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EFFECT OF EXTRACTION TEMPERATURES ON *IN VITRO* ANTIOXIDANT ACTIVITIES OF POLYSACCHARIDES FROM *OPHIOCORDYCEPS SOBOLIFERA*

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Abstract. The objective of this paper is to evaluate the effects of extraction temperatures on *in vitro* antioxidant potential of polysaccharides from the *Ophiocordyceps sobolifera.* The antioxidant capacity of *Ophiocordyceps sobolifera* polysaccharides was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a radical scavenger and the total antioxidant capacity methods. The results show that the highest antioxidant activity of the polysaccharides could be attained at the extraction temperature of 80°C. The total antioxidant capacity of polysaccharides from *Ophiocordyceps sobolifera* is from 0.1183 ± 0.0011 to 0.1454 ± 0.0021 mg GA/g or from 0.1105 ± 0.0004 to 0.1215 ± 0.0006 μmol AS/g. The polysaccharides have the most potent antioxidant activity with the lowest half-maximal inhibitory concentration (IC_{50}) value ranging from 0.97 mg/mL to 1.06 mg/mL.

Keywords: *Ophiocordyceps sobolifera*, polysaccharides, extraction temperature, antioxidant activity

1 Introduction

Ophiocordyceps sobolifera (syn *Cordyceps sobolifera*) belonging to the *Cordyceps* genus is an entomogenous fungi species that is parasitic on wingless cicada nymphs. A previous report indicated that *Ophiocordyceps sobolifera* significantly exhibited HIV-1 reverse transcriptase inhibitory activity [1]. In addition, Chiu et al. reported that the polysaccharide extract of *Ophiocordyceps sobolifera* attenuates renal injury in endotoxemic rats [2].

The natural polysaccharides collected from biomass continue to attract the attention of researchers due to their apparent advantages such as biocompatibility nontoxicity and have many uses in the pharmaceutic field and food [3]. The investigation of antioxidant activities of polysaccharides is an important process for their application or further research and development. Li et al. [4] and Wang et al. [5] investigated the extraction optimization of polysaccharides with antioxidant activities, but no studies on the effect of the extraction temperatures on antioxidant activities of polysaccharides from *Ophiocordyceps sobolifera* were found.

The objective of this paper is to design experiments to evaluate the effects of extraction temperatures on the *in vitro* antioxidant activities of polysaccharides from *Ophiocordyceps sobolifera.* The antioxidant potential of the polysaccharides was evaluated as total antioxidant activity and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity.

2 Experimental

2.1 Material

Ophiocordyceps sobolifera was purchased from the United States (Aloha Medicinals, 2300 Arrowhead

Drive, Carson City, United States) and grown on biomass in Nha Trang province, Vietnam. It was then taxonomically identified at the Institute of Microbiology and Biotechnology, Ha Noi National University, Vietnam.

2.2 Extraction of polysaccharides

The extraction of polysaccharides (PS) was carried out through two main steps: extraction of polysaccharides from *Ophiocordyceps sobolifera* and precipitation of polysaccharides with ethanol [4, 5]. The powder samples (3 g) were dispersed in 150 mL of distilled water. The extraction temperature was set at 60, 70, 80, 90, and 100 °C to examine the influence of extraction temperature on the yield of the polysaccharides when other extraction conditions are as follows: extraction time 3 h and 3 replications. When the extraction process of polysaccharides was accomplished, the mixture was cooled to room temperature using cold water and centrifuged. The supernatant was concentrated in a rotary evaporator under reduced pressure to receive an extract solution. Ethanol 96% was added to the concentrated extract solution to precipitate polysaccharides completely (the ratio of ethanol 96% to extract volume 4:1). The resulting precipitation was collected using centrifugation and then washed sequentially with cold ethanol and acetone. Finally, the product was vacuum-dried at 40 °C to yield crude water-soluble PS powder [2].

2.3 Qualitative and quantitative analysis of water soluble polysaccharides

Polysaccharides were examined using the phenol-sulfuric acid colorimetric method with D-glucose as a standard at a wavelength of 490 nm [6]. The standard curve equation of D-glucose is $Y = 0.0082 \times X - 0.0004$, $R = 0.9993$. The content of pure polysaccharides was calculated as follows:

Content of pure PS (%)

$$= \frac{OD + 0.0004}{0.0082} \times V \times \frac{100}{m \times (1 - W)} \times \frac{162}{180}$$

where OD is the optical density of the sample; V is the volume of sample; m is the mass of the sample; W is the moisture content of the sample.

Data analyses were performed using the Statistical Analysis System on Excel software and Origin 8.0.

2.4 Evaluation of the total antioxidant activity using the phospho-molybdenum method

The total antioxidant activity of the samples was determined according to Nair et al. [7] with certain modifications. In brief, a 0.3 mL aliquot of the sample was mixed with 3 mL of a reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate), and then the mixture was incubated at 95 °C for 90 min. The mixture was then cooled to 25 °C, and the absorbance was measured at a wavelength of 695 nm against a blank that contains 3 mL of the reagent solution without the sample. The total antioxidant activity is expressed as the number of equivalents of gallic acid (GA) [8] and ascorbic acid (AS) [9] (with concentrations of between 0.1 and 0.5 mg/mL).

2.5 Evaluation of DPPH radical scavenging activity

The DPPH free radical scavenging activity of each sample was determined using a Jasco V-630 Spectrophotometer according to Nair et al. [7] and Wong et al. [10] with certain modifications. The samples were dissolved in 1.5 mL methanol at various concentrations (25, 50, 75, and 100 μg/mL) and mixed with 1.5 mL of 100 μM DPPH (100 μM DPPH dissolved in methanol before use). The reaction mixture was shaken for one minute and

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incubated at room temperature for 30 minutes. The absorbance was then measured at a wavelength of 517 nm. Three milliliters of methanol was used as a blank sample. The radical scavenging activity was evaluated using the IC_{50} value [11].

3 Results and discussion

3.1 Effect of extraction temperature on the yield of the polysaccharides from *O. sobolifera*

The extraction yields of polysaccharides at various temperatures (from 60 to 100 °C) were evaluated (Table 1). The highest yield of polysaccharides is 4.516%, obtained at 100 °C.

It is speculated that at high temperatures, the cell membrane structure and viscosity of the polysaccharide solution may be affected. The membrane was easily damaged at high temperatures, and, therefore, polysaccharides enter the solution. Furthermore, at a higher temperature, the viscosity of the extract solution decreases, thus increasing the solubility of polysaccharides, which, in turn, accelerates the release and dissolution of these compounds.

Table 1. The effect of extraction temperature on the PS obtained

Extraction temperature (°C)	Percentage of pure PS (%)
60	3.579 ± 0.088
70	3.985 ± 0.010
80	4.066 ± 0.144
90	4.327 ± 0.096
100	4.516 ± 0.099

3.2 Antioxidant activity assay of polysaccharides

Total antioxidant capacity

In this study, the total antioxidant capacity was determined by assessing the electron-donating capacity of the sample using the phospho-molybdenum method. In principle, this method is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the formation of a green Mo(V) complex at a low pH with maximal absorbance at 695 nm. A high absorbance value indicates that the sample possesses high antioxidant activity [7].

Fig. 1 shows the effect of extraction temperature on the antioxidant activities of the polysaccharides. The results indicate that the antioxidant activities of the polysaccharides significantly increase with the increase in concentration from 0.1 to 1.5 mg/mL. We could observe that the antioxidant activities of the polysaccharides are proportional to extraction temperature between 60 °C and 80 °C. After this point, the antioxidant activities of the polysaccharides decrease.

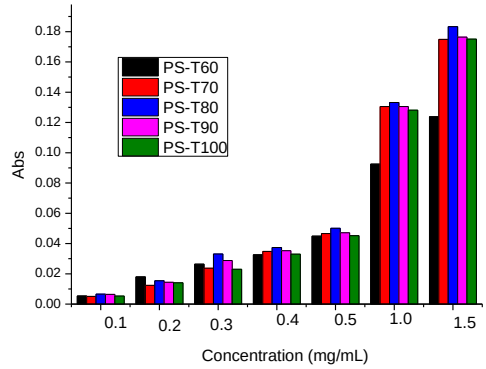

Fig. 1. Antioxidant activity of polysaccharides of *Ophiocordyceps sobolifera* in total antioxidant capacity model

In our opinion, the properties and activities of polysaccharides are related to their space structure and molecular weight. The increase of the extraction temperature may enhance the yield of the polysaccharides and the molecular weight of the extracted polysaccharides [3]. Maybe, high-molecular-weight polysaccharides are unfavorable for antioxidant activity, and as a result, the antioxidant activity of polysaccharides, instead, decreases.

The antioxidant capacity is expressed as the number of equivalents of gallic acid or ascorbic acid (the standard curve equation of gallic acid: Abs = 0.7820 × C_{GA} + 0.1648, R = 0.9966; and the standard curve equation of ascorbic acid: Abs = 4.5974 × C_{AS} – 0.3231, R = 0.9952). The total antioxidant capacity of the polysaccharides at the concentration of 1.5 mg/mL is from 0.1183 ± 0.0011 to 0.1454 ± 0.0021 mg GA/g or from 0.1105 ± 0.0004 to 0.1215 ± 0.0006 μmol AS/g (Table 2). This result suggests that the polysaccharides have antioxidant capacity.

DPPH radical scavenging activity

It can be seen that the DPPH radical scavenging activities of polysaccharides of *Ophiocordyceps sobolifera* increase with the polysaccharide concentration (Table 3). The DPPH radical scavenging activity at the concentration of 2.5 mg/mL is over 75%. The IC_{50} values of polysaccharides range from 0.97 mg/mL to 1.06 mg/mL. Thus, the polysaccharides of *Ophiocordyceps sobolifera* have potential antioxidant properties.

Table 2. Total antioxidant capacity (TAC) of polysaccharides of *Ophiocordyceps sobolifera*

Sample	mg GA/g	μmol AS/g
PS-T60	0.1183 ± 0.0011	0.1105 ± 0.0004
PS-T70	0.1393 ± 0.0009	0.1190 ± 0.0007
PS-T80	0.1454 ± 0.0021	0.1215 ± 0.0006
PS-T90	0.1404 ± 0.0007	0.1194 ± 0.0009
PS-T100	0.1400 ± 0.0013	0.1193 ± 0.0002

Table 3. DPPH radical scavenging activity rates of polysaccharides of *Ophiocordyceps sobolifera*

Sample	Concentration (mg/mL)						IC_{50} (mg/mL)
	0.4	0.6	0.8	1	2	2.5	
PS-T60	21.02	27.08	31.42	48.57	72.67	75.71	1.06 ± 0.01
PS-T70	18.58	28.57	31.44	49.02	69.53	81.74	1.05 ± 0.02
PS-T80	16.52	23.79	40.68	51.36	78.75	93.85	0.97 ± 0.01
PS-T90	15.52	26.20	40.90	50.87	79.12	91.75	0.99 ± 0.01
PS-T100	10.68	27.62	32.65	48.45	78.16	91.28	1.05 ± 0.01

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The DPPH radical scavenging activities of the polysaccharides from *Ophiocordyceps sobolifera* are also comparable with those of other medicinal fungi, which are in either the same species or between species in the same genus mentioned in the literature. The DPPH radical scavenging activity at the concentration of 1.0 mg/mL of polysaccharides is lower than that of *Cordyceps sobolifera* polysaccharides (55.6% at 1 mg/mL) [12]. It can be seen that the DPPH radical scavenging activity of the polysaccharides is higher than that of both *C. sinensis* polysaccharides (IC_{50}: 1.23 mg/mL) [13] and *C. militaris* polysaccharides (IC_{50}: 1.15 mg/mL) [14].

4 Conclusions

The experimental results may provide theoretical basis for further systematical research and development of *Ophiocordyceps sobolifera* polysaccharides. The content and antioxidant activities of polysaccharides are related to the extraction temperature. The highest antioxidant activity of the polysaccharides could be obtained from the extraction temperature of 80 °C. At this temperature, the total antioxidant capacity of polysaccharide from *Ophiocordyceps sobolifera* is 0.1454 ± 0.0021 mg GA/g or 0.1215 ± 0.0006 μmol AS/g. The IC_{50} value of polysaccharides ranges from 0.97 mg/mL to 1.06 mg/mL. The results of antioxidant activity assay of polysaccharides show that the *Ophiocordyceps sobolifera* polysaccharides have appreciable antioxidant activity *in vitro*.

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