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Optimization of Hot-Water Extraction of Polysaccharides from *Lycium chinense* Leaves: Process Modeling and Response Surface Methodology

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ABSTRACT

Plant polysaccharides have attracted considerable research interest due to their diverse bioactive properties, including immunomodulatory, antioxidant, and hypoglycemic effects. Among phytochemical sources, *Lycium* species are recognized as polysaccharide-rich plants, yet reports specifically addressing the extraction optimization of *Lycium chinense* leaf polysaccharides from Southwest China remain limited. This study optimizes the hot-water extraction of polysaccharides from *L. chinense* leaves using single-factor experiments and response surface methodology (RSM). Key parameters, including liquid-to-solid ratio (1:30–1:50), temperature (60–80 °C), and duration (1–3 h), were evaluated. RSM determined optimal conditions of liquid-to-solid ratio 1:44.69, temperature 71.14 °C, and time 1.72 h, with a predicted crude extract yield of 6.81%. Verification under practical conditions (ratio 1:45, 70 °C, 1.5 h) achieved a yield of 6.77 ± 0.004%, confirming model reliability (R^2 = 0.9876). Qualitative tests provided preliminary indications that the extract was predominantly composed of non-reducing, non-starch polysaccharides. The optimized hot-water extraction method provides an efficient laboratory-scale platform for obtaining crude polysaccharide extracts from *L. chinense* leaves. While the present study focuses on extraction process optimization, the crude extract obtained may serve as a starting material for future investigations into compositional characterization, bioactivity profiling, and potential applications in functional ingredient development.

1. INTRODUCTION

Lycium chinense (Mill.), commonly known as large-leaf wolfberry, is a leaf-utilization species within the *Lycium* genus, with its principal utilized parts being leaves and buds. In China, this plant is recognized by various common names, including large-leaf wolfberry, wolfberry vegetable, and wolfberry leaf buds. Young stems and leaves are consumed as vegetables or brewed as tea, and the plant functions as a versatile herb for both therapeutic and culinary purposes, with widespread adoption in recent traditional and modern applications. The species is predominantly distributed in southern China, particularly in Guangdong, Zhejiang, and Guangxi provinces, with limited occurrence in northern regions [1]. In Southwest China, the plants are characterized by vigorous growth under warm, humid climatic conditions, producing soft, pendulous branches and broad, deep-green leaves (4–6 cm long, 1–2 cm wide). The fruits are typically uncommon and small, and the young leaf buds are locally known as wolfberry buds. Recent germplasm evaluations of leaf-utilization wolfberries have revealed significant variation in agronomic traits among cultivars [1, 2], underscoring the importance of regional specificity in understanding their biochemical composition. *Lycium chinense* leaves contain diverse bioactive compounds and have been associated with multiple health-promoting functions, including anti-inflammatory regulation, visual support, fatigue reduction, and modulation of hepatic and renal function [1, 3]. Current research on leaf-utilization wolfberries has led to significant advancements in surveying planting resources, selecting new cultivars, optimizing cultivation techniques, and developing commercial products [4].

Plant polysaccharides have been extensively studied as functional ingredients in food and pharmaceutical industries due to their diverse bioactive properties, including immunomodulatory, antioxidant, and hypoglycemic effects [5-7]. Among the phytochemicals present in *Lycium* species, polysaccharides are recognized as key bioactive components [8]. Recent systematic reviews and meta-analyses have demonstrated that *Lycium barbarum* polysaccharides (LBP) exert significant regulatory effects on serum triglyceride levels,

fasting blood glucose, and lipoprotein concentrations, underscoring their potential for preventing and treating chronic metabolic diseases within traditional Chinese medicine and functional food applications [5]. While these findings primarily concern *L. barbarum*, they provide a comparative reference for understanding the potential bioactivity of *L. chinense* leaf polysaccharides (LCP), given the taxonomic proximity and shared polysaccharide-rich composition of both species.

Response surface methodology (RSM) is a well-established statistical optimization strategy commonly employed to refine extraction processes and improve polysaccharide recovery from diverse biological resources. RSM has been coupled with various extraction methodologies, including ultrasound-assisted extraction, microwave-aided extraction, and conventional hot-water extraction, to enhance yield and efficiency [9-11]. This approach has been successfully applied to optimize polysaccharide extraction from various botanical materials, including wolfberries [12] and mulberry leaves [13], as well as from rice bran polysaccharides, which exhibit notable antioxidant and antimicrobial properties [14]. Notably, the extraction of polysaccharides from *L. barbarum* leaves has been systematically investigated and optimized using ultrasonic-microwave combined treatment, yielding novel polysaccharide fractions with enhanced hypoglycemic and antioxidant activities [9, 15], demonstrating the potential of hybrid extraction techniques. RSM has also improved polysaccharide extraction from edible mushrooms such as chestnut mushroom (*Agrocybe aegerita*) [10] and Reishi (*Ganoderma lucidum*) [16], as well as marine macroalgae [17]. Compared with the previous optimization, a study that focused on LCP extraction and its hypoglycemic activity [18], the present study specifically targets leaves from Southwest China and employs a cost-effective hot-water extraction method that may be suitable for larger-scale production of polysaccharides.

Comparative studies on different extraction methods have revealed significant variations in the biological activities of obtained polysaccharides, emphasizing the critical importance of method selection for specific applications [10, 19]. Currently, extensive research has focused on extracting polysaccharides from the leaves of *Lycium* species [9, 15, 18], and optimization methodologies such as RSM have been employed to enhance enzyme production through microbial fermentation [20]. However, reports specifically addressing the extraction optimization of polysaccharides from *L. chinense* leaves cultivated in Southwest China remain limited [21]. Southwest China, particularly Guizhou Province, harbors diverse medicinal plant resources such as *Rosa roxburghii*, which has been extensively studied for its polysaccharide content and pharmacological activities [22]. In contrast, *L. chinense* from this region remains understudied. Therefore, this study combines single-factor analysis and RSM to optimize the hot-water extraction of LCP, establishing a process foundation for subsequent purification [23], structural characterization, and bioactivity investigations.

2. MATERIAL AND METHOD

2.1 Plant material and preparation

Mature *L. chinense* leaves were collected in October 2023 from a cultivated field in Tongren City, Guizhou Province, Southwest China (latitude 27.79 °N, longitude 109.21 °E, altitude 430 m). The plant material was botanically identified with reference to a voucher specimen (No. TRU-LC-2023-10) deposited at the Herbarium of Tongren University, China. Fully expanded mature leaves (third to fourth leaf pair from the apex) were selected, washed with distilled water, oven-dried at 60 ℃ for 24 h, ground into a fine powder, and sieved through a 60-mesh screen. The powder was stored in sealed glass containers at 4 ℃ for no more than 3 months before extraction.

Lycium chinense leaves were selected based on their traditional use as functional food ingredients and emerging evidence of immunomodulatory polysaccharides. The hot-water extraction method was chosen for its operational simplicity, low equipment requirements, and absence of organic solvent residues, which make it potentially suitable for larger-scale applications [8].

2.2 Experimental methods

2.2.1 Single-factor experiments and general extraction procedure

Single-factor experiments were conducted to determine the appropriate ranges of key extraction parameters for LCP extraction. The effects of liquid-to-solid ratio (1:20–1:60, g/mL), extraction temperature (60–100 ℃), and extraction time (1–5 h) were investigated individually. In each experiment, one factor varied while the other two were kept constant. All experiments were performed in triplicate, and the crude extract yield (calculated as the mass of dried crude extract relative to the mass of raw leaf powder, expressed as a percentage) was used as the response variable.

Table 1. Process parameters for hot-water extraction and ethanol precipitation of crude *L. chinense* leaf polysaccharides (LCP)

Parameter	Value/Range
Leaf powder mass per batch	5.0 g
Solvent	Distilled water
liquid-to-solid ratio	1:20–1:60 (single-factor); 1:30–1:50 (RSM)
Extraction temperature	60–100 ℃ (single-factor); 60–80 ℃ (RSM)
Extraction time	1–5 h (single-factor); 1–3 h (RSM)
Stirring speed	300 rpm
Extraction cycles	1
Centrifugation	8,000 × g, 5 min
Ethanol precipitation concentration	80% (v/v)
Precipitation temperature/time	4 ℃, 12 h
Washing solvent	95% ethanol
Washing cycles	2
Freeze-drying conditions	Pre-freezing: -25 ℃, 4 h; Desorption: 35 ℃, 10 h
Storage after freeze-drying	Desiccator at room temperature

Notes: RSM: Response surface methodology.

For each experimental run, 5.0 g of *L. chinense* leaf powder was mixed with distilled water at the designated liquid-to-solid ratio and extracted at the specified temperature and time under constant magnetic stirring at 300 rpm. A single extraction cycle was performed for each run. After extraction, the mixture was centrifuged at 8,000 × g for 5 min, and the supernatant was collected. Anhydrous ethanol was added to the supernatant to a final concentration of 80% (v/v). The mixture was allowed to stand at 4 ℃ for 12 h to precipitate polysaccharides. The precipitate was recovered by centrifugation (8,000 × g, 5 min), washed twice with 95%

ethanol, and dried under controlled freeze-drying conditions (pre-freezing at -25 ℃ for 4 h followed by desorption at 35 ℃ for 10 h). The resulting product was stored in a desiccator at room temperature and designated as crude LCP. The detailed process parameters are summarized in Table 1.

The results obtained from the single-factor experiments were used to define the factor ranges and central values for subsequent RSM optimization.

2.2.2 Response surface methodology

Based on the results of the single-factor experiments, RSM was employed to further optimize the extraction conditions [24]. A Box–Behnken design (BBD) was constructed using Design-Expert software version 13.0 (Stat-Ease Inc., Minneapolis, USA). The independent variables were liquid-to-solid ratio, extraction temperature, and extraction time, while crude extract yield was defined as the response variable [24]. The coded and actual levels of the experimental factors are presented in Table 2.

Table 2. Coded and actual levels of independent variables for Box–Behnken design (BBD)

Coded Level	Factors		
	A: Liquid-to-Solid Ratio (g/mL)	B: Temperature (℃)	C: Time (h)
-1	30	60	1
0	40	70	2
1	50	80	3

2.2.3 Hot-water extraction of *L. chinense* leaf polysaccharides

The extraction procedure followed the general protocol described in Section 2.2.1 and Table 1, with experimental conditions specified by the single-factor or RSM design (see Table 2 for coded and actual levels). The stepwise workflow is illustrated in Figure 1.

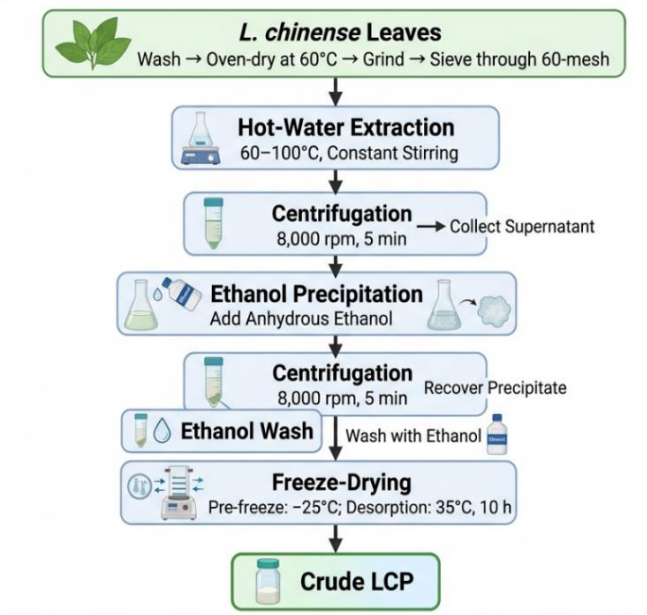


Figure 1. Stepwise workflow for hot-water extraction and ethanol precipitation of crude polysaccharides from *L. chinense* leaves

2.2.4 Determination of crude extract yield

The crude extract yield was calculated using Eq. (1):

$$\text{Crude extract yield (\%)} = (m/M) \times 100 \quad (1)$$

where, m is the total mass of the dried crude extract (mg) obtained after ethanol precipitation and freeze-drying, and M is the mass of the raw *L. chinense* leaf powder (mg).

It should be noted that the phenol–sulfuric acid method was employed to monitor the carbohydrate content in the extraction process; however, the reported yield represents the total crude extract mass rather than purified polysaccharide mass, as the crude extract may contain proteins, phenolics, ash, and other co-extracted impurities.

2.2.5 Preliminary qualitative characterization of crude *L. chinense* leaf polysaccharides

The crude LCP obtained under optimized conditions was subjected to preliminary qualitative analysis. The Molisch test was used to confirm the presence of carbohydrate compounds, indicated by the formation of a purple ring at the interface between the sample solution and concentrated sulfuric acid. The Fehling test was used to detect reducing sugars; the absence of a brick-red precipitate indicated the absence of reducing carbohydrates. The iodine–potassium iodide (I–KI) test was used to detect starch contamination; the absence of blue coloration indicated that starch was not present in the extract.

2.3 Statistical analysis, model development, and verification

Data were processed using Excel 2019 (Microsoft Corporation, Redmond, WA, USA), with single-factor experiment results analyzed via one-way Analysis of Variance (ANOVA) in SPSS 22.0 (IBM Corporation, Armonk, NY, USA). The significance threshold was set at $P < 0.05$, with high significance defined as $p < 0.01$. Design-Expert 13.0 (Stat-Ease, Inc., Minneapolis, MN, USA) facilitated RSM design. The coefficient of determination (R^2) and adjusted coefficient of determination (R^2adj) were used to evaluate model reliability, while the coefficient of variation (CV) assessed reproducibility. The adequacy precision (Adeq Precision) was computed to measure the signal-to-noise ratio, with a value greater than 4 indicating adequate model discrimination.

To further verify the reliability of the model, additional experiments were conducted under the predicted optimal extraction conditions. The experimental crude extract yield obtained under these conditions was compared with the value predicted by the model to evaluate the accuracy and predictive ability of the RSM optimization. The agreement between experimental and predicted values confirmed the validity of the developed model.

3. RESULTS

3.1 Single-factor experiment

The crude extract yield increased from 6.66 ± 0.01% to 6.85 ± 0.04% as the liquid-to-solid ratio varied from 1:20 to 1:50, peaking at 1:50 (6.85 ± 0.04%). At 1:60, the yield declined to 6.60 ± 0.04%, indicating that the optimal ratio was reached at 1:50 and excessive solvent volume resulted in a modest dilution-related decrease. One-way ANOVA revealed significant differences among ratios ($F = 48.36$, $p < 0.001$),

with the 1:50 group significantly higher than those at 1:20, 1:30 and 1:60 but not significantly different from 1:40 (p < 0.05, Duncan's test) (Figure 2). Extraction time exhibited a marked non-linear trend, peaking at 2 h (6.85 ± 0.10%) within the 1–3 h range. The yield dropped sharply to its lowest level at 3 h (5.36 ± 0.08%) and then partially recovered at 4 h (6.37 ± 0.09%) and 5 h (6.45 ± 0.08%), suggesting a dynamic balance between polysaccharide solubilization and degradation or reprecipitation during prolonged aqueous heating. Significant differences were detected among time points (F = 146.23, p = 0.001), with 2 h significantly higher than all other time points (p < 0.05) (Figure 3). Temperature exerted a positive effect on yield across the tested range (Figure 4): the yield increased from 6.30 ± 0.03% at 60 ℃ to 7.25 ± 0.09% at 100 ℃, with no significant difference between 70 ℃ and 80 ℃, or between 90 ℃ and 100 ℃ (F = 72.47, p < 0.001, Duncan's test), indicating that the yield gain gradually plateaued at elevated temperatures. Considering energy consumption, operational cost, and the potential risk of thermal degradation of heat-sensitive polysaccharide structures at temperatures approaching 100 ℃, a moderate temperature range was adopted for subsequent optimization. Based on these results, the parameter ranges of 1:30–1:50 for liquid-to-solid ratio, 60–80 ℃ for temperature, and 1–3 h for extraction time were selected for subsequent RSM optimization. These ranges were chosen to bracket the single-factor optima (1:50 and 2 h) while excluding conditions associated with pronounced yield decline or instability (ratio > 1:50, temperature > 80 ℃, time > 2 h), thereby ensuring that the RSM design operated within a region of high yield stability.

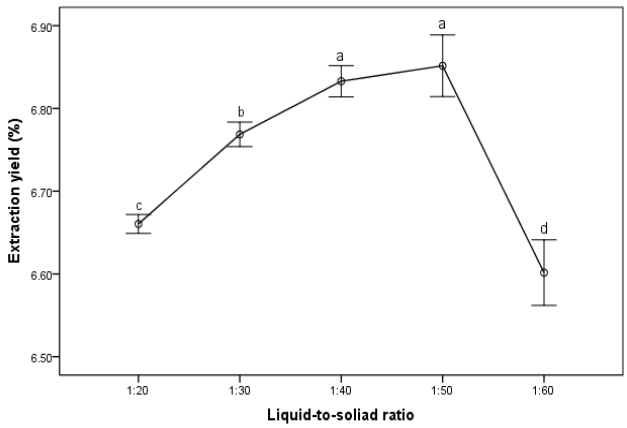


Figure 2. Effect of liquid-to-solid ratio on crude extract yield from *L. chinense* leaves in single-factor tests

Notes: Values are means ± SD (n = 3). Different lowercase letters indicate significant differences (one-way Analysis of Variance (ANOVA), Duncan's test, p < 0.05).

3.2 Response surface methodology optimization

The BBD was employed to evaluate interaction effects between variables. The experimental design and corresponding yields are presented in Table 3.

Multiple regression analysis fitted a second-order polynomial equation in terms of coded variables as Eq. (2):

$$Y = -14.6812 + 0.1070A + 0.5184B + 0.6400C - 0.0005AB + 0.0073AC + 0.005BC - 0.0009A^2 - 0.0036B^2 - 0.3567C^2 \quad (2)$$

where, A, B, and C denote the independent values of liquid-to-solid ratio, temperature, and time, respectively, with their coded levels (−1, 0, and +1) and corresponding actual values as defined in Table 2.

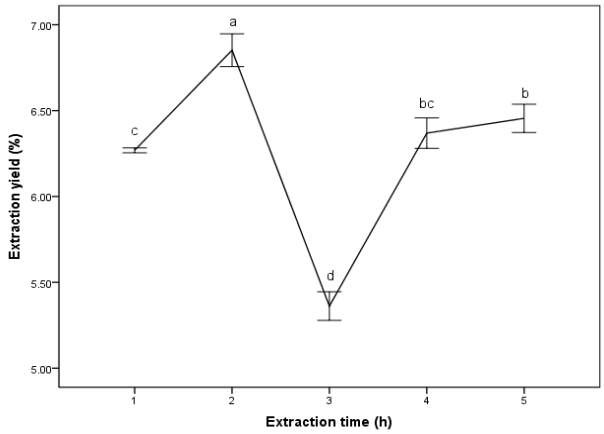


Figure 3. Influence of extraction time on crude extract yield from *L. chinense* leaves

Notes: Values are means ± SD (n = 3). Different lowercase letters indicate significant differences (one-way Analysis of Variance (ANOVA), Duncan's test, p < 0.05).

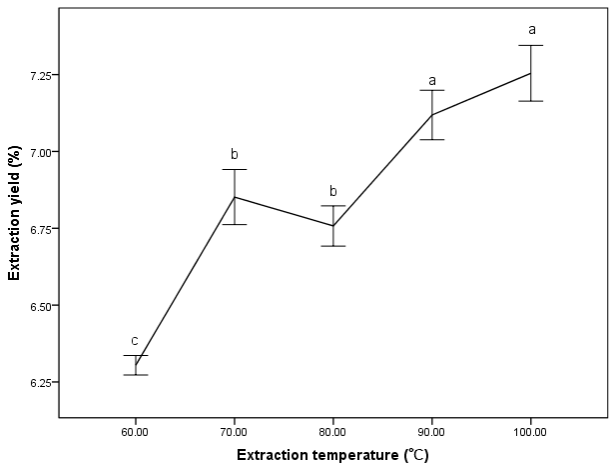


Figure 4. Impact of extraction temperature on crude extract yield from *L. chinense* leaves

Notes: Values are means ± SD (n = 3). Different lowercase letters indicate significant differences (one-way Analysis of Variance (ANOVA), Duncan's test, p < 0.05).

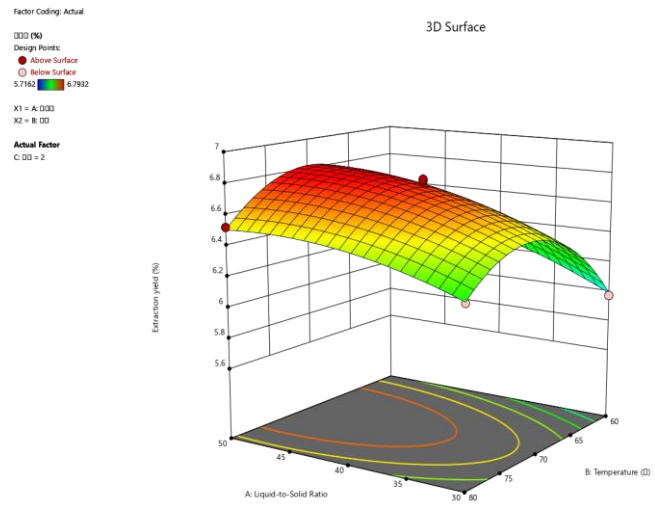
Table 3. Box–Behnken experimental design and measured yields of *L. chinense* leaf polysaccharides (LCP)

Std	*A* (coded)	*B* (coded)	*C* (coded)	*Y* (%)
1	−1	−1	0	5.97
2	1	−1	0	6.43
3	−1	1	0	6.26
4	1	1	0	6.52
5	−1	0	−1	6.33
6	1	0	−1	6.5
7	−1	0	1	5.95
8	1	0	1	6.41
9	0	−1	−1	6.17
10	0	1	−1	6.24
11	0	−1	1	5.72
12	0	1	1	5.98
13	0	0	0	6.75
14	0	0	0	6.78
15	0	0	0	6.79
16	0	0	0	6.72
17	0	0	0	6.66

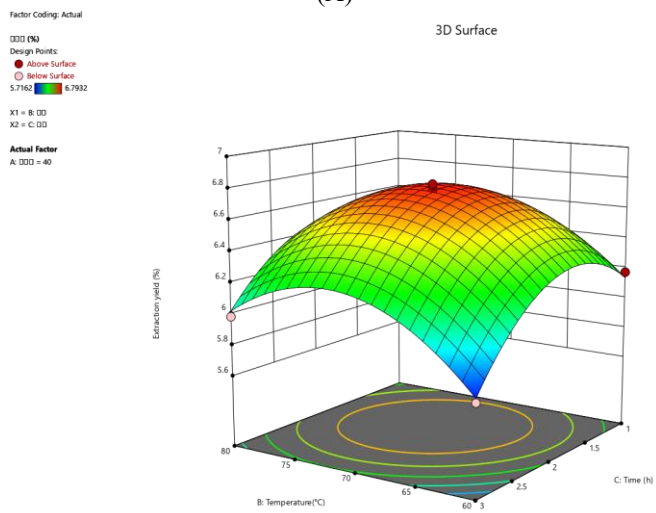
ANOVA results (Table 4) confirmed the model's high significance ($p < 0.0001$) and reliability ($R^2 = 0.9876$, $R^2_{adj} = 0.9716$). The lack-of-fit test was non-significant ($p = 0.4750$). The CV = 0.87% demonstrated excellent reproducibility. The adequacy precision was 23.5472.

Table 4. Analysis of Variance (ANOVA) results for the quadratic model

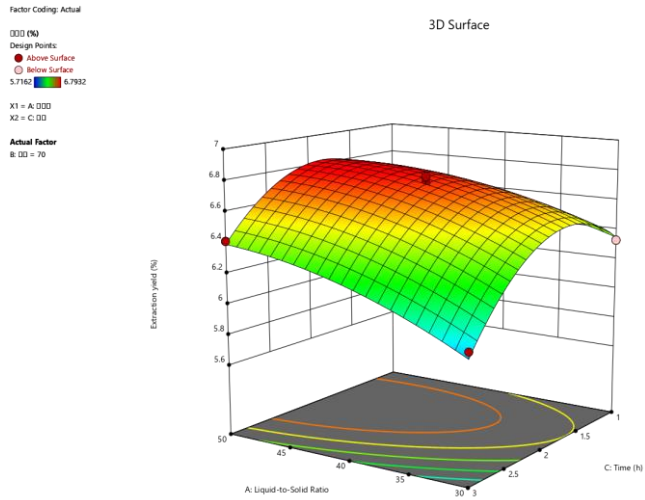
Factor	Sum Sq	*df*	Mean Sq	*F-stat*	*Sig. (p)*
Model	1.70	9	0.1890	61.81	< 0.0001
A	0.2260	1	0.2260	73.89	< 0.0001
B	0.0626	1	0.0626	20.47	0.0027
C	0.1722	1	0.1722	56.31	0.0001
AB	0.0100	1	0.0100	3.27	0.1135
AC	0.0210	1	0.0210	6.88	0.0343
BC	0.0100	1	0.0100	3.27	0.1135
A^2	0.0319	1	0.0319	10.44	0.0144
B^2	0.5361	1	0.5361	175.32	< 0.0001
C^2	0.5358	1	0.5358	175.20	< 0.0001
Residual	0.0214	7	0.0031		
Lack of fit	0.0092	3	0.0031	1.01	0.4750
Pure Error	0.0122	4	0.0030		
Cor Total	1.72	16			
R^2	0.9876	R^2_{adj}	0.9716		
Adeq Precision	23.5472	CV%	0.8686		



(A)

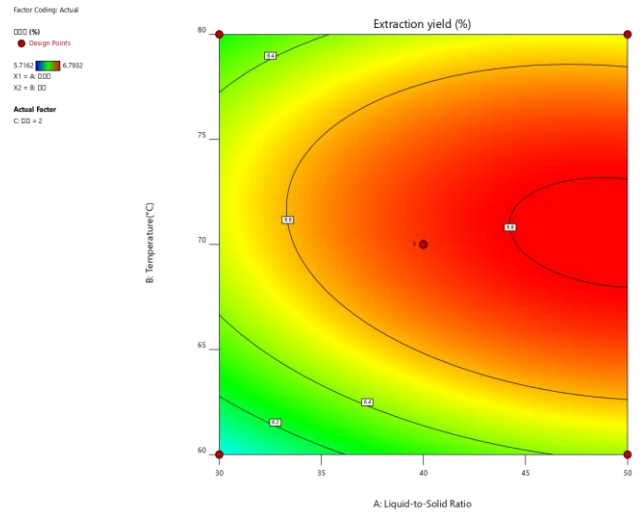


(B)

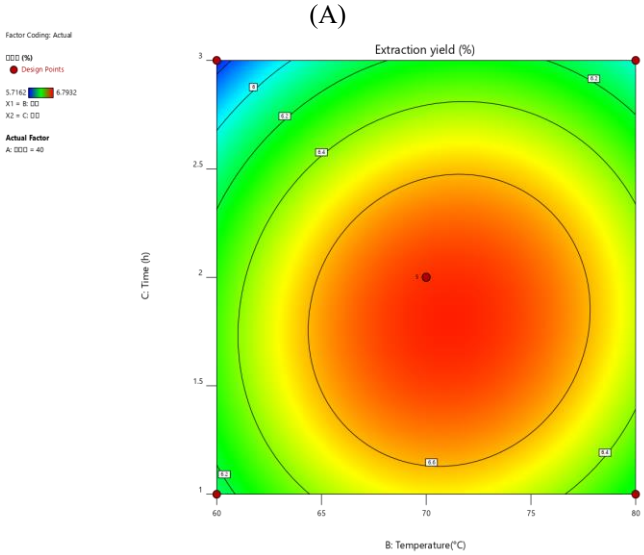


(C)

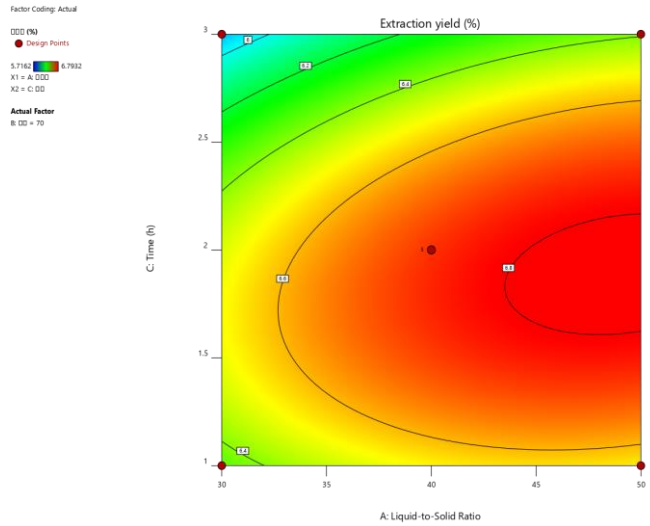
Figure 5. Three-dimensional plots of response surfaces depicting the combined effects of extraction factors on crude extract yield from *L. chinense* leaves. (A) Liquid-solid ratio × temperature interaction, (B) temperature × time interaction, (C) liquid-solid ratio × time interaction



(A)



(B)



(C)

Figure 6. Contour plots showing effects of extraction temperature, duration, and liquid-to-solid ratio on crude extract yield from *L. chinense* leaves. (A) Liquid-solid ratio × temperature interaction, (B) temperature × time interaction, (C) liquid-solid ratio × time interaction

The response surface plots (Figure 5) and contour plots (Figure 6) illustrate the combined effects of extraction parameters on crude extract yield. Optimal conditions were determined as a liquid-to-solid ratio of 1:44.69, a temperature of 71.14 ℃, and a duration of 1.72 h, predicting a maximum yield of 6.81%. For practical application, these values were adjusted to a ratio of 1:45, 70 ℃, and 1.5 h in the following validation experiment.

3.3 Model validation and qualitative characterization

Three independent validation batches were conducted under adjusted practical conditions (liquid-to-solid ratio 1:45, temperature 70 ℃, time 1.5 h). The model-predicted crude extract yield under these practical conditions was calculated as 6.79% by substituting the coded values (A = 0.897, B = -0.114, C = -0.559) into Eq. (2). The experimental yield of 6.77 ± 0.004% (mean ± SD, n = 3) showed close agreement with the predicted value, with a relative error of 0.29%, confirming the model's predictive capacity.

Qualitative analyses were performed to provide preliminary indications of the chemical composition of the extracted polysaccharides. The Molisch test produced a violet ring at the interface between the sample solution and concentrated sulfuric acid, consistent with the presence of carbohydrates as the principal constituents of the extract. The Fehling test showed no brick-red precipitate, suggesting the absence of detectable reducing sugars under the test conditions, while the I–KI test showed no blue coloration, indicating that iodine-responsive starch was not detected. These results provide preliminary indications that the crude LCP extract is predominantly composed of non-reducing, non-starch-type polysaccharides, though further quantitative and structural characterization is required for definitive classification.

The crude LCP obtained under optimized conditions was used directly for qualitative characterization without further purification, as the present study focused on extraction optimization rather than purification. Consequently, comprehensive compositional characterization–including total sugar purity, protein, total phenolics, and ash contents–was not performed, and these parameters remain to be determined in future work. Future studies should also determine monosaccharide composition and molecular weight distribution to support structure–activity relationship investigations.

4. DISCUSSION

4.1 Single-factor experimental analysis

The observed increase in crude extract yield with increasing liquid-to-solid ratio up to 1:50 (Figure 2) reflects the critical role of solvent volume in facilitating mass transfer during polysaccharide dissolution. Beyond this optimal ratio, the marginal decline in yield may be attributed to dilution effects reducing the collision frequency between solvent and substrate, or potential thermal degradation of polysaccharides under excessive solvent conditions, as reported for *L. barbarum* polysaccharide extraction [9, 15]. However, the present study did not directly measure structural changes or degradation products, and this explanation remains speculative. The non-linear response of extraction time (Figure 3), with yield peaking at 2 h followed by fluctuations, suggests a dynamic equilibrium between polysaccharide solubilization and degradation. This pattern aligns with observations in *Epimedium brevicornu* polysaccharide extraction, where prolonged exposure to aqueous conditions promotes reprecipitation or hydrolytic cleavage of glycosidic bonds [19, 25]. The temperature-dependent yield profile (Figure 4), characterized by an initial increase followed by a positive effect on yield across the tested range with a gradual plateau at elevated temperatures, indicates the thermal sensitivity of LCP. This phenomenon is consistent with the thermal instability of complex heteropolysaccharides, wherein excessive heat disrupts non-covalent interactions and glycosidic linkages, as documented in marine algal polysaccharide systems [17]. The moderate range of 60–80 ℃ was therefore adopted for RSM optimization to balance extraction yield, energy efficiency, and the preservation of structural integrity.

It is important to acknowledge that the yields reported herein represent crude extract yields rather than purified polysaccharide yields. The crude extracts obtained under optimized conditions may contain co-extracted proteins, phenolic compounds, ash, and other impurities. Without quantification of total sugar purity, the actual polysaccharide content may be substantially lower than the reported crude extract yield. Future studies should determine the total sugar content, protein content, and ash content of the crude extract to enable accurate calculation of true polysaccharide recovery.

4.2 Response surface methodology model reliability and parameter interactions

The relative importance of the three factors in influencing crude extract yield, as evidenced by the F-values (Table 4), follows the order liquid-to-solid ratio (A) > extraction time (C) > temperature (B). Factor A exhibited the highest F-value (73.89), indicating it as the most influential parameter, followed by C (F = 56.31) and B (F = 20.47). This hierarchy suggests that solvent volume plays a primary role in facilitating polysaccharide dissolution, while extraction time and temperature serve as secondary modulators. This

hierarchy is consistent with established mass transfer theory, wherein sufficient solvent volume ensures adequate concentration gradients to drive polysaccharide diffusion from the plant matrix into the aqueous phase, while prolonged extraction time allows progressive disruption of cell wall matrices and enhanced release of intracellular polysaccharides [9]. The statistically significant interaction between liquid-to-solid ratio and time (*AC*; $p = 0.0343$) suggests that the effect of solvent volume on yield is modulated by extraction duration. This interaction is depicted in the response surface topography (Figures 5(C) and 6(C)), where the yield contour lines exhibit steeper gradients along the time axis at higher liquid-to-solid ratios. The non-significant AB and BC interactions ($p > 0.05$) indicate that temperature and time, as well as ratio and temperature, do not exhibit synergistic effects under the tested conditions. This pattern implies that extraction time operates primarily as an independent factor governing overall extraction duration rather than modulating the efficacy of other parameters, a pattern similarly observed in *Ganoderma lucidum* polysaccharide optimization [16]. The close agreement between predicted and experimental yields under adjusted practical conditions validates the model's predictive capacity and supports its applicability for process design and potential scale-up.

4.3 Comparison with previous extraction methods

It should be noted that the majority of published studies on *Lycium* polysaccharides have focused on *L. barbarum* fruit or leaf extracts. Direct extrapolation of *L. barbarum* polysaccharide properties to *L. chinense* should be made with caution, as differences in species, plant part, and geographic origin may influence chemical composition and bioactivity. The yield achieved by the optimized hot-water extraction method is comparable to polysaccharide extracts from other botanical sources currently investigated for functional applications. Compared with existing methods such as ultrasonic-assisted or enzymatic extraction [11, 19], the hot-water extraction optimized herein offers distinct advantages for larger-scale applications: operational simplicity, low equipment requirements, absence of organic solvent residues, and cost-effectiveness. These characteristics make the method potentially suitable for industrial applications where cost and operational simplicity are critical considerations [8]. The regional specificity of *L. chinense* from Southwest China, characterized by hot, humid climates and distinctive morphological features, underscores the need to investigate cultivar-specific polysaccharide profiles and their bioactivity, which may be influenced by local environmental factors [2, 21].

4.4 Chemical characterization and application suitability

Plant polysaccharides have attracted increasing attention as functional ingredients due to their reported immunomodulatory and antioxidant properties [6, 26, 27]. In the present study, the crude extract was found to be predominantly composed of non-reducing, non-starch polysaccharides based on qualitative analyses. This characteristic is consistent with previous reports describing *Lycium*-derived polysaccharides as complex heteropolysaccharides with notable bioactive potential [8, 9]. However, the present study did not determine the monosaccharide composition, molecular weight distribution, or linkage patterns of the polysaccharide fraction, and structural characterization remains necessary for understanding structure--activity relationships.

Furthermore, the crude extract yield reported in this study (6.77%) does not equate to pure polysaccharide yield. The crude extract likely contains proteins, phenolics, ash, and other non-carbohydrate components. Without quantitative determination of total sugar purity, the true polysaccharide content–and consequently the actual polysaccharide recovery–cannot be accurately estimated. We explicitly acknowledge this limitation and recommend that future studies perform comprehensive compositional analysis (total sugar, protein, phenolics, and ash contents) to standardize the extract quality prior to any bioactivity assessment.

The non-reducing and non-starch nature of the crude extract, as preliminarily indicated by qualitative tests, suggests potential thermal stability; however, no thermal stability or storage tests were conducted. The assessment of the extract's suitability for further processing remains a subject for future investigation. The crude extract characterized herein requires further in vitro and in vivo evaluation to determine its biological activities. Hybrid techniques (e.g., ultrasound-assisted hot-water extraction) could be explored to increase extraction yield without compromising polysaccharide integrity [27], particularly if enhanced production efficiency is required for scale-up applications.

5. CONCLUSIONS

This research employed single-factor experiments combined with RSM to optimize the hot-water extraction of crude polysaccharide extracts from *L. chinense* leaves. Initial single-factor experiments identified that liquid-to-solid ratio (1:30–1:50), extraction temperature (60–80 ℃), and processing time (1–3 h) significantly influenced crude extract yield. RSM refinement determined optimal conditions as a liquid-to-solid ratio of 1:44.69, a temperature of 71.14 ℃, and a time of 1.72 h, predicting a yield of 6.81%. Experimental validation under adjusted practical conditions (ratio 1:45, 70 ℃, 1.5 h) achieved 6.77 ± 0.004%, closely aligning with the model's prediction and confirming its reliability ($R^2 = 0.9876$) for process design and potential scale-up. These results demonstrate that the established RSM model is robust and suitable for guiding laboratory-scale extraction optimization and potential scale-up considerations.

Overall, the optimized hot-water extraction method provides an efficient and cost-effective approach for obtaining crude polysaccharide extracts from *L. chinense* leaves native to Southwest China. The present work establishes a process optimization foundation for LCP extraction; however, comprehensive compositional characterization (total sugar purity, protein, phenolics, ash), structural analysis, and biological activity evaluations remain necessary to fully characterize the extract and support its potential application in functional ingredient development.

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REFERENCES

[1] Zhang, B., Duan, L.Y., Qin, K., et al. (2024). Analysis and comprehensive evaluation of agronomic characters of 93 varieties of *Lycium* L. Shaanxi Journal of Agricultural Sciences, 70(4): 55-62. http://sxnykx.cnjournals.com/ch/reader/view_abstract.aspx?file_no=20240412.

[2] Li, Y.H., Yang, L., Nan, X.X., Wang, Y.L., Huang, Z.M. (2021). Comparison of nutritional components and yield of new varieties of different leaf-growing wolfberries. Jiangsu Agricultural Sciences, 49: 113-116. https://doi.org/10.15889/j.issn.1002-1302.2021.13.022

[3] Lan, T., Duan, G., Qi, Y., Almezgagi, M., Fan, G., Ma, Y. (2024). Exploration of chemical compositions in different germplasm wolfberry using UPLC-MS/MS and evaluation of the in vitro anti-inflammatory activity of quercetin. Frontiers in Pharmacology, 15: 1426944. https://doi.org/10.3389/fphar.2024.1426944

[4] Zhu, Y.C., Qian, T., Zhou, X.B., Cheng, L., Jin, Z.C. (2022). Application status and industrial development strategy of leaf-growing wolfberries. Modern Horticulture, 45(24): 37-38. https://doi.org/10.3969/j.issn.1006-4958.2022.24.014

[5] Zhou, B., Xia, H., Yang, L., Wang, S., Sun, G. (2022). The effect of *Lycium barbarum* polysaccharide on the glucose and lipid metabolism: A systematic review and meta-analysis. Journal of the American Nutrition Association, 41(6): 617-625. https://doi.org/10.1080/07315724.2021.1925996

[6] Yin, M., Zhang, Y., Li, H. (2019). Advances in research on immunoregulation of macrophages by plant polysaccharides. Frontiers in Immunology, 10: 145. https://doi.org/10.3389/fimmu.2019.00145

[7] Masci, A., Carradori, S., Casadei, M.A., et al. (2018). *Lycium barbarum* polysaccharides: Extraction, purification, structural characterisation and evidence about hypoglycaemic and hypolipidaemic effects. A review. Food Chemistry, 254: 377-389. https://doi.org/10.1016/j.foodchem.2018.01.176

[8] Wang, J., Li, S., Zhang, H., Zhang, X. (2024). A review of *Lycium barbarum* polysaccharides: Extraction, purification, structural-property relationships, and bioactive molecular mechanisms. Carbohydrate Research, 544: 109230. https://doi.org/10.1016/j.carres.2024.109230

[9] Quan, N., Wang, Y., Li, G., et al. (2023). Ultrasound–microwave combined extraction of novel polysaccharide fractions from *Lycium barbarum* leaves and their in vitro hypoglycemic and antioxidant activities. Molecules, 28(9): 3880. https://doi.org/10.3390/molecules28093880

[10] Du, B., Peng, F., et al. (2022). Optimization extraction and antioxidant activity of crude polysaccharide from chestnut mushroom (*Agrocybe aegerita*) by accelerated solvent extraction combined with response surface methodology (ASE-RSM). Molecules, 27(8): 2380. https://doi.org/10.3390/molecules27082380

[11] Xie, J.H., Shen, M.Y., Xie, M.Y., et al. (2012). Ultrasonic-assisted extraction, antimicrobial and antioxidant activities of *Cyclocarya paliurus* (Batal.) Iljinskaja polysaccharides. Carbohydrate Polymers, 89(1): 177-184. https://doi.org/10.1016/j.carbpol.2012.02.068

[12] Zhang, J., Jia, S., Liu, Y., Wu, S., Ran, J. (2011). Optimization of enzyme-assisted extraction of the *Lycium barbarum* polysaccharides using response surface methodology. Carbohydrate Polymers, 86(2): 1089-1092. https://doi.org/10.1016/j.carbpol.2011.06.027

[13] Wang, T., Zou, X., Zhang, H., et al. (2025). Ultrasound-assisted extraction of polysaccharides from mulberry leaves using response surface methodology: Purification and component identification of extract. Molecules, 30(8): 1747. https://doi.org/10.3390/molecules30081747

[14] Surin, S., Seesuriyachan, P., Thakeow, P., You, S., Phimolsiripol, Y. (2018). Antioxidant and antimicrobial properties of polysaccharides from rice brans. Chiang Mai Journal of Science, 45(3): 1372-1382.

[15] Quan, N. (2018). Isolation, purification, structural characterization and hypoglycemic activity analysis of *Lycium barbarum* leaf polysaccharides. Ph.D. dissertation. Shaanxi Normal University, Xi'an, China.

[16] Zheng, S., Zhang, W., Liu, S. (2020). Optimization of ultrasonic-assisted extraction of polysaccharides and triterpenoids from the medicinal mushroom *Ganoderma lucidum* and evaluation of their in vitro antioxidant capacities. PLOS ONE, 15(12): e0244749. https://doi.org/10.1371/journal.pone.0244749

[17] Tian, H., Liu, H., Song, W., Zhu, L., Yin, X. (2021). Polysaccharide from *Caulerpa lentillifera*: Extraction optimization with response surface methodology, structure and antioxidant activities. Natural Product Research, 35(20): 3417-3425. https://doi.org/10.1080/14786419.2019.1700507

[18] Jiang, L., Mei, L.J., Liu, Z.G., et al. (2013). Extraction and hypoglycemic effect of crude polysaccharides from Chinese wolfberry (Lycium chinense) leaves. Food Science, 34: 42-46.

[19] Yang, J., Zhang, H., Cao, X., et al. (2017). Enzymatic water extraction of polysaccharides from Epimedium brevicornu and their antioxidant activity and protective effect against DNA damage. Journal of Food Biochemistry, 41(1): e12298. https://doi.org/10.1111/jfbc.12298

[20] Adetiloye, O.A., Solomon, B.O., Omolaiye, J.A., Betiku, E. (2025). Optimization of thermostable amylolytic enzyme production from *Bacillus cereus* isolated from a recreational warm spring via Box Behnken design and response surface methodology. Microbial Cell Factories, 24(1): 87. https://doi.org/10.1186/s12934-025-02709-w

[21] Fan, J.D., Xu, Q.Z., Mei, J., Lu, D.W., Yao, Q.F., Fan, Z.J. (2021). Nutritional components of wolfberry leaf tea in Fanjing Mountain. Food Industry, 42(10): 324-328.

[22] Yin, C., Zhang, Y., Zhang, L., et al. (2024). Exploring Rosa roxburghii Tratt polysaccharides: From extraction to application potential in functional products - An in-depth review. International Journal of Biological Macromolecules, 280: 135543. https://doi.org/10.1016/j.ijbiomac.2024.135543

[23] Pan, F., Li, S., Zhu, X., et al. (2022). Purification and the effects on structure and bioactivity for polysaccharide from Actinidia valvata Dunn. using macroporous

adsorption resin. Food Science and Technology, 42: e99721. https://doi.org/10.1590/fst.99721
[24] Bezerra, M.A., Santelli, R.E., Oliveira, E.P., Villar, L.S., Escaleira, L.A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta, 76(5): 965-977. https://doi.org/10.1016/j.talanta.2008.05.019
[25] Zhang, H., Niu, L., Yang, X., Li, L. (2014). Analysis of water-soluble polysaccharides in an edible medicinal plant epimedium: Method development, validation, and application. Journal of AOAC International, 97(3): 784-790. https://doi.org/10.5740/jaoacint.12-379
[26] Mohammadi, G., Hafezieh, M., Karimi, A.A., et al. (2022). The synergistic effects of plant polysaccharide and Pediococcus acidilactici as a synbiotic additive on growth, antioxidant status, immune response, and resistance of Nile tilapia (Oreochromis niloticus) against Aeromonas hydrophila. Fish & Shellfish Immunology, 120: 304-313. https://doi.org/10.1016/j.fsi.2021.11.028
[27] Mohan, K., Ravichandran, S., Muralisankar, T., et al. (2019). Application of marine-derived polysaccharides as immunostimulants in aquaculture: A review of current knowledge and further perspectives. Fish & Shellfish Immunology, 86: 1177-1193. https://doi.org/10.1016/j.fsi.2018.12.072