

OPTIMIZATION OF ULTRA-HIGH PRESSURE-ASSISTED DEEP EUTECTIC SOLVENT EXTRACTION AND ANTIOXIDANT PROPERTIES OF POLYSACCHARIDES FROM *ERIOBOTRYAE FOLIUM* L.

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Abstract

This study employed ultra-high pressure-assisted deep eutectic solvent (UHP-DES) extraction to obtain polysaccharides from *Eriobotryae folium*. Based on the selection of the optimal DES system, single-factor experiments and response surface methodology (RSM) were applied to optimize the extraction process of polysaccharides from *E. folium*, followed by an evaluation of their antioxidant activities. The results showed that the optimal extraction conditions were a DES water content of 45%, an extraction pressure of 380 MPa, and an extraction time of 10 minutes. Under these optimized conditions, the yield of polysaccharides from *E. folium* reached $(6.18 \pm 0.11)\%$. Furthermore, the polysaccharides extracted by UHP-DES exhibited strong scavenging activities against both DPPH· and ·OH free radicals. These findings indicate that ultra-high pressure-assisted DES extraction is a suitable and effective method for the preparation of polysaccharides from *E. folium*.

Introduction

The dried leaves of *Eriobotrya japonica* (Thunb.) Lindl. (Rosaceae), is a newly approved novel food material by the National Health Commission of China and is rich in bioactive constituents, including polysaccharides (Fu *et al.* 2019, triterpenoids (Jin *et al.* 2024), phenolic acids (Khouya *et al.* 2022), and flavonoids (Wang *et al.* 2024). Among these components, polysaccharides from *E. folium* exhibit various functional properties, such as antioxidant (Yao *et al.* 2023), hypoglycemic (Zhao *et al.* 2024), and hypolipidemic activities (Mokhtari *et al.* 2024). Therefore, the extraction of polysaccharides from *E. folium* is of great significance for the efficient utilization and value-added development of this plant resource.

At present, the extraction of polysaccharides from *E. folium* is mainly carried out using the conventional water extraction–alcohol precipitation method (Wang *et al.* 2021). In addition, auxiliary techniques such as microwave-assisted extraction (Fu *et al.* 2020) and ultrasonic-assisted extraction (Li *et al.* 2025) have been applied to enhance the extraction efficiency. However, the traditional water extraction–alcohol precipitation method is characterized by long extraction times and low yields. Although microwave and ultrasonic-assisted extraction methods can shorten extraction time and improve yield, the heat generated during processing may cause structural alterations of polysaccharides, thereby affecting their functional properties (Dong *et al.* 2024).

Deep eutectic solvents (DES) are formed by mixing two or more hydrogen bond donors and acceptors at specific molar ratios. These solvents exhibit high solubility for polysaccharides, along with advantages such as low cost and environmental friendliness (Pei *et al.* 2024). In recent years, DES have been increasingly applied in the extraction of plant polysaccharides (Zhang *et al.* 2024, Wang *et al.* 2024). Ultra-high pressure extraction (UPE), a non-thermal extraction technology operating at pressures above 100 MPa, enhances extraction efficiency by disrupting plant cell

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structures through high pressure and has also been applied to the extraction of plant polysaccharides in recent years (Lin *et al.* 2022, Huang *et al.* 2023, Meng *et al.* 2023). However, studies on ultra-high pressure-assisted DES extraction of polysaccharides from *E. folium* remain scarce.

Therefore, in this study, ultra-high pressure-assisted DES extraction was employed to extract polysaccharides from *E. folium*. The effects of key parameters, including DES water content, extraction pressure, and extraction time, on polysaccharide yield were optimized. In addition, the antioxidant properties of the extracted polysaccharides were systematically investigated. This work aims to provide theoretical support for the efficient utilization and further development of *E. folium* resources.

Materials and Methods

2L Ultra-High Pressure Processing Unit (L2 Series, Huataisenmiao Ultra-High Pressure Equipment Engineering Technology Co. Ltd., Tianjin), FR224CN Electronic Analytical Balance (OHAUS Instruments (Shanghai) Co. Ltd.), RE-2000B Rotary Evaporator (Shanghai Yarong Biochemical Instrument Factory), Avanti J-26s XPI High-Speed Refrigerated Centrifuge (Beckman Coulter, USA), EU2600-A UV-Vis Spectrophotometer (Angla Instrument Co. Ltd., Shanghai) was used in the experiment.

Eriobotryae folium was collected from Haikou, Hainan Province, China, and authenticated as the leaves of *E. japonica* (Thunb.) Lindl. by Prof. Wang Huamin. The leaves were dried at 60°C to a moisture content of 5%, pulverized, and passed through a 0.178 mm sieve prior to use. Choline chloride, glycerol, 1,4-butanediol, ethylene glycol, ethanol, urea, lactic acid, ferrous sulfate, salicylic acid, and hydrogen peroxide were all of analytical grade. 1,1-Diphenyl-2-picrylhydrazyl (DPPH, purity ≥ 98%) was obtained from Shanghai Yuanye Bio-Technology Co. Ltd.

Based on preliminary experiments, choline chloride was selected as the hydrogen bond acceptor (HBA) and was separately combined with five hydrogen bond donors (HBDs), namely glycerol (polyol), 1,4-butanediol (polyol), ethylene glycol (polyol), urea (amide), and lactic acid (carboxylic acid), at a molar ratio of 1 : 2. The mixtures were placed in round-bottom flasks and stirred at 85°C for 2 hrs until a homogeneous and transparent liquid was obtained, yielding DES-1, DES-2, DES-3, DES-4, and DES-5, respectively. Under a solid–liquid ratio of 1 : 30 (g/ml), different DESs were used to extract polysaccharides from *E. folium* with a DES water content of 40%, an ultra-high pressure of 400 MPa, and an extraction time of 10 min. The effects of different DES systems on the extraction yield of polysaccharides from *E. folium* were then compared.

Ten grams of pulverized *E. folium* powder were weighed and mixed with different DES solvents at a solid–liquid ratio of 1 : 30 (g/ml). After thorough mixing, the mixtures were sealed in high-pressure polyethylene plastic bags and placed into the sample chamber of an ultra-high pressure extraction unit. Extraction was carried out at the preset pressure for a specified duration. After extraction, the samples were centrifuged at 8000 r/min for 15 min, and the supernatant was collected for polysaccharide content determination. The extraction yield of polysaccharides from *E. folium* was calculated according to Eq. 1.

$$Y = (m_2 / m_1) \times 100\% \dots\dots\dots (1)$$

Where Y represents the extraction yield of polysaccharides from *Eriobotryae Folium* (%), m_1 is the mass of the raw *E. folium* material (g), and m_2 is the mass of polysaccharides extracted by ultra-high pressure (g).

The polysaccharide content in *E. folium* was determined using the phenol–sulfuric acid method, as previously reported (Peng *et al.* 2021).

Using the extraction yield of polysaccharides from *E. folium* as the evaluation index, single-factor experiments were conducted with the selected optimal DES system. On the basis of a DES water content of 40%, an extraction pressure of 400 MPa, and an extraction time of 10 min, the effects of DES water content, extraction pressure, and extraction time on polysaccharide yield were investigated individually.

Based on the appropriate conditions preliminarily determined by the single-factor experiments, DES water content (A), extraction pressure (B), and extraction time (C) were selected as independent variables, while the extraction yield of polysaccharides from *E. folium* was taken as the response value. A response surface optimization experiment was then carried out, and the levels of the experimental factors are shown in Table 1. In the subsequent response surface experiments, the A, B, C columns in Table 2 use the coded levels (−1, 0, 1) as defined in Table 1.

Table 1. Level of factors in response surface experiment.

Level	Factor		
	A DES water content (%)	B Extraction pressure (MPa)	C Extraction time (min)
−1	35	350	9
0	40	400	10
1	45	450	11

The extract of *E. folium* was concentrated to a certain volume, and three volumes of 75% ethanol were added. The mixture was homogenized and left to stand at 4°C for 24 hrs. The precipitate was collected, vacuum-dried, and then dissolved to prepare polysaccharide solutions at concentrations of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml.

For the DPPH radical scavenging assay, 2 ml of each polysaccharide solution was mixed with 2 ml of DPPH solution (0.2 mmol/l) and thoroughly vortexed. The mixture was incubated at 25°C in the dark for 30 min, after which the absorbance was measured at 517 nm. Vitamin C solutions at equivalent concentrations were used as a positive control. The DPPH scavenging rate (D_1) was calculated as Eq. 2.

$$D_1 (\%) = (1 - (A_1 - A_2) / A_0) \times 100\% \dots\dots\dots(2)$$

Where A_0 is the absorbance of the DPPH solution alone, A_1 is the absorbance of the mixture containing both sample and DPPH, and A_2 is the absorbance of the sample solution alone.

The extract of *E. folium* was concentrated to a certain volume, and three volumes of 75% ethanol (based on the volume of the concentrated extract) were added. The mixture was homogenized and left to stand at 4°C for 24 hrs. The precipitate was collected, vacuum-dried, and then dissolved to prepare polysaccharide solutions at concentrations of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml (based on solution volume).

For the hydroxyl radical ($\cdot\text{OH}$) scavenging assay, 2 ml of each polysaccharide solution was mixed with 1 ml of FeSO_4 solution (5 mmol/l), 1 ml of salicylic acid–ethanol solution, and 1 ml of H_2O_2 solution. The mixture was thoroughly vortexed and incubated at 37°C in the dark for 30 min. The absorbance was measured at 510 nm. Vitamin C solutions at equivalent concentrations were used as a positive control. The hydroxyl radical scavenging rate (D_2) was determined by the Eq. 3.

$$D_2 (\%) = [1 - (A_4 - A_5)/A_3] \times 100\% \dots\dots\dots(3)$$

Where A_3 is the absorbance of the system containing only pure water, A_4 is the absorbance of the reaction mixture containing sample, FeSO_4 , salicylic acid–ethanol, and H_2O_2 , and A_5 is the absorbance of the sample solution alone.

In the single-factor experiments for polysaccharide extraction and the DPPH/ $\bullet\text{OH}$ scavenging activity assays, each data point was determined in triplicate ($n=3$), and the results were expressed as mean $\pm$ standard deviation (Mean $\pm$ SD). All statistical significance tests were performed using SPSS 26.0 software. Statistical significance letters were assigned based on the multiple comparison results of Duncan's multiple range test, with a significance level of $P < 0.05$. The experimental design and data analysis for response surface methodology were carried out using Design-Expert 13.0 software

Results and Discussion

As shown in Fig. 1, among the five DES systems tested, the choline chloride–ethylene glycol system (DES-3) achieved the highest extraction yield of polysaccharides from *Eriobotryae Folium*, reaching 6.12%. This result can be attributed to the differing polarities of the DES systems; the polarity of DES-3 closely matches that of *E. folium* polysaccharides, and according to the “like dissolves like” principle, this enhances polysaccharide solubility. In addition, the viscosity characteristics of different DES systems vary, and the moderate viscosity of DES-3 facilitates polysaccharide diffusion (Huang *et al.* 2020). Therefore, DES-3 was selected as the solvent for the subsequent optimization of polysaccharide extraction from *E. folium*.

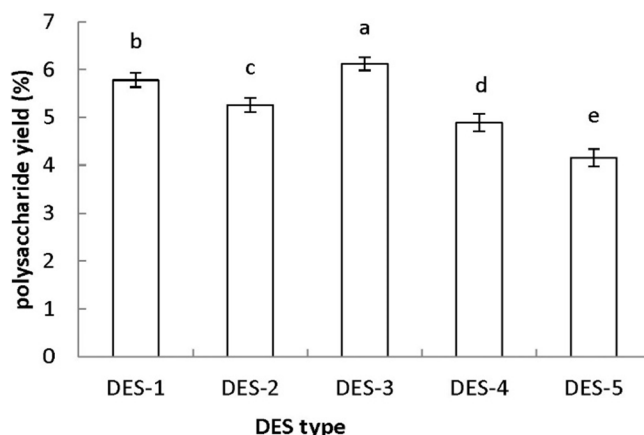


Fig. 1. Effect of different DES types on polysaccharide yield from *Eriobotryae folium*.

The univariate experimental results revealed significant insights into the optimization of polysaccharide extraction using aqueous DES under UHP. Each factor–water content, pressure, and time–exhibited a non-linear relationship with extraction yield, underscoring the complexity of the underlying physiochemical processes.

As depicted in Fig. 2(a), the polysaccharide yield from *E. folium* increased with rising DES water content up to 40%; however, further increases beyond 40% led to a decline in yield. This behavior can be explained by two competing effects: increasing water content reduces the viscosity of the DES, which facilitates polysaccharide diffusion, but excessive water disrupts the hydrogen-bonding network of the DES, thereby weakening the extraction efficiency (Chen *et al.* 2021). Based on these results, water contents of 35, 40, and 45% were selected for the subsequent response surface experiments.

The extraction yield of polysaccharides from *E. folium* increased continuously as the extraction pressure rose from 100 MPa to 400 MPa, reaching a maximum yield of 6.12% at 400 MPa Fig. 2(b). This increase is attributed to high pressure causing disruption of cell walls, membranes, and organelles, which facilitates the release of polysaccharides from the cells. However, when the pressure was further increased from 400 MPa to 500 MPa, the polysaccharide yield decreased. Excessive pressure leads to over-disruption of cells, releasing a large amount of impurities that interfere with polysaccharide release (Bai *et al.* 2024). Therefore, extraction pressures of 350, 400, and 450 MPa were selected for the subsequent response surface experiments.

As shown in Fig. 2(c), the polysaccharide yield from *E. folium* increased with prolonged extraction time, reaching the highest yield at 10 min. Beyond 10 min, further extension of the extraction time did not result in a significant increase in yield. Under ultra-high pressure conditions, the dissolution and diffusion of polysaccharides in the DES solvent require a certain period, but once the extraction time exceeds 10 min, these processes reach equilibrium, and the yield plateaus. Therefore, extraction times of 9, 10, and 11 min were selected for the subsequent response surface experiments.

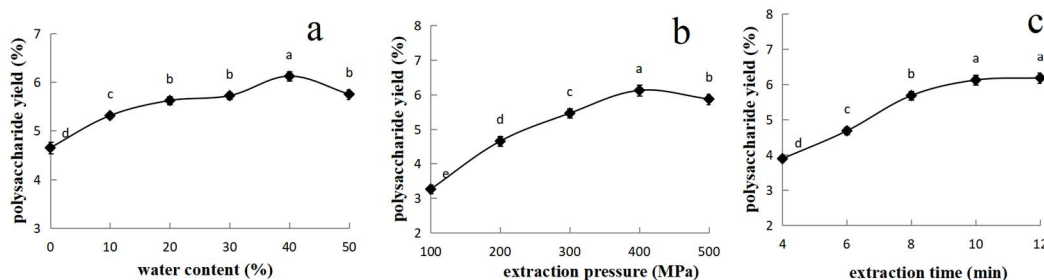


Fig. 2. Impact of different DES parameters on the extraction yields of polysaccharide.

Table 2. Design and results of response surface test.

Run	Water content/%	Extraction pressure /MPa	Extraction time /min	Polysaccharide yield /%
1	0	0	0	6.15
2	0	-1	-1	4.66
3	0	0	0	5.87
4	-1	1	0	5.69
5	0	1	1	5.21
6	1	0	1	4.65
7	-1	0	-1	4.96
8	0	-1	1	4.58
9	0	0	0	6.17
10	0	1	-1	5.05
11	1	0	-1	5.12
12	-1	-1	0	4.78
13	1	1	0	5.37
14	0	0	0	6.16
15	1	-1	0	6.09
16	-1	0	1	4.83
17	0	0	0	6.11

The response surface experimental design and results for the extraction of polysaccharides from *E. folium* are presented in Table 2. Using Design Expert software, the data in Table 2 were fitted, resulting in the following regression equation for the extraction yield of polysaccharides from *E. folium* (Y):

$$Y = 6.09 + 0.12A + 0.15B - 0.065C - 0.41AB - 0.085AC + 0.06BC - 0.30A^2 - 0.31B^2 - 0.90C^2$$

The experimental data from Table 2 were analyzed using Design Expert software, with the ANOVA results presented in Table 3. The regression model exhibited statistical significance ($P < 0.05$), indicating high reliability and accuracy, while the lack-of-fit test was non-significant ($P > 0.05$), confirming minimal model error. In the fitted equation, the terms AB, A^2 , B^2 , and C^2 all had P-values < 0.05 , demonstrating that these factors and their interactions significantly affect polysaccharide yield. The model showed a coefficient of determination $R^2 = 94.35\%$, indicating a good fit between predicted and actual values, and an adjusted R^2 of 87.09%, suggesting that the model can predict the majority of observed variation, confirming its reasonableness.

The response surface plots for the interaction effects of water content (A), extraction pressure (B), and extraction time (C) are shown in Fig. 3.

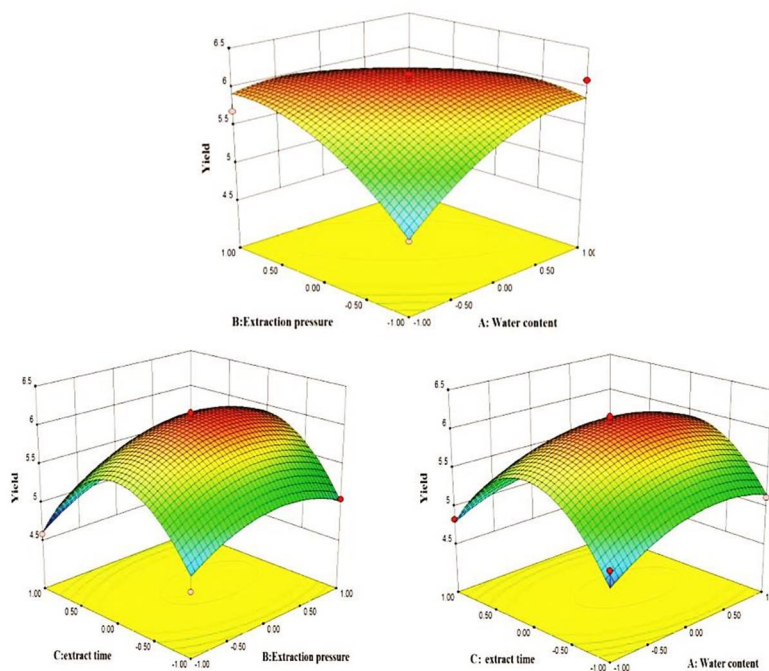


Fig. 3. Response surface plots for the interaction effects of water content (A), extraction pressure (B), and extraction time (C).

The response surface for the A-B interaction exhibits the steepest slope, indicating that the interaction between water content and extraction pressure has a significant effect on the extraction yield. The surface shows a pronounced convex shape with a clear extreme point. In contrast, the A-C interaction surface is relatively flat, suggesting a weaker interaction between extraction time and water content. The B-C interaction surface displays a slope between the two, indicating some degree of interaction, though less pronounced than that between A and B.

Optimization based on the model predicted the best extraction conditions as DES water content 45.00%, extraction pressure 379.06 MPa, and extraction time 9.90 min, with a predicted polysaccharide yield of 6.17%. For practical operation, these parameters were adjusted to 45% water content, 380 MPa pressure, and 10 min extraction time. Under these optimized conditions, three replicate experiments were conducted, yielding an average polysaccharide extraction rate of $(6.18 \pm 0.11) \%$, closely matching the predicted value. This validates the model's accuracy and demonstrates its practical applicability for guiding the extraction of polysaccharides from *E. folium*.

Table 3. Variance analysis of the modela.

Source of variance	Sum of squares	Degree of freedom	Mean square	F-Value	P-Value	Significance
Model	5.60	9	0.62	13.00	0.0014	*
A	0.12	1	0.12	2.46	0.1610	
B	0.18	1	0.18	3.82	0.0915	
C	0.034	1	0.034	0.71	0.4285	
AB	0.66	1	0.66	13.87	0.0074	*
AC	0.029	1	0.029	0.60	0.4626	
BC	0.014	1	0.014	0.30	0.6004	
A ²	0.37	1	0.37	7.77	0.0270	*
B ²	0.41	1	0.41	8.58	0.0221	*
C ²	3.45	1	3.45	72.00	< 0.0001	*
Residual	0.34	7	0.048			
Lack of fit	0.27	3	0.090	5.68	0.0633	
Pure error	0.064	4	0.016			
Total deviation	5.93	16				

* = indicates a significant effect ($P < 0.05$).

DPPH scavenging results are shown in Fig. 4(a). As shown in the figure, the DPPH radical scavenging ability of *E. folium* polysaccharides increased significantly with concentration up to 0.6 mg/ml ($P < 0.05$). Beyond 0.6 mg/ml, further increases in concentration did not result in a significant improvement in scavenging activity ($P > 0.05$). At 0.6 mg/ml, the polysaccharides achieved a DPPH scavenging rate of 63.5%, demonstrating substantial antioxidant capacity. However, compared with vitamin C solutions at the same concentration, the scavenging ability of the polysaccharides remained lower than that of vitamin C.

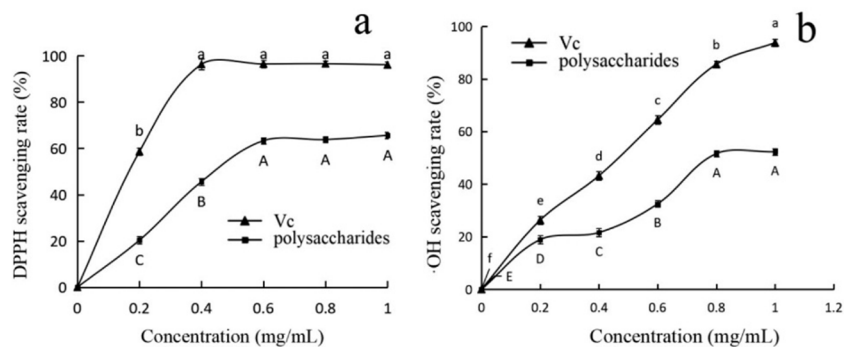


Fig. 4. Impacts of concentration on DPPH scavenging (a) and OH scavenging (b). Different lowercase letters indicate significant differences in scavenging rate at the same concentration ($P < 0.05$); different uppercase letters indicate significant differences in scavenging rate of the same polysaccharide at different concentrations ($P < 0.05$).

OH scavenging results are shown in Fig. 4(b). As shown in the figure, the $\cdot\text{OH}$ radical scavenging ability of *E. folium* polysaccharides increased with concentration up to 0.8 mg/ml, but beyond this concentration, further increases did not result in a significant improvement in scavenging activity ($P > 0.05$). At 0.8 mg/ml, the polysaccharides achieved a $\cdot\text{OH}$ scavenging rate of 51.6%, demonstrating substantial antioxidant capacity. However, compared with vitamin C solutions at the same concentration, the scavenging ability of the polysaccharides remained lower than that of vitamin C.

The optimal conditions for extracting *E. folium* polysaccharides using ultra-high pressure-assisted DES were determined as 45% DES water content, 380 MPa extraction pressure, and 10 min extraction time. Under these optimized conditions, three replicate experiments yielded a polysaccharide extraction rate of $(6.18 \pm 0.11) \%$. The *E. folium* polysaccharides obtained via UHP-DES exhibited increasing DPPH and $\cdot\text{OH}$ radical scavenging activities with rising polysaccharide concentration, demonstrating substantial antioxidant capacity against both DPPH and $\cdot\text{OH}$ radicals. These results indicate that UHP-assisted DES extraction is a suitable and effective method for the preparation of *E. folium* polysaccharides.

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