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Antioxidant Activity *in Vitro* of Polysaccharide Extracted by Ultrasound with Different Powers from *Ophiopogon japonicus*

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Abstract

The polysaccharides were extracted by different power ultrasound from *Ophiopogon japonicus*. The extraction yield and *in vitro* antioxidant activities including scavenging effect on hydroxyl radical, superoxide anion free radicals and DPPH were investigated. The results showed that with the increase of ultrasonic power, the yield of the polysaccharides decreased first and then increased. The antioxidant activity of polysaccharides increased first and then decreased with the increase of ultrasonic power. When the power was 400 W, the scavenging effect on superoxide anion and DPPH of the polysaccharides was the strongest, and the scavenging ability of hydroxyl radical was the strongest when the power was 560 W. Therefore, different ultrasonic power could affect the extraction yield and antioxidant activity of *Ophiopogon japonicus* polysaccharide. It will provide theoretical basis and experimental support for the application of ultrasonic extraction on polysaccharides from *Ophiopogon japonicus*.

Keywords

Ultrasound, *Ophiopogon japonicus*, Antioxidant Activity

1. Introduction

With the development of research on polysaccharides, there comes forth a great deal of extraction methods at present, which depend on different extraction techniques such as hot water, enzyme, microwave, ultrasonic and so on [1] [2]. Ultrasonic extraction was a timesaving and highly active extraction method depending on its unique physical and chemical effects, which was researched in the

academe extensively [3] [4], but it was very rare to study the effect of ultrasound with different power on the yield and activity. So, researches on the effect of ultrasound with different parameters on polysaccharide will give a better insight into the influence of ultrasonic extraction on polysaccharides.

As a traditional Chinese medicine, the plant *Ophiopogon japonicus* was used nourishing the heart and fornourishing Yin, the treatment of dryness, constipation, dry cough, insomnia, etc. [5] [6]. The main chemical constituents were isoflavones, volatile oils, saponins, inorganic elements, alcohols and polysaccharides [7] [8]. The polysaccharide was one of the main active components in *Ophiopogon japonicus*, which has the functions of anti-fatigue, hypoglycemic activity, anti-radiation, auxiliary inhibition of tumor and so on [9] [10] [11]. In this research, ultrasound with different power was employed to extract polysaccharides from *Ophiopogon japonicus*. The yield and *in vitro* antioxidant activities of the polysaccharides extracted by ultrasound were studied comparatively. The results can provide experimental foundation for further studying the effect of ultrasound on the structure and activity of polysaccharides.

2. Materials and Methods

2.1. Plant Material, Chemicals and Reagents

Roots of *Ophiopogon japonicus* were collected in Zhejiang province, China. All other chemicals used were of analytical grade.

2.2. Extraction and Isolation of Polysaccharides

The roots of *Ophiopogon japonicus* were dried at 60˚C, crushed, soaked with 95% ethanol to remove pigments and small lipophilic molecules, and then dried again at 60˚C. The residue was extracted twice by ultrasound assisted extraction apparatus (JY92-II, Ningbo Xinzhi Biological Scientific Technology Co., China). The ultrasonic conditions were as follows: the ultrasonic treatment lasted for 10 s, intermittent time was 15 s; repeated 90 times, ultrasonic power was 80 W, 240 W, 400 W, 560 W and 720 W, respectively [12]. The filtrate was concentrated and then 3 volumes of 95% ethanol were added to precipitate polysaccharides. The precipitate was acquired by centrifugation and dissolved by distilled water, then dialyzed (MWCO 3500, Sigma Corp.). Finally the solution was concentrated and lyophilized to obtain polysaccharides. No absorption was observed at 280 nm and 260 nm in the UV absorption spectra of these polysaccharides extracted by the five powers ultrasound, which demonstrated the absence of protein and nucleic acid in the polysaccharides.

2.3. Scavenging Effect on Hydroxyl Radical of the Polysaccharides

The ability of the polysaccharides to scavenge hydroxyl radical was determined by the method of Smirnoff and Cumbes with some modification [13]. 1 mL of polysaccharide samples (at different concentrations) were dissolved in 2 mL 50 mM sodium phosphate buffer (pH 7.4), mixed with 7.5 mM $FeSO_4$, 5 mM phenanth-

roline and 0.1% H_2O_2. The solutions were incubated at 37˚C for 1 h and then the absorbance was detected at 510 nm. The scavenging effect of the hydroxyl radical was calculated as follows: scavenging effect (%) = [1 − (A_{sample} − $A_{sample\ blank}$)/$A_{control}$] × 100, where A_{sample} is the absorbance of the test group in the hydroxyl radical generation system, $A_{control}$ is the absorbance of the control group and $A_{sample\ blank}$ is the absorbance of the samples only. Ascorbic acid was used as a positive control in this study.

It was found that the maximum absorption wavelength was 510 nm through full-wavelength scanning (Figure 1). So the anti-oxidation measurement was conducted at 510 nm.

2.4. Determination of Superoxide Anion Scavenging Activity

The ability of *Ophiopogon japonicus* polysaccharides to scavenge superoxide anion was tested according to the pyrogallol autoxidation method [14]. With some modification in this experiment, the reaction was performed in 4.5 mL 50 mM Tris-HCl buffer (pH 8.2), which contained 3 mM pyrogallol solution and the samples to be detected at different concentrations. The change speed (A/min) of absorbance of the reactive solution was measured at 325 nm. The scavenging effect of superoxide anion production was calculated as follows: scavenging effect (%) = (A − B)/A × 100, where A is the change speed of absorbance of the control group and B is the change speed of absorbance of the test sample in the superoxide anion generation system. Ascorbic acid was used as a positive control in this study.

2.5. Determination of 1,1-Diphenyl-2-Picrylhydrazyl Free Radical (DPPH) Scavenging Activity of the Polysaccharides

The DPPH radical scavenging activity of the polysaccharides was measured according to the method described in the literature [15] with some modifications. Polysaccharide samples were dissolved in doubly distilled water at 0.25, 0.5, 1, 2, 4, and 8 mg/mL. 1 mL of the sample was mixed with 2 mL 0.1 mM DPPH

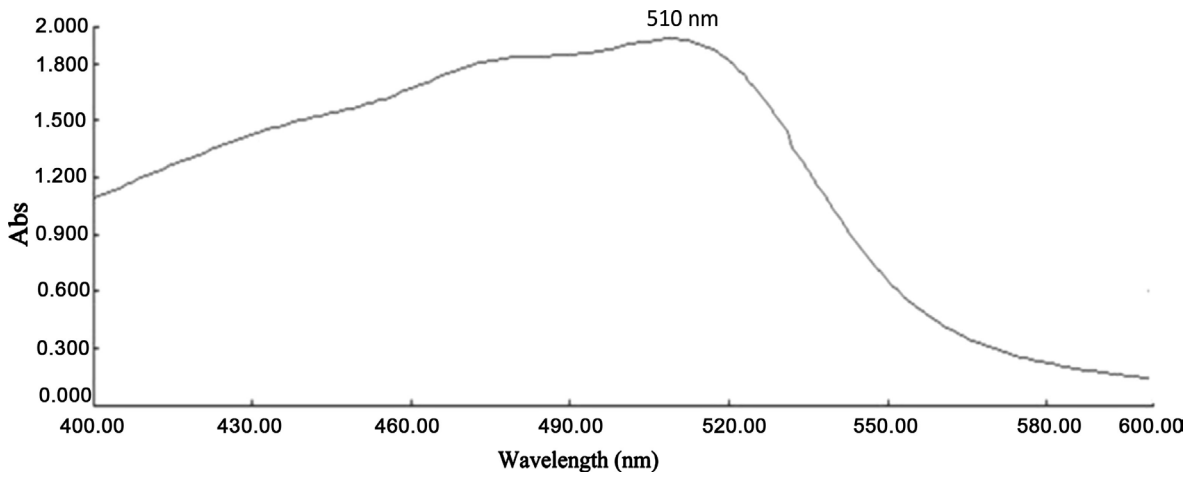

Figure 1. The ultraviolet spectrogram of phenanthroline Fe^{2+} reaction system

(freshly prepared) in 50% ethanol. The mixture was incubated at 25˚C for 30 min in the dark after shaking well, and then the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture demonstrated higher free radical scavenging capacity. Doubly distilled water was used as a negative control and ascorbic acid was used as a positive control. The experiment was carried out in triplicate and averaged. The scavenging capacity of the DPPH radical was calculated by the following formula: scavenging effect (%) = $(1 - A_{sample}/A_{control}) \times 100$, where A_{sample} is the absorbance of the test sample, and $A_{control}$ is the negative control without the polysaccharide sample.

2.6. Statistical Analysis

All data were presented as means ± standard deviation (SD) of three replications. Statistical analyses were performed using SPSS 12.0 software package and one-way analysis of variance.

3. Results and Discussion

3.1. Extraction Results of Polysaccharide from *Ophiopogon japonicus*

Ultrasound with five different powers was used to extract the polysaccharides from *Ophiopogon japonicus*. The results showed the polysaccharides had the following characteristics: white powder, fresh scent, easy absorption of moisture in the air, stronger viscosity, easily soluble in water, greater solubility. The yield of the polysaccharide relative to *Ophiopogon japonicus* powder was 11.87%, 8.74%, 7.5%, 4.06% and 8.23% extracted by ultrasonic extraction power with 80 W, 240 W, 400 W, 560 W and 720 W respectively (Figure 2). The total sugar content in *Ophiopogon japonicus* polysaccharide was 88%, 82%, 80%, 77% and 84% respectively by ultraviolet spectrophotometer. It indicated that the extraction yield of *Ophiopogon japonicus* polysaccharides decreased first and then increased with the increase of ultrasonic power. This could be because with the

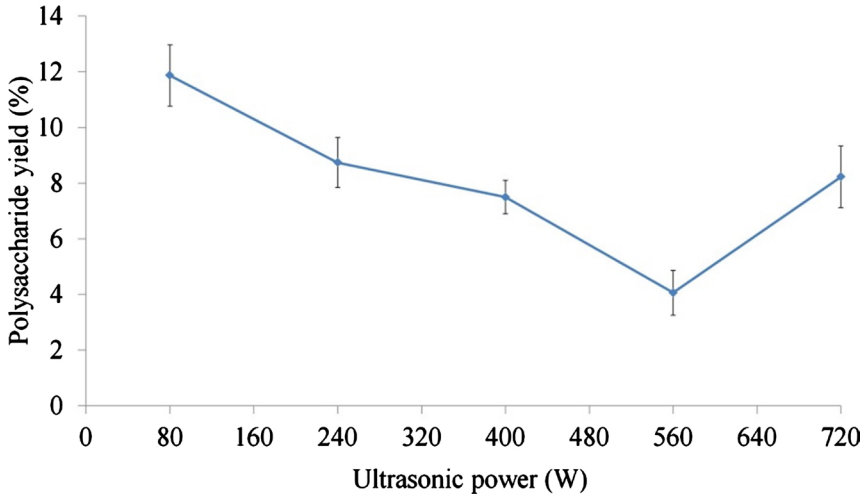

Figure 2. The extraction yield of *Ophiopogon japonicus* polysaccharide.

increase of ultrasonic power, some of the polysaccharides were degraded under the huge ultrasonic energy, resulting in the reduction of extraction yield, and with the further increase of ultrasonic power, cell walls were damaged by ultrasonic energy and more polysaccharides that cannot be extracted with small power were extracted.

3.2. Scavenging Effect on Hydroxyl Radical of the Polysaccharides

The scavenging effects on hydroxyl radical of five polysaccharides extracted by different power ultrasound from *Ophiopogon japonicus* were tested. The experimental results were shown in Figure 3. The five kinds of the polysaccharides extracted by ultrasound with different power had certain scavenging effect on hydroxyl radical. As the concentration increases, the scavenging effect on hydroxyl radical increased. The scavenging effects on hydroxyl radical of the polysaccharides extracted by ultrasound with different power were different at the same concentration. The scavenging effects on hydroxyl radical of polysaccharides increased first and then decreased with the increase of ultrasonic power. When the ultrasonic power was 560 W and the concentration of 8 mg/mL, the scavenging effect (32.19%) hydroxyl radical was the highest and it was lower when the ultrasonic power was 80 W.

3.3. Superoxide Anion Scavenging Activity of the Polysaccharides

Superoxide anion free radicals can react with almost all organic compounds in cells, causing a series of chain reactions that destroy proteins, nucleic acids, amino acids and lipids, and further damage the structure and function of cells. The scavenging effects on superoxide anion free radicals of the polysaccharides extracted by different power ultrasound were shown in Figure 4. The results

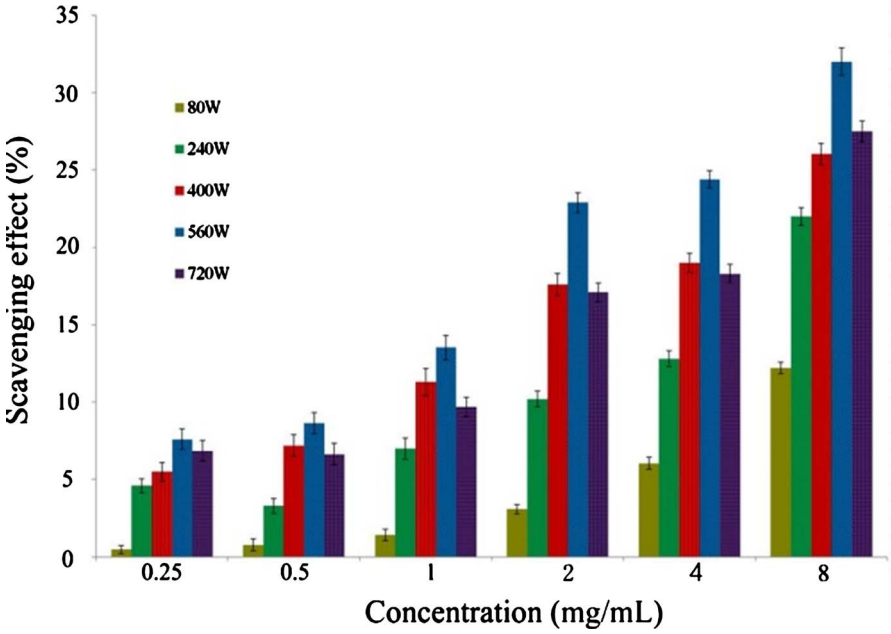

Figure 3. The scavenging effect on hydroxyl radical of the polysaccharides.

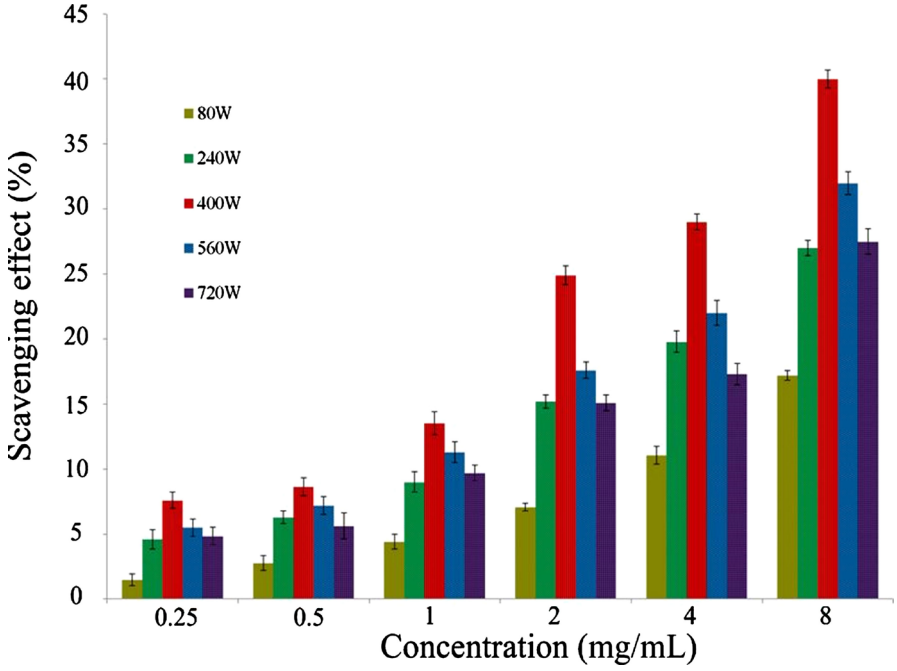

Figure 4. The scavenging effect on superoxide anion of the polysaccharides.

showed that the scavenging effect on superoxide anion radicals increased with the concentration increased. The scavenging effects on superoxide anion radicals of the polysaccharides extracted by ultrasound with different power were discrepant. The scavenging effect (40.23%) on superoxide anion was the highest when the ultrasonic power was 400 W and the concentration of 8 mg/mL.

3.4. DPPH Scavenging Activity of the Polysaccharides

The DPPH free radical is a stable radical with a maximum absorption at 517 nm, can readily undergo scavenging by an antioxidant. So it has been widely used for evaluating the free radical scavenging activities of natural compounds [16]. The DPPH radical scavenging activities of the polysaccharides extracted by different power ultrasound were shown in **Figure 5**. The results indicated that DPPH scavenging activity was caused by different concentrations of the polysaccharides. And ultrasonic extraction power had influence on the DPPH scavenging activity of the polysaccharides. The scavenging effects on DPPH of polysaccharides increased first and then decreased with the increase of ultrasonic power. The scavenging effect was higher when the ultrasonic power was 400 W and 560 W.

Therefore, different ultrasonic extraction power could lead to different antioxidant activity of polysaccharide. This may be because more active polysaccharides were extracted by increasing ultrasonic power. As the ultrasonic power continues to increase, the antioxidant activity decreased, which may be caused by the destruction of the structure of active polysaccharides by the high ultrasonic power. It also suggests that the ultrasonic power of 400 W may be the best power to extract active polysaccharide from *Ophiopogon japonicus*.

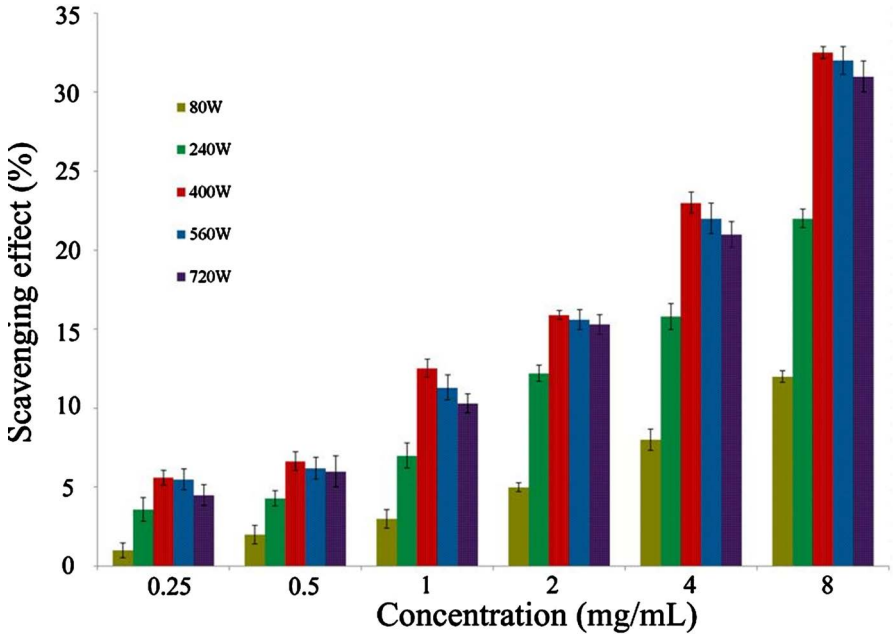

Figure 5. The scavenging effect on DPPH of the polysaccharides.

4. Conclusion

The extraction yield and antioxidant activity of the polysaccharides extracted by five different ultrasonic powers from *Ophiopogon japonicus* were investigated. It was found that the ultrasonic extraction power could have an impact on the extraction yield and the antioxidant activity of polysaccharide. With the increase of ultrasonic power, the extraction yield of the polysaccharides decreased first and then increased. With the increase of ultrasonic power, the antioxidant activity of the polysaccharides from *Ophiopogon japonicus* increased first and then decreased. When the ultrasonic power was 400 W, the antioxidant activity was the highest. In conclusion, the selection of ultrasonic power was crucial to the extraction of plant effective components, and the selection of appropriate ultrasonic power was particularly important in the extraction of plant effective components. The study will provide certain theoretical basis and experimental support for the application of power ultrasonic in the extraction of plant effective components.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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