical assay

The hydroxyl radical assay was measured by the method of Ghiselli with a minor modification [6]. Samples were dissolved in distilled water at 0(control), 1, 5, 10, 20, 40 or 60 mg/mL. The sample solution (0.1 mL) was mixed with 0.6 mL of reaction buffer [0.2 M phosphate buffer (pH 7.4), 2.67 mM deoxyribose, and 0.13 mM EDTA], and then 0.2 mL of 0.4 mM ferrous ammonium sulfate, 0.05 mL of 2.0 mM ascorbic acid and 0.05 mL of 20 mM H_2O_2 were added to the reaction solution. The reaction solution was incubated for 15 min at 37 ℃, and 1 mL of 1% thiobarbituric acid and 1 mL of 2.0% trichloroacetic acid were added to the mixture. The mixture was boiled for 15 min and cooled on ice. The absorbance of the mixture was measured at 532 nm. Percent inhibition of hydroxyl radical was calculated as (1 - absorbance of sample/absorbance of control) × 100%.

DPPH assay

The scavenging abilities for DPPH of extracts were investigated. Briefly, samples were dissolved in distilled water at 0, 40, 20, 10, 5, 2.5 and 0.5 mg/mL. The sample solution (0.1 mL) or distilled water (0.1 mL, control) was mixed with 2 mL of 0.3 mM DPPH, was incubated for 20 min at 25 ℃. The absorbance of the mixture was measured at 517 nm [7]. The scavenging ability for DPPH of sample was calculated using the equation (1 - absorbance of sample/absorbance of control) × 100%.

Results and Discussion

Scavenging activity of superoxide radical

Superoxide radicals were generated in a PMS/NADH system for being assayed in the reduction of NBT. Fig. 2 shows that the inhibitory effect of several extracts from *Tremella* indicated a concentration-dependent, radical-scavenging activity at all tested concentrations.

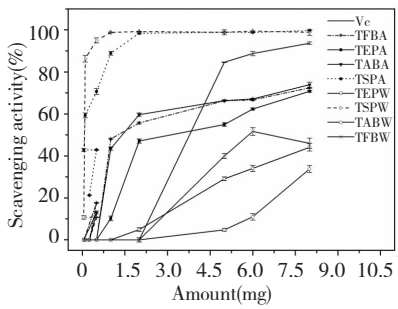

Fig. 1 Scavenging effects of extracts on superoxide radicals

TSPW and TSPA have higher scavenging effects than Vc (Fig. 1). The scavenging effects increased with increasing concentration. Scavenging effect of TSPW was 10.71% to 95.07% at amount of 0.05 to 0.5 mg, that of TSPA was 42.86% to 88.87% at amount of 0.05 to 1 mg, and that of Vitamin C was about 0 at amount of 0.05 to 1 mg. The results prove that crude extracts from *Tremella samguinea Peng* have significant effect

on scavenging superoxide radical, and that is more pronounced than that Vitamin C. In addition, extracts TFBA, TEPA and TABA from acid isolation have higher scavenging activities than the extracts from water isolation.

Scavenging activity of hydroxyl radical

Hydroxyl radicals, generated by reaction of iron – EDTA complex with H_2O_2 in the presence of ascorbic acid, attack deoxyribose to form products that, upon heating with 2-thiobarbituric acid under acid conditions, yield a pink tint. Added hydroxyl radical scavengers compete with deoxyribose for the resulted hydroxyl radicals and diminish tint formation [8]. The above mentioned model was used to measure inhibitory activities of all fractions on hydroxyl radicals.

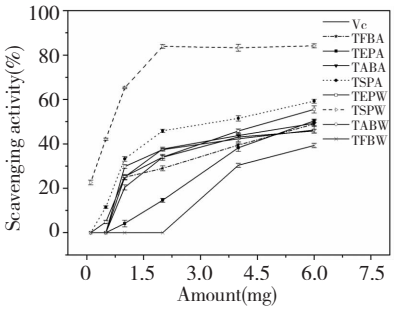

Fig. 2 Scavenging effects of extracts on hydroxyl radical

As shown in Fig. 2, several extracts were all found to have the abilities to scavenge hydroxyl radical. TSPW has the highest activity, at the amount of between 0.1 mg and 6 mg, the effect on scavenging hydroxyl radical of TSPW was 22.59% to 84.2%, which is higher than Vc. TSPA has also the higher activity than Vc, the scavenging activity of TSPA was 0 to 59.23% at the amount of between 0.1 mg and 6 mg, and Vitamin C for hydroxyl radical was 0 to 45.8%. Compared to the above result, TSPW and TSPA have stronger scavenging activities for superoxide radical than Vitamin C. Our data on the activities of scavenging superoxide suggests that *Tremella samguinea Peng* is likely to be explored as novel potential antioxidants.

Scavenging activity of DPPH

Fig. 3 depicted the scavenging power of DPPH of Vc, and Fig. 4 depicted the scavenging power of DPPH of TSPW. These results indicate that TSPA has scavenging power for DPPH, another extracts haven't scavenging power, and TSPA can be explored as novel potential antioxidants.

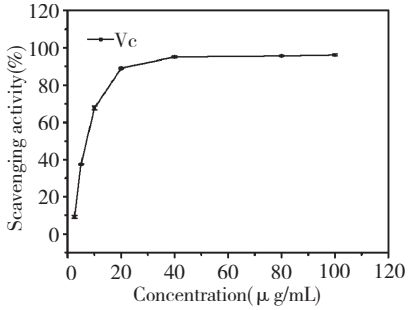

Fig. 3 Scavenging effects of Vc on DPPH

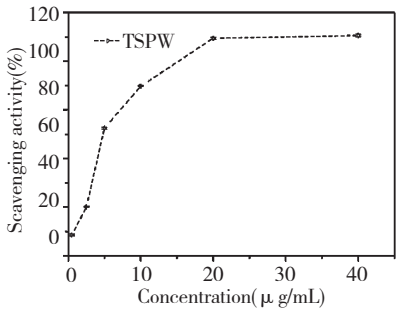

Fig. 4 Scavenging effects of TSPW on DPPH

Conclusion

According to the results above, it is concluded that the crude extracts were obtained from *Tremella* and their antioxidant activities were studied. Both TSPA and TSPW from *Tremella samguinea Peng* have higher scavenging activities of hydroxyl radical and superoxide radicals than Vc, and TSPA has also a scavenging activity for DPPH.

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