Optimization of Enzymatic Extraction Process of Polysaccharides from *Pseudostellaria heterophylla* Fibrous Roots by Response Surface Methodology and Its Pilot Application

Huiqing PAN[1], Qi ZHAO[2], Yanda ZHANG[1,3*], Rongping YANG[2]

1. Fujian Provincial Enterprise Technology Center of Fujian Beidi Pharmaceutical Co., Ltd., Ningde 355300, China; 2. Fujian Beidi Pharmaceutical Co., Ltd., Ningde 355300, China; 3. Zherong County TaiZiShen Association, Ningde 355300, China

Abstract [**Objectives**] To study and optimize the process conditions of enzymatic hydrolysis technology for extracting polysaccharides from *Pseudostellaria heterophylla* fibrous roots and its application in workshop pilot tests. [**Methods**] *P. heterophylla* fibrous roots were taken as the matrix material, and Box Behnken design was used to analyze the extraction time, composite enzyme addition amount, and liquid – solid ratio for response surface optimization experiments, and then applied to the pilot extraction of *P. heterophylla* fibrous roots. [**Results**] Response surface analysis showed that all factors had a significant impact on the experimental indicators. The optimal extraction process conditions for polysaccharides from *P. heterophylla* fibrous roots were extraction time of 2.7 h, compound enzyme addition of 2.5%, and liquid – solid ratio of 32. The yield of polysaccharides from *P. heterophylla* fibrous roots was 4.83%. The water extraction process of *P. heterophylla* fibrous roots extraction pilot was used as the control group for response surface optimization of the pilot experiment. The optimization results showed that the extraction time was 3 h, the amount of composite enzyme added was 2.5%, and the liquid – solid ratio was 28. The polysaccharide yield was 4.75%, an increase of 4.63% compared to the control group. [**Conclusions**] This paper could provide feasibility for the innovation of enzymatic hydrolysis technology for *P. heterophylla* fibrous roots and its workshop pilot practice application, as well as a reference for the industrial application of its medicinal resources.

Key words *Pseudostellaria heterophylla* fibrous roots, Polysaccharides, Enzymatic extraction, Pilot production, Response surface optimization

1 Introduction

Pseudostellariae Radix is dry root tuber of *Pseudostellaria heterophylla*, the Caryophyllaceae plant, which belongs to the nourishing traditional Chinese medicine. It was first recorded in the Qing Dynasty's Thoroughly Revised Materia Medica. *P. heterophylla* is mainly produced in Fujian, Guizhou, and Jiangsu[1–2], and its production in Zherong is relatively large[3]. The medicinal ingredients of *P. heterophylla* are rich in polysaccharides, saponins, cyclic peptides and other components, which have both medicinal and nutritional effects[4]. Clinical trials have proved that *P. heterophylla* has the effects of invigorating the spleen and lungs[5], anti-oxidation[6–7], anti-inflammatory[8], immune regulation[9], and strain probiotics[10]. The literature reports that *P. heterophylla* polysaccharide has significant effects of enhancing immunity[11], cell differentiation[12], anti-oxidation[7], and auxiliary treatment of diabetes[13]. Traditional use of *P. heterophylla* is taking root tubers as medicine, and its fibrous roots are discarded. However, the biomass of *P. heterophylla* fibrous roots accounts for 14.93% of the dry weight of root tubers[14]. At the same time, research found that the basic substances of *P. heterophylla* fibrous roots are basically the same as those of root tubers, and their effects are similar[15–16]. Therefore, timely development and comprehensive utilization of matrix resources from *P. heterophylla* fibrous roots have positive practical significance for the sustainable development of *P. heterophylla* industry chain. The search found that there are many literature studies on the extraction process and optimization of *P. heterophylla*, mainly focusing on water extraction and alcohol precipitation[17], ultrasonic extraction[18], enzymatic hydrolysis[19], and high-pressure steam method[20]. However, there is relatively little research on the extraction process of *P. heterophylla* fibrous roots, and there are no literature reports on the application of enzymatic hydrolysis technology in the extraction and pilot production of *P. heterophylla* fibrous roots. Response surface methodology (RSM) is a method of finding the optimal process parameters by conducting mathematical modeling and statistical regression analysis through deterministic experiments[21]. This paper intended to use RSM to optimize the process of enzymatic hydrolysis technology for extracting polysaccharides from *P. heterophylla* fibrous roots, explored the feasibility of enzymatic extraction technology in the extraction and pilot production of *P. heterophylla* fibrous roots, and provide new technological approaches and ideas for the comprehensive application of traditional Chinese medicine resource utilization and industrialization of *P. heterophylla* fibrous roots.

2 Materials and methods

2.1 Test materials and instruments The material was from Fujian Beidi Pharmaceutical Co., Ltd., and was collected from the GAP base of *P. heterophylla* cultivation in Zherong County. After collecting the sample, impurities were removed, and it was dried in a drying oven at 60 ℃ for later use. The reagents anhydrous glucose, phenol, sulfuric acid, and anhydrous ethanol were all domestically produced AR; cellulase, protease.

The experimental instruments included a UV spectrophotome-

Received: May 21, 2024 Accepted: September 12, 2024

Supported by Special Project of Central Leading Local Science and Technology Development (2021L3030); Regional Development Project of Fujian Provincial Science and Technology Plan (2022N3017).

* Corresponding author. E-mail: 958757567@qq.com

ter (Shanghai Yuanxi Instrument Co., Ltd.), a thermostatic water bath pot (Tianjin Taiste Instrument Co., Ltd.), a grinder (Wuhan Haina Electric Appliance Co., Ltd.), a drying oven (Beijing Kewei Yongxing Instrument Co., Ltd.), and an electronic balance (Shimazu Manufacturing Institute).

2.2 Experimental methods After being crushed by a grinder with 30 meshes, the dried product of *P. heterophylla* fibrous roots was weighed to a certain mass and stirred according to a certain liquid – solid ratio. Composite enzymes (cellulase : protease = 2 : 1) were added in proportion, and the extraction temperature was controlled at 50 ℃, pH was 5. The extraction frequency was once. The response surface optimization experiment was conducted for a certain period of time, and the evaluation index was the level of polysaccharide yield from *P. heterophylla* fibrous roots.

Based on the research results of response surface methodology optimization for enzymatic extraction of polysaccharides from *P. heterophylla* fibrous roots, the experiment was magnified by 100 times, and a pilot experiment was conducted, with the evaluation index being the yield of polysaccharides from *P. heterophylla* fibrous roots. Among them, the water extraction process of the pilot plant for extracting *P. heterophylla* fibrous roots was used as the control group. The process conditions of the control group were as follows: the liquid – solid ratio of *P. heterophylla* fibrous roots was 1 : 20, the extraction time was 3 h, the extraction was performed twice, and the pH was 7.6. Finally, polysaccharides indicator was detected and recorded.

2.3 Analysis methods The method for determining the content of polysaccharides from *P. heterophylla* fibrous roots referred to the literature[22]. A certain amount of glucose standard was weighed and prepared into a 0.01 mg/mL of standard solution for testing. Different volumes of the test solution were added one by one in ten test tubes, diluted to 1 mL with distilled water, so that the sugar concentration in the test tubes was 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 μg/mL. Then, 1 mL of 5% phenol solution was added and mixed. 5 mL of concentrated sulfuric acid was slowly added, carefully mixed, and heated in a boiling water bath for 15 min. After cooling, the absorbance of each tube's test solution at a wavelength of 490 nm was measured. The sugar concentration was used as the *x*-axis, and the absorbance was used as the *y*-axis. A standard curve was drawn using plotting software, and a regression equation was derived. The regression equation of the obtained standard curve was $y = 0.008\,4x + 0.004\,7$, $R^2 = 0.999\,3$, and there was a good linear relationship between glucose concentration and absorbance.

P. heterophylla fibrous roots dried to constant weight were taken and crushed with 30 meshes, and a certain volume of distilled water was added. It was set into a water bath, heated and boiled for a certain time. After filtering, the filtrates were merged, reduced pressure and concentrated to 30 mL, and 4 times of 95% ethanol was added. It was stood in the refrigerator until the supernatant was clarified, and was washed with 95% ethanol and anhydrous ethanol. Then, it was dried in a 50 ℃ oven to obtain crude polysaccharides. The content of the obtained crude polysaccharides from *P. heterophylla* fibrous roots was determined using the phenol-sulfuric acid method (as mentioned above), and its *OD* value was determined. The sugar content was calculated based on the standard curve.

Yield of polysaccharides from *P. heterophylla* fibrous roots (%) = [Mass of polysaccharides from *P. heterophylla* fibrous roots(g)/Mass of *P. heterophylla* fibrous roots raw materials(g)] ×100%.

2.4 Experimental design Based on the single factor experimental research in the early stage of the project, three influencing factors of Box Behnken (BBD) design, namely extraction time (A), compound enzyme addition amount (B), and liquid – solid ratio (C), were comprehensively analyzed and determined. The yield of polysaccharides from *P. heterophylla* fibrous roots (Y) was used as the indicator factor, and the response surface analysis software was Design-Expert 8.0.6. The three factors in the experiment were all taken at three levels and coded with (−1, 0, 1). The response surface test factors and their levels were shown in Table 1.

Table 1 Factors and levels of experimental design

Factor	Coding	Encoding level		
		−1	0	1
Extraction time//h	A	2	2.5	3
Compound enzyme addition amount//%	B	1	2	3
Liquid – solid ratio//%	C	20	30	40

3 Results and analysis

3.1 Design and results of response surface experiment The response surface experiment results of enzymatic extraction of polysaccharides from *P. heterophylla* fibrous roots were shown in Table 2.

Table 2 Experimental design and results of Box-Behnken

Test No.	Extraction time A//h	Compound enzyme addition amount B//%	Liquid – solid ratio C	Polysaccharides yield//%
1	−1	0	1	3.59
2	−1	−1	0	3.12
3	−1	1	0	3.73
4	1	0	1	4.37
5	0	0	0	4.43
6	1	−1	0	4.08
7	1	0	−1	4.22
8	0	−1	1	3.83
9	0	0	0	4.61
10	−1	0	−1	3.27
11	0	0	0	4.52
12	0	1	−1	4.17
13	0	1	1	4.48
14	1	1	0	4.50
15	0	0	0	4.77
16	0	−1	−1	3.70
17	0	0	0	4.59

3.2 Mathematical model establishment and analysis of variance Using Design-Expert8.0.6 software, regression fitting and calculation were carried out on the experimental data of polysaccharides content from *P. heterophylla* fibrous roots in Table 2, and a quadratic response surface regression model was established. The equation was $Y = 4.58 + 0.43A + 0.27B + 0.11C - 0.048AB - 0.042AC + 0.045BC - 0.45A^2 - 0.27B^2 - 0.26C^2$, $R^2 = 0.962$. The significance test and analysis of variance were performed on the regression model, and the results were shown in Table 3. Seen from Table 3, the regression model $P < 0.0001$, indicating that the regression effect of the model was extremely significant. The misfit term of model was not significant ($P = 0.9943 > 0.05$), indicating that the constructed model had statistical significance, and the model analysis results were relatively stable, which can better predict the changes in polysaccharides content from *P. heterophylla* fibrous roots in the actual treatment. The $R^2 = 0.962$ of the regression equation indicated a high degree of fit and a relatively small error. In addition, according to the model statistical analysis data of extraction time (A) and compound enzyme addition amount (B), the P values of the two factors were all less than 0.000 1, which were extremely significant. The liquid - solid ratio (C) had a significant impact ($P < 0.05$). At the same time, the order of the F values of the three factors can indicate the order of their influence on the yield of polysaccharides from *P. heterophylla* fibrous roots, namely: A > B > C. Therefore, this model can be used for statistical analysis of the yield of polysaccharides from *P. heterophylla* fibrous roots.

3.3 Response surface analysis of model The software Design Expert produced response surface maps and contour maps among various factors for studying regression models, which can be used to evaluate the pairwise interaction of the three factors on the polysaccharides content from *P. heterophylla* fibrous roots and determine the optimal level range of the three factors. The contour characteristics of contour maps can intuitively reflect the strength of the interaction between two factors, with elliptical contour lines indicating more significant interaction between factors and circular contour lines indicating less significant interaction[21].

Fig. 1 showed the interaction effect of experimental factors extraction time (A) and compound enzyme addition amount (B) on the yield of polysaccharides from *P. heterophylla* fibrous roots (Y). The results showed that the interaction between the two factors was not significant, but the effect of A was significantly greater than B. When A and B were at low water levels, the Y value was the smallest. As the factor level increased, the Y value gradually increased. Fig. 2 showed the interaction effect of experimental factors the amount of composite enzyme added (B) and the liquid - solid ratio (C) on the yield of polysaccharides from *P. heterophylla* fibrous roots (Y). The results showed that the interaction between the two factors was not significant, but the effect of B was significantly greater than C. Fig. 3 showed the interaction effect of extraction time (A) and liquid - solid ratio (C) on the yield of polysaccharides from *P. heterophylla* fibrous roots (Y). The results showed that the interaction between the two factors was not significant, but the effect of A was significantly greater than C. When A and C were at low water levels, the Y value was the smallest, and gradually increased with the increase of factor level values.

Table 3 Variance analysis of regression model

Source	Sum of squares	Freedom	Mean square	F value	P value
Model	3.840 0	9	0.430	46.51	< 0.000 1
A	1.500 0	1	1.500	163.05	< 0.000 1
B	0.580 0	1	0.580	62.96	< 0.000 1
C	0.100 0	1	0.100	11.28	0.012 1
AB	9.03E-03	1	9.03E-03	0.98	0.354 4
AC	7.23E-03	1	7.23E-03	0.79	0.404 4
BC	8.10E-03	1	8.10E-03	0.88	0.378 8
A2	0.870 0	1	0.870	94.77	< 0.000 1
B2	0.310 0	1	0.310	33.94	0.000 6
C2	0.300 0	1	0.300	32.71	0.000 7
Residual	0.064 0	7	9.18E-03	–	–
Misfitting	1.13E-03	3	3.75E-04	0.024	0.994 3
Error	0.063 0	4	0.016	–	–
Sum	3.910 0	16	–	–	–
R^2	0.983 6	–	–	–	–
R^2_{Adj}	0.962 4	–	–	–	–

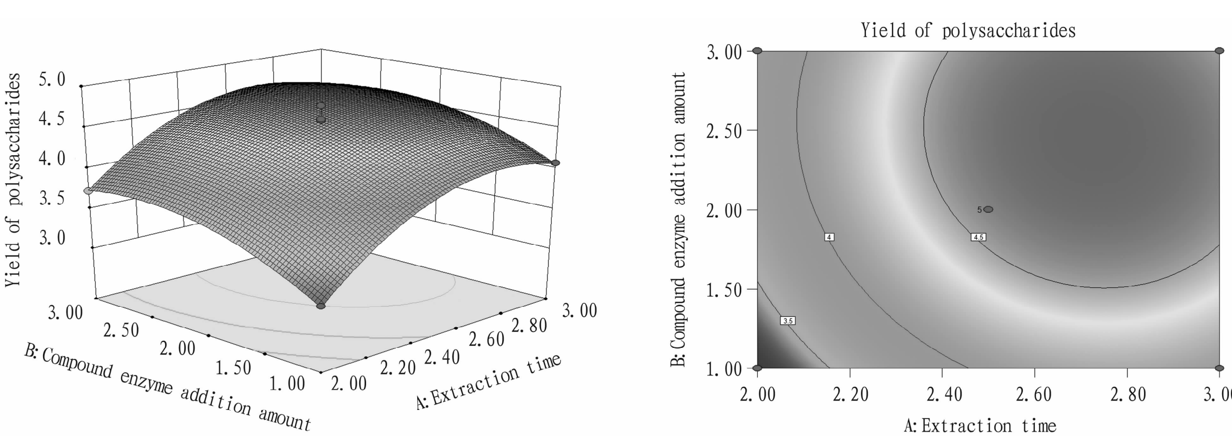

Fig. 1 Response surface and contour lines of the interaction between extraction time and compound enzyme addition amount

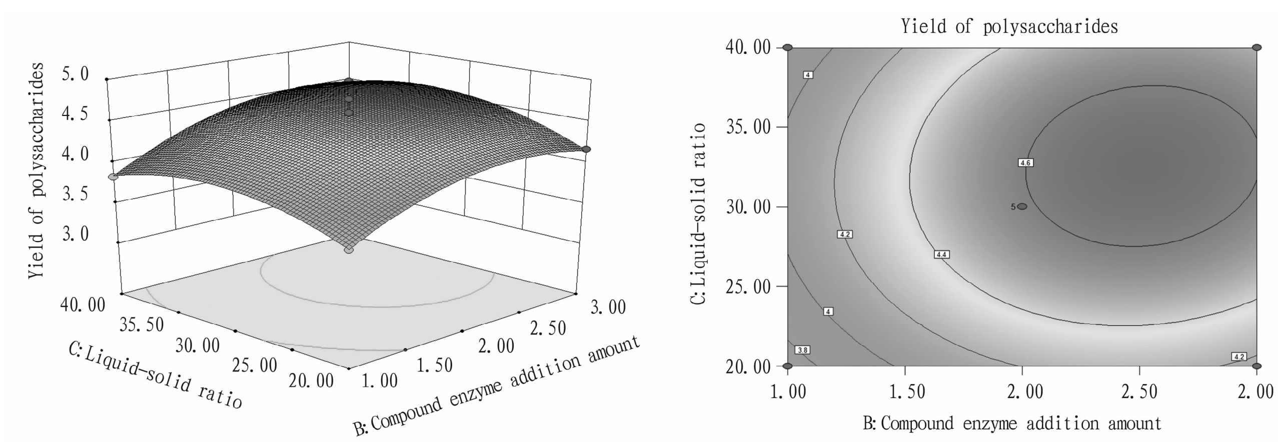

Fig. 2 Response surface and contour lines of the interaction between compound enzyme addition amount and liquid – solid ratio

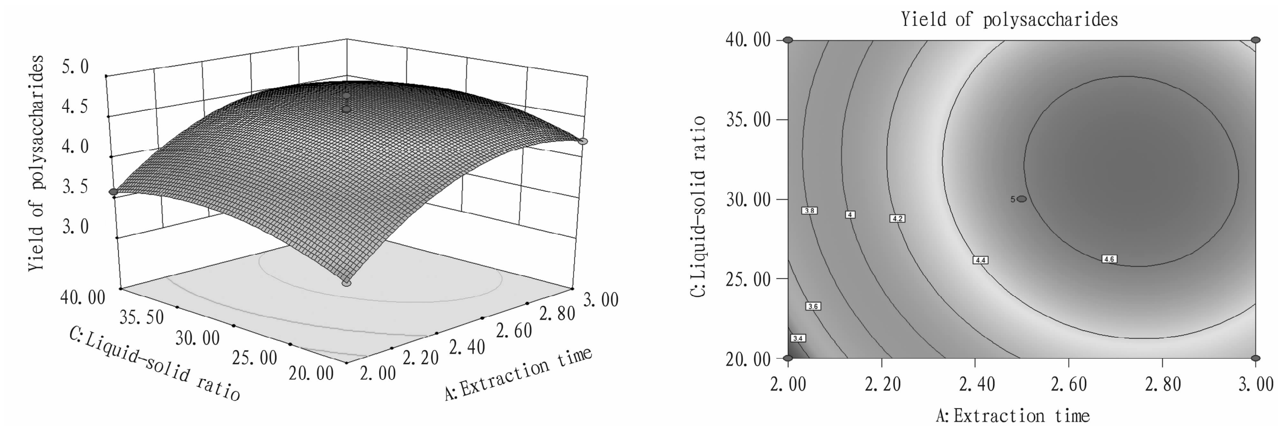

Fig. 3 Response surface and contour lines of the interaction between extraction time and liquid – solid ratio

3.4 Optimization and validation of process condition Using Design Expert software analysis, it was found that the optimal extraction process for polysaccharides from *P. heterophylla* fibrous roots was as follows: extraction time of 2.72 h, addition of 2.47% composite enzyme, and liquid – solid ratio of 32.18. To facilitate operation, the conditions were adjusted to extraction time of 2.7 h, addition of 2.5% composite enzyme, and liquid – solid ratio of 32. Under these optimal conditions, the average yield of polysaccharides from *P. heterophylla* fibrous roots in three repeated experiments was 4.83%, with an error of less than 1.5% compared to the optimal yield value analyzed by the model theory, indicating that the model had good accuracy and repeatability.

3.5 Pilot experiment results of response surface optimization process Based on the research results of response surface methodology optimization for enzymatic extraction of polysaccharides from *P. heterophylla* fibrous roots, the experiment was magnified by 100 times, and a pilot experiment was conducted. The pre-experimental process was as follows: drying *P. heterophylla* fibrous roots at 60 ℃, grinding with a fineness of 30 meshes, adding 2.5% composite enzyme, a solid-liquid ratio of 1 : 32, extraction temperature of 50 ℃, pH of 5, extraction time of 2.7 h, and extraction frequency of once. The yield of polysaccharides from *P. heterophylla* fibrous roots was experimentally investigated. Based on the actual production situation of the factory and the principle of easy operation, three key factors were adjusted, and the polysaccharides yield index was evaluated. The maximum value of the pilot experiment was 4.75%. The factor values under these conditions were extraction time of 3 h, compound enzyme addition of 2.5%, and liquid – solid ratio of 28. The pilot process for extracting *P. heterophylla* fibrous roots was 30 meshes, compound enzyme addition of 2.5%, liquid – solid ratio of 1 : 28, extraction temperature of 50 ℃, pH of 5, time of 3 h, and extraction frequency of once. At the same time, the water extraction process of *P. heterophylla* fibrous roots was taken as control group during the pilot experiment, and the average yield of polysaccharides was 4.54%. Under the pilot conditions, the yield of polysaccharides from the enzymatic hydrolysis process of *P. heterophylla* fibrous roots was higher than that of the control group, and the yield of polysaccharides from *P. heterophylla* fibrous roots increased by 4.63% compared to the control group. The results showed the specific feasibility of the enzymatic hydrolysis process of *P. heterophylla* fibrous roots in the pilot test.

4 Conclusions and discussion

Based on Box-Behnken design and analysis, a mathematical model was constructed using *P. heterophylla* fibrous roots as the research object. The results showed that all experimental factors significantly affected the polysaccharides content from *P. heterophylla* fibrous roots. The optimized process conditions were extraction time of 2.7 h, composite enzyme addition of 2.5%, liquid – solid ratio of 32. The yield of polysaccharides from *P. heterophylla* fibrous roots was 4.83%, indicating the feasibility of using a composite enzyme combination of cellulose and protease in polysaccharides extraction technology from *P. heterophylla* fibrous roots. Fang Wenqing *et al.* [19, 23] studied the cellulase extraction process of polysaccharides from *P. heterophylla* root tuber and extract residue of its root tubers, respectively. Although *P. heterophylla* fibrous roots is a substrate material for traditional Chinese medicine attached to *P. heterophylla* root tubers, both the single factor experiment of cellulase and the optimization experiment of composite enzyme response surface in this study have confirmed that cellulase also had a significant effect on the extraction of polysaccharides from *P. heterophylla* fibrous roots. This also provided data support for the enzymatic extraction of *P. heterophylla* integrated Chinese herbal medicine matrix (root tubers and fibrous roots), and reference for the efficient utilization of *P. heterophylla* resources in the future. At the same time, the industrial production of traditional Chinese medicine is the technological core of modern Chinese medicine manufacturing industry and one of the strong manifestations of market competitiveness. It is particularly important to achieve efficient production of resources and extract preparation of *P. heterophylla* resources. Based on the optimization process of response surface methodology, this paper conducted pilot experiment. The optimization results showed that the extraction time was 3 h, the amount of composite enzyme added was 2.5%, and the liquid – solid ratio was 28. The polysaccharides yield was 4.75%, an increase of 4.63% compared to the control group. Therefore, this paper provided feasibility for the innovation of enzymatic hydrolysis technology for *P. heterophylla* fibrous roots and its workshop pilot practice application, with significant significance, as well as a reference for the industrial application of its medicinal resources.

References

[1] MEI W, CHEN L, HUANG X, *et al.* Rapid authentication of *Pseudostellaria heterophylla* (Taizishen) from different regions by Raman spectroscopy coupled with chemometric methods[J]. Journal of Luminescence, 2018: S0022231317314059.

[2] ZHANG YD, PAN HQ, LIN HG, *et al.* Mapping knowledge domains of the patent information of *Pseudostellaria heterophylla*[J]. Journal of Anhui Agricultural Sciences, 2019, 47(6): 234 –239. (in Chinese).

[3] YAN PP, JING Y, LI YX, *et al.* The analysis basing on identification of Zherong Radix pseudostellariae of HPLC fingerprint[J]. Advanced Materials Research, 2014(955 –959): 843 –847.

[4] HU DJ, SHAKERIAN F, ZHAO J, *et al.* Chemistry, pharmacology and analysis of *Pseudostellaria heterophylla*: A mini-review[J]. Chinese Medicine, 2019, 14(1): 21.

[5] YANG Q, CAI X, HUANG M, *et al.* Immunomodulatory effects of *Pseudostellaria heterophylla* peptide on spleen lymphocytes via a Ca^{2+}/CaN/NFATc1/IFN-γ pathway[J]. Food Funct, 2019(10): 3466 – 3476.

[6] NG TB, LIU F, WANG HX. The antioxidant effects of aqueous and organic extracts of *Panax quinquefolium*, *Panax notoginseng*, *Codonopsis pilosula*, *Pseudostellaria heterophylla* and *Glehnia littoralis*[J]. Journal of Ethnopharmacology, 2004, 93(2): 285 –288.

[7] ZHANG YD, PAN HQ, MA YF, *et al.* Meta-analysis of the effects of *Pseudostellaria heterophylla* extraction on the anti-oxidative stress in animals[J]. Feed Research, 2019(10): 122 –128. (in Chinese).

[8] CHOI YY, KIM MH, AHN KS, *et al.* Immunomodulatory effects of *Pseudostellaria heterophylla* (Miquel) Pax on regulation of Th1/Th2 levels in mice with atopic dermatitis[J]. Molecular Medicine Reports, 2017, 15(2): 649 –656.

[9] YANG Q, CAI X, HUANG M, *et al.* A specific peptide with immunomodulatory activity from *Pseudostellaria heterophylla* and the action mechanism[J]. Journal of Functional Foods, 2020(68): 1 –9.

[10] HQ P, YD Z, ZJ L, *et al.* *In vitro* probiotic effect of *Pseudostellaria heterophylla* fibrous roots on feed bacillus subtilis and preliminary study on its solid fermentation[J]. Animal Husbandry and Feed Science, 2019, 11(2): 72 –76.

[11] ZHANG LJ, WANG Z, LIAO SG. Preliminary study on the immunomodulatory activity of *Pseudostellaria* polysaccharides on macrophage cell line RAW264.7[J]. Chinese Wild Plant Resources, 2018, 37(4): 14 – 17. (in Chinese).

[12] WONG CK, LEUNG KN, FUNG KP, *et al.* Effects of *Pseudostellaria heterophylla* on proliferation and differentiation of murine bone marrow cells[J]. Immunopharmacology and Immunotoxicology 1994, 16(1): 71 –84.

[13] HU J, PANG W, CHEN J, *et al.* Hypoglycemic effect of polysaccharides with different molecular weight of *Pseudostellaria heterophylla*[J]. Bmc Complementary & Alternative Medicine, 2013, 13(1): 267.

[14] DING CX. Quality evaluation and related pharmacodynamics research of *Pseudostellaria heterophylla*[D]. Fuzhou: Fujian University of Traditional Chinese Medicine, 2013. (in Chinese).

[15] DING CH, LIN PL, ZENG JW, *et al.* Determination of polysaccharides and total saponins in root tuber and fibrous root of *Pseudostellaria heterophylla*[J]. Journal of Fujian College of Traditional Chinese Medicine, 2012, 22(3): 40 –43. (in Chinese).

[16] ZENG LN, YUAN YH, HUANG SY, *et al.* A comparison of effective medicinal ingredients content of *Pseudostellaria heterophlla* in different harvest times and medicinal parts[J]. Fujian Forestry, 2014(2): 25 – 26. (in Chinese).

[17] KAN YJ, SHI PP, JIANG C, *et al.* Effects of different extraction methods on content of polysaccharide from *Pseudostellaria heterophylla*[J]. Chinese Journal of Ethnomedicine and Ethnopharmacy, 2019, 28(14): 41 –43. (in Chinese).

[18] XUE JY, SHI H. Optimization of ultrasonic extraction process of polysaccharides from *Pseudostellaria heterophylla*[J]. Modern Agricultural Science and Technology, 2023(6): 189 –192. (in Chinese).

[19] LI L, YANG XY, WANG JL, *et al.* Optimization of the extraction process of crude polysaccharides from pseudostellariae radix tuber roots by cellulase[J]. Food Engineering, 2021(2): 30 –32. (in Chinese).

[20] PAN HQ, ZHANG ZK, ZHANG YD, *et al.* Optimization of steam explosion process condition for extracting polysaccharides from *Pseudostellaria heterophylla* by response surface methodology[J]. Agricultural Science & Technology, 2020, 21(2): 41 –46.

[21] SODEIFIAN G, ARDESTANI NS, SAJADIAN SA. Extraction of seed oil from *Diospyros lotus* optimized using response surface methodology[J]. Journal of Forestry Research, 2019, 30(2): 709 –719.

[22] LIN QM, PANG WS, ZENG J, *et al.* Experimental study on the extracts and polysaccharides of different sarieties of *Pseudostellaria heterophylla* from Zhelong Fujian Province[J]. Chinese Journal of Ethnomedicine and Ethnopharmacy, 2019, 28(3): 25 –27. (in Chinese).

[23] FANG WQ, KONG YT, AN FP, *et al.* Optimization of response surface method for polysaccharides extraction from Radix Pseudostellariae residues[J]. Immunological Journal, 2018, 34(9): 791 –798. (in Chinese).