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**Research Article**

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# Extraction Optimization and Antioxidant Activity of Polysaccharides from *Cyclocarya paliurus*

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**ABSTRACT**

*Cyclocarya paliurus (Batal.) Iljinsk (C. paliurus) is a well-known medicinal plant in China for regulating blood glucose of diabetic and is commonly called 'sweet tea tree'. The aim of this study was to optimize the extraction condition using $L_9(3)^4$ orthogonal experimental design by reflux extraction and investigate the antioxidant activity of polysaccharides from C. paliurus. Four factors and three levels each factor were designed to determine the optimum parameters for the extraction and the total reducing power determination was used to study the antioxidation of the polysaccharides from C. paliurus. The results showed that the optimum extraction time was 2.5 h, times were 2, temperature was 90 °C and material/solvent ratio was 1:15 (g/mL). Under this condition, maximum yields of 4.95% could be obtained. The reducing power test confirmed the antioxidant activity of polysaccharides from C. paliurus. In summary, reflux extraction is a good, simple and efficient method for the extraction of polysaccharides from C. paliurus and the polysaccharides possess an antioxidant activity.*

**Keywords:** *Cyclocarya paliurus*; Polysaccharides; Orthogonal experiment; Extraction

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## INTRODUCTION

*Cyclocarya paliurus (Batal.) Iljinskaja*, grown on cloudy and foggy highlands in southern China, is the sole species in its genus. Over the years, leaves of *C. paliurus* have been widely used for the treatment of diabetes mellitus and identified with its anti-diabetic activity by more recent research [1,2]. The extract of *C. paliurus* exhibited significant blood sugar reducing activity [3,4] and some studies demonstrate the anti-hyperlipidemic effect of *C. paliurus* and the inhibition of apolipoprotein B48 overproduction [5,6]. Moreover, extracts from leaves of *C. paliurus* beneficially regulated adipokine expression against inflammatory insult and ameliorated insulin resistance [2]. Our previous study shows that *C. paliurus* extracts possess a good antioxidant activity and can alleviate diabetic nephropathy by inhibiting oxidative stress and aldose reductase[7]. Chemical studies have shown that *C. paliurus* contains polysaccharides, flavonoids, triterpenoids, steroids and phenolic compounds [8,9]. As the research on carbohydrate attracts increasing attention, polysaccharides in *C. paliurus* have gradually become a research focus [10]. Extraction is a critical process for isolating polysaccharides effectively in plants. The reflux extraction method is a widely-used method with the advantage of simplicity, stability and efficiency. In the present study, we aimed to develop an optimum extraction condition of polysaccharides from *C. paliurus* by reflux extraction and study the antioxidant activity of the polysaccharides. We used orthogonal experimental design to determine the optimum extraction parameters for polysaccharides from *C. paliurus*. Several factors including the extraction time, times, temperature and the ratio of material to solvent were taken into consideration.

## EXPERIMENTAL SECTION

### Chemicals and instruments

Glucose (UV，purity≥98%, Shanghai Baoman Biotechnology Co., Ltd., China), all other chemicals and reagents

used were of analytical grade. Instruments used in this experiment were the same as our previous study [11].

**Plant material**

*Cyclocarya paliurus (Batal.) Iljinsk* (CP) leaves were obtained and authenticated as our previous study [11].

**Extraction of polysaccharides from *Cyclocarya paliurus***

Approximately 3 g of powdered CP was extracted with doubly distilled water in a round bottom flask. Under the experimental conditions, the aqueous extract of CP was obtained. After concentrated, the solution was precipitated with four times as its volume of 95% ethanol at overnight. The precipitation was filtered and purified with anhydrous ethanol, and then freeze-dried. Finally, a brown powder was obtained and marked as polysaccharides from CP (hereafter referred to as PCP)

**Determination of polysaccharides contents**

The polysaccharides contents were determined by phenol-sulfuric acid colorimetry method with D-glucose as a standard. The procedure was performed according to the method of Masuko [12] with a minor modification. In brief, 1.0 mL of the extracts prepared at various concentrations was reacted with 1.0 mL of 5% phenol solution and 5.0 mL of concentrated sulfuric acid was added immediately. The solution was then mixed and placed for 15 min at 45 $^{o}$C. Finally, the absorbance of the mixture was measured at 490 nm, parallel test 3 times.

**Single factor experiment design**

The leaves of CP were air-dried and smashed prior to use and stored at room temperature. Four factors were designed to study the influence of experiment condition on the yields of PCP and the levels were shown in table 1. Yields of the polysaccharides were calculated as follows:

Yield of PCP (%) = (polysaccharides contents/materials quantities) × 100%

**Table 1: Factors and levels of Single factor experiment design**

| Extraction time(h) | Extraction times | Temperatures(°C) | Material/solvent |
|---|---|---|---|
| 1 | 1 | 50 | 1:10 |
| 1.5 | 2 | 60 | 1:15 |
| 2 | 3 | 70 | 1:20 |
| 2.5 | 4 | 80 | 1:25 |
| 3 | 5 | 90 | 1:30 |

**Orthogonal experimental design**

An orthogonal experiment [$L_9(3)^4$] design was used to optimize the extraction parameter of PCP by reflux extraction[13,14]. As seen in Table 2, nine experiments were conducted with four factors, extraction time, temperatures, extraction times, the ratio of material to solvent water and three levels per factor on the basis of single-factor experiments. The optimum extraction process of PCP was investigated.

**Table 2: Design of orthogonal experiment [$L_9(3)^4$]**

| Levels | Factors | | | |
|---|---|---|---|---|
| | A | B | C | D |
| 1 | 1.5 | 70 | 1 | 01:15 |
| 2 | 2 | 80 | 2 | 01:20 |
| 3 | 2.5 | 90 | 3 | 01:25 |

A: Extraction time (h); B: Extraction temperature ($^{o}$C); C: extraction times; D: Ratio of material to solvent (g/mL).

**Antioxidant activity of PCP**

Antioxidant activity of the extraction was measured according to our previous study [15]. 2.0 mL of PCP with various concentrations (0-5 mg/mL) were mixed with 0.2 mL of phosphate buffer (0.2 mol/L, pH 6.8). After the addition of 2.0 mL 1% potassium ferricyanide, the mixture was incubated at 50 $^{o}$C for 20 min. The solution was cooled immediately by tap water and 2.0 mL of 10% trichloroacetic acid was added. After centrifuged at 3742 g for 10 min, 5 mL of the supernatant was mixed with 4.0 mL of distilled water and 1.0 mL of 0.1% ferric chloride. Then the solution was placed for 10 min at room temperature. Finally, the absorbance was measured at 700 nm. A higher absorbance indicated a higher reducing power activity, parallel test 3 times.

## RESULTS AND DISCUSSION

### Single-factor experimental analysis

To determine the effect of extraction time on the extraction yield of PCP, experiments were carried out twice, at 90°C and the material to solvent ratio was 1:20. To determine the effect of extraction times on the extraction yield of PCP, experiments were carried out with a material to solvent ratio of 1:20, at 90°C, 1h. To determine the effect of temperature on the extraction yield of PCP, experiments were carried out twice, 2h each time and the material to solvent ratio was 1:20. To determine the effect of material to solvent ratio on the extraction yield of PCP, experiments were carried out twice, 2h each time and the temperature were set at 90°C. The results are shown in Fig. 1 to Fig. 4. Under different extraction conditions, the yields of PCP varied from 1.97% to 5.22%. It was indicated that the four factors all had important influences on the extraction yield of PCP and it was necessary to optimize the extraction condition of PCP.

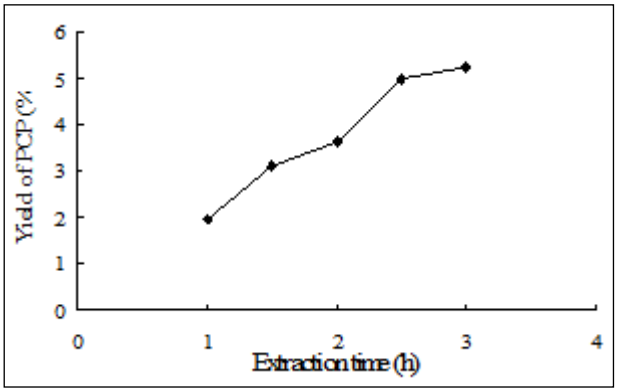


**Figure1: Effect of extraction time on the extraction yield of PCP**

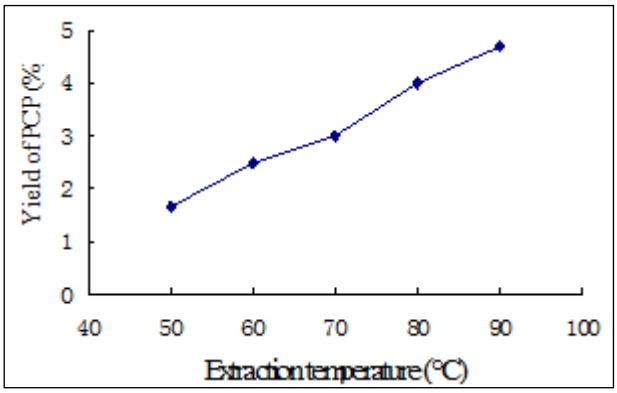


**Figure 2: Effect of temperature on the extraction yield of PCP**

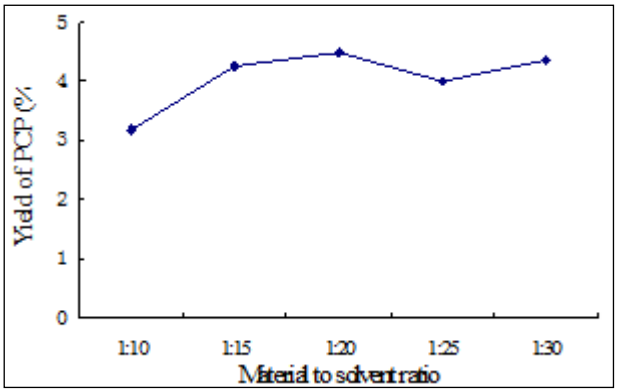


**Figure 3: Effect of material to solvent ratio on the extraction yield of PCP**

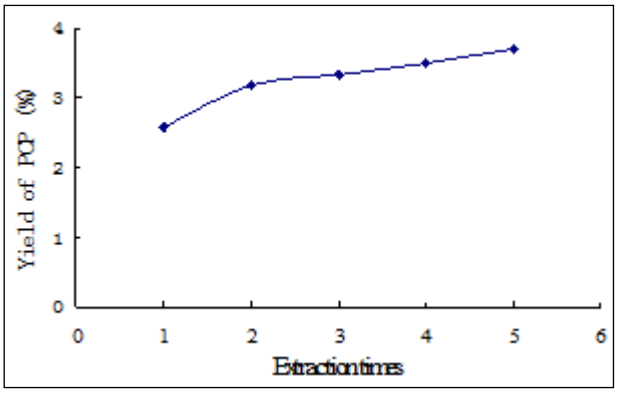


**Figure 4: Effect of Extraction times on the extraction yield of PCP**

**Orthogonal analysis**

Taguchi-based orthogonal array experimental design is a widely used and powerful optimization technique that allows optimization with minimum number of experiments. Thus, by this method, it is possible to reduce the time and cost for experimental investigations and improve the extraction efficiency. In the present study, four factors and three levels per factor were defined, and nine experiments were conducted. The results are shown in Table 3. All results of the design were expressed as the mean of three parallel tests. According to the range values (R), the influence of different factors on the yield of polysaccharides was A > B > C > D. Thus, the optimal condition was a combination of A3B3C2D1, namely, extracted twice, at 90 °C, 2.5 h and the material to solvent ratio was 1:15. Under this condition, an extraction yield of 4.95% was achieved.

Table 3 Design and results of the $L_9(3)^4$ orthogonal experiment

| Number | A | B | C | D | Yield of PCP (%) |
|---|---|---|---|---|---|
| 1 | 1.5 | 70 | 1 | 01:15 | 2.62 |
| 2 | 1.5 | 80 | 2 | 01:20 | 2.93 |
| 3 | 1.5 | 90 | 3 | 01:25 | 3.11 |
| 4 | 2 | 70 | 2 | 01:25 | 3.96 |
| 5 | 2 | 80 | 3 | 01:15 | 4.19 |
| 6 | 2 | 90 | 1 | 01:20 | 3.98 |
| 7 | 2.5 | 70 | 3 | 01:20 | 4.57 |
| 8 | 2.5 | 80 | 1 | 01:25 | 4.62 |
| 9 | 2.5 | 90 | 2 | 01:15 | 4.95 |
| $\overline{k_1}$ | 2.89 | 3.72 | 3.74 | 3.92 | |
| $\overline{k_1}$ | 4.04 | 3.91 | 3.95 | 3.83 | |
| $\overline{k_1}$ | 4.71 | 4.01 | 3.96 | 3.9 | |
| R | 1.83 | 0.3 | 0.22 | 0.09 | |

A: Extraction time (h); B: Extraction temperature (°C); C: extraction times; D: Ratio of material to solvent (g/mL).

**Antioxidant activity of PCP**

The reducing power is one of the most commonly used methods for measure the antioxidant activity of a compound. As shown in Fig.5, the reducing power increased as the concentrations of PCP increased. The absorbance at 700 nm indicated that the PCP prepared under experiment conditions possessed an antioxidant activity and could provide a natural antioxidant material.

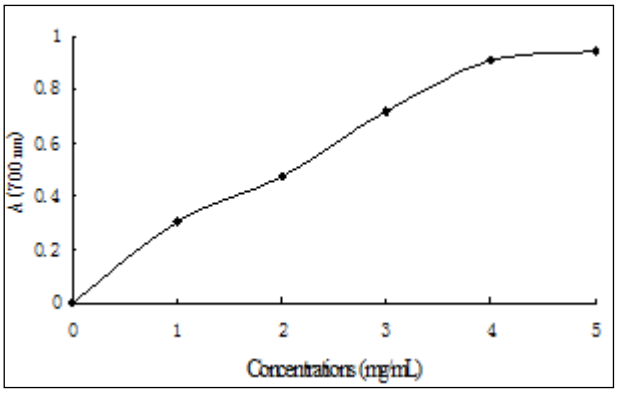


**Figure 5: Antioxidant activity of polysaccharides from *Cyclocarya paliurus***

## CONCLUSION

The hot water reflux extraction and ethanol sedimentation method has been suggested as a good extraction process for isolating polysaccharides from *C. paliurus*. Through the orthogonal experimental, an optimized reflux extraction method was developed with extraction times 2, temperature 90 °C, extraction time 2.5 h and the material to solvent ratio 1:15. The reducing power test showed the antioxidant activity of polysaccharides. This research provides a reference for the large-scale extraction of polysaccharides from *C. paliurus*. Hence, *C. paliurus* might be explored as a new source of natural antioxidant product.

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