

# Optimization of Loganin Extraction from *Viburnum erosum* Thunb. by Response Surface Methodology

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**Abstract** [Objectives] This study was conducted to efficiently extract loganin from *Viburnum erosum* Thunb. [Methods] Ultrasound-assisted extraction coupled with response surface methodology was employed. The extraction was carried out with 60% methanol as the solvent and loganin extraction rate as the evaluation index. The effects of liquid-to-solid ratio, extraction temperature, and extraction time on loganin extraction rate were investigated through single-factor experiments. Subsequently, a Box-Behnken experimental design was implemented based on the single-factor results to optimize the extraction process. [Results] Under the conditions of liquid-to-solid ratio of 6 mL/g, extraction temperature of 39 °C, and extraction time of 88 min, the loganin extraction rate was 4.199%, which agreed with the predicted value of 4.271%. The order of three factors affecting the extraction rate of loganin in *V. erosum* Thunb was liquid-to-solid ratio > extraction time > extraction temperature. The response surface methodology demonstrates high stability and feasibility for optimizing the extraction process, and the proposed protocol achieves efficient loganin extraction from *V. erosum* Thunb. [Conclusions] This study provides a novel approach for extracting iridoid glycosides from *V. erosum* Thunb. and expands the plant sources of loganin.

**Key words** *Viburnum erosum* Thunb.; Loganin; Ultrasonic-assisted extraction; Single-factor experiment; Response surface

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*Viburnum* plants, belonging to the Adoxaceae family, comprise approximately 200 species distributed worldwide. Due to their remarkable ecological adaptability and ornamental value, they are renowned in international horticulture as "versatile landscaping shrubs". Hubei Province in central China serves as a significant distribution area for this genus, with the Enshi Tujia and Miao Autonomous Prefecture in southwestern Hubei being particularly notable. This region is distributed with 28 taxonomic units (including 22 original species and 6 varieties), accounting for over 65% of the total *Viburnum* species in the province (43 species)<sup>[1]</sup>. As a representative species of *Viburnum* in the Caprifoliaceae family, *Viburnum erosum* Thunb. is a highly adaptable deciduous shrub that grows on mountain slopes, in forests, or thickets at elevations of 300 – 1 800 m. It is distributed across East, Central, and Southwest China, as well as in Shaanxi, Guangdong, and Guangxi<sup>[2]</sup>. *V. erosum* Thunb. not only possesses significant ornamental value but also considerable medicinal importance. It has the effects of detoxifying, eliminating damp toxin and relieving itching.

Studies have shown that plants of the genus *Viburnum* are rich

in structurally diverse bioactive components, including a wide range of terpenoids, flavonoids, and phenolic compounds<sup>[3]</sup>. In 2011, Li *et al.*<sup>[4]</sup> isolated loganin from the *Viburnum* genus for the first time by employing various chromatographic methods and spectroscopic techniques. Loganin is a natural product with extensive biological activities, which has been proven to exert neuroprotective, anti-inflammatory, immunomodulatory, anti-tumor and anti-diabetic complication effects<sup>[5–7]</sup>. For inflammation-related diseases, Loganin can alleviate intestinal barrier damage and inflammatory responses in septic rats by reducing the activity of the Rho A/ROCK1 signaling pathway<sup>[8–9]</sup>. In oral squamous cell carcinoma, it precisely regulates the growth, proliferation, migration, and apoptosis of cancer cells by intervening in the circ\_0030018/miR-142-5p signaling pathway<sup>[10–12]</sup>. Loganin directly binds to and activates AMPK $\alpha$ , which reduces the expression of key factors in the fatty acid synthesis pathway, such as SREBP-1 and FASN, thereby modulating lipid metabolism in hepatocytes<sup>[13–15]</sup>. In diabetic retinopathy (DR), it can also target the RhoA/ROCK signaling pathway to significantly reduce the level of inflammatory factors and mitigate oxidative stress injury, thereby effectively alleviating retinal pathological changes in DR rats<sup>[21]</sup>. Currently, ultrasound-assisted extraction is primarily employed to obtain iridoid glycosides such as loganin. The cavitation effect generated by ultrasound causes physical rupture of plant cells, facilitating the release of target components. This process is not only operationally convenient and time-efficient, but also achieves high extraction yields while avoiding the degradation of active compounds by high temperatures. As an efficient and environmentally friendly extraction method, it is widely used for the extraction of active ingredients

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from plants<sup>[16]</sup>.

Currently, research on the extraction of active components from *V. erosum* Thunb. remains unexplored, and no relevant studies have been published to date. In this study, an extraction process for loganin was established, aiming to provide a scientific basis for the development of the medicinal value of *V. erosum* Thunb. and offer new approaches for expanding natural sources of loganin.

## Materials and Methods

### Materials and reagents

*V. erosum* Thunb. was collected from the Enshi region of Hubei Province and identified by associate professor Cui Lingjun from the College of Forestry and Horticulture at Hubei Minzu University as *V. erosum* Thunb. of the Adoxaceae family. Fresh, mature and young leaves of *V. erosum* without pest damage or spots were selected, washed, and dried at 40 °C under vacuum conditions. After drying, the leaves were ground and passed through a 60-mesh sieve for subsequent use.

The reagents used in this experiment included methanol (analytically pure, Sinopharm Chemical Reagent Co., Ltd.), deionized water, and loganin standard (purity ≥ 98%, Shanghai Shifeng Biological technology Co., Ltd.).

### Experimental instruments

The instruments used included a SHZ-D circulating water vacuum pump (Zhengzhou Greatwall Scientific Industrial & Trade Co., Ltd.), a DZF-6020A vacuum drying oven (Shandong Ru Yi Scientific Instrument Co., Ltd.), a YB-300 high-speed multi-functional grinder (Yongkang Sufeng Industrial and Trading Co., Ltd.), a PS-40 ultrasonic cleaner, an FA1004 electronic balance (Shanghai Xiniu Leibo Instrument Co., Ltd.), and an LC-20 semi-preparative high-performance liquid chromatography system (Shimadzu Corporation).

### Experimental design

Due to the low molecular weight and hydrophilic nature of iridoid compounds, methanol, ethanol, and water are commonly used as extraction solvents. When methanol is used as the extraction solvent, the yield is the highest. Therefore, methanol was selected as the extraction solvent for this study<sup>[22]</sup>.

**Preparation of loganin standard solution** A 3 mg sample of loganin standard was accurately weighed, dissolved in methanol, and diluted to a final volume of 10 ml to prepare a loganin standard solution with a mass concentration of 0.3 mg/ml.

**Preparation of loganin extract** A 0.1 g sample of *V. erosum* Thunb. powder was accurately weighed, and 5 ml of 60% methanol solution (liquid-to-solid ratio 50 : 1) was added. The mixture was subjected to ultrasonic extraction at 50 °C for 30 min, and the extraction process was repeated twice. The two extraction filtrates were combined, concentrated to dryness by rotary evaporation, and then dissolved in methanol. The solution was diluted to a final

volume of 10 ml and filtered through a 0.22 μm microporous membrane to obtain the test solution.

**Chromatographic conditions** Chromatographic separation was performed on a Shimadzu LC-20 HPLC system equipped with an Extend-C<sub>18</sub> column (5 μm, 4.6 mm × 250 mm). The mobile phase consisted of methanol-water (40 : 60, v/v) delivered at a flow rate of 0.8 ml/min, with the column temperature maintained at 25 °C.

Detection was carried out at a UV wavelength of 238 nm with an injection volume of 15 μl.

**System applicability test** The loganin standard solution from section "Preparation of loganin standard solution" and the test solution from section "Preparation of loganin extract" were measured and injected according to the chromatographic conditions specified in section "Chromatographic conditions".

**Instrument precision** The standard solution specified in section "Preparation of loganin standard solution" was measured and injected six times consecutively under the chromatographic conditions described in section "Chromatographic conditions". The relative standard deviation (RSD) of the peak areas was calculated.

**Linear relationship investigation** The standard solution from section "Preparation of loganin standard solution" was measured and diluted with methanol to prepare standard solutions with mass concentrations of 0.3, 0.1, 0.06, 0.03, 0.01, 0.006, and 0.003 mg/ml. Injection analysis was performed under the chromatographic conditions specified in section "Chromatographic conditions" and the chromatographic peak areas corresponding to each concentration point were recorded. A linear regression equation was established with the standard concentration as X and the peak area as Y.

**Repeatability test** Six portions of *V. erosum* Thunb. powder were weighed, with precise weights of 0.101 0, 0.101 1, 0.100 9, 0.101 1, 0.100 9, and 0.101 3 g, respectively. Test solutions were prepared according to the conditions specified in section "Preparation of loganin extract", and injection analysis was performed under the chromatographic conditions described in section "Chromatographic conditions". Six parallel injections were completed, and the relative standard deviation (RSD) of the peak areas was calculated.

**Stability test** A 0.100 6 g sample of *V. erosum* Thunb. powder was accurately weighed and used to prepare the test solution under the conditions specified in section "Preparation of loganin extract". The solution was injected for analysis at 0, 2, 4, 8, 12, and 24 h under the chromatographic conditions described in section "Chromatographic conditions", and the relative standard deviation (RSD) of the peak areas was calculated.

**Recovery test** Six test solutions were prepared in parallel according to the method described in section "Preparation of loganin extract". A certain amount of loganin standard equivalent to that present in the sample was added to each solution. Analysis was

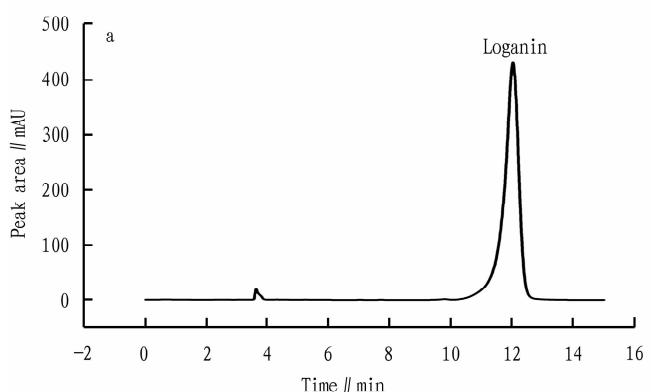
then performed under the chromatographic conditions specified in section "Chromatographic conditions", and the recovery rate was calculated.

### Single-factor experiments

(1) Methanol concentration: A 0.1 g sample of *V. erosum* Thunb. powder was weighed and extracted with fixed parameters of liquid-to-solid ratio 10 ml/g, extraction temperature 30 °C, and extraction time 60 min. Methanol concentrations of 20%, 40%, 60%, 80%, and 100% were tested. After treatment, the extraction rate of loganin was determined by high-performance liquid chromatography.

(2) Liquid-to-solid ratio: A 0.1 g sample of *V. erosum* Thunb. powder was weighed and extracted with the optimal methanol concentration, while keeping the extraction temperature at 30 °C and extraction time at 60 min. Liquid-to-solid ratios of 5, 10, 15, 20, and 25 ml/g were tested. After treatment, the extraction rate of loganin was determined by HPLC.

(3) Extraction temperature: A 0.1 g sample of *V. erosum* Thunb. powder was weighed and extracted with the optimal methanol concentration and liquid-to-solid ratio, while maintaining a constant extraction time of 60 min. Extraction temperatures of 20, 30, 40, 50, and 60 °C were tested. After treatment, the extraction rate of loganin was determined by HPLC.



a. Chromatogram of loganin standard; b. Chromatogram of loganin test solution.

Fig. 1 The chromatogram of loganin standard and test solution at 238 nm

### Results of precision test

The RSD for precision was 1.78% ( $n=6$ ), which is below 2%, indicating that the instrument precision is satisfactory.

### Results of linear relationship investigation

Successively, 20  $\mu$ l of standard solutions at different concentrations were quantitatively analyzed by HPLC. Using the mass concentration (mg/ml) of the standard solution as the X-axis and the loganin peak area as the Y-axis, a standard curve for loganin was generated (as shown in Fig. 2). The linear regression equation obtained was  $Y = 3.4759 + 490.0538X$ , with  $R^2 = 0.9991$ , indicating a strong linear relationship between loganin concentration and peak area within the mass concentration range of 0.003 – 0.300 mg/ml.

(4) Extraction time: A 0.1 g sample of *V. erosum* Thunb. powder was weighed and extracted with the optimal methanol concentration, liquid-to-solid ratio, and extraction temperature. The extraction time was set as 30, 60, 90, 120, and 150 min, respectively. After treatment, the extraction rate of loganin was determined by HPLC.

**Response surface experiment** Based on the results of single-factor experiments, the liquid-to-solid ratio (A), extraction temperature (B), and extraction time (C), which had significant effects on the extraction rate of loganin, were selected as factors for investigation. A three-factor three-level Box-Behnken experimental design was employed.

## Results and Analysis

### Results of system applicability test

Under the chromatographic conditions specified in "Chromatographic conditions", equal volumes of standard solution and test solution were analyzed by HPLC. Comparison of the chromatograms of loganin standard and test sample at 238 nm (as shown in Fig. 1) revealed a distinct loganin peak in the *V. erosum* Thunb. test solution under these conditions. The loganin peak was well separated, demonstrating good specificity of the method.

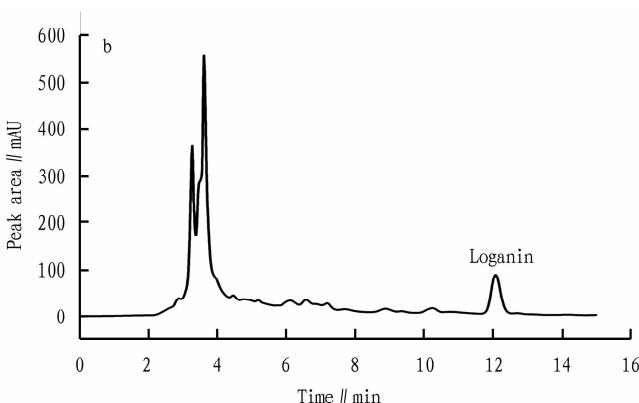


Fig. 2 The standard curve of loganin

## Results of repeatability test

The repeatability *RSD* was 1.88% ( $n = 6$ ), which is below 2%, indicating good repeatability of the method.

## Results of stability test

Within 24 h, the stability *RSD* was 1.28%, falling between -2% and 2%, indicating good solution stability.

## Results of recovery test

The average recovery rate of loganin was 99.63%, and the *RSD* was 1.42%, falling between -2% and 2%, demonstrating good accuracy of the method.

## Analysis of single-factor experiment results

### Effect of methanol concentration on the extraction rate of loganin

As shown in Fig. 3a, the extraction rate of loganin initially increased and then decreased with methanol concentration increasing. The optimal extraction rate was achieved at a methanol concentration of 80%. The underlying reason lies in that the polarity of the extraction solvent matches that of the target compound. According to the "like dissolves like" principle, target molecules are more likely to diffuse from the plant cell matrix into the extraction solution under such conditions. Increasing or decreasing the solvent ratio would lead to a greater mismatch in polarity between the extraction solution and the target molecules. Additionally, the increased dissolution of non-target substances, particularly lipophilic compounds, in the solvent may impede the effective extraction of the target molecules<sup>[17]</sup>. Therefore, 80% methanol was selected as the optimal extraction solvent.

### Effect of liquid-to-solid ratio on the extraction rate of loganin

As shown in Fig. 3b, the extraction efficiency reached its peak at a liquid-to-solid ratio of 10 : 1. Deviating from this ratio, either

higher or lower, resulted in reduced extraction efficiency. Increasing the solvent beyond the 10 : 1 ratio led to a decline in extraction efficiency, as the target molecules had already been nearly fully extracted. An increase in the liquid-to-solid ratio leads to higher moisture content during filtration and evaporation, prolonging treatment time and potentially resulting in loss of loganin. Furthermore, the increased dissolution of impurity molecules may interfere with the solubility of loganin<sup>[18]</sup>. Therefore, a ratio of 10 : 1 was selected as the appropriate liquid-to-solid ratio.

### Effect of extraction temperature on the extraction rate of loganin

As shown in Fig. 3c, the extraction efficiency reached its peak at 40 °C. Below 40 °C, the increase in temperature enhanced the solubility of the target compound in the solvent. However, beyond 40 °C, the dissolution of other substances with similar polarity increased sharply, which may have reduced the solubility of the target compound. Therefore, 40 °C was selected as the optimal extraction temperature.

### Effect of extraction time on the extraction rate of loganin

As shown in Fig. 3d, an ultrasonic extraction time of 60 min achieved the highest extraction rate of loganin. An excessively short extraction time resulted in insufficient cell wall disruption and incomplete release of loganin, thereby negatively affecting the extraction yield<sup>[19]</sup>. Moreover, the dissolved amount of loganin increased with prolonged ultrasonic time within 60 min, and tended to stabilize after reaching saturation. Excessive extraction duration may reduce the extraction efficiency of loganin due to increased dissolution of impurities, leading to a decline in the extraction rate. Therefore, 60 min was determined to be the optimal ultrasonic extraction time.

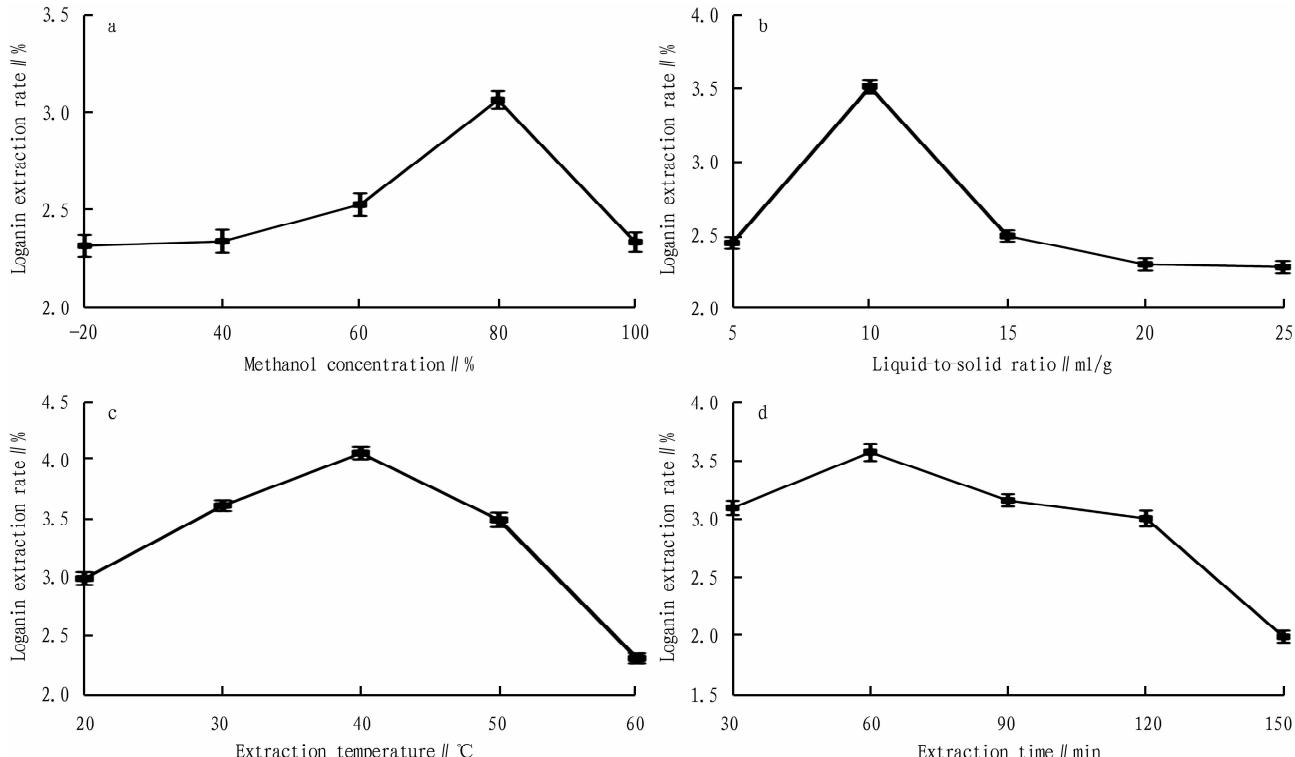


Fig. 3 The influence of various factors on the content of loganin

## Results and analysis of response surface experiment

**Response surface experiments** Based on the results of the single-factor experiments, a Box-Behnken experimental design was implemented, selecting three factors: liquid-to-solid ratio (A), extraction temperature (B), and extraction time (C). The factors and their corresponding levels for the response surface experiment are presented in Table 1. According to the design presented in Table 1, a response surface experiment was conducted, comprising 17 sets of loganin extraction tests. Each set was performed in triplicate, and the average value was taken as the extraction yield of

loganin (Y). The results are presented in Table 2. Additionally, variance analysis was performed on the experimental data, with the results shown in Table 3.

**Table 1** Box-Behnken factors and levels

| Level | Factor    |         |          |
|-------|-----------|---------|----------|
|       | A // ml/g | B // °C | C // min |
| -1    | 5         | 30      | 30       |
| 0     | 10        | 40      | 60       |
| 1     | 15        | 50      | 90       |

**Table 2** Box-Behnken experimental design and results

| No. | Factor    |         |          | Y // % | Factor    |         |          | Y // % |       |
|-----|-----------|---------|----------|--------|-----------|---------|----------|--------|-------|
|     | A // ml/g | B // °C | C // min |        | A // ml/g | B // °C | C // min |        |       |
| 1   | -1        | -1      | 0        | 3.265  | 10        | 0       | 1        | -1     | 3.492 |
| 2   | 1         | -1      | 0        | 2.801  | 11        | 0       | -1       | 1      | 3.531 |
| 3   | -1        | 1       | 0        | 2.919  | 12        | 0       | 1        | 1      | 3.464 |
| 4   | 1         | 1       | 0        | 2.864  | 13        | 0       | 0        | 0      | 4.162 |
| 5   | -1        | 0       | -1       | 3.713  | 14        | 0       | 0        | 0      | 4.091 |
| 6   | 1         | 0       | -1       | 4.096  | 15        | 0       | 0        | 0      | 4.099 |
| 7   | -1        | 0       | 1        | 4.196  | 16        | 0       | 0        | 0      | 4.092 |
| 8   | 1         | 0       | 1        | 3.576  | 17        | 0       | 0        | 0      | 3.936 |
| 9   | 0         | -1      | -1       | 3.293  |           |         |          |        |       |

As shown in Table 2, the actual value of the loganin extraction yield (Y) was calculated using following formula:

$$Y = 4.08 - 0.0945A - 0.0189B + 0.0216C + 0.1022AB - 0.2508AC - 0.0665BC - 0.3318A^2 - 0.7820B^2 + 0.1510C^2.$$

**Table 3** Analysis of variance

| Experiment No.       | Sum of squares | df | Mean square | F value | P value | Significance          |
|----------------------|----------------|----|-------------|---------|---------|-----------------------|
| Model                | 3.58           | 9  | 0.3983      | 41.55   | <0.0001 | Extremely significant |
| A                    | 0.0714         | 1  | 0.0714      | 7.45    | 0.0293  | Significant           |
| B                    | 0.0029         | 1  | 0.0029      | 0.2973  | 0.6025  |                       |
| C                    | 0.0037         | 1  | 0.0037      | 0.3903  | 0.5520  |                       |
| AB                   | 0.0418         | 1  | 0.0418      | 4.36    | 0.0751  |                       |
| AC                   | 0.2515         | 1  | 0.2515      | 26.24   | 0.0014  | Extremely significant |
| BC                   | 0.0177         | 1  | 0.0177      | 1.85    | 0.2165  |                       |
| $A^2$                | 0.4634         | 1  | 0.4634      | 48.34   | 0.0002  | Extremely significant |
| $B^2$                | 2.57           | 1  | 2.57        | 268.61  | <0.0001 | Extremely significant |
| $C^2$                | 0.0960         | 1  | 0.0960      | 10.02   | 0.0158  | Significant           |
| Residual             | 0.0671         | 7  | 0.0096      |         |         |                       |
| Lack of fit          | 0.0391         | 3  | 0.0130      | 1.86    | 0.2769  | Non-significant       |
| Absolute error       | 0.0280         | 4  | 0.0070      |         |         |                       |
| Total sum of squares | 3.65           | 16 |             |         |         |                       |

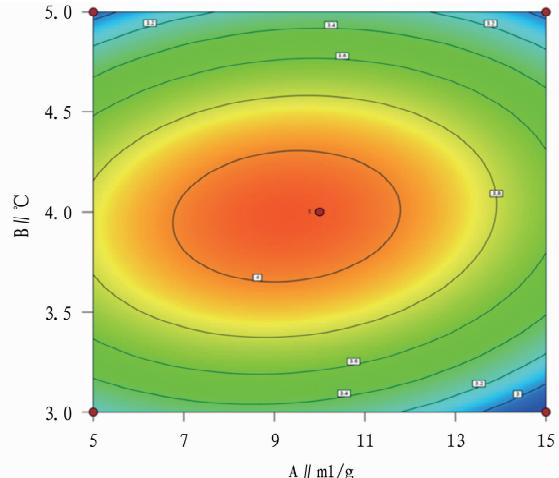
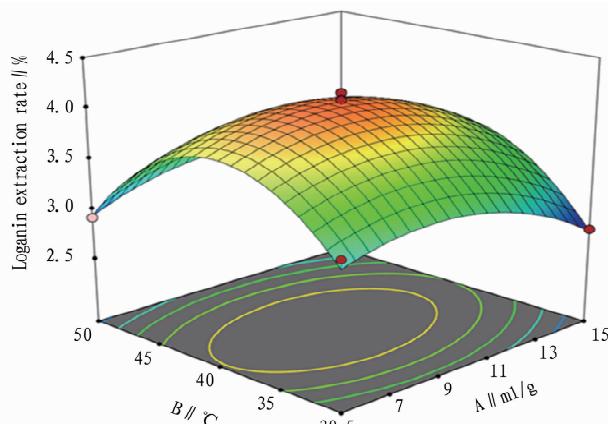
As shown in Table 3, the quadratic multivariate regression model demonstrates significant goodness-of-fit and robustness in predicting the extraction yield of loganin under different treatment conditions. The correlation coefficient ( $R^2 = 0.9816$ ) approaches 1, indicating that 98.16% of the variation in the response variable can be explained by the independent variables, confirming that the model possesses both high explanatory power and predictive capability. Moreover, the lack-of-fit analysis ( $F = 1.86$ ,  $P = 0.2769$ ) shows a  $P$ -value above the significance threshold (0.05), further demonstrating good agreement between the experimental data and the model. Based on the  $F$ -values and  $P$ -values of various factors and their interactions, the ANOVA results indicated that the

liquid-to-solid ratio (A), extraction temperature (B), and extraction time (C) exhibited significant nonlinear functional relationships with the yield of loganin. The order of their effects, ranked by  $F$ -value, was: A ( $F = 18.6$ ) > C ( $F = 12.3$ ) > B ( $F = 5.8$ ), meaning liquid-to-solid ratio > extraction time > extraction temperature.

**Response surface analysis** Response surface methodology was employed to optimize three factors, *i.e.*, liquid-to-solid ratio (A), extraction temperature (B), and extraction time (C), with the aim of improving the extraction rate of loganin from *V. erosum* Thunb. Through Box-Behnken experimental design and analysis of variance, the influence patterns of these three factors on the

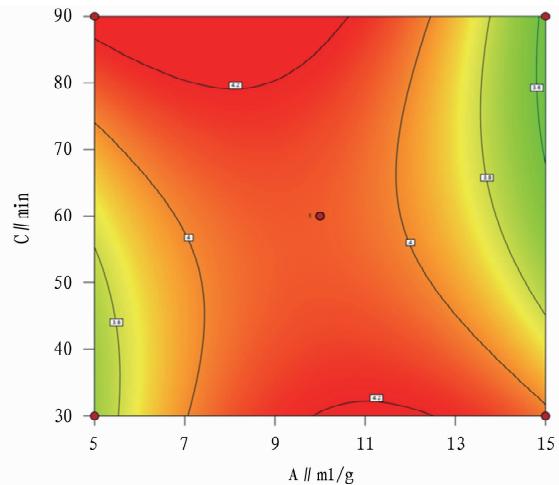
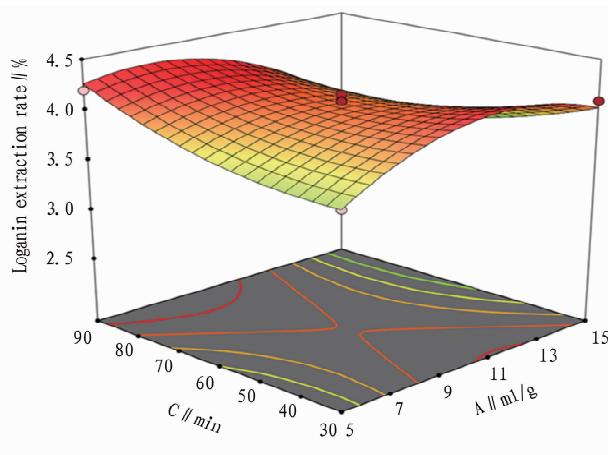
extraction rate of loganin were elucidated. The response surface and contour plots systematically illustrated the interactions among

the three factors and their relative impacts on the extraction rate of loganin, with the results presented in Fig. 4 – Fig. 6.



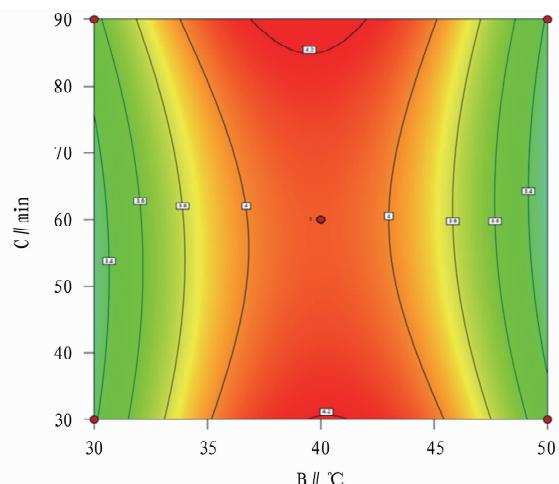
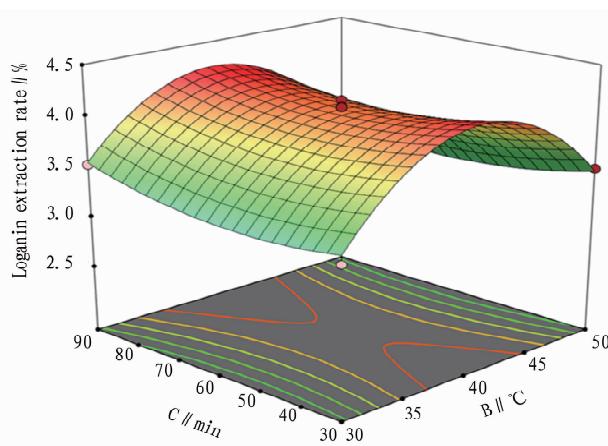
a. Response surface of A and B; b. Contour lines of A and B.

**Fig. 4 Response surface and contour plots of the interaction between liquid-to-solid ratio (A) and extraction temperature (B)**



a. Response surface of A and C; b. Contour lines of A and C.

**Fig. 5 Response surface and contour plots of the interaction between liquid-to-solid ratio (A) and extraction time (C)**



a. Response surface of B and C; b. Contour lines of B and C.

**Fig. 6 Response surface and contour plots of the interaction between extraction temperature (B) and extraction time (C)**

As shown in Fig. 4, the contour lines are elliptical, indicating a certain degree of interaction between the liquid-to-solid ratio and extraction temperature on the extraction rate of loganin, though the interaction is not significant ( $P = 0.075 > 0.05$ )<sup>[20]</sup>. From Fig. 5, the interaction between the liquid-to-solid ratio and extraction time is the most pronounced in the response surface curve, exhibiting a highly significant effect on the extraction rate of loganin ( $P = 0.0014 < 0.01$ ). Fig. 6 shows saddle-shaped contour lines, indicating a certain interaction between extraction temperature and extraction time on the extraction rate of loganin, though the interaction is not significant ( $P = 0.2165 > 0.05$ ). Further analysis revealed that the significance of the interactions among the three factors followed the order: AC > AB > BC.

**Results of validation experiment** Using Design Expert 13 software, the theoretically optimal extraction conditions for this process were determined as follows: liquid-to-solid ratio of 5.546 ml/g, extraction temperature of 38.911 °C, and extraction time of 88.244 min. The model predicted a maximum loganin extraction rate of 4.271%. To validate the model's accuracy and account for practical constraints, the process parameters were adjusted to a liquid-to-solid ratio of 6 ml/g, an extraction temperature of 39 °C, and an extraction time of 88 min. Triplicate parallel experiments conducted under these adjusted conditions yielded an average loganin extraction rate of 4.199%. The close agreement between the experimental and predicted results confirms that response surface methodology is a feasible and efficient approach for optimizing the ultrasonic extraction of loganin.

## Conclusions and Discussion

This study utilized the Box-Behnken response surface methodology to optimize three factors, *i. e.*, liquid-to-solid ratio, extraction temperature, and extraction time, for determining the optimal process for ultrasound-assisted extraction of loganin from *V. erosum* Thunb. Among these factors, the liquid-to-solid ratio had the greatest impact on the extraction rate of loganin, followed by the extraction time, while the extraction temperature had the least effect. Under the optimal extraction conditions, the extraction rate of loganin reached 4.271%.

In this study, the ultrasound-assisted extraction of loganin from *V. erosum* Thunb. was investigated, providing a research foundation and direction for the preparation of other functional compounds from this plant. Subsequent research will focus on evaluating the anti-inflammatory effects of the loganin extract to validate its potential as a natural anti-inflammatory component. Building on this experimental work, further studies will also explore the extraction of other constituents from *V. erosum* Thunb., such as  $\beta$ -sitosterol, ursolic acid, and daucosterol, to develop other medicinal components.

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## The effectiveness evaluation of curriculum reform requires in-depth reflection

Current assessments of the effectiveness of teaching reforms in universities often remain limited to short-term indicators such as course satisfaction and student project presentations. There is a lack of longitudinal studies tracking the long-term impact on graduates' career development and design practices. In the future, it is necessary to establish more scientific and long-term evaluation mechanisms. Through methods such as alumni interviews, employer feedback, and case studies, a comprehensive assessment of the actual impact of curriculum reform on students' professional competencies and industry development should be conducted. This will provide a basis for the continuous optimization of the reform.

## Conclusions and Prospects

The Nature-based Solutions-oriented reform of landscape design courses for postgraduate represents an active response to current ecological and environmental challenges as well as industry talent demands. Through systematic reform measures including restructuring the curriculum system, innovating teaching methods, and refining evaluation mechanisms, the effective cultivation of advanced landscape design professionals capable of meeting future challenges can be achieved. The curriculum reform framework proposed in this paper emphasizes the deep integration of ecological knowledge and design practice, the balanced development of scientific thinking and artistic expression, and the equal importance of system analysis and innovative problem-solving ability. It offers a new direction for the development of graduate education in landscape design.

Reflecting on the reform practice of the course *Landscape Design* and *Environmental Protection* at Zhongyuan University of Technology, some successful experiences are gradually taking shape. For example, the "experts in the classroom" model has effectively bridged academia and industry, and the introduction of the CDIO educational philosophy has provided a framework for cultivating project management skills throughout the entire process. These diverse explorations collectively enrich the content of graduate education in landscape design and offer varied reference options for universities with different foundations and characteristics.

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Looking ahead, Nature-based Solutions-oriented landscape design education still needs continuous deepening in several aspects. It is essential to further strengthen interaction with the forefront of scientific research, promptly integrating new discoveries from fields such as ecology and geography into teaching content. Active exploration is needed for the application methods of digital technologies throughout the entire process of Nature-based Solutions design to enhance the precision and adaptability of designs. Attention must be paid to issues of design justice and inclusivity, ensuring that Nature-based Solutions benefit all social groups, particularly vulnerable communities and environmental justice areas.

The fundamental goal of landscape design education is to cultivate professional forces capable of leading industry transformation and promoting sustainable development. Through curriculum reform based on Nature-based Solutions, a new generation of landscape designers is being nurtured. They not only have solid design skills and ecological knowledge, but also possess system thinking, interdisciplinary collaboration, and innovative problem-solving abilities. These professionals are poised to play a significant role in the great journey of China's ecological civilization construction.

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