Optimization of Enzyme-assisted Extraction of Polyphyllins from Paris polyphylla var. yunnanensis Rhizomes Using Response Surface Methodology

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Abstract

Paris polyphylla Smith var. *yunnanensis* (Franch.) Hand.-Mazz. (*P. polyphylla* var. *yunnanensis*) is a perennial herb of the genus *Paris*. As an important medicinal resource, *P. polyphylla* var. *Yunnanensis* is facing exhaustion due to the high demand and its specific growth characteristics. To efficiently utilize its resources, the response surface methodology (RSM) was utilized to optimize the enzyme-assisted extraction process of polyphyllins from its rhizome, with the total extraction content of polyphyllin I, II, and VII as the evaluation index. The optimal conditions were as follows: extraction temperature of 52°C, extraction time of 34 min, and solid-to-liquid ratio of 1:19 g/mL. Under these conditions, the total content of the three polyphyllins was 29.70 mg/g, which was close to the predicted value of 29.90 mg/g and represented an increase of 27.63% over the control group. The analysis of variance (ANOVA) showed that the RSM model exhibited a good fit, and the Box-Behnken design (BBD) could be applied to optimize the extraction process of polyphyllins. This study provides a theoretical basis and a reference approach for the efficient utilization of *P. polyphylla* var. y*unnanensis* resources.

1. Introduction

Paris polyphylla Smith var. *yunnanensis* (Franch.) Hand.-Mazz. (*P. polyphylla* var. *yunnanensis*) is a perennial herb of the genus *Paris*, which is mainly distributed in the southwestern region of China. Its dried rhizome, "Chonglou", has been recorded as an important traditional Chinese medicine in the *Pharmacopoeia of the People's Republic of China*[1]. *P. polyphylla* var. *yunnanensi*s has a wide range of pharmacological effects and serves as a core raw material for well-known Chinese patent medicines, such as “Yunnan Baiyao”, “Gong Xue Ning”, “Jidesheng Sheyao Tablets”[2, 3]. Modern pharmacological studies have shown that *P. polyphylla* var. *yunnanensis* contains various chemical components, including steroidal saponins, flavonoids, phytosterols, etc. Among them, polyphyllin I (PPI), II (PPII), and VII (PPVII) are the main active ingredients of *P. polyphylla* var. y*unnanensis* (Fig. 1).

Their total content (≥ 0.6%) is one of the important indicators for evaluating the quality of *P. polyphylla* var. *yunnanensis* in the *Chinese Pharmacopoeia* (2020 edition)[2, 4]. In addition, polyphyllins exhibit multiple pharmacological properties, including anti-tumor, anti-viral, hemostatic, anti-bacterial, and anti-inflammatory effects[5, 6]. Studies of PPI in various types of cancers have shown that it exerts a broad range of anti-tumor effects, including inducing cell apoptosis, inducing cell cycle arrest, and anti-angiogenesis. In the targeted therapy of breast cancer, gastric cancer, and ovarian cancer, PPI exhibits significant anticancer properties[7, 8]. PPII has anti-proliferation properties in liver cancer cells, lung cancer cells, and colorectal cancer cells and can induce apoptosis of liver cancer cells[9, 10]. PPVII can induce apoptosis of lung cancer cells and has a unique anti-inflammatory effect both in vitro and in vivo[11]. As pharmacological research advances, *P. polyphylla* var. *yunnanensis* has been widely used in the medical field, and the market demand for it keeps rising. However, due to factors such as over-excavation, ecological environment changes, and its unique growth characteristics, the natural resources of *P. polyphylla* var. *yunnanensis* have been on the verge of exhaustion in the past few decades[12].

Therefore, it is necessary to explore efficient methods for extracting polyphyllins and achieve the sustainable utilization of *P. polyphylla* var. *yunnanensis* resources.

Traditional extraction methods suffer from drawbacks such as long extraction time, low extraction efficiency, and poor-quality extracts. Meanwhile, apart from soluble compounds, plant cell walls also contain some poorly soluble polysaccharides, such as pectin, hemicellulose, and starch, which are difficult to leach out using traditional extraction methods[13, 14]. In the field of biomolecule extraction technology, enzymes serve as ideal biocatalysts that can hydrolyze or disrupt plant cell walls, thereby releasing the intracellular components and significantly increasing the extraction rate of target compounds. Moreover, compared with other extraction methods, enzyme-assisted extraction is more environmentally friendly, has a shorter extraction time, and is easier to operate. Therefore, this method has gradually replaced the traditional solvent extraction method and become an efficient and environmentally friendly extraction method[15, 16].

Currently, enzymes such as cellulase, β-glucosidase, and pectinase are widely used in the extraction of bioactive substances from plants[17]. Cellulase is a hydrolytic enzyme that randomly cleaves internal bonds within cellulose chains, eventually releasing the glucose monomers[18, 19]. β-glucosidase is ubiquitous in nature, and for some bioactive components in plants that exist in the form of glycosides, it can hydrolyze glycosidic bonds to release β-D glucosyl residues, thereby improving their extraction efficiency[20, 21]. Pectinase is a crucial depolymerase that can decompose pectin into galacturonic acid and simultaneously reduce the viscosity of cells, making the resulting solution clearer and facilitating subsequent processing and separation[19, 22]. Given their functions, we screened the enzyme most effective at enhancing the extraction of polyphyllins from among the three enzymes mentioned above for further research.

The efficiency of extracting chemical components from plants is affected by various factors. When using enzymes to assist in the extraction of polyphyllins, factors such as temperature, time, and solid-to-liquid ratio usually have an impact on the extraction efficiency. Therefore, optimizing these conditions is crucial for improving the efficiency of enzyme-assisted extraction[23]. Response surface methodology (RSM) is a mathematical and statistical technique widely used to optimize extraction processes, which is commonly employed for modeling and optimizing the extraction of bioactive compounds. Compared with single-variable optimization methods, RSM can evaluate interactions between various factors and predict the optimal performance conditions with fewer experiments, thereby saving time and costs[24–26].

This study used the rhizomes of *P. polyphylla* var. *yunnanensis* as experimental materials and set extraction time, extraction temperature, and solid-to-liquid ratio as three experimental variables. The Box-Behnken Design (BBD) response surface methodology was employed to optimize the enzyme-assisted extraction of PPI, PPII, and PPVII. The objective was to enhance the extraction efficiency of polyphyllins from *P. polyphylla* var. *yunnanensis*, thereby promoting the sustainable utilization of its medicinal

resources and addressing the growing market demand. This study provides a theoretical basis and practical guidance for the sustainable utilization of *P. polyphylla* var. *yunnanensis* resources.

2. Results and Analysis

2.1. Preliminary Experiment

A preliminary experiment was conducted to evaluate the effects of enzyme types (cellulase, β-glucosidase, and pectinase) and concentrations (0.5 and 1 mg/mL) on the contents of three polyphyllins. As shown in Fig. 2, all experimental groups exhibited significantly higher content of PPI, PPII, and PPVII compared to the control group ($p < 0.05$). Specifically, PPI showed the highest content when treated with 1mg/mL β-glucosidase (Fig. 2A). The content of PPII in both 0.5 mg/mL pectinase and 1 mg/mL β-glucosidase was significantly higher than that in other treatments, with no significant difference between them (Fig. 2B). For PPVII, although exhibit no significant differences among enzyme treatments, the 0.5 mg/mL pectinase group showed a tendency toward higher values (Fig. 2C).

Although 1 mg/mL β-glucosidase exhibited slightly higher efficiency, 0.5 mg/mL pectinase was selected as the optimal choice for subsequent enzyme-assisted extraction experiments after comprehensive consideration of extraction efficiency and industrial production. Mechanistically, pectinase specifically hydrolyzes the α-1,4-galactosidic bonds in polygalacturonic acid, a major component of the plant cell wall matrix, thereby facilitating cell wall disintegration and enhancing the release of polyphyllins[27]. Economically, the unit activity cost of β-glucosidase is approximately 7–10 times higher than that of pectinase[28, 29]. In large-scale extraction processes, the incremental yield gains from β-glucosidase are outweighed by its prohibitive costs. Therefore, 0.5 mg/mL pectinase was identified as the optimal choice.

2.2. Single Factor Experiments

Three variables were investigated: extraction time (30, 60, 90, and 120 min), extraction temperature (40, 45, 50, and 55°C), and solid-to-liquid ratio (1:5, 1:10, 1:20, and 1:30 g/mL). To evaluate the effects of each factor on the content of PPI, PPII, and PPVII, only one variable was altered at a time while keeping other parameters constant.

Enzyme-assisted extraction time is a critical determinant of extraction efficiency, as it influences the interaction between the solvent and target compounds[30]. As shown in Fig. 3A, all three polyphyllins reached their maximum content at 30 minutes. When the extraction time exceeded 30 minutes, the content consistently declined, which can be attributed to the time-dependent degradation of polyphyllins under prolonged treatment[31]. Based on these findings, 30 minutes was selected as the central value for subsequent RSM experiments, with 15 and 60 min designated as the low and high levels, respectively.

As shown in Fig. 3B, enzyme-assisted extraction temperature significantly influenced the contents of PPI, PPII, and PPVII. As the temperature rose from 40°C to 45°C, the contents of the three polyphyllins increased significantly. This phenomenon is likely due to enhanced enzyme activity and molecular motion at elevated hydrolysis temperatures, which accelerate the mass transfer of intracellular substances[32]. When the temperature was further increased to 55°C, there was no significant difference in the contents of PPI and PPII. However, the content of PPVII first increased and then decreased within the range of 45–55°C, reaching its maximum at 50°C. Structurally, saponins in plants have diverse structures due to the presence of different sugars at different locations and orientations. The structural differences among polyphyllin I, II, and VII may affect their stability at different temperatures, thereby leading to variations in the changing trends of saponin contents[33, 34]. Therefore, 50°C was set as the central value, with 40 and 60°C designated as the low-level and high-level values, respectively.

The solid-liquid ratio significantly influences the efficiency of enzyme-assisted extraction. Generally, a high solvent-to-sample ratio can reduce enzyme activity and stability due to excessive water dilution, thereby decreasing the degradation of plant cell structures by enzymes and causing the extraction amount to stabilize or decline[35, 36]. As shown in Fig. 3C, no obvious differences were observed among the treatments for PPI, PPII, and PPVII with increasing solid-liquid ratios. To systematically investigate the interactive effects of extraction conditions on the content of polyphyllins, a relatively wide range of experimental levels was selected. Specifically, solid-liquid ratios of 1:5 and 1:30 g/mL were chosen as the low and high levels for the response surface methodology test.

2.3 Establishment of the Regression Equation and ANOVA

The response surface experimental design and results are presented in Table 1. To analyze the effect of independent variables on three polyphyllins, the experimental data were fitted through multiple regression analysis using Design-Expert 13 software.

$$Y = 29.84 + 0.3007A - 0.2609B + 0.1311C - 0.0931AB + 0.2463AC + 0.2425BC - 0.6228A^2 - 0.4871B^2 - 0.4571C^2$$

where A represents solid-to-liquid ratio, B represents extraction time, C represents extraction temperature. In this equation, the absolute values of the coefficients directly reflect the influence magnitude of each factor on the response value, while the positive or negative signs of the coefficients indicate the direction of the effect.

Table 1
Box-Behnken experimental design and results of polyphyllins

Run	A Solid-to-liquid ratio (g/mL)	B Time (min)	C Temperature (℃)	Polyphyllins content (mg/g)
1	-1	-1	0	28.62
2	1	-1	0	29.39
3	-1	1	0	28.25
4	1	1	0	28.64
5	-1	0	-1	28.53
6	1	0	-1	28.66
7	-1	0	1	28.36
8	1	0	1	29.48
9	0	-1	-1	29.27
10	0	1	-1	28.31
11	0	-1	1	28.99
12	0	1	1	28.99
13	0	0	0	29.75
14	0	0	0	29.88
15	0	0	0	29.90
16	0	0	0	29.86
17	0	0	0	29.79

To validate the effectiveness of the constructed model, analysis of variance (ANOVA) was performed. One of the fundamental assumptions for ANOVA validity is that the residuals follow a normal distribution[37]. As shown in Fig. 4A, the sample points are uniformly distributed around the best-fit line in the normal probability plot of the residuals. This distribution pattern indicates that the residuals exhibit a normal distribution without obvious deviation. As shown in Fig. 4B, the fitted line between the model-predicted values and experimental actual values is nearly linear. This indicates good consistency between the second-order polynomial regression model and experimental results, reflecting the excellent fitting performance of the model. According to the ANOVA results in Table 2, the regression model is highly significant ($p < 0.01$), and the lack of fit is not significant ($p = 0.4553 > 0.05$), thus confirming the validity of the quadratic model. The correlation coefficient R^2 of the model is 0.9952, which indicates that

there is an excellent correlation between experimental and predicted values. Meanwhile, the low coefficient of variation (CV) value (0.22%) signifies the high precision and reliability of the experimental data. The results of the model's significance test show that the main terms A and B, as well as the square terms A^2, B^2 and C^2, exhibit extremely significant effects ($p < 0.001$). The main term C and the interaction terms AC and BC reach a highly significant level ($p < 0.01$). The effects of the interaction term AB reach a significant level ($p < 0.05$). The effects of each factor on the extraction yield of polyphyllins can be ranked in the following order: solid-to-liquid ratio (A) > time (B) > temperature (C).

Table 2
Analysis of variance (ANOVA) for the regression equation of total polyphyllins

Source	Sum of Squares	df	Mean Square	F-Value	p-Value
Model	5.8300	9	0.6480	159.8800	< 0.0001***
A-Solid-to-liquid ratio	0.7235	1	0.7235	178.5000	< 0.0001***
B-Extraction time	0.5444	1	0.5444	134.3300	< 0.0001***
C-Extraction temperature	0.1374	1	0.1374	33.9100	0.0006**
AB	0.0347	1	0.0347	8.5500	0.0222*
AC	0.2427	1	0.2427	59.8700	0.0001**
BC	0.2351	1	0.2351	58.0100	0.0001**
A^2	1.6300	1	1.6300	402.9400	< 0.0001***
B^2	0.9990	1	0.9990	246.4700	< 0.0001***
C^2	0.8799	1	0.8799	217.1000	< 0.0001***
Residual	0.0284	7	0.0041		
Lack of fit	0.0126	3	0.0042	1.0700	0.4553
Pure error	0.0157	4	0.0039		
Cor total	5.8600	16			

* indicates significant difference ($p < 0.05$); ** indicates highly significant ($p < 0.01$); *** indicates extremely significant ($p < 0.0001$).

2.4 Analysis of Response Surface Interactions

The three-dimensional (3D) and two-dimensional (2D) response surface plots from regression analysis visually illustrate the interactions among factors (Fig. 5). Generally, the intensity of factor interactions can be intuitively assessed by curve characteristics: steeper response surface slopes, elliptical contour shapes, and denser contour distributions indicate stronger interactive effects on the response value[38–40]. Comparative analysis of two-factor interactions revealed that the response surface curves of

extraction time and solid-to-liquid ratio (Fig. 5A), solid-to-liquid ratio and extraction temperature (Fig. 5B), and extraction time and temperature (Fig. 5C) were all steep, with their 2D contour plots exhibiting elliptical shapes and dense distributions. This indicates significant interactive effects among the three factors on polyphyllins. As shown in Fig. 5A, the content of polyphyllins first increased and then decreased with increasing solid-to-liquid ratio and extraction time, reaching its maximum at moderate levels of both factors. In Fig. 5B, the steeper 3D surface of the solid-to-liquid ratio compared to temperature shows a more dominant effect on polyphyllins under their interactive influence. Figure 5C demonstrates a decline in the content of polyphyllins during prolonged extraction, likely due to structural degradation of polyphyllins under high-temperature conditions over extended periods, combined with gradual decline in enzyme activity that reduces the release of polyphyllins[41].

2.5 Model Validation

A three-factor, three-level BBD was employed to optimize the extraction process of polyphyllins. The optimal parameters were determined as follows: extraction time of 34.38 min, extraction temperature of 51.69°C, and solid-to-liquid ratio of 1:19.18 g/mL. Under these conditions, the theoretical total content of the three polyphyllins (PPI, PPII, and PPVII) was 29.90 mg/g. Considering the convenience and feasibility of practical operations, the parameters were adjusted to extraction time of 34 min, extraction temperature of 52°C, and solid-to-liquid ratio of 1:19 g/mL. Three parallel validation experiments were conducted under the adjusted conditions (Table 3). The total content of polyphyllins was 29.70 mg/g (RSD = 0.79%), which closely matched the predicted value with a relative error of 0.67%. Compared with the control group without added enzyme, the content was increased by 27.63%. The results demonstrate excellent consistency between experimental and predicted values, validating the high fitting accuracy of the RSM model. Therefore, this model can be effectively applied to optimize the extraction process of polyphyllins from *P. polyphylla* var. y*unnanensis*.

Table 3
Results of enzyme-assisted extraction validation tests.

Group	Content* (mg/g)	Predicted content (mg/g)	RSD (%)	Increase rate (%)
Control group	23.27 ± 0.10	-	0.41	27.63
Experimental group	29.70 ± 0.24	29.90	0.79	

* Each value is the mean of triplicate measure, n = 3.

3. Materials and Methods

3.1 Materials and Reagents

The *P. polyphylla* var. *yunnanensis* plants were purchased from Kunming Jiange Chinese Herbal Medicine Planting Co., Ltd. (Kunming, China). The rhizomes of fresh plants were collected, washed to remove

impurities and soil, and air-dried. Fresh weight was recorded, followed by drying in a thermostatic drying oven (DHG-9145A, Shanghai Yiheng Scientific Instruments Co., Ltd., Shanghai, China) at 50°C. The dried rhizomes were then powdered using a grinder (BJ-800A, Shanghai Baijie Industrial Co., Ltd., Shanghai, China), and sieved to remove incompletely crushed particles.

Pectinase, cellulase, and β-glucosidase were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Analytical-grade ethanol and methanol were obtained from Guangdong Guanghua Technology Co., Ltd. (Guangdong, China). Chromatographic acetonitrile was purchased from Beijing J&K Scientific Co., Ltd. (Beijing, China). Reference standards of PPI, PPII and PPVII (purity > 98%) were purchased from Shanghai Standard Biotech Co., Ltd. (Shanghai, China).

3.2 Determination of Polyphyllin Content

An external standard method was employed for quantitative analysis in this experiment. Reference standards (PPI, PPII, and PPVII) were dissolved in methanol to prepare 1 mg/mL solutions, which were then serially diluted to 0.5, 0.25, 0.125, and 0.0625 mg/mL. The standard curves were plotted with the concentration of PPI, PPII, and PPVII as the X-axis and the peak area as the Y-axis. The results are shown in Fig. 6, and the regression equations are as follows:

$y_1 = 3928.7x + 37.466$ ($R^2 = 0.9996$)

$y_2 = 2899x + 25.202$ ($R^2 = 0.9992$)

$y_3 = 3078.7x + 37.704$ ($R^2 = 0.9998$)

where Y_1 is PPI, Y_2 is PPII,Y_3 is PPVII, and X is the independent variable.

The content of PPI, PPII, and PPVII was detected using high performance liquid chromatography (HPLC) (Agilent Technologies, Inc., Santa Clara, CA, USA). The instrument was equipped with a quaternary pump (G1311A, GER), an autosampler (G1329A, GER), a DAD UV detector (G1315D, GER), and an EC-C18 column (250 mm × 4.6 mm id; 4 μm). The detection parameters were set as follows: ultrapure water as mobile phase A and acetonitrile as mobile phase B; injection volume of 10 μL; detection wavelength set at 203 nm; column temperature set at 30°C; flow rate controlled at 1.0 mL/min; and analysis time of 25 min. The elution program is shown in Table 4.

Table 4
HPLC elution program

Time(min)	Water (%)	Acetonitrile (%)
0	57	43
13	57	43
14	45	55
25	45	55

3.3 Preliminary Experiment

A total of 0.5 g rhizome powder was treated with 5 mL of enzyme solutions containing pectinase, cellulase, and β-glucosidase at concentrations of 0.5 mg/mL and 1 mg/mL, respectively. The mixture was extracted at 50°C for 30 minutes using an ultrasonic cleaner (K5210HP, Shanghai KD Ultrasonic Instrument Co., Ltd., China). After the extraction was completed, the mixture was centrifuged at 12,000 rpm for 10 minutes to collect the supernatant. The remaining precipitate was further extracted three times with 5 mL of 75% ethanol. The extract was then concentrated to dryness at 50°C using a rotary evaporator (N-1300, Shanghai Ailang Instrument Co., Ltd., China), and the residue was dissolved with 6 mL of 75% ethanol. Extraction with 75% ethanol without enzyme treatment was performed as a control. All sample solutions were filtered through a 0.22 μm microporous membrane before HPLC.

3.4 Single Factor Experiment

A total of 0.5 g rhizome powder was transferred into a centrifuge tube, and enzyme solution was added at specific solid-to-liquid ratio (1:5, 1:10, 1:20, 1:30 g/mL). Enzyme-assisted extraction was performed in an ultrasonic cleaner at specific temperature (40, 45, 50, 55°C) and time (30, 60, 90, 120 min), and then the supernatant was collected after centrifugation. The subsequent procedures (including ethanol extraction, concentration, and filtration) were performed as described in Section 3.3 and the experimental design is shown in Fig. 7.

3.5 Response Surface Design

Based on the results of preliminary experiments and single-factor experiments, a three-factor, three-level BBD was employed to optimize extraction parameters (Table 5). The experiment used 0.5 mg/mL pectinase as the catalyst, with solid-to-liquid ratio (A), extraction time (B), and extraction temperature (C) as independent variables, and the total content of PPI, PPII, and PPVII as the response variable. In the single-factor experiments, PPI, PPII, and PPVII were analyzed individually to determine the optimal operational ranges for each factor, thereby providing a foundation for subsequent response surface modeling. During the response surface design phase, the total PPI, PPII, and PPVII content was designated as the response variable, aligning with the *Chinese Pharmacopoeia* (2020 Edition) requirement that their total content not be less than 0.60%.

Table 5
Experimental factors and level design

Level	Factor		
	A (g/mL)	B (min)	C (℃)
-1	1:5.0	15.0	40.0
0	1:17.5	37.5	50.0
1	1:30.0	60.0	60.0

4. Conclusion

In this study, based on single-factor experiments for pectinase-assisted extraction of polyphyllins, the BBD was employed to optimize the enzyme-assisted extraction parameters of polyphyllins from the rhizome of *P. polyphylla* var. y*unnanensis.* The optimal extraction parameters were identified as: extraction time of 34 minutes, extraction temperature of 52°C, and solid-to-liquid ratio of 1:19 g/mL. The total content of PPI, PPII, and PPVII was 29.70 mg/g (RSD = 0.79%) under the conditions, which closely matched the predicted value of 29.90 mg/g with a relative error of 0.67%. In addition, their total content increased by 27.63% compared to the control group. This confirms excellent model fitting and demonstrates that the BBD is a reliable method for optimizing extraction processes of polyphyllins. This study provides a theoretical foundation and practical guidance for the efficient utilization of *P. polyphylla* var. y*unnanensis* in pharmaceutical, cosmetic, and food industries.

Declarations

Author contributions: Conceptualization and methodology, R.S. and X.H.; investigation, L.D., Y.J. and L.C.; resources and data curation, R.S and P.L.; writing—original draft preparation, L.D. and P.L.; writing—review and editing, L.D., P.L. and R.S.; supervision, R.S. and X.H. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest: The authors declare no competing interests.

Data availability: All data generated or analysed during this study are included in this published article.

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Figures

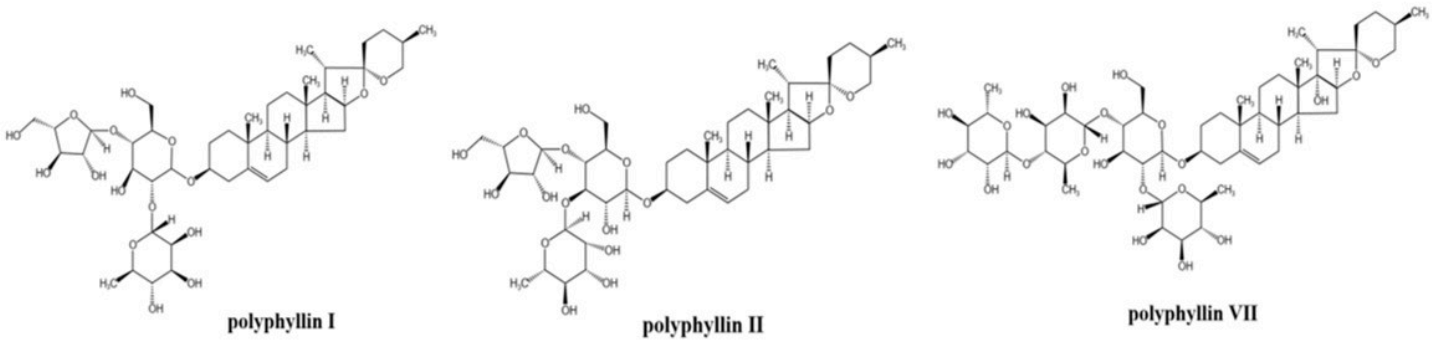

Figure 1

Chemical structures of PPI, PPII, and PPVII

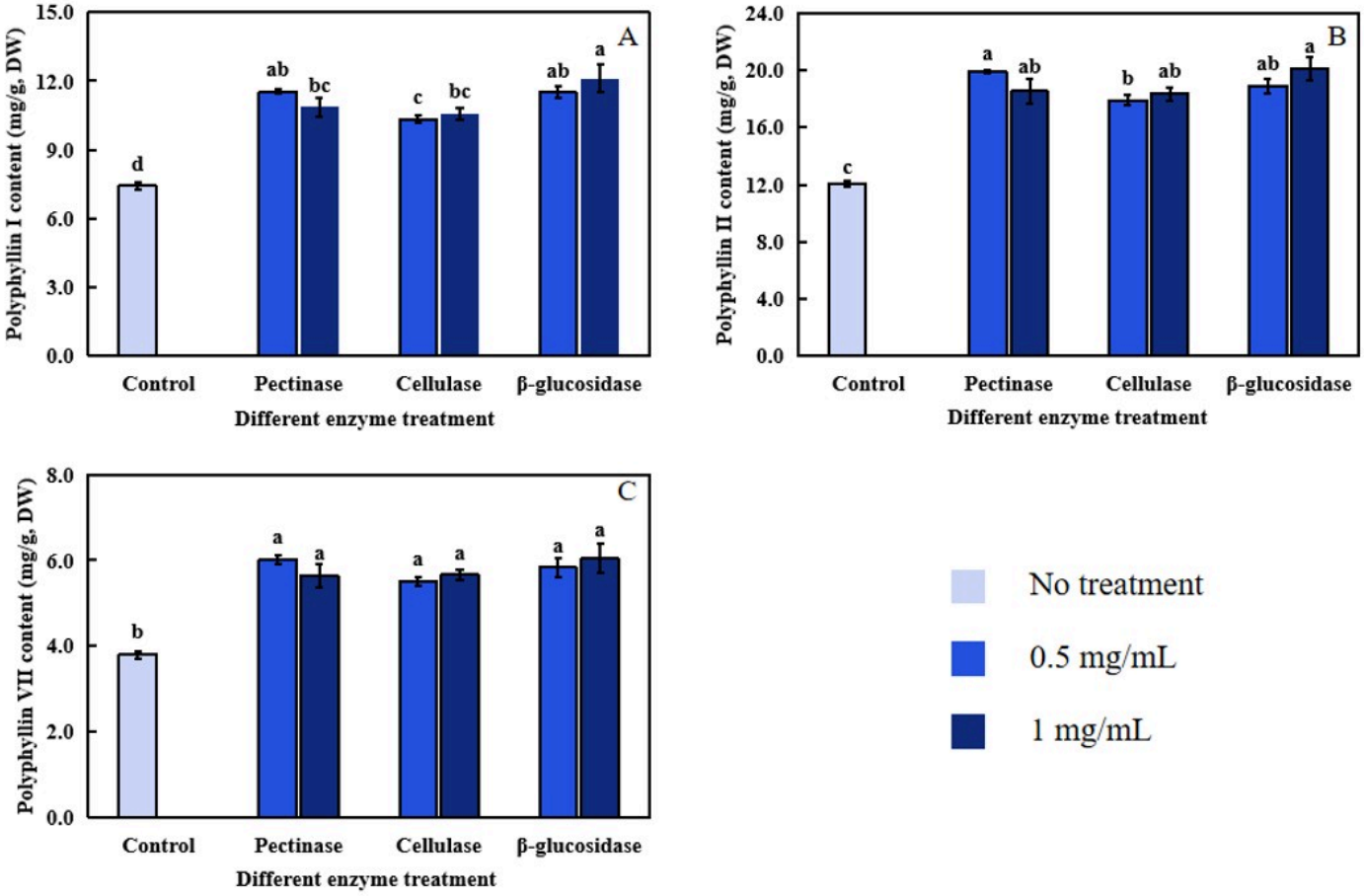

Figure 2

Effect of enzyme types and enzyme concentration on the contents of PP□(A), PP□(B) and PPVII(C). Different lower cases above the bar graph represent significant differences among polyphyllins content ($p < 0.05$). Each value is the mean of triplicate measure, n = 3

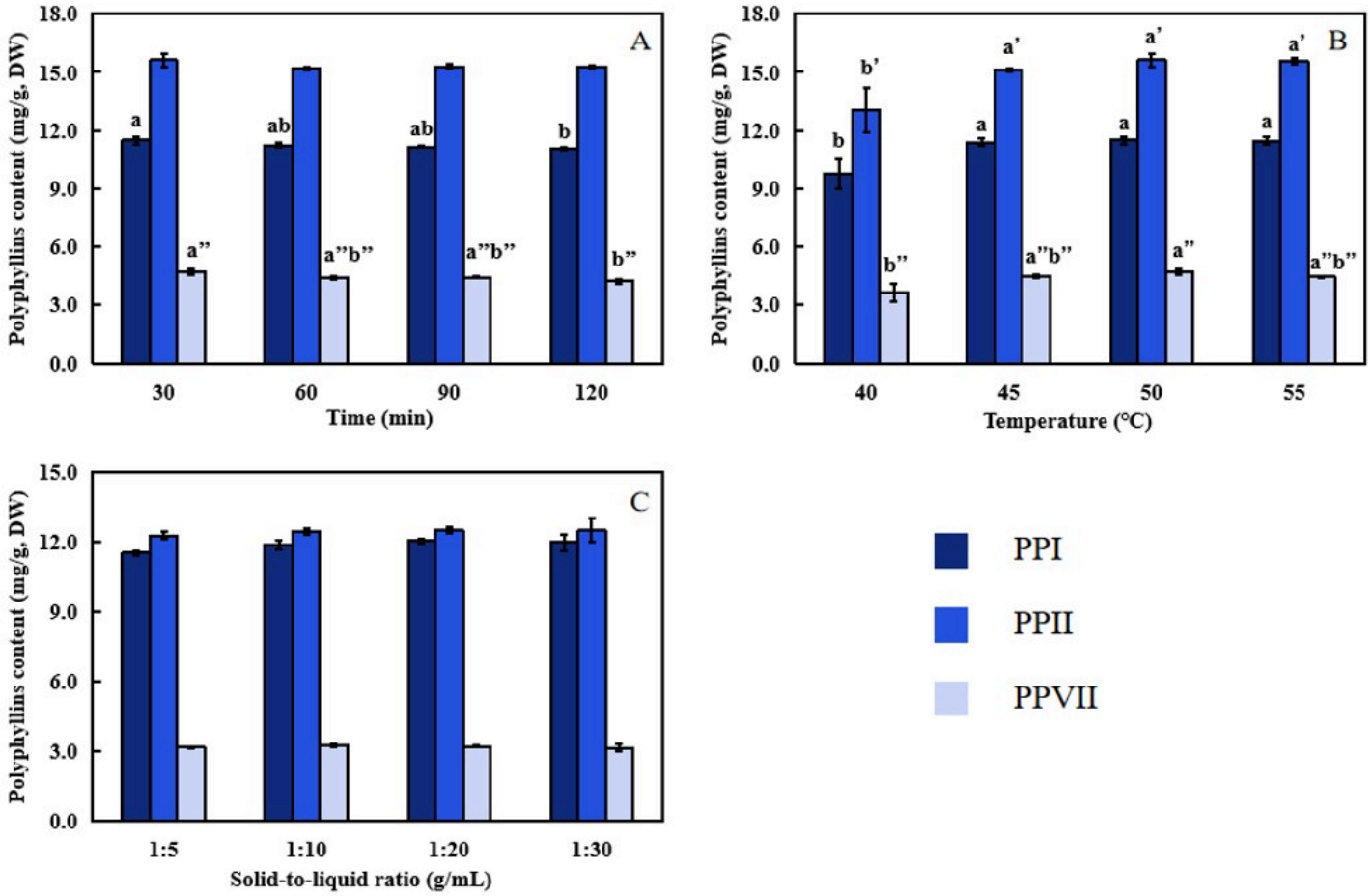

Figure 3

Effects of extraction time(A), extraction temperature(B) and solid-to-liquid ratio(C) on the content of PPI, PPII and PPVII. Different lower cases above the bar graph represent significant differences among saponins content ($p < 0.05$). Each value is the mean of triplicate measure, n = 3

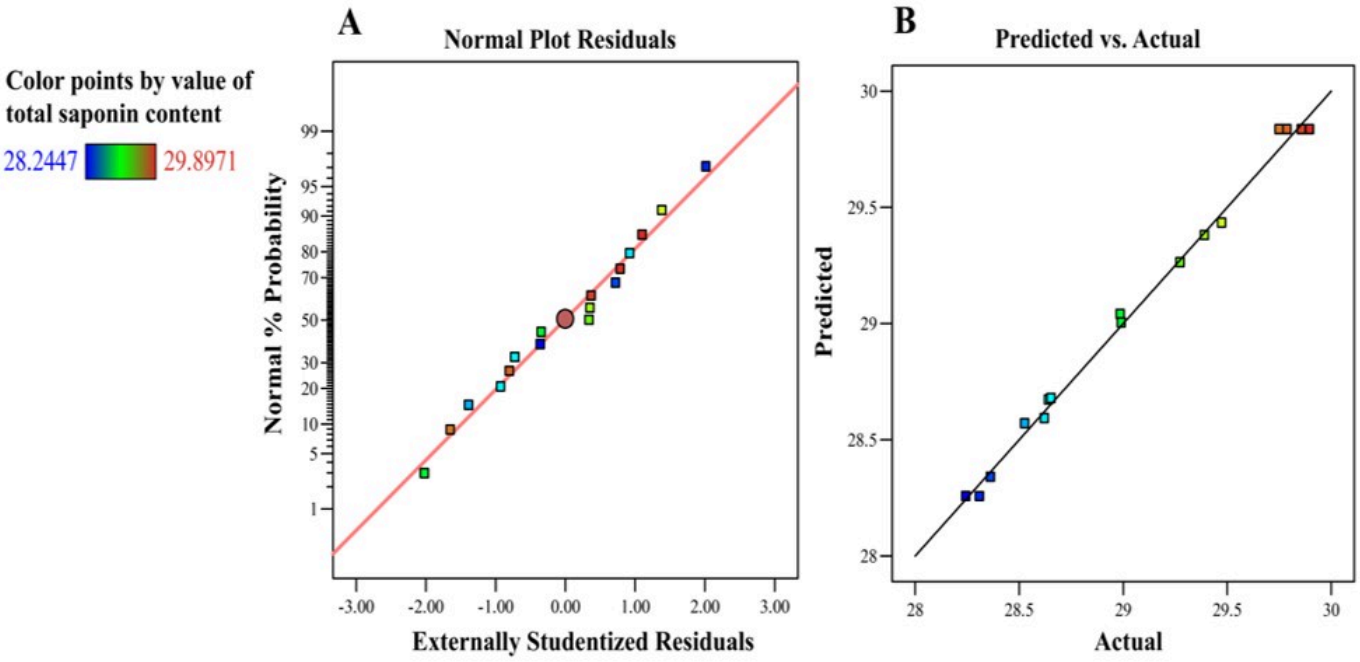

Figure 4

Normal plot for residuals and predicted vs. actual values of total polyphyllins content

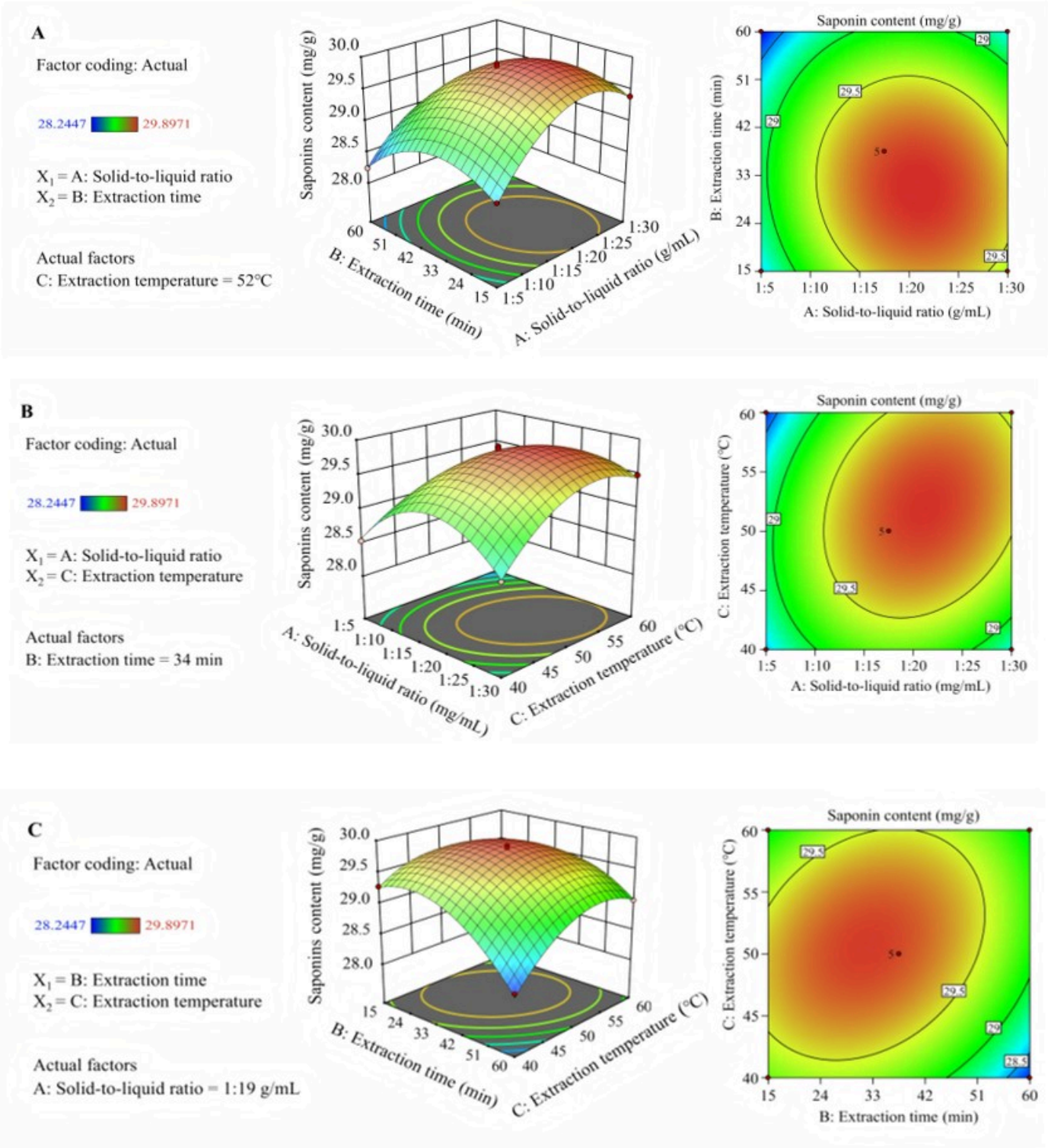

Figure 5

Response surface curve plots of the interactive effects of different factors on polyphyllins. (A)Interaction between extraction time and solid-to-liquid ratio; (B)Interaction between solid-to-liquid ratio and extraction temperature extraction time; (C)Interaction between extraction time and extraction temperature.

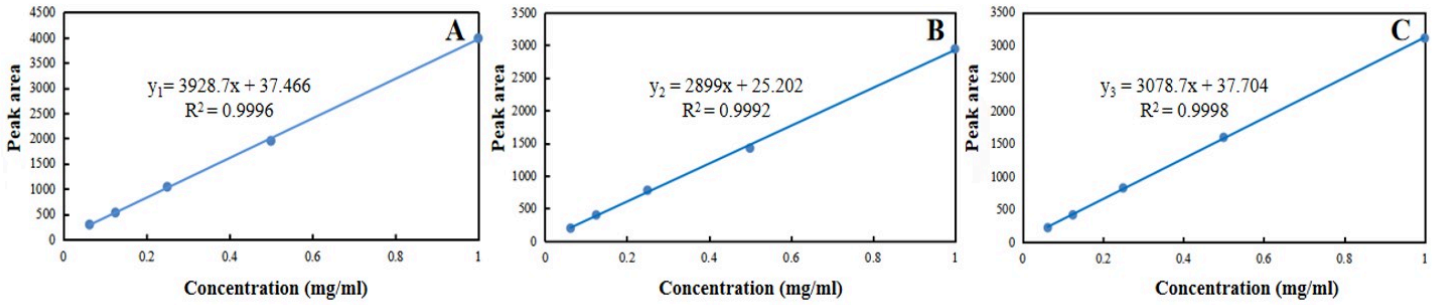

Figure 6

Standard curves of PPI (A) , PP□ (B), and PP□ (C)

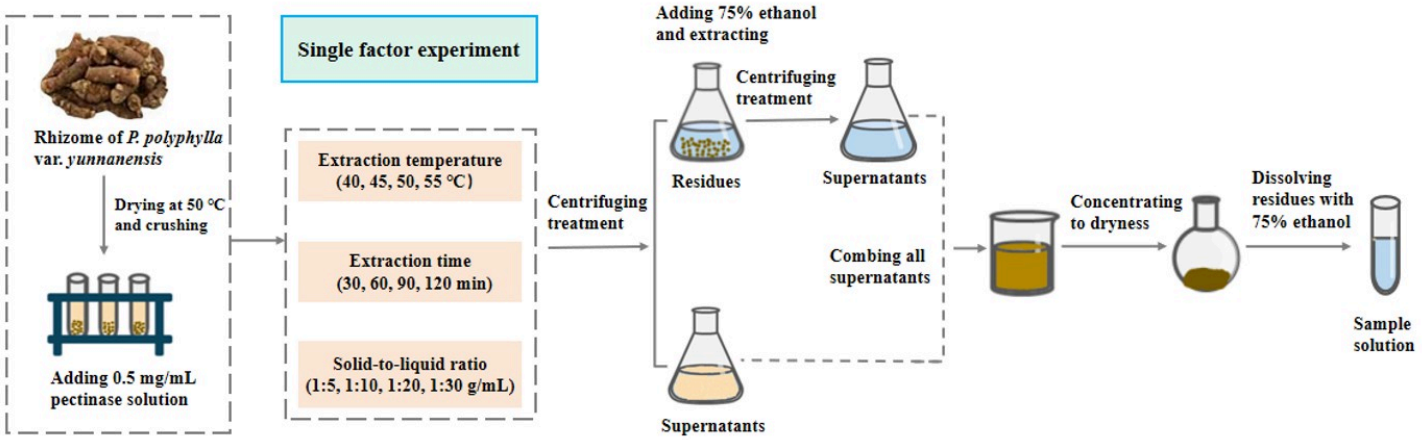

Figure 7

Experimental scheme for extraction of polyphyllins