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Study on extraction technology of flavonoids from *Cyclocarya paliurus* by orthogonal design

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ABSTRACT

Cyclocarya paliurus (Batal.) Iljinsk (C. paliurus) is a very important medicinal herb in traditional Chinese medicine for the treatment of diabetes mellitus. The aim of this study was to develop an optimum extraction method of flavonoids from C. paliurus by reflux extraction. Orthogonal experimental design was used to determine the optimal extraction parameters for flavonoids from C. paliurus. The results showed that the maximum yields of flavonoids (31.16±0.96) mg/g could be obtained when the extraction time was 2 h, the extraction temperature was 80 °C, the ratio of material to solvent was 1:30 (g/mL) and 80% ethanol was used. In summary, this study provides an efficient reflux extraction for flavonoids from C. paliurus, which can be used as natural products in the pharmaceutical and functional food industries.

Keywords: *Cyclocarya paliurus*, Flavonoids, Reflux extraction, Orthogonal experimental design

INTRODUCTION

Cyclocarya paliurus (Batal.) Iljinsk, (*C. paliurus*) commonly known as "sweet tea tree," is a kind of medicinal herb which is widely used in China to treat diabetes mellitus. In addition, *C. paliurus* antihyperglycemic herbal tea is the first health tea from China which has been approved by the United States Food and Drug Administration [1-2]. Many studies have demonstrated that *C. paliurus* possesses a variety of bioactivities, including hypoglycemic, antihypertensive, hypolipidemic, antioxidant activity, *etc* [3-4]. Chemical studies have shown that the plant contains polysaccharides, flavonoids, triterpenoids, protein, steroids, saponins and phenolic compounds [5-6].

As the research on natural products attracts increasing attention, flavonoids in *C. paliurus* have gradually become a research focus [7]. Extraction is a critical process for isolating the effective components in plants. A variety of innovative methods are used for extracting flavonoids from *C. paliurus* [8-9]. While the reflux extraction method is still widely used due to its simplicity, stability and efficiency. Despite its widespread use, the inappropriate conditions such as high extraction temperature or long extraction time may destroy the structure of the flavonoids in *C. paliurus* and thus decrease their bioactivities [10]. In the present study, we aimed to develop an optimum extraction method of flavonoids from *C. paliurus* by reflux extraction. We used orthogonal experimental design to determine the optimal extraction parameters for flavonoids from *C. paliurus*. Several factors including the concentration of solvent ethanol, extraction time, extraction temperature and the ratio of material to extraction solvent were taken into consideration.

EXPERIMENTAL SECTION

Chemicals and Instruments

UV8000 UV–visible spectrophotometer (Shanghai Precision Instrument Co., Ltd, China.); RE 52-86A rotary evaporator (Shanghai Yarong Biochemical Instrument Factory, China.). Rutin (HPLC，purity≥98%, Shanghai

Ronghe Medicine Science and Technology Development Co., Ltd., China.). All other chemicals and reagents used were of analytical grade.

Plant material

Cyclocarya paliurus (Batal.) Iljinsk leaves were collected in Zhangjiajie, Hunan province, China and authenticated by Dr. Chongmei Xu from the Department of Pharmacognosy of Weifang Medical University.

Single factor experiment design

Under defined extraction time (0.5, 1, 2, 3, 4 h), temperatures (50, 60, 70, 80 and 90 °C), ethanol concentrations (50%, 60%, 70%, 80% and 90%) and material to extraction solvent ratios (1:10, 1:20, 1:30, 1:40 and 1:50 g/mL), approximately 5 g of the pretreated *C. paliurus* powder was extracted. Then, the extracted solution was filtered and concentrated using a rotary evaporator at reduced pressure to make a final volume of 100mL.

Orthogonal array experimental design

An orthogonal experiment [$L_9(3)^4$] test design was used to optimize the extraction conditions of flavonoids from *C. paliurus* by reflux extraction[11,12]. As seen in Table 1, nine experiments were conducted with four factors, and three levels per factor on the basis of single-factor experiments. The effects of extraction time, the extraction temperature, the ratio of material to extraction solvent and the best concentration of ethanol on extraction yield were investigated.

Table 1: Design of orthogonal experiment

Levels	Factors			
	A	B	C	D
1	60	70	1	1:20
2	70	80	2	1:30
3	80	90	3	1:40

A: Ethanol concentration (%); B: Extraction temperature (^oC); C: Extraction time (h); D: Extraction ratio of material/solvent (g/mL).

Determination of flavonoid contents

Flavonoids contents were determined using the aluminum chloride colorimetric method. The procedure was performed according to the method of Hsieh [13] with a minor modification. In brief, 0.5 mL of the extracts prepared at various concentrations (0.01, 0.05, 0.10, 0.50 and 1.00 mg/mL) were reacted with 0.40 mL of 5% sodium nitrite, and the solution was mixed and placed for 6 min. Then 0.40 mL of 10% aluminum chloride was added and another 6 min was needed after mixing. After the addition of 4.00 mL of 4% sodium hydroxide, the mixture was diluted to 10.00 mL with 95% ethanol and placed for 10 min at room temperature. Finally, the absorbance of the mixture was measured at 390 nm. Rutin was used as the standard.

RESULTS AND DISCUSSION

Single-factor experimental analysis

Effect of extraction time on the extraction yield of flavonoids

To determine the effect of extraction time on the extraction yield of flavonoids, experiments were carried out in a round-bottom flask at 80°C with 70% ethanol and a material/solvent ratio of 1:30. The result in Fig. 1 indicates that the yield of flavonoids increased rapidly before 2 h and slowly from 2 to 4 h.

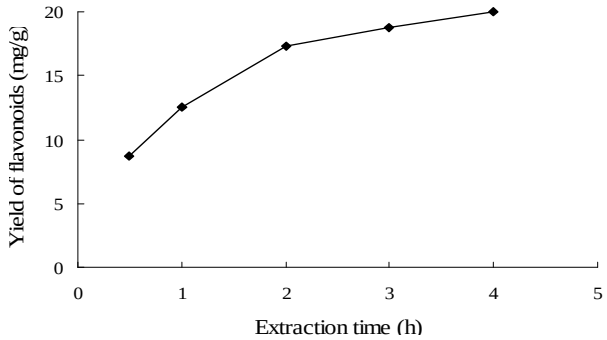

Fig.1. Effect of extraction time on the extraction yield of flavonoids

Effect of temperature on the extraction yield of flavonoids

The temperature is another important factor affecting the yield of flavonoids. Therefore, in the present study, temperatures of 50, 60, 70, 80 and 90°C were evaluated for their influence on the yield of flavonoids. As shown in Fig.2, when the extraction time, ethanol concentration and material/solvent ratio were set at 2 h, 70% and 1:30 g/mL, respectively, the flavonoids extraction yield increased from 14.49% to 18.56% as the temperature increased from 50°C to 80°C, while decreased at 90°C. This phenomenon may be caused by the thermal instability of some flavonoids.

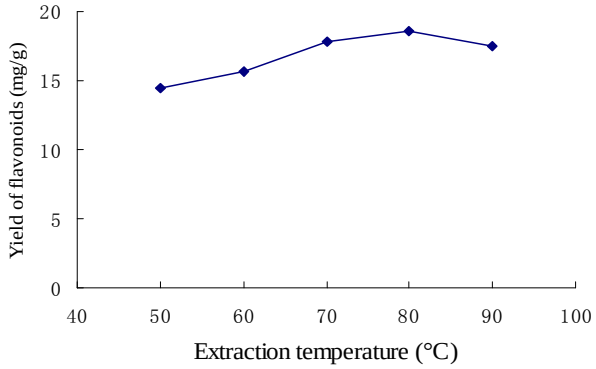

Fig.2. Effect of temperature on the extraction yield of flavonoids

Effect of material/solvent ratio on the extraction yield of flavonoids

As shown in Fig.3, the effect of the material/solvent ratio on the yield of flavonoids was investigated. Here, the material/solvent ratio ranged from 1:10 to 1:50 (g/mL), while the other extraction parameters were as follows: 70% ethanol, extraction temperature 50°C and treatment time 2 h. The results showed that the maximum yield of flavonoids was increased as the material/solvent ratio increased, but increased slowly when the material/solvent ratio was over 1:30.

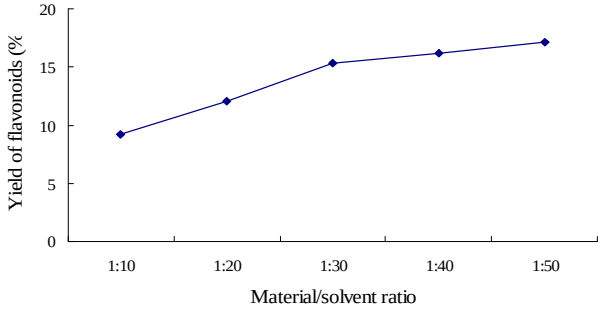

Fig.3. Effect of material/solvent ratio on the extraction yield of flavonoids

Effect of ethanol concentration on the extraction yield of flavonoids

Because of the complexity of the chemical structure of flavonoids, the effect of the ethanol concentrations on the yield of flavonoids was investigated. Here, the ethanol concentrations ranged from 50% to 90%, while the other extraction parameters were as follows: extraction temperature 50 °C, treatment time 2 h and material/solvent ratio 1:30. The results in Fig.4 showed that the maximum yield of flavonoids was increased as the material/liquid ratio increased, but increased slowly when the material/solvent ratio was over 1:30.

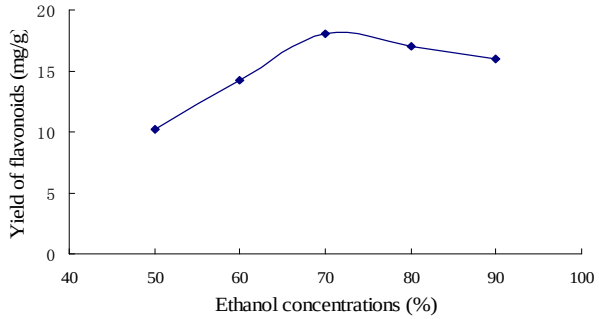

Fig.4. Effect of ethanol concentration on the extraction yield of flavonoids

Orthogonal analysis

To reduce the number of experimental trials and understand the interactions between factors, an orthogonal array experimental design was used to optimize the best extraction of flavonoids from *C. paliurus*. The extraction time, extraction temperature, concentration of ethanol and the ratio of material to solvent were considered as important factors during the extraction process of the samples. The orthogonal experimental design and the range analysis are shown in Table 2. According to the range values (R), the influence of the different factors on the yield of flavonoids was B>A>D>C. Thus, the optimal condition was a combination of A3B2C2D2, namely, 80% ethanol, 80 °C, 2 h and a material/solvent ratio of 1:30. Under these conditions, an extraction yield of (31.16±0.96) mg/g was achieved.

Table 2: Design and results of orthogonal experiment

Number	A	B	C	D	Yield of flavonoids (mg/g)
1	60	70	1	1:20	20.26
2	60	80	2	1:30	26.52
3	60	90	3	1:40	25.05
4	70	70	2	1:40	24.01
5	70	80	3	1:20	26.50
6	70	90	1	1:30	26.87
7	80	70	3	1:30	25.00
8	80	80	1	1:40	28.99
9	80	90	2	1:20	28.20
$\overline{k_1}$	23.94	23.09	25.37	24.99	
$\overline{k_2}$	25.79	27.34	26.24	26.13	
$\overline{k_3}$	27.40	26.71	25.52	26.02	
R	3.45	4.25	0.87	1.14	

A: Ethanol concentration (%); B: Extraction temperature (°C); C: Extraction time (h); D: Extraction proportion of sample/solvent (g/mL).

CONCLUSION

The reflux extraction has been suggested as a good extraction process for isolating flavonoids from *C. paliurus*. Through the orthogonal experimental, four factors including the extraction time, extraction temperature, the best concentration of ethanol and the ratio of material to solvent for reflux extraction of *C. paliurus* were optimized. This research provides a reference for the large-scale extraction of flavonoids from *C. paliurus*. Hence, *C. paliurus* might be explored as a new source of natural product.

Acknowledgments

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