RESEARCH ARTICLE

Ultrasonic-assisted extraction of saponins from *Phytolacca acinosa* Roxb. roots and seasonal variation of saponin content

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Phytolacca acinosa* Roxb., a perennial herb in the Phytolaccaceae family, is renowned for its rich medicinal properties. It is commonly used for its effects in diuresis, reducing edema, promoting bowel movement, detoxification, and resolving masses. Saponins, the bioactive compounds found in the roots of *Phytolacca acinosa* Roxb., hold significant medicinal value. As research on the functional properties of saponins in the roots of *Phytolacca acinosa* Roxb. progresses, there is an increasing demand for identifying the most effective extraction methods. This study aimed to optimize the extraction process of saponin from the roots of *Phytolacca acinosa* Roxb. using single factor experiments. The Box-Behnken response surface design was employed to refine the extraction technique. The study also compared the effectiveness of proposed method with other three different extraction methods and investigated how the saponin content in the roots of *Phytolacca acinosa* Roxb. varied from different harvesting seasons. The results showed that the optimal extraction conditions for ultrasonic-assisted extraction were 28 minutes of ultrasonic time, 62°C, 1:22 solid-to-liquid ratio (g/mL), 70% ethanol, and 200 W ultrasonic power. Under these conditions, the saponin yield was 3.63 ± 0.017 mg/g, which was 2.29 mg/g higher than the ethanol reflux method and 0.86 mg/g higher than the microwave-assisted extraction method. Saponin content was the highest in the spring and autumn with the lowest levels observed in January and the highest concentration in October. This study had guiding significance for improving the added value of *Phytolacca acinosa* Roxb. and selecting the best harvesting period and had important reference value for subsequent research.

Keywords: response surface methodology; ultrasonic extraction; *Phytolacca acinosa* Roxb.; saponin; seasonal variation in content.

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Introduction

In China, the *Phytolacca* L. genus comprises four species including *Phytolacca acinosa* Roxb. (Common Phytolacca), *Phytolacca americana* L. (American Phytolacca), *Phytolacca japonica* Makino (Japanese Phytolacca), and *Phytolacca polyandra* Batal. (Multiflora Phytolacca) [1-3]. According to the 2020 edition of the Chinese Pharmacopoeia, the medicinal herb "Shanglu" refers to the dried roots of either *Phytolacca acinosa* or *Phytolacca americana* [4]. Modern pharmacological studies have revealed that plants of the *Phytolacca* genus exhibit significant anti-inflammatory, anti-tumor, antibacterial, antifungal, and antimalarial activities and are commonly used to treat conditions such as edema, nephritis, hepatitis, and malaria [5, 6].

The chemical composition of plants in the *Phytolacca* genus is complex with numerous compounds identified to date including saponins [7], proteins [8], polysaccharides [9], flavonoids [10], phenolic acids [11], and sterols [12]. Among them, saponins are the most abundant chemical constituents in *Phytolacca* species. They are not only structurally diverse but also exhibit a wide range of pharmacological activities [8]. As research on these compounds continues to advance, there is an increasing need to identify the most efficient extraction methods for *Phytolacca* saponins. The current extraction methods for saponins were limited from issues such as low efficiency and poor selectivity [13]. Traditional extraction processes can easily lead to loss of active ingredients and excessive impurities, which hinders the acquisition of high-purity, high-quality active ingredients required for subsequent research and development [14]. Ultrasonic extraction utilizes the cavitation effect of ultrasound to break down plant cell walls, facilitating the release of internal saponins. This method boasts several advantages including high energy efficiency, preservation of active compounds, and high product yield [15, 16]. Although some studies have reported on saponin extraction from the roots of *Phytolacca acinosa* Roxb. [17], there is a lack of systematic research specifically on ultrasonic extraction of saponin from the roots of *Phytolacca acinosa* Roxb.

Yongzhou, Hunan, China is a key production area for *Phytolacca acinosa* Roxb. and has seen limited comprehensive utilization of the plant. Geographic and seasonal variations are significant factors influencing the saponin content in *Phytolacca acinosa* Roxb., yet data on the saponin content and its seasonal distribution remains scarce [18, 19]. To address this gap, this study employed ultrasonic-assisted extraction of saponin from the roots of *Phytolacca acinosa* Roxb. in Yongzhou area to investigate the optimal extraction process. The study also compared the effects of ultrasonic-assisted, heated reflux, and microwave-assisted extraction methods. Additionally, saponin content in the roots of *Phytolacca acinosa* Roxb. from different harvesting seasons was measured, providing valuable insights for the further development and rational harvest timing of *Phytolacca acinosa* Roxb. in Yongzhou, facilitating its application in the pharmaceutical field.

Materials and methods

Preparation of the standard curve

Ten milligrams of *Phytolacca acinosa* Roxb. saponin A standard were accurately weighed, dissolved in methanol, and diluted to a final volume of 10 mL to prepare a standard solution with a concentration of 1.0 mg/mL [20]. Aliquots of 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mL of the standard solution were precisely pipetted into separate stoppered test tubes. Methanol was added to each tube to bring the total volume to 1.0 mL. To each reaction tube, 0.5 mL of 8.0% vanillin-ethanol solution was added followed by 8.5 mL of 77% sulfuric acid to initiate color development. After mixing well, the reaction was placed in a 60°C water bath for 10 minutes, then cooled down in an ice bath for 15 minutes with shaking after each step. The solvent only was used as the blank. The absorbance of the solution was measured at 450 nm using a SP-754 UV-Vis spectrophotometer (Shanghai Spectrum Instruments Co., Ltd., Shanghai, China). Linear regression of absorbance (Y) against the *Phytolacca acinosa* Roxb. saponin A concentration (X, mg/mL) was performed, and the regression equation was obtained as below.

$Y = 2.436\ 9X + 0.18918$ ($R^2 = 0.995$)

Determination of saponin content in the roots of *Phytolacca acinosa* Roxb.

An accurate weight of 0.1 g of crude *Phytolacca acinosa* Roxb. saponin was dissolved in methanol and the volume was adjusted to 50 mL. Then, 2.0 mL of this solution was transferred and further diluted with methanol to a final volume of 50 mL. 1.0 mL of the diluted solution was taken, and the saponin content (N) was determined using the same method to obtain the standard curve

described above. The saponin yield was calculated as follows.

$$N\ (mg/g) = \frac{c \times V \times D}{M} \quad (1)$$

where *c* was the concentration of the diluted sample solution and calculated from the standard curve (mg/mL). *V* was the volume of the initial solution. *D* was the dilution factor. *M* was the mass of the powdered roots of *Phytolacca acinosa* Roxb. (g).

Single factor experiment on saponin extraction from the roots of *Phytolacca acinosa* Roxb.

250 g powdered roots of *Phytolacca acinosa* Roxb. was accurately weighed and placed into a round-bottom flask. Petroleum ether was added at a solid-to-liquid ratio of 1:20 followed by heating the mixture at 60°C under a SUNNE SN-DZTW-5000mL reflux (Shanghai SUNNE Instrument Equipment Co., Ltd., Shanghai, China) for two 30 minutes extraction cycles. The solution was then filtered, and the residue was dried in a 60°C air-drying oven before being ground into a powder. 10 g of defatted powdered roots of *Phytolacca acinosa* Roxb. was mixed with 70% ethanol at a fixed material-to-solvent ratio of 1:25. The extraction was carried out using GENTLEMAN F-040ST ultrasonic instrument (Shenzhen Fuyang Technology Group Co., Ltd., Shenzhen, Guangdong, China) at 70°C for 20 minutes with an ultrasonic power of 250 W. Extraction was performed sequentially under various conditions as the material-to-solvent ratios of 1:10, 1:15, 1:20, 1:25, 1:30 (g/mL), ethanol concentrations of 40, 50, 60, 70, 80%, ultrasonic temperatures of 45, 50, 55, 60, 65°C, ultrasonic times of 15, 20, 25, 30, 35 minutes, and ultrasonic powers of 100, 150, 200, 250, 300 W. The saponin crude extract was filtered and concentrated under reduced pressure using SUNNE SN-RE-201D rotary evaporator (Shanghai SUNNE Instrument Equipment Co., Ltd., Shanghai, China) and dried using Yiheng DZF-6020 vacuum oven (Shanghai Yiheng Technology Instrument Co., Ltd., Shanghai, China) to obtain crude saponin. The yield of saponin from the roots of *Phytolacca acinosa* Roxb. was then determined to evaluate the influence of various experimental factors on saponin yield.

Optimization of ultrasonic-assisted extraction conditions for saponin from the roots of *Phytolacca acinosa* Roxb.

Based on the results of the single factor experiments, the saponin yield was selected as the response variable. Factors that had a significant impact on the saponin yield were chosen as independent variables. The extraction process was then optimized using the response surface methodology (RSM) with a central composite design. After fixation of the ethanol concentration at 70% and ultrasound power at 200 W, three factors including ultrasonic time (A), ultrasonic temperature (B), and solid-liquid ratio (C) were selected for the response surface design. The specific factors and their levels were shown in Table 1.

Table 1. Response surface experimental factors and levels.

Factors	Levels		
	-1	0	1
A (min)	20	25	30
B (°C)	55	60	65
C (g/mL)	1:15	1:20	1:25

Comparison of extraction methods

1. Ethanol heating reflux extraction

The saponin extraction was done by using 80% ethanol as the solvent, a solid-to-liquid ratio of 1:20, and heating at 80°C for 2 hours [24]. The resulting extract was concentrated and dried to obtain crude saponin from the roots of *Phytolacca acinosa* Roxb before calculation.

2. Microwave-assisted extraction

Using 75% ethanol as the solvent and a solid-to-liquid ratio of 1:25 g/mL, extraction was carried out at 70°C and 500 W microwave power for 80 minutes [19]. The resulting extract was concentrated and dried to obtain crude saponin

from the roots of *Phytolacca acinosa* Roxb. Before the determination of saponin yield.

Influence of seasonal variation on saponin content in the roots of *Phytolacca acinosa* Roxb.
Twelve samples of powdered roots of *Phytolacca acinosa* Roxb. collected from March 2023 to February 2024 were processed to determine the saponin content. The measurement was repeated three times, and statistical analysis was conducted based on month and season.

Data analysis
Experimental data were presented as mean ± standard deviation (Mean ± SD). The data were processed using Design Expert 8.0.6 (StatEase, Minneapolis, MN, USA), SPSS 17.0 (IBM, Armonk, NY, USA), and Microsoft Office Excel 2010 (Microsoft, Redmond, WA, USA). T-test and an analysis of variance (ANOVA) were conducted for statistical analysis. Origin Pro2021 (OriginLab, Northampton, MA, USA) was used for graphing.

Results and discussion

Single factor experiment results
The results showed that, with the increase in solid-to-liquid ratio, the saponin yield from the roots of *Phytolacca acinosa* Roxb. first increased and then decreased. The maximum yield was achieved when the solid-to-liquid ratio was 1:20. After that, further increasing the ratio caused the yield to decline (Figure 1a). As the solid-to-liquid ratio increased, the contact area between the solute and solvent expanded. The increased solvent volume enlarged the diffusion space, promoting molecular movement and enhancing the yield of active substances. However, when the solid-to-liquid ratio exceeded 1:20, the saponin yield reached a saturation point, and excessive solvent hindered mass transfer, thus reducing the saponin yield from the roots of *Phytolacca acinosa* Roxb. [21]. Therefore, a solid-to-liquid ratio of 1:20 was deemed optimal. When ethanol concentration increased, the saponin yield from the roots of *Phytolacca acinosa* Roxb. first increased and then decreased (Figure 1b), which might be because, at lower ethanol concentrations, the rate of extraction of active components was relatively slow. Increasing the ethanol concentration effectively enhanced the saponin yield. However, when the ethanol concentration exceeded 70%, the polarity of the solution decreased, leading to the leaching of lipophilic impurities from the cells. These impurities then competed with the active components for the solvent, resulting in a decline in the saponin yield [22]. Therefore, the optimal ethanol concentration was 70%. The saponin yield increased progressively with the rise of ultrasound temperature. The highest yield was achieved at 60°C, after which further temperature increase led to a decline in the total saponin yield (Figure 1c). When the ultrasound temperature was below 60°C, elevating the temperature promoted molecular motion and enhanced the ultrasonic cavitation effect, thereby improving extraction efficiency [23]. However, when the temperature exceeded 60°C, the decrease in saponin yield might be attributed to the instability of the saponin-active compounds under heat, as high temperatures could cause decomposition or structural changes in the chemical constituents [24]. Therefore, the optimal ultrasound temperature was 60°C. With the prolongation of ultrasound duration, the saponin yield initially increased and then decreased with the maximum yield obtained at an ultrasound time of 25 minutes. When the ultrasound time was less than 25 minutes, the saponin yield increased significantly. However, after 25 minutes, the yield gradually declined (Figure 1d), which might be due to the initially large concentration gradient between the extraction solvent and the extract, and the ease with which saponins were extracted from the outer cells of *Phytolacca acinosa* Roxb. [25]. As the concentration gradient decreased and substances were extracted from inside of the cells, the extraction rate slowed down [26]. Therefore, the optimal ultrasound duration was 25 minutes. When ultrasound power increased, the saponin yield initially increased and then decreased. The extraction of saponins reached near saturation at an ultrasound power of 200 W

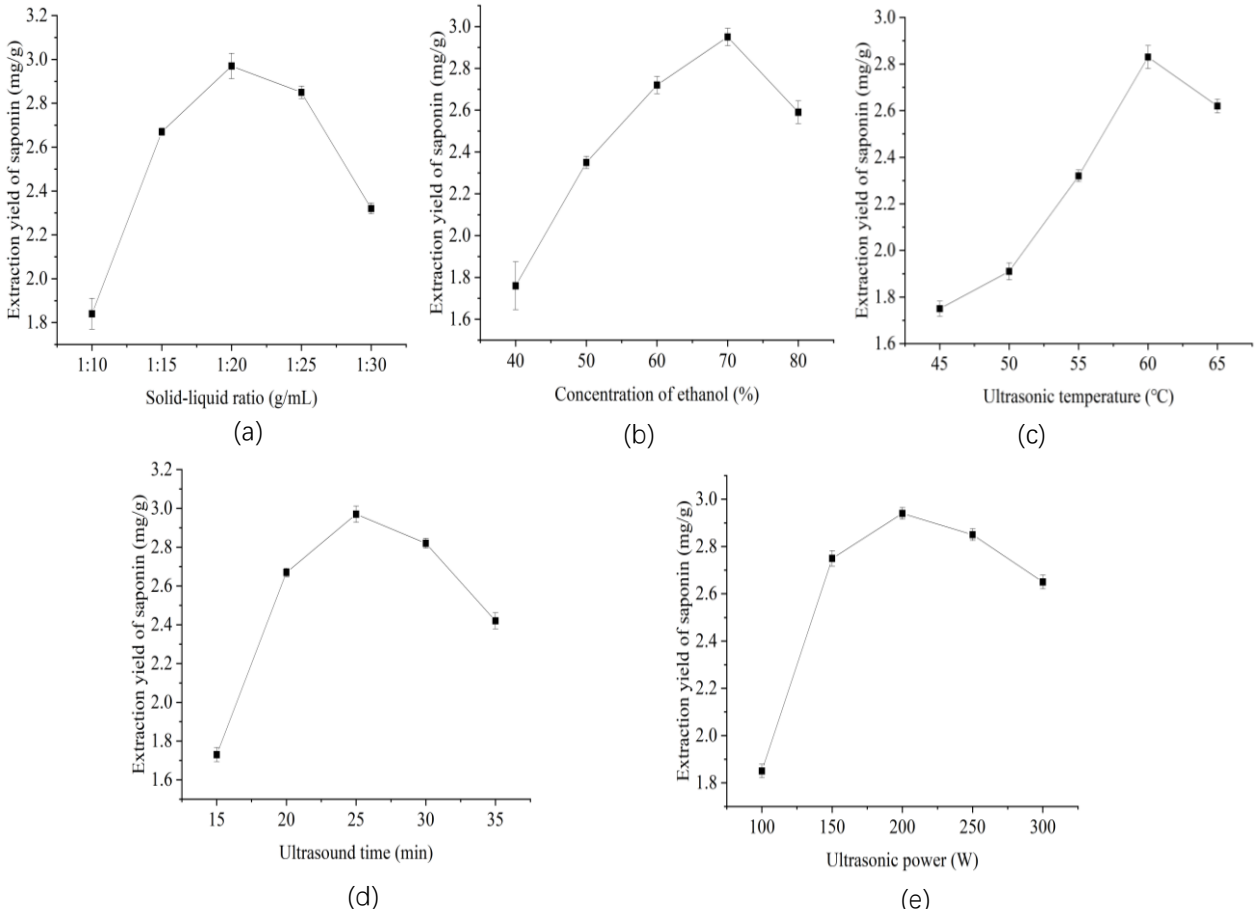


Figure 1. Single factor experiment results.

(Figure 1e). Further increases in ultrasound power resulted in a gradual decline in the total saponin yield, which could be attributed to the enhanced cavitation effect that generated significant energy and shear forces, facilitating the release of saponins from the cells into the extraction solvent. However, beyond 200 W, the decrease in saponin yield might be due to excessive shear forces disrupting the structure of the saponins, and the elevated temperature could also lead to their decomposition [27, 28]. From the perspective of improving extraction efficiency and cost-effectiveness, an ultrasound power of 200 W was considered optimal.

Response surface experiment results

1. Development of the data model

Design-Expert 8.0.6 statistical software was used for response surface analysis (Table 2). A second-order polynomial regression was performed to model the saponin yield from the roots of *Phytolacca acinosa* Roxb. The resulting second-order polynomial regression model equation was shown below.

$$Y = 3.41 + 0.79A + 0.6B + 0.47C + 0.3AB + 0.11AC + 0.028BC - 0.7A^2 - 0.98B^2 - 0.82C^2$$

where Y was a function of ultrasonic time (A), ultrasonic temperature (B), and solid-liquid ratio (C).

2. Analysis of variance for the regression model

The evaluation results of the regression model equation were shown in Table 3. The significance value of the model (P value) was less than 0.0001, and the F value was 54.74, indicating that the equation model was statistically significant. The

Table 2. Response surface experimental results.

Experiment No.	Ultrasonic time (A)	Ultrasonic temperature (B)	Solid-liquid ratio (C)	Saponin yield (mg/g)
1	0	1	1	2.95 ± 0.021
2	0	-1	1	1.43 ± 0.018
3	0	0	0	3.53 ± 0.023
4	0	1	-1	1.73 ± 0.034
5	1	1	0	3.32 ± 0.028
6	0	0	0	3.49 ± 0.043
7	-1	1	0	1.09 ± 0.011
8	1	0	1	3.12 ± 0.015
9	-1	0	-1	0.86 ± 0.008
10	1	-1	0	1.76 ± 0.019
11	1	0	-1	2.18 ± 0.017
12	0	0	0	3.28 ± 0.036
13	-1	0	1	1.38 ± 0.022
14	0	0	0	3.45 ± 0.048
15	0	-1	-1	0.32 ± 0.007
16	0	0	0	3.29 ± 0.051
17	-1	-1	0	0.75 ± 0.009

Table 3. Analysis of variance for the regression model.

Source	Sum of squares	df	Mean square	*F* vale	*P vale*
Model	20.06	9	2.23	54.74	< 0.000 1
A	4.96	1	4.96	121.87	< 0.000 1
B	2.92	1	2.92	71.64	< 0.000 1
C	1.8	1	1.8	44.11	0.0003
AB	0.37	1	0.37	9.14	0.0193
AC	0.044	1	0.044	1.08	0.3326
BC	3.025E-003	1	3.025E-003	0.074	0.7930
A^2	2.06	1	2.06	50.72	0.0002
B^2	4.03	1	4.03	98.88	< 0.000 1
C^2	2.85	1	2.85	70.02	< 0.000 1
Residual	0.28	7	0.041		
Lack of fit	0.23	3	0.077	5.74	0.062
Pure error	0.054	4	0.013		
Cor total	20.34	16			
	R^2 = 0.9860			R^2_{adj} = 0.9680	

lack of fit value was 0.062, which was greater than 0.05, suggesting that the model's discrepancy from the experimental data was minimal. The adjusted coefficient of determination (R^2_{adj}) was 0.9680, meaning that the model explained 96.80% of the variation in the response. The regression coefficient (R^2) was 0.9860, indicating a good fit of the model and a small experimental error. Therefore, this regression equation model was valid and could be used to replace actual experimental data for analysis and prediction of experimental results. Furthermore, the *P* values for P_A, P_B, P_C, P_A^2, P_B^2, and P_C^2 were all less than 0.01, and P_{AB} was less than 0.05, indicating that the main effects of ultrasonic time, ultrasonic temperature, and

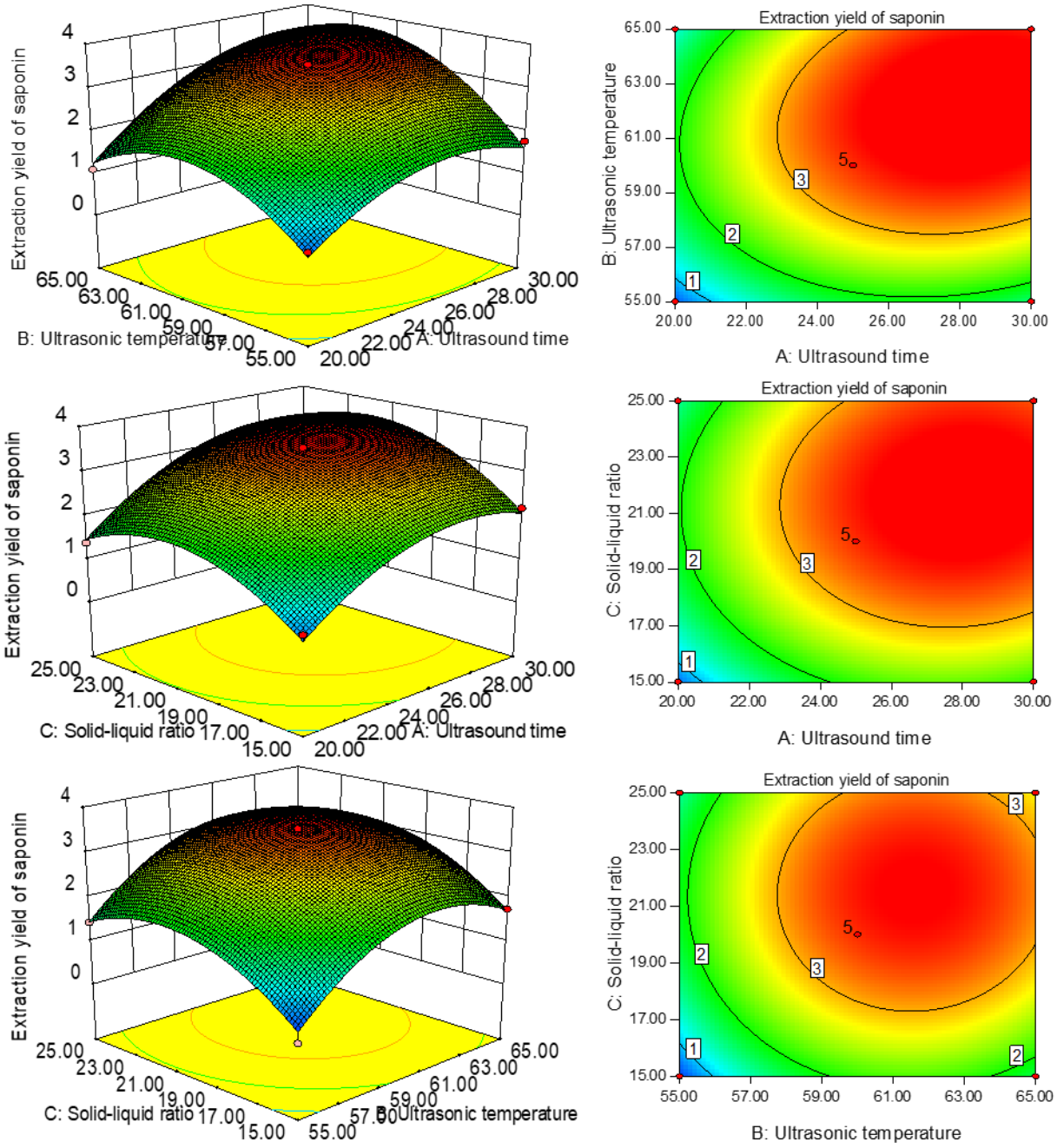


Figure 2. 3D plot of interactions between the three factors.

solid-liquid ratio, as well as the interaction terms between ultrasonic time and temperature, and between ultrasonic time and solid-liquid ratio, as well as the quadratic terms for all three factors, were statistically significant.

3. Response surface analysis

The interactions between the three factors were simulated using Design-Expert 8.0.6 software, and the response surface plots were shown in Figure 2. The extent to which each factor influenced the response value could be reflected by the steepness of the 3D response surface plot. The steeper the response surface, the stronger

the interaction between the two factors. The strength of the interaction between the two factors could also be inferred from the shape of the contour plot. The more elliptical the contour, the more significant the interaction between the two factors. The results showed that the interaction surface between A (ultrasonic time) and B (ultrasonic temperature) was the steepest, indicating that their interaction had the most pronounced effect on the saponin yield from the roots of *Phytolacca acinosa* Roxb.

4. Optimization of extraction conditions and validation experiment

Based on the analysis of the regression model, the optimal extraction conditions for saponin from the roots of *Phytolacca acinosa* Roxb. were determined as ultrasonic time of 28.39 minutes, ultrasonic temperature of 62.10°C, and a solid-liquid ratio of 1:21.69 (g/mL). Taking practical considerations into account, the optimal extraction conditions were adjusted to ultrasonic time of 28 minutes, ultrasonic temperature of 62°C, and a solid-liquid ratio of 1:22 (g/mL). Under these conditions, three parallel validation experiments were conducted, and the average actual saponin yield from the roots of *Phytolacca acinosa* Roxb. was found to be 3.63 ± 0.017 mg/g.

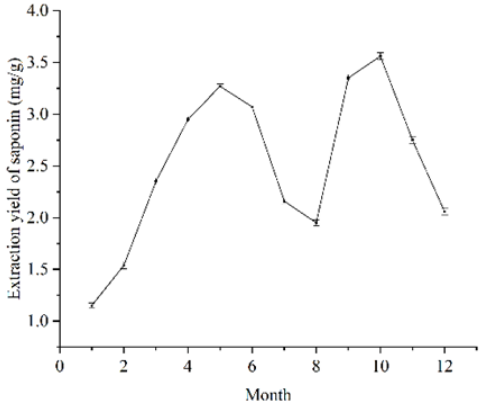
Comparison of extraction methods

The saponin yield from the roots of *Phytolacca acinosa* Roxb. using ultrasonic-assisted extraction was 3.63 ± 0.017 mg/g, while the yield using ethanol reflux extraction was 1.34 ± 0.068 mg/g, and the yield using microwave-assisted extraction was 2.77 ± 0.079 mg/g. The ultrasonic-assisted extraction method yielded 2.29 mg/g and 0.86 mg/g higher than that of the ethanol heating reflux extraction method and microwave-assisted extraction method, respectively. Moreover, this method required a lower extraction temperature and shorter time, which was beneficial for energy conservation. Therefore, compared to the other two extraction methods, ultrasonic extraction demonstrated a clear advantage for extracting saponin from the roots of *Phytolacca acinosa* Roxb.

Seasonal variation of saponin content

The results showed that there were distinct differences in the saponin content of the roots of *Phytolacca acinosa* Roxb. harvested in different months (Figure 3A). From January to May and August to October, the saponin content in the roots showed an increasing trend, while in the remaining months, the content exhibited a decreasing trend. The saponin content was the highest in May and October with the lowest content observed in January. Furthermore, when categorized by season, the saponin content in the roots of *Phytolacca acinosa* Roxb. was the highest during the autumn months from September to November and the lowest during the winter months from December to February (Figure 3B).

A.



B.

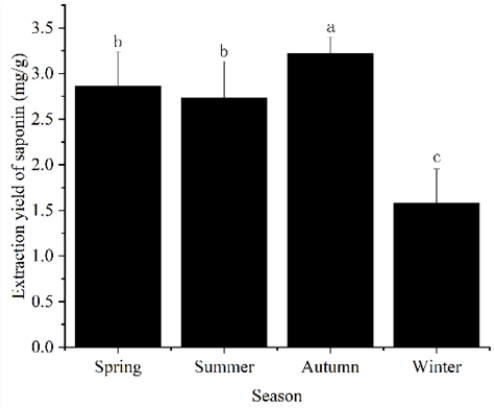


Figure 3. Seasonal variation of saponin content in the roots of *Phytolacca acinosa* Roxb. Different lowercase letters indicated significant differences ($P < 0.05$).

Conclusion

The optimal extraction conditions for saponin yield from the roots of *Phytolacca acinosa* Roxb. using ultrasonic-assisted extraction were 28 minutes of ultrasonic time at 62°C with solid-liquid ratio of 1:22 (g/mL), ethanol concentration of 70%, and ultrasonic power of 200 W. Under these conditions, the yield of saponin from the roots of *Phytolacca acinosa* Roxb. was 3.63 ± 0.017 mg/g. Ultrasonic-assisted extraction method demonstrated significant advantages in extracting saponins from the roots of *Phytolacca acinosa* Roxb. The saponin content in the roots of *Phytolacca acinosa* Roxb. varied with the harvesting month. The highest saponin content was observed in May and October, while the lowest one was in January. Autumn was therefore considered the most suitable season for harvesting the roots of *Phytolacca acinosa* Roxb.

References

1. Ma HY, WangY. 2025. Research progress on the chemical constituents and biological activities of *Phytolacca* plants. M Chem Rsch. 2025(04):18-21.
2. Wang AJ, Guan JD, Cai CT, Li JZ, Wen JJ, Jin CH. 2024. Pharmacological effects and mechanism of esculentoside A. Medicinal Plant. 15(4):26-28.
3. Wang X, Tang JY, Zhang XG, Zeng HL. 2024. The protective effect of esculentoside A on MPL/lpr mice by upregulating the expression of CD19+IL-35+breg cells and interleukin-35. Int Arch Allergy Imm. 11:11.
4. Phytolaccae Radix. 2020. In Pharmacopoeia of the People's Republic of China. Volume 1. Edited by the National Pharmacopoeia Committee. China: Chinese Pharmacopoeia Commission.
5. Li YF, Yao GT. 2011. Review on pharmacological effects and toxicity of *Phytolaccae*. Chin J Exp Tradit Med Formulae. 17(13):248.
6. Song LQ, Zheng MM. 2012. Research comment on the treatment of renal edema by Chinese medicine. Acta Chinese Medicine. 27(4):457.
7. Desta KT, Abd EL, Aty AM. 2021. Triterpenoid and saponin rich *Phytolacca dodecandra* L Herit (Endod): A review on its phytochemistry and pharmacological properties. Mini RevMed Chem. 21(1):23.
8. Barbieri L, Aron GM, Irvin JD, Stirpe F.1982. Purification and partial characterization of another form of the antiviral protein from the seeds of *Phytolacca americana* L. (pokeweed). Biochem J. 203(1):55-59.
9. Wang ZL, Chen HS, Zheng QY, Qian DH, Zhang JP. 1990. Isolation and purification of polysaccharides from *Phytolaccae radix*. Academic Journal of Naval Medical University. 11(1):56.
10. Ma XP, Zhang WF, Yi P, Lan JJ, Xia B, Jiang S, *et al*. 2017. Novel flavones from the root of *Phytolacca acinosa* Roxb. Chem Biodivers. 14(12):1612.
11. Bylka W, Matlawska I. 2001. Flawonoids and free phenolic acids from *Phytolacca americana* L. leaves. Acta Pol Pharm. 58(1):69.
12. Gao HM, Liu JX, Wang ZM, Wang WH. 2009. Phytolacacinoside A, a new triterpenoid saponin from *Phytolacca acinosa* Roxb. J Asian Nat Prod Res. 11(5):433.
13. Feng HQ, Xu XF, Yang HW, Shen BY, Hu J, Li CZ. 2016. Comparative studies on different extraction process of saponin from quinoa seeds. Sci Technol Food Ind. 37(21):216-221,227.
14. Lei T, Zhao L, Jiang C, Zhou CQ, Han J, Wang G. 2024. Comparative study on different extraction methods of twelve hour total saponins from the medicine ginseng research. Ginseng Res. 36(05):37-39.
15. Wei Q, Zhang YH. 2023. Ultrasound-assisted polysaccharide extraction from *Cercis chinensis* and properties, antioxidant activity of polysaccharide. Ultrason Sonochem. 96:106422.
16. Otero P, Carpena M, Garcia-Oliveira P, Lvarez JE, Lage MNP. 2023. Seaweed polysaccharides: Emerging extraction technologies, chemical modifications and bioactive properties. Crit Rev Food Sci Nutr. 63(13):1901-1929.
17. Yan RL, Mao LY, Liao Y, Huang GW, Xie ZD, He XY. 2017. Microwave assisted extraction of seed oil and root saponin from *Phytolacca americana* and their physicochemical properties as well as insecticidal activity. Nat Prod Res Develop. 29(7):1218-1223.
18. Zhou JC, Zhou GY, Liu JA, He YA, Dong WT. 2017. Antifungal mechanis of the ethyi acetate extracts from *Phytolacca americana* and *Ageratum conyzoides* against *Colletotrichum gloeosporioides* of dalbergia odorifera. Plant Prot. 43(4):46-50.
19. Pei LX, Ji BY, Chen SQ, Sun LP. 2017. Quality of *Phytolaccae radix* from different sources in Henan. Chin J Exp Tradit Med. 23(2):48-52.
20. Xie QL, Chen Z, Pan Y, Yang JJ. 2019. Different dry means on the contents of total saponins and esculentoside A of *Phytolacca americana*. Hubei Agricultural Sciences. 58(8):126-128.
21. Zhen SY, Chen S, Geng S, Zhang H, Chen YS, Liu BG. 2022. Ultrasound-assisted natural deep eutectic solvent extraction and bioactivities of flavonoids in *Ampelopsis grossedentata* leaves. Foods. 11(5):668.
22. Tian Y, Mao JS, Li L, Xue J. 2024. Optimization of *Aralia chinensis* L. saponin extraction process. Chemical Engineer. 38(12):1-5, 14.
23. Sun SJ, Fu ML, Lou SH, Li DD, Han X, Gao S, *et al*. 2024. Optimization of flavonoids extracted from hawthorn (*Crataegus pinnatifida*) by ultrasonic-assisted deep eutectic solvent. Food Biosci. 59:103767.
24. Kong F, Li L, Liu YJ. 2020. Ultrasonic-assisted deep eutectic eolvents extraction of total flavonoids from apple leaves. Sci Technol Food Ind. 41(14):134-139, 147.

25. Gong QY, Guo ZD, Sun ZH, Gong JG, Wei FY. 2020. Graphene oxide-assisted ethanol reflux extraction of total flavonoids from *Ginkgo biloba* Leaves: Study of kinetics and mechanism. Chemicke Zvesti. 74(3):971-984.
26. Lei QR, Wang XJ, Hong YN, Pan H, Fan YR. 2024. Optimization of ultrasonic assisted low melting solvent extraction process for total saponins of ginseng. Chin Tradit Pat Med. 46(10):3426-3430.
27. Li YB, Pan ZC, Wang BS, Yu W, Song SW, Feng HH, *et al*. 2020. Ultrasound-assisted extraction of bioactive alkaloids from *Phellodendri amuren* sis cortex using deep ertectic solvent aqueous solutions. New J Chem. 44(22):9172-9178.
28. Chen HH, Tarun BW, Li GF, Luo ZS. 2020. Enzymolysis-ultrasound-assisted surfactant extraction and antioxidant activity of saponins from *Panax notoginseng* flowers. Food Ferment Ind. 46(20):178-186.