Effects of Cultivation Methods on Yield and Quality of *Radix scutellariae*

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Abstract [**Objectives**] To investigate the effects of three cultivation methods-level bed, high ridge, and ridge cultivation-on the yield and quality of *Radix scutellariae*, providing a basis for establishing its Good Agricultural Practice (GAP) technical system. [**Methods**] Utilizing two-year-old *R. scutellariae* plants from the cultivation base in Kushan Township, Ju County as the experimental material, yield-related indicators including root length, root diameter, branch number, and fresh weight were measured at harvest time. The baicalin content in the roots was determined using High-Performance Liquid Chromatography (HPLC), and the differences in effects among the cultivation methods were analyzed. [**Results**] *R. scutellariae* cultivated using the ridge method exhibited significantly superior root length compared to both level bed and high ridge cultivation. Its branch number was significantly higher than that under level bed cultivation. The baicalin content reached 13.17%, surpassing that achieved with high ridge (12.73%) and level bed (11.87%) cultivation. Based on a comprehensive evaluation of agronomic traits and active constituent content, *R. scutellariae* cultivated using the ridge method demonstrated significant superiority in both yield and quality over those grown under high ridge or level bed conditions. [**Conclusions**] Ridge cultivation effectively promotes root growth in *R. scutellariae*, enhances medicinal material yield and baicalin content. This method is recommended for widespread application in artificial cultivation and provides a scientific foundation for establishing *R. scutellariae* GAP standards.

Key words *Radix scutellariae*, Baicalin, Cultivation method, HPLC

0 Introduction

Radix scutellariae is a commonly used traditional Chinese medicine, first documented in the *Shennong Bencao Jing* (*Classic of Materia Medica*) and classified as a medium-grade herb. It is the dried root of *Scutellaria baicalensis* Georgi, a member of the Labiatae family[1]. In recent years, due to increasing demand for *R. scutellariae* and the over-exploitation of wild resources, cultivated *R. scutellariae* has become the primary source of commercial medicinal material. Cultivation methods significantly influence plant growth, development, and yield formation. Therefore, investigating the impact of different cultivation methods on the yield and quality of *R. scutellariae* is crucial for ensuring and enhancing the quality of *R. scutellariae* medicinal material. To achieve the objectives of medicinal materials being "safe, effective, stable, and controllable" and to ensure clinical therapeutic efficacy, this study systematically compared the effects of three cultivation methods—level bed, high ridge, and ridge cultivation—on *R. scutellariae* yield (as indicated by root length, diameter, branching, and fresh weight) and quality (as determined by baicalin content). The aim is to provide a basis for selecting appropriate cultivation techniques for the artificial planting of *R. scutellariae* and for establishing its Good Agricultural Practice (GAP) standards.

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1 Materials and methods

1.1 Materials

1.1.1 Plant material source. The experimental material consisted of *R. scutellariae* plants provided by the *R. scutellariae* cultivation base in Kushan Township, Ju County.

1.1.2 Instruments. Lihe Brand Electric Thermostatic Incubator (Medical Equipment Factory, Shandong Weifang Pharmaceutical Group Co., Ltd.); KQ-250E Medical Ultrasonic Cleaner (Kunshan Ultrasonic Instruments Co., Ltd.); FA1104 Top-Loading Electronic Balance (Shanghai Balance Instrument Factory); Shimadzu LC-10ATvp High-Performance Liquid Chromatography (HPLC) System (comprising LC-10ATvp high-pressure constant-flow pump, SPD-10Avp UV detector); PHENOMENEX Chromatography Column; 25 μL Microsyringe.

1.1.3 Reagents. 95% Ethanol (Analytical Reagent Grade, Linzi Tiande Fine Chemical Research Institute, Zibo); Anhydrous Ethanol (Analytical Reagent Grade, Linzi Tiande Fine Chemical Research Institute, Zibo); Methanol (HPLC Grade, Tianjin Concord Technology Co., Ltd.); Baicalin Reference Standard (National Institutes for Food and Drug Control, China).

1.2 Methods

1.2.1 Sample preparation. Roots of two-year-old *R. scutellariae* plants were excavated at harvest time, sun-dried under natural

conditions, and then cleaned to remove residual stems, weeds, and soil impurities. The cleaned roots were subsequently pulverized using a grinder, sieved, and stored for later use.

1.2.2 Chromatographic conditions. Column: PHENOMENEX C_{18} column; Mobile phase: Methanol – Water – Phosphoric Acid (47 ∶ 53 ∶ 0.2, *v/v/v*); Detection wavelength: 280 nm; Column temperature: 25 ℃; Flow rate: 1.0 mL/min; Detection sensitivity: 0.01 AUFS; Theoretical plate number: Not less than 2 500; Injection volume: 10 μL.

1.2.3 Preparation of reference solution. Baicalin reference standard, previously dried under reduced pressure at 60 ℃ for 4 h, was accurately weighed (0.006 0 g) and transferred to a 100 mL volumetric flask. The solution was then diluted to volume with the appropriate solvent (typically methanol or mobile phase), resulting in a baicalin reference solution with a concentration of 60 μg/mL.

1.2.4 Preparation of test solution. Based on methodological comparisons (ultrasonic extraction vs. heat reflux extraction), ultrasonic extraction with 70% ethanol twice, each for 40 min, was determined to be the method for preparing the test solution. Approximately 0.3 g of the sample powder was accurately weighed and extracted according to the method described above. The extract was filtered, diluted to the appropriate volume in a volumetric flask, and then filtered through a 0.45 μm microporous membrane prior to injection into the HPLC system.

1.2.5 Construction of calibration curve and linearity study. Under the chromatographic conditions specified above, precisely measured volumes (6, 8, 10, 12, and 14 μL) of the baicalin reference solution were injected into the HPLC system. A calibration curve was constructed by plotting the peak area (A) of baicalin against its concentration (c, μg/mL). The resulting linear regression equation was $A = 164\ 151c + 1\ 796\ 129$ ($r = 0.999\ 5$). The results demonstrated a good linear relationship between the peak area and baicalin concentration within the range of 0.386 to 0.784 μg/mL, meeting the requirements for quantitative analysis.

1.2.6 Precision test. The same *R. scutellariae* test solution was injected consecutively six times (10 μL per injection). The peak area of baicalin was measured each time, and the relative standard deviation (*RSD*) was calculated. The results showed an *RSD* of 1.40% ($n = 6$) for the peak areas, indicating good precision of the instrument.

1.2.7 Stability test. The same *R. scutellariae* test solution was stored at room temperature. Aliquots (10 μL) were injected into the HPLC system at 0, 2, 6, 12, 18, and 24 h. The baicalin peak area was measured at each time point, and the *RSD* was calculated. The results showed an *RSD* of 1.31% ($n = 6$) for the peak areas over the 24-hour period, indicating good stability of the baicalin test solution within 24 h.

1.2.8 Accuracy test (standard addition method). Six portions of *R. scutellariae* powder (approximately 0.3 g each), with known baicalin content, were accurately weighed. Approximately 30 mg of baicalin reference standard was added to each portion. Test solutions were then prepared according to the extraction method described subsequently, and the baicalin content was determined. The recovery rate was calculated using the formula:

$$\text{Recovery}\ (\%) = [(N - L)/M] \times 100\%$$

where L is the amount of the analyte originally present in the sample, M is the amount of the reference standard added, and N is the total amount measured.

As shown in Table 1, the average recovery rate was 97.28% with an *RSD* of 1.40% ($n = 6$). This indicates good accuracy of the method, fulfilling the requirements for quantitative analysis.

Table 1 Results of the standard addition recovery test

Sample No.	L	M	N	Recovery %	Average recovery // %	*RSD* %
1	0.042 0	0.028 8	0.069 7	96.18	97.28	1.40
2	0.045 7	0.029 2	0.074 6	98.97		
3	0.043 1	0.030 3	0.072 4	96.70		
4	0.044 5	0.031 0	0.074 1	95.48		
5	0.049 9	0.029 8	0.079 2	98.32		
6	0.046 7	0.030 0	0.075 1	98.00		

1.2.9 Selection of extraction method[2-3]. Approximately 0.3 g of *R. scutellariae* powder was accurately weighed and placed into a stoppered conical flask. 40 mL of 70% ethanol was added, and extraction was performed using four different methods (Table 2). After extraction, the solution was allowed to cool, filtered, and the filtrate was transferred to a 100 mL volumetric flask. The container and residue were washed with a small amount of 70% ethanol, and the washings were combined with the filtrate in the volumetric flask. The solution was then diluted to volume with 70% ethanol and mixed well. 1 mL of this solution was precisely transferred to a 10 mL volumetric flask, diluted to volume with methanol, mixed, filtered through a 0.45 μm microporous membrane, yielding the test solution for HPLC injection and determination of baicalin content.

Table 2 Comparative analysis of results from different extraction methods

Method No.	Method	$X \pm SD$ (%)
①	Ultrasonic extraction twice, 30 min each time	15.99 ± 0.016 B*
②	Ultrasonic extraction twice, 40 min each time	17.60 ± 0.412 A
③	Ultrasonic extraction twice, 60 min each time	15.48 ± 0.009 C
④	Heat reflux extraction for 3 h	17.70 ± 0.202 A

NOTE SAS analysis at $\alpha = 0.05$. Different uppercase letters indicate significant differences between groups. The same below.

The results in Table 2 show that the baicalin extraction yields of Method ② and Method ④ showed no significant difference, and both were significantly higher than those of Method ① and Method ③; Considering both extraction efficiency and operational simplicity, Method ② (ultrasonic extraction twice, 40 min each time) was

selected as the extraction method for baicalin in this experiment.

1.2.10 Determination of baicalin content. Using the selected extraction method described above, test solutions were prepared separately from the roots of *R. scutellariae* cultivated under level bed, high ridge, and ridge conditions. Precisely 10 μL[4] of both the reference solution and each test solution were injected into the HPLC system under the chromatographic conditions specified in Section **1.2.2**. The baicalin content (%) was calculated using the external standard method with single-point calibration.

1.2.11 Measurement of agronomic traits. Thirty *R. scutellariae* plants exhibiting uniform growth were selected from each of the three cultivation methods. Root length (cm) was measured using a tape measure, root diameter (mm) was measured using vernier calipers, the number of branches was counted manually, and root fresh weight (g) was measured using an electronic balance. The mean ± standard deviation ($X \pm SD$) was calculated for each trait. Significant differences among the cultivation methods were analyzed using SAS software at a significance level of $P = 0.05$.

2 Results and analysis

2.1 Effects of different cultivation methods on agronomic traits of *R. scutellariae* roots The effects of the three cultivation methods on root length, diameter, branch number, and fresh weight of *R. scutellariae* are presented in Table 3. As shown in Table 3, there were no significant differences in root diameter or root fresh weight among the three cultivation methods. Root length under ridge cultivation was significantly greater than that under both level bed and high ridge cultivation, while no significant difference was observed between level bed and high ridge cultivation. The number of branches under both high ridge and ridge cultivation was significantly higher than that under level bed cultivation, with no significant difference between high ridge and ridge cultivation. Considering the comprehensive agronomic performance, ridge cultivation promoted longitudinal root growth, increased branch number, and enhanced the appearance quality of the medicinal material, demonstrating superiority over both level bed and high ridge cultivation.

Table 3 Effects of different cultivation methods on agronomic traits of *Radix scutellariae* roots ($X \pm SD$)

Cultivation method	Root length//cm	Root diameter//mm	Number of branches	Fresh weight//g
High ridge	27.00 ± 4.81 B*	12.86 ± 4.62 A	5.26 ± 2.79 A	25.70 ± 13.82 A
Ridge	30.66 ± 6.44 A	13.66 ± 4.32 A	5.50 ± 3.92 A	30.86 ± 16.16 A
Level bed	26.00 ± 4.72 B	12.48 ± 3.00 A	2.56 ± 1.43 B	25.10 ± 13.43 A

2.2 Effects of different cultivation methods on baicalin content in *R. scutellariae* roots The effects of the three cultivation methods on the baicalin content in *R. scutellariae* roots are shown in Table 4. As indicated in Table 4, *R. scutellariae* cultivated using the ridge method exhibited the highest baicalin content (13.17%), followed by high ridge cultivation (12.73%), with level bed cultivation yielding the lowest content (11.87%). Significance analysis revealed no significant difference in baicalin content between ridge and high ridge cultivation, and both were significantly higher than that under level bed cultivation.

Table 4 Effects of different cultivation methods on baicalin content in *Radix scutellariae* roots ($X \pm SD$, %)

Cultivation method	Baicalin content//%	Significance
High ridge	12.73 ± 0.382	A*
Ridge	13.17 ± 0.250	A
Level bed	11.87 ± 0.297	B

2.3 Comprehensive evaluation of different cultivation methods Integrating the analysis of agronomic traits and active constituent content: *R. scutellariae* under level bed cultivation had shorter roots, fewer branches, and the lowest baicalin content, representing the poorest overall performance. While high ridge cultivation yielded relatively high baicalin content, its root length was significantly shorter than that achieved with ridge cultivation. Ridge cultivation not only resulted in the best agronomic traits, such as root length and branch number, but also produced the highest baicalin content among the three methods. It demonstrated significant advantages in both yield potential (agronomic traits) and medicinal quality (active components), establishing it as the optimal choice among the three cultivation methods.

3 Conclusions and discussion

The results of this study demonstrate that cultivation methods significantly influence both the yield and quality of *R. scutellariae*. Ridge cultivation, by enhancing soil temperature, aeration, and drainage, creates a more favorable environment for the longitudinal growth and development of the *R. scutellariae* root system. This leads to superior performance in yield indicators such as root length and branch number, while also resulting in the highest baicalin content in the medicinal material. This validates the traditional empirical view that ridge cultivation is beneficial for *R. scutellariae* growth.

Therefore, for the standardized artificial cultivation of *R. scutellariae*, the ridge cultivation method is recommended to simultaneously enhance both the yield and intrinsic quality of the medicinal material. These findings provide crucial experimental evidence for establishing high-yield, high-quality cultivation techniques for *R. scutellariae* and for formulating its GAP standards.

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2024), to fruit composition, bioactive chemicals, and industrial applications[8,17,19,21]. This signals a field transitioning from basic inquiry to targeted development. Future frontiers will likely be defined by: (i) deepening the functional genomics of lipid pathways; (ii) comprehensive biochemical characterization of fruit compounds; and (iii) scaling applied uses through optimized oil extraction and value-chain integration.

4 Conclusions

This first bibliometric study of *I. polycarpa* research (1994 – 2024) delineates a rapidly evolving field characterized by significant growth since 2016, yet one that remains geographically concentrated within China with limited international collaboration. The intellectual structure, founded on studies of sexual differentiation and lipid metabolism, reveals a clear trajectory from basic science toward applied research. A notable divergence exists: international efforts prioritize fundamental biological mechanisms, while Chinese research is strongly application-oriented, focusing on oil extraction and utilization. Three major research fronts have emerged-lipid biosynthesis and functional genomics, fruit composition and bioactivity, and industrial applications.

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