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Optimization of Extraction and Multi-Component Quantification of Alkaloids from Lindera aggregata

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ABSTRACT

Lindera aggregata is a traditional medicinal plant rich in bioactive alkaloids. The lack of systematic methods for extraction and quality control hinders its standardized application. This study presents an integrated approach combining response surface methodology (RSM)-based extraction optimization with simultaneous high-performance liquid chromatography (HPLC) quantification of its major alkaloids. Extraction was optimized by single-factor and Box-Behnken designs. A sensitive HPLC method was developed and validated to simultaneously determine norisoboldine, boldine, and reticuline using an Inertsil ODS-SP column with gradient elution (acetonitrile / 0.1% triethylamine, pH 3.0) at 235 nm. Optimal conditions were: 57.5% ethanol, solid to liquid ratio 1:30.5 g/mL, 99.8 min, 82.3 °C, yielding 10.173 mg/g total alkaloids. The HPLC method was validated (specificity, linearity, precision, accuracy). Contents were: norisoboldine 6.028 ± 0.046, boldine 1.628 ± 0.038, reticuline 1.034 ± 0.039 mg/g. This work provides an integrated quality by design framework, combining process optimization with multi component analysis for reproducible and quality controlled alkaloid extracts.

Keywords: Lindera aggregata; alkaloids; response surface methodology; extraction optimization; HPLC; simultaneous quantification.

INTRODUCITON

Lindera aggregata (Sims) Kosterm. (Lauraceae), known as “Wu Yao” in traditional Chinese medicine, is historically valued for its warming properties and its efficacy in promoting qi circulation, alleviating pain, and treating conditions such as abdominal distension and hernia[1-2]. Modern pharmacological research has corroborated these traditional uses, revealing a spectrum of bioactivities—including anti-inflammatory[3], analgesic[4], and antioxidant effects[5]—that are attributed to its complex phytochemical profile comprising volatile oils, sesquiterpenes, and notably, isoquinoline alkaloids.1

Among these constituents, isoquinoline alkaloids-particularly norisoboldine, boldine, and reticuline—have drawn significant research interest due to their marked pharmacological properties[6]. For instance, norisoboldine exhibits potent immunomodulatory and anti-arthritic effects[7-8], while boldine is renowned for its robust antioxidant and cardioprotective activities[9]. Consequently, these alkaloids serve as critical bioactive markers and key determinants of the quality and therapeutic efficacy of *L. aggregata*.

Nevertheless, the standardized preparation of *L. aggregata* alkaloid extracts faces two persistent methodological hurdles. First, conventional extraction protocols often depend on empirical or onefactoratatime approaches, which overlook the complex interactions among critical variables (e.g., solvent concentration, temperature, time). This leads to suboptimal extraction yields, poor batchtobatch reproducibility, and inefficient resource utilization[10]. Second, prevailing qualitycontrol practices remain rudimentary, frequently restricted to the assay of total alkaloids or a single marker compound. Such simplified approaches fail to reflect the multicomponent, synergydriven nature of herbal medicine, thereby creating a disconnect between chemical characterization and holistic quality assessment[11].

To bridge this gap, an integrated strategy that combines systematic process optimization with precise multicomponent analysis is imperative. RSM provides a powerful statistical framework for modeling and optimizing processes with multiple interacting variables by explicitly elucidating their interaction effects[12]. When coupled with a robust analytical technique such as HPLC, it enables the establishment of a reliable "processquality" linkage—a cornerstone for the standardization of herbal extracts[13-14]. Unlike previous studies on *L. aggregata* that employed single-factor or orthogonal designs for alkaloids extraction[15], this work integrates RSM-based optimization with simultaneous HPLC quantification of three major alkaloids, providing a more comprehensive standardization strategy.

Accordingly, the objectives of this work were twofold: (1) to systematically optimize the extraction of total alkaloids from *L. aggregata* using RSM, aiming to maximize yield through the evaluation of key parameter interactions; and (2) to establish and rigorously validate a sensitive, reproducible HPLC method for the simultaneous quantification of three principal bioactive alkaloids—norisoboldine, boldine, and reticuline. By merging advanced process engineering with targeted phytochemical profiling, this work aims to deliver a scientifically rigorous and transferable framework for the qualityassured production of *L. aggregata* alkaloids, thereby facilitating their consistent application in pharmacological research and future product development.

MATERIALS AND METHODS

Plant Material

Fresh roots of *L. aggregata* were collected in Taizhou City, Zhejiang Province, China. The botanical identity was authenticated by Prof. Peng Xin (Ningbo Institute of Chinese Materia Medica) as the tuberous root of *L. aggregata*. A corresponding voucher specimen (No. ZJPC-MM-2023-001) was deposited in the Herbarium of the Institute of Materia Medica, Zhejiang Pharmaceutical University. To preserve the integrity of the chemical constituents, the fresh roots were steamed for 5 min to inactivate enzymes. Subsequently, they were dried at 50 °C, ground to a fine powder, passed through a 100-mesh sieve, and stored in a desiccator for later use.

Chemicals and Reagents

High-purity reference standards (≥98%) of norisoboldine, boldine, and reticuline were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Berberine hydrochloride (≥98%), used to calibrate the total alkaloid assay, originated from the National Institutes for Food and Drug Control (Beijing, China). Methanol, ethanol, hydrochloric acid, sodium hydroxide, glacial acetic acid, and dichloromethane were analytical-grade reagents. HPLC-grade acetonitrile was purchased from Sigma-Aldrich (St. Louis, MO, USA). All aqueous solutions utilized commercially available purified water (Wahaha®).

Extraction and Spectrophotometric Determination of Total Alkaloids

Total alkaloid quantification relied on spectrophotometry, employing bromocresol green (BCG) for complexation, as referenced[16-17].

Berberine hydrochloride served as the calibration standard. To construct the calibration curve, berberine hydrochloride concentrations spanning 10 to 50 µg/mL were prepared, yielding a linear relationship ($Y = 0.0164X - 0.0061$, $R^2 = 0.9997$) where Y and X represent absorbance and standard concentration, respectively.

For sample preparation, reflux extraction with ethanol was applied to approximately 1.0 g of powdered starting material. The subsequent extract underwent acid-base partitioning: following evaporation, the residue was dissolved in 2% HCl, adjusted to pH 10–11, and the alkaloids were back-extracted into dichloromethane. After evaporating the combined organic layers, the residue was reconstituted in a defined volume of dichloromethane for analysis. Absorbance measurements at 412 nm enabled the calculation of total alkaloid content, reported as mg of berberine hydrochloride equivalents per gram of dry material (mg/g). Each sample was analyzed in triplicate, and results were expressed as mean ± SD.

Optimization of the Extraction Process

Preliminary single-factor screening was performed to delineate the experimental domain for systematic optimization. Four process variables—ethanol concentration, solid-to-liquid ratio, extraction time, and temperature—were evaluated individually. The yield profiles from these one-variable-at-a-time experiments identified proximate optimal ranges for each parameter, which subsequently defined the factor levels for the response surface design.

Multivariate optimization was subsequently conducted via a four-factor, three-level Box-Behnken design (BBD), implemented in Design-Expert® software (Version 13.0). Table 1 summarizes the independent variables and their corresponding coded levels, while the total alkaloid yield served as the designated response (Y). Following data acquisition, a second-order polynomial model was generated through regression fitting. The statistical robustness of this model, including the significance of both main factor effects and their interactions, was then assessed through analysis of variance (ANOVA).

Table 1. Independent variables and their coded levels employed in the Box-Behnken experimental design

Level			**Factor**	
	Ethanol volume (%)	**L/S ratio (B)** (min)	**Extraction time (C)** (D)	**Extraction temperature (°C)**
-1	40	25:1	60	75
0	50	30:1	80	80
1	60	35:1	100	85

HPLC Analysis of Three Specific Alkaloids

Preparation of Standard and Sample Solutions

Individual stock solutions of norisoboldine (5.894 mg/mL), boldine (2.024 mg/mL), and reticuline (1.954 mg/mL) were prepared in methanol. A mixed working standard solution, with target concentrations of 294.7, 101.2, and 97.7 µg/mL for norisoboldine, boldine, and reticuline respectively, was generated through appropriate dilution of the stock solutions using methanol.

For sample preparation, approximately 0.1 g of the powdered material was accurately weighed and subjected to reflux extraction with 10 mL of 60% (v/v) ethanol at 80°C for 100 min. After cooling, any solvent loss was compensated, and the resulting extract was filtered through a 0.22 µm nylon membrane prior to chromatographic injection. Each sample was prepared and analyzed in triplicate. The alkaloid content was calculated from each individual peak area, and the results were expressed as mean ± SD.

Chromatographic Conditions and Analysis

HPLC analysis was performed on a Shimadzu LC-20AT system. Separations were carried out on an Inertsil ODSSP C_{11} column (150 mm × 4.6 mm i.d., 5 µm particle size) thermostatted at 40 °C. The binary mobile phase consisted of (A) 0.1% (v/v) triethylamine in water, adjusted to pH 3.0 with glacial acetic acid, and (B) acetonitrile. A gradient elution scheme was executed at a constant flow of 0.9 mL·min$_1$^{1} according to the following timetable: 0–0.1 min, 10% B; 0.1–15 min, 10% $_1$ 20% B; 15–25 min, 20% $_1$ 60% B; 25–40 min, 60% $_1$ 100% B. Following each injection, the column was re-equilibrated to the initial solvent composition for 10 min prior to the next analysis. The detection wavelength was 235 nm, and a 20 μL injection volume was used. Under these optimized conditions, the three target alkaloids—norisoboldine, boldine, and reticuline—were successfully separated, as shown in the representative chromatograms (Fig. 1).

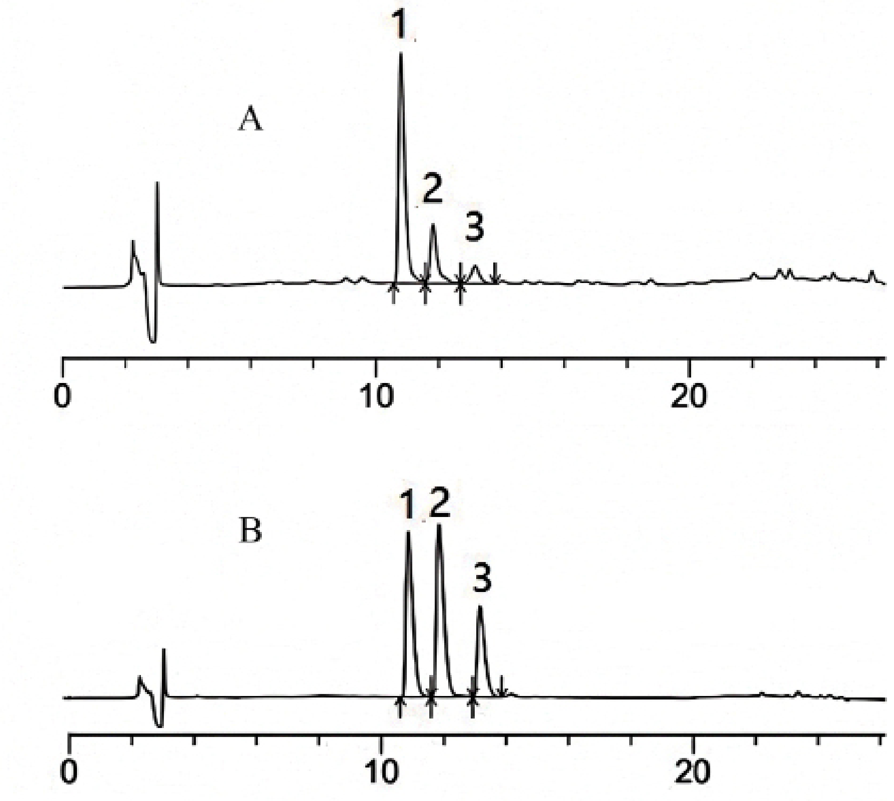

Fig. 1: HPLC chromatograms of L. aggregata extract (A) and mixed reference standards (B). Peaks: 1, Norisoboldine; 2, Boldine; 3, Reticuline. (Source: Original data from this study; enerated using Shimadzu LabSolutions software.)

RESULTS AND DISCUSSION

Single-factor Experiments for Optimization of Total Alkaloids Extraction

The influence of four critical parameters on the extraction efficiency of total alkaloids from *L. aggregata* was initially assessed through single-factor experiments (Fig. 2).

Fig. 2A shows that the extraction yield initially rose in conjunction with increasing ethanol concentration, peaking at approximately 50% (v/v), before exhibiting a marked decline. This unimodal pattern is characteristic of medium-polarity compounds such as isoquinoline alkaloids. Lower ethanol concentrations favor the extraction of more polar constituents, whereas excessively high ethanol levels reduce solvent polarity, thereby decreasing the solubility of the target alkaloids and simultaneously promoting the co-extraction of non-polar impurities, which collectively lowers the apparent yield[18]. Based on these results, ethanol concentrations of 40%,

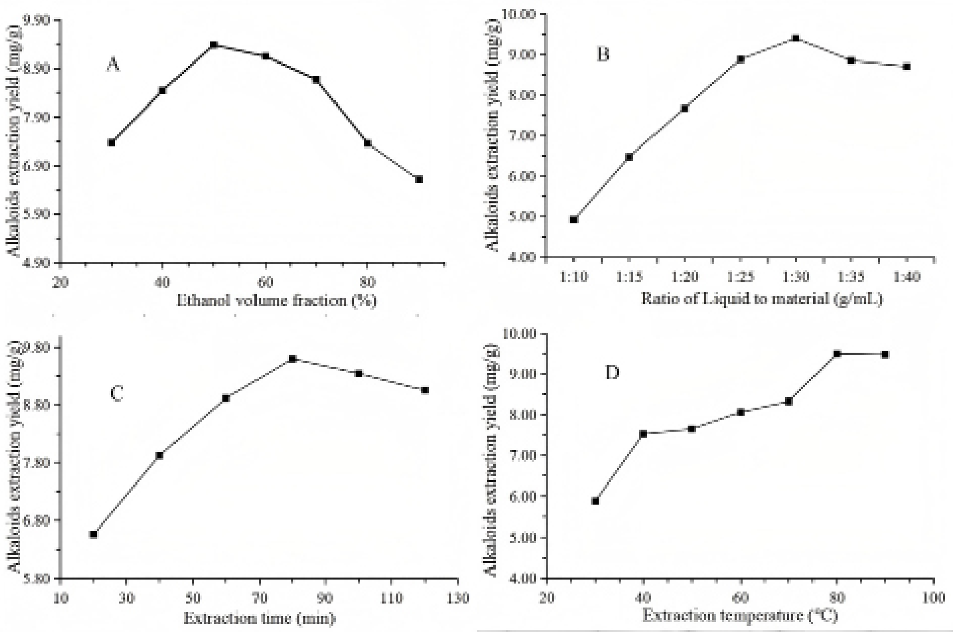

Fig. 2. Effects of single factors on the extraction yield of alkaloids from *L. aggregata.* (A) Ethanol concentration. (B) Solid-to-liquid ratio. (C) Extraction time. (D) Extraction temperature

50%, and 60% were chosen as the level settings for the following response surface design.

The influence of solidtoliquid ratio is presented in **Fig. 2B**. The yield increased sharply when the ratio was expanded from 10:1 to 30:1 (mL/g), after which further increases led to a gradual plateau followed by a slight decrease. An appropriate increase in solvent volume enhances the concentration gradient between the plant matrix and the bulk solution, thus driving mass transfer. However, beyond an optimum point, the dilution effect becomes dominant, and the marginal gain in yield no longer justifies the substantial increase in solvent consumption and downstream processing costs[19]. Consequently, ratios of 25:1, 30:1, and 35:1 mL/g were chosen for further optimization.

Fig. 2C illustrates the effect of extraction time. The yield rose steadily during the first 80 min, indicating an ongoing masstransfer process. Extending the extraction beyond this period resulted in a reduction of yield. This decline may be attributed to the potential degradation or structural transformation of certain thermolabile alkaloids under prolonged heating, or simply that the extraction had reached equilibrium[20]. Therefore, extraction times of 60, 80, and 100 min were selected as the three experimental levels.

Regarding extraction temperature (**Fig. 2D**), the yield increased markedly from 30 °C to 80 °C, above which no further significant increase was observed. Elevated temperature reduces solvent viscosity and surface tension, increases molecular kinetic energy, and enhances both solubility and diffusion rates, thereby improving extraction efficiency. Nevertheless, excessively high temperatures can induce the thermal decomposition of sensitive bioactive compounds, such as specific isoquinoline alkaloids, leading to irreversible yield loss[21]. Hence, temperatures of 75 °C, 80 °C, and 85 °C were chosen as the central levels for the subsequent response surface investigation.

The identified optimal ranges for each parameter were logically set as the central (0) levels in the forthcoming Box–Behnken response surface design, ensuring that the experimental domain encompassed the region of maximum interest.

Analysis of RSM Results

The experimental data from the Box-Behnken design were fitted to a second-order polynomial model. The regression model demonstrated high statistical significance, as evidenced by ANOVA ($p = 0.0017$). Furthermore, the lack-of-fit test was non-significant ($p = 0.1386$), supporting the adequacy of the model fit. The model explained 84.19% of the response variation ($R^2 = 0.8419$, adjusted $R^2 = 0.6839$).

The resulting coded regression equation for the total alkaloid yield (Y) is presented below: $Y = 9.25 + 0.5708A - 0.3642B + 0.4450C + 0.1967D + 0.2875AB + 0.1275AC + 0.5725AD + 0.2150BC - 0.0350BD - 0.1075CD - 0.6601A^2 - 0.3176B^2 + 0.2837C^2 - 0.8663D^2$

Where Y is the total alkaloid yield (mg/g), and A, B, C, D are the coded variables for ethanol concentration, solid-to-liquid ratio, extraction time, and temperature, respectively[22].

Evaluation of the individual model terms revealed that the linear coefficients for ethanol concentration (A) and extraction time (C), the quadratic terms A^2 and D^2, and the interaction term AD were statistically significant ($p < 0.05$). Notably, the quadratic effect of temperature (D^2) was the most pronounced, highlighting a strong curvilinear relationship. Based on the magnitude of the linear

Table 2: Experimental layout and corresponding outcomes from the response surface methodology

Test Number	Code Value of Box-Behnkenn Test Ethanol Volume Fraction (%)	Liquid-to-Solid Ratio (g/mL)	Extraction Time (min)	Extraction Temperature(°C)	Total Alkaloid Content (mg/g)
1	-1	1	0	0	7.05
2	0	-1	0	-1	7.51
3	1	-1	0	0	9.6
4	0	0	0	0	9.05
5	0	0	0	0	8.95
6	0	-1	1	0	9.65
7	1	1	0	0	9.15
8	0	1	0	-1	6.95
9	0	1	-1	0	8.45
10	0	0	0	0	9.33
11	0	0	0	0	9.15
12	1	0	0	-1	7.55
13	1	0	1	0	9.45
14	0	1	0	1	7.75
15	0	1	1	0	9.55
16	0	0	0	0	9.75
17	-1	0	1	0	8.25
18	1	0	-1	0	8.44
19	1	0	0	1	8.95
20	0	-1	0	1	8.45
21	0	0	1	-1	9.71
22	0	-1	-1	0	9.41
23	-1	0	-1	0	7.75
24	-1	0	0	1	6.85
25	-1	-1	0	0	8.65
26	-1	0	0	-1	7.74
27	0	0	1	1	9.55
28	0	0	-1	-1	8.25
29	0	0	-1	1	8.52

Table 3: ANOVA of the regression model.

Source of Variance	Sum of Squares	Freedom	Mean Square	F-value	P-value
Model	18.87	14	1.35	5.33	0.0017
A	3.91	1	3.91	15.47	0.0015
B	1.59	1	1.59	6.3	0.0250
C	2.38	1	2.38	9.4	0.0084
D	0.4641	1	0.4641	1.84	0.1969
AB	0.3306	1	0.3306	1.31	0.2719
AC	0.065	1	0.065	0.2573	0.6199
AD	1.31	1	1.31	5.19	0.0390
BC	0.1849	1	0.1849	0.7315	0.4068
BD	0.0049	1	0.0049	0.0194	0.8912
CD	0.0462	1	0.0462	0.1829	0.6754
A^2	2.83	1	2.83	11.18	0.0048
B^2	0.6542	1	0.6542	2.59	0.13
C^2	0.5219	1	0.5219	2.06	0.1727
D^2	4.87	1	4.87	19.26	0.0006
Residual	3.54	14	0.2528		
Lack of Fit	3.14	10	0.3142	3.17	0.1386
Pure Error	0.3963	4	0.0991		
Cor Total	22.41	28			

Note:*Significant (P $_1$ 0.05);** Very significant (P $_1$ 0.01) .

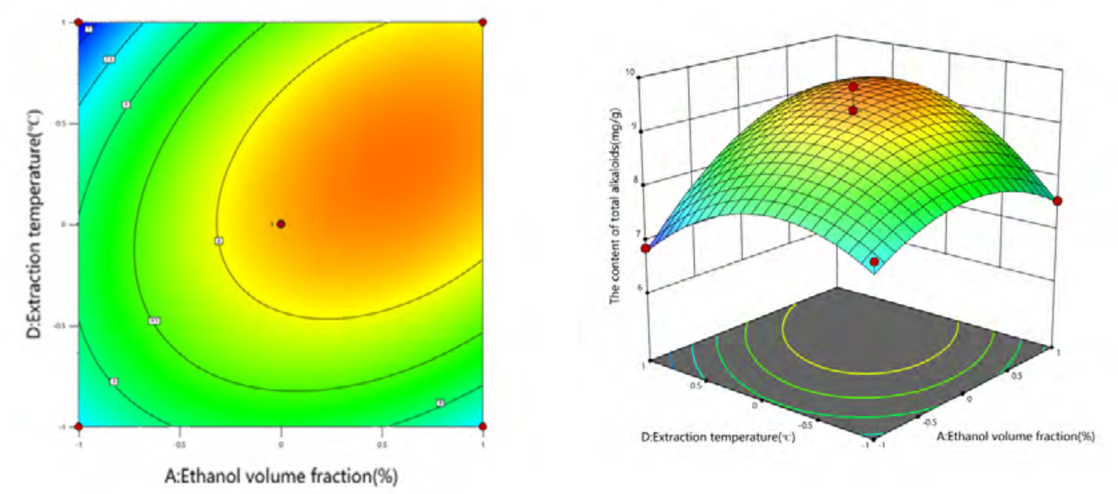

Fig. 3. Response surface plot for the interaction of ethanol concentration and temperature on total alkaloid yield.

coefficients, the factors can be ranked in order of their primary influence: A exerted the greatest effect, followed by C, then B, with D showing the least linear influence.

The interactive effect between ethanol concentration and temperature (AD) was the only significant interaction (p = 0.0390). The corresponding response surface plot (Fig. 3) exhibited a distinct bell-shaped curvature with elliptical contours, indicating a clear synergistic relationship. Within an optimal range, simultaneously increasing both parameters enhanced the yield, beyond which a decline was observed. From a practical perspective, this synergistic interaction allows the use of lower ethanol concentrations at elevated temperatures without compromising yield—an advantage for industrial scale-up where solvent reduction is often preferred over energy savings. All remaining two-factor interaction terms were found to be non-significant ($p > 0.05$), as evidenced by their relatively flat response surfaces, implying that these factors act largely independently within the studied domain[23].

In summary, the extraction process is governed by a combination of linear, quadratic, and interactive effects. The linear influence of ethanol concentration, its synergistic interaction with temperature (AD), and the pronounced quadratic effect of temperature itself were identified as the key determinants of extraction efficiency. The established model is statistically robust, provides a good fit to the experimental data, and reliably captures the non-linear dynamics of the system, thereby forming a solid foundation for process optimization.

Determination of Optimal Conditions and Model Validation

The response surface model predicted the following optimal conditions for maximum total alkaloid yield: 57.5% ethanol, a solid-to-liquid ratio of 30.5 mL/g, 99.8 min, and 82.3 °C, corresponding to a predicted yield of 10.173 mg/g.

To validate these predictions, three independent verification experiments were conducted. The mean experimental yield was 10.051 ± 0.26 mg/g (RSD = 2.62%), demonstrating excellent repeatability. The relative error between the experimental mean and the predicted value was 1.21%, confirming the high predictive accuracy of the model and the robustness of the optimized process.

Method Validation for the HPLC Assay

The established HPLC method underwent validation following recognized guidelines, which included assessment of system suitability, linearity, precision, repeatability, stability, and accuracy[24-25]. Prior to sample analysis, system suitability was confirmed: the theoretical plate number for the norisoboldine peak exceeded 10,000, the resolution between adjacent critical peak pairs was greater than 1.5, and tailing factors were below 1.2, meeting standard chromatographic requirements.

Linearity

Excellent linear relationships were established for all three alkaloids within their specified concentration ranges. The corresponding regression equations, correlation coefficients (R), along with their respective linear ranges, are compiled in Table 4.

Table 4:Calibration data for the three alkaloid reference standards

Component	Linear Regression Equation	R	Linear Range(μg/mL)
Norisoboldine	$y = 34249x - 43325$	0.9994	7.37-294.70
Boldine	$y = 35838x + 26906$	0.9995	2.35-101.20
Reticuline	$y = 18982x + 79275$	0.9992	2.44-97.70

Precision

Intra-day precision was evaluated by calculating the relative standard deviation (RSD) based on peak areas from six replicate injections. The resulting RSD values were 1.27% for norisoboldine, 1.88% for boldine, and 0.79% for reticuline.

Repeatability

Method repeatability was assessed by analyzing six independently prepared sample solutions. The RSDs of the determined contents were 3.16% for norisoboldine, 2.54% for boldine, and 2.47% for reticuline.

Stability

The short-term stability of the prepared sample solution at room temperature was monitored over 24 h. The RSDs of peak areas across different

Table 5:Results of the recovery test for norisoboldine, boldine, and reticuline (n=6)

Component	m(Sample) /g	m(Content) /mg	m(Added) /mg	m(Measured) /mg	Recovery/ %	Average	RSD/%
Norisoboldine	0.1048	0.6320	0.6000	1.2182	97.70	98.88	2.21
	0.0987	0.5951	0.6000	1.1784	97.22		
	0.1021	0.6154	0.6000	1.2097	99.06		
	0.1026	0.6184	0.6000	1.2014	97.16		
	0.0984	0.5936	0.6000	1.1888	99.20		
	0.1029	0.6203	0.6000	1.2381	102.97		
Boldine	0.1027	0.1671	0.1600	0.3181	94.35	97.87	2.43
	0.0996	0.1622	0.1600	0.3175	97.08		
	0.1048	0.1705	0.1600	0.3246	96.29		
	0.1030	0.1676	0.1600	0.3288	100.73		
	0.0987	0.1607	0.1600	0.3197	99.35		
	0.1042	0.1696	0.1600	0.3287	99.42		
Reticuline	0.1026	0.1060	0.1000	0.2058	99.75	101.18	2.16
	0.0996	0.1030	0.1000	0.2039	100.95		
	0.1031	0.1066	0.1000	0.2092	102.60		
	0.1027	0.1062	0.1000	0.2039	97.69		
	0.0999	0.1033	0.1000	0.2069	103.64		
	0.1029	0.1064	0.1000	0.2088	102.44		

Quantification of Target Alkaloids in the Optimized Extract

Table 6:Contents of three target alkaloids in the optimized *L. aggregata* extract (n = 3).

Alkaloid	Content (mg/g, mean ± SD)	RSD (%)
Norisoboldine	6.028 ± 0.046	0.76
Boldine	1.628 ± 0.038	2.33
Reticuline	1.034 ± 0.039	3.77

time points were 1.39% for norisoboldine, 1.77% for boldine, and 2.11% for reticuline, confirming acceptable short-term stability.

Accuracy

Accuracy was evaluated via a standard addition recovery test. The average recovery rates (n = 6) were 98.88% for norisoboldine, 97.87% for boldine, and 101.18% for reticuline, with corresponding RSDs of 2.21%, 2.43%, and 2.16%, respectively (Table 5).

Quantification of Target Alkaloids in the Optimized Extract

The contents of the three target alkaloids in the extract obtained under the optimized conditions were determined using the validated HPLC method. As summarized in Table 6, norisoboldine was found to be the predominant alkaloid with a content of 6.028 ± 0.046 mg/g, which was approximately 3.7-fold and 5.8-fold higher than that of boldine (1.628 ± 0.038 mg/g) and reticuline (1.034 ± 0.039 mg/g), respectively (mean ± SD, n = 3). The relative standard deviations (RSDs) for the triplicate measurements were 0.76%, 2.33%, and 3.77% for norisoboldine, boldine, and reticuline, confirming the high precision of the quantification. This distinct abundance profile underscores norisoboldine as the major alkaloidal constituent in the prepared extract.

This study establishes an integrated "process–quality" framework for *L. aggregata* by combining multivariate extraction optimization with multicomponent chemical analysis, moving beyond empirical, singlefactor approaches.

The RSM model identified the interactive effect of ethanol concentration and temperature as the key determinant of total alkaloid yield. This nonlinearity, where excessive levels of either parameter reduce efficiency, highlights the necessity of multivariate statistical optimization over traditional onefactoratatime methods for complex plant matrices[26].

A validated HPLC method was established to enable the concurrent quantification of three key alkaloids. Notably, using total alkaloid yield as the optimization response proved effective, as it cooptimized the extraction of the specific targets—norisoboldine, boldine, and reticuline—indicating correlated extraction behaviors. The optimized extract showed superior total alkaloid yield (10.173 mg/g), with norisoboldine as the predominant constituent (6.028 mg/g).

The quantified alkaloid profile is pharmacologically relevant, as the three target compounds —norisoboldine, boldine, and reticuline—are well-documented contributors to the anti-inflammatory, antioxidant, and immunomodulatory effects of *L. aggregata*[27–30]. Thus, monitoring these three markers establishes a pharmacologicallyrelevant quality control strategy, ensuring extracts contain the key efficacydriving compounds.

In conclusion, this work provides a sciencedriven, qualitybydesign framework for producing standardized *L. aggregata* extracts. The established "process–quality" link ensures batch consistency for reliable research. Future work should scale up the process, validate in vivo efficacy, and expand the analytical fingerprint.

CONCLUSION

This study delivers an integrated, scientificallygrounded platform for the qualitybydesign production of bioactive alkaloids from *L. aggregata*. By employing response surface methodology, we elucidated the nonlinear dynamics and critical synergies governing extraction efficiency, advancing beyond empirical optimization. Concurrently, a validated HPLC method was established for the simultaneous quantification of three pharmacologically relevant alkaloids—norisoboldine, boldine, and reticuline—providing a multiconstituent chemical benchmark directly linked to the herb's bioactivity.

The optimized process yielded an extract with norisoboldine as the predominant component, aligning chemical composition with mechanistic drivers of efficacy. More broadly, the established "process–quality" correlation model offers a transferable framework for the standardization of complex botanical extracts. This work not only ensures the batchtobatch reproducibility essential for rigorous scientific inquiry but also sets a precedent for linking multivariate process engineering with targeted phytochemical analysis in herbal medicine. The standardized extract produced herein serves as a reliable and wellcharacterized material for advancing pharmacological research and paves the way for the development of consistent, evidencebased herbal products. With its full analytical characterization and validated process parameters, this platform is readily adaptable for industrial quality control and the development of phytopharmaceutical preparations.

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REFERENCES

1. Chen, F. Y.; Liu, Y.; Xie, D.; Luo, Y. M. *Chin. J. Chin. Mater. Med.* **2023**, *48*, 5719-5726.
2. Lv, Y. B.; Zou, Y. F.; Zhang, X. D.; Liu, B. R.; Peng, X.; Chu, C. *Front. Nutr.* **2023**, *9*, 1071276.
3. Wang, X. L.; Zhang, X.; Xie, W.; Wang, Y. Y.; Zang, S. X.; Ban, Z. Y.; Gao, Y. G. *Brain Res.* **2025**, *1846*, 149295.

4. Peng, X.; Luo, Y. Y.; Wang, J.; Ji, T.; Yuan, L. X.; Kai, G. Y. *Food Res. Int.* 2020, *138*, 109799.
5. Zhao, X.; Tan, M. M.; He, Y. C.; Pan, Y.; Fan, X. L.; Ye, L. Y.; Fu, L. Q. *Oxid. Med. Cell. Longev.* **2022**, *2022*, 6256450.
6. Tao, Y.; Deng, Y. L.; Wang, P. *J. Ethnopharmacol.* **2023**, *318*, 116954.
7. Wu, B. S.; Wu, J. N.; Bai, X. X.; Zhou, Q.; Wang, X. P. *J. Ethnopharmacol.* **2025**, *354*, 120479.
8. Zhang, X.; Wang, X. L.; Wang, Y. Y.; Ma, X. Y.; Geng, Y. Y.; Zang, S. X.; Ban, Z. Y.; Jia, Y. G.; Gao, Y. G. *Clin. Exp. Pharmacol. Physiol.* **2025**, *52*, e70033.
9. Tekin, E.; Kip, G.; Küçük, A. *Ren. Fail.* **2025**, *47*, 2558098.
10. Shen, W.; Zhang, Y. W. J. *Food Saf. Qual.* **2022**, *13*, 1256-1263.
11. Wen, S. S.; Liu, Y. J.; Liu, Y. L.; Zhao, Y.; Xu, X. X.; Guo, C. C.; Zhang, N. *RSC Adv.* **2024**, *14*, 36101-36114.
12. Selvakumarasamy, S.; Mahesh, J. S.; Kamble, D. B.; Rengaraju, B. *Chem. Pap.* **2025**, 79, 1-22.
13. Liang, Y. T.; Dai, L. F.; Guo, R. Y.; Ma, X. Q.; Zheng, X. Y.; Feng, Y. N. *Chem. Adhes.* **2022**, *44*, 341-344.
14. Xun, G.; Zhang, Y. F.; Li, Y. X.; Fu, Y.; Wang, Q. *J. Shenyang Pharm. Univ.* **2025**, *42*, 412-422.1
15. Zhang, J.; Liu, B. J.; Li, C.; Wu, W. Y.; Du, B. *Jiangsu Agric. Sci.* **2014**, *42*, 266-267.
16. Wang, H. W.; Peng, H. X.; Ding, W. W.; Zhang, Z. Y.; Zheng, Y. D.; Chen, S. K.; Chen, X. Y.; Qu, Q. W.; Liu, Y. Y.; Li, Y. H. *Ultrason. Sonochem.* **2025**, *119*, 107396.
17. Rambo, F. D.; Biegelmeyer, R.; Toson, S. N. *Ind. Crops Prod.* **2019**, *133*, 250-258.
18. Shi, R. Z.; Zhao, Y.; Li, L.; Yin, Z. L.; Xu, Y. N.; Liu, Y. *J. Yunnan Univ. Chin. Med.* **2025**, *48*, 69-74.
19. Zhang, Y. C.; Zhao, Y.; Han, L. X.; Liu, S. F.; Tian, Y.; Gao, X. Y. *Food Sci. Technol.* **2024**, *49*, 218-227.
20. Liu, D. J.; Guo, L. L.; Tian, L. J.; Li, L.; Yang, T. Z.; Zhang, H. M.; Li, L. L.; Xia, J. Sci. Technol. Food Ind. **2023**, *44*, 195-203.
21. Chen, L.; Liang, Q. L.; Wang, H.; Zheng, W. H.; Yang, X. J.; Ma, L. Chem. Ind. For. Prod. **2025**, *45*, 53-62.
22. Sun, L.; Li, T. J.; Yang, X. X.; Bao, Y. R.; Wang, S.; Meng, X. S. *Chin. J. Pharm.* **2025**, *56*, 929-936.
23. Lin, Q.; Jiang, W. S.; Li, Y. J.; He, S. Y. *Mod. Food* **2022**, *28*, 82-91.
24. Wu, T. Q.; Li, H. J.; Chen, L.; Gao, J.; Yang, M. Y.; Wu, Y.; Wen, J. G.; Zhang, Y.; Liu, *J. Chin. J. Pharm. Anal.* **2025**, *45*, 571-577.
25. Liu, Z. H.; Ren, L. C. C.; Wang, Y.; Dong, Q.; Wang, H. L.; Hu, N. *WCJPS* **2024**, *39*, 321-323.
26. Guo, J. J.; Li, Y. X.; Fan, Z. Y.; Chen, X. T.; Wang, Q.; Fu, Q. *Nat. Prod. Res.* Dev. **2022**, *34*, 1181-1188.
27. Zhang, Q.; Fang, Y.; Lv, C.; Zhu, Y.; Xia, Y.; Wei, Z.; Dai, Y. *FASEB J.* **2022**, *36*, e22230.
28. Li, S. M.; Yan, W. L.; Sun, K. N.; Miao, J. Y.; Liu, Z. C.; Xu, J. Y.; Zhang, Q. *Mol. Nutr. Food Res.* **2024**, *68*, e2400193.
29. Sáez, C. J.; Burrell, C. J.; Cahill, M. C. Front. *Pharmacol.* **2024**, *15*, 1427147.
30. Alghamdi, A. H.; Alghamdi, S. S.; Zahrani, A. H. M.; Trivilegio, T.; Bahattab, S.; AlRoshody, R.; Alhaidan, Y.; Alghamdi, A. R.; Nasri, M. S. *Curr. Issues Mol. Biol.* **2025**, *47*, 810.