Phyton-
International Journal of
Experimental Botany

Tech Science Press

http://doi.org/10.32604/phyton.2026.082999

ARTICLE

Optimization and Sensory Evaluation of the Volatile Oil Extracts from *Peucedanum praeruptorum* Using Response Surface Methodology and HS-SPME-GC/MS Detection

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Received: 26 March 2026; Accepted: 23 April 2026; Published: 27 May 2026

ABSTRACT: Essential oil is one of the main active ingredients in the medicinal herb *Peucedanum praeruptorum*. However, limited studies have been conducted on the quality evaluation of this essential oil. Here, we performed an optimization and evaluation of *P. praeruptorum* volatile oil extraction using response surface methodology. Partial least squares discriminant analysis (PLS-DA) was employed to screen relevant biomarkers. A sensory evaluation of the volatile oil components of *P. praeruptorum* was conducted across different time periods, and aroma differences were examined. The optimal extraction process involved an extraction time of 4 h, a soaking time of 2 h, a liquid-to-solid ratio of 12 mL/g and an extraction temperature of 132°C. HS-SPME-GC/MS analysis of the volatile oil composition from different origins, periods and plant parts identified various chemical components, including terpenes, aldehydes, hydrocarbons and ketones. The main components identified were composed of α-pinene, (-)-β-pinene, 3-carene, β-myrcene, D-limonene, β-phellandrene, and other terpenes. The principal component analysis (PCA) revealed clear differences from different origins and time periods. The PLS-DA identified 39 key differential components in the aboveground parts and 20 in the underground parts with variable importance in projection (VIP) values > 1. The sensory evaluation analysis described the aroma of *P. praeruptorum* volatile oil as grassy, woody, spicy, earthy and reminiscent of pine resin, with the most prominent note being spicy. This was highly correlated with β-pinene, which accounted for the highest proportion. The prominent spicy note was found to be derived from three components: (–)-β-pinene, β-phellandrene, and o-cymene. Our study provides a scientific basis for the evaluation, development and application of volatile oil from *P. praeruptorum*.

KEYWORDS: Volatile oil; terpenes; GC-MS; response surface method; sensory evaluation

1 Introduction

Peucedani Radix is derived from the dried root of *Peucedanum praeruptorum* crude materials. Apiales are mostly aromatic and rich in characteristic secondary metabolites such as volatile oils and coumarins. They are one of the taxa of great research value in medicinal resources. As a classic phlegm-relieving and cough traditional Chinese medicine, *P. praeruptorum*'s medicinal history dates back thousands of years, and its traditional efficacy has been continuously verified by generations of clinical practice. The volatile oil synthesized in its body is an important plant secondary metabolite that gives it a special aroma and plays a key role in plant's defense, growth and development and environmental adaptation.

It has both medicinal value and significance in plant physiological research, and is an important carrier connecting traditional medicinal practice with modern botany [1]. It has a variety of pharmacological effects, including treating wind-heat colds (acute upper respiratory tract infection caused by a viral or bacterial infection) and helping with coughs and phlegm (mucous or serous exudate secreted by irritation of the respiratory mucosa). *P. praeruptorum* also exhibits anti-inflammatory and anti-tumor benefits [1,2]. The volatile oil of *P. praeruptorum* comprises many components, primarily sesquiterpenes, alkanes and aromatic compounds. A number of studies have reported its volatile constituents at differing periods of harvest and discovered that the oil contains high levels of olefins, esters, alcohols, aldehydes, and ketones. Notably, α-pinene, caryophyllene, phellandrene, and β-pinene are present at particularly high levels [3,4]. These components are present in the essential oils of many plants and exhibit various pharmacological activities, including anticoagulant, antitumor, anxiolytic and antibacterial properties [5–9]. At present, there are no unified standards for the extraction time, temperature, soaking time, or liquid-to-material ratio for the volatile oil of *P. praeruptorum*. This lack of standardisation in the extraction process leads to significant variability in extraction processes across different laboratories, which directly affects the scientific validity and reproducibility of studies investigating the pharmacodynamic mechanism of its volatile oil. Moreover, the existing extraction methods suffer from excessively long extraction times and high energy consumption, thereby increasing both the time and economic costs of laboratory research. Systematically investigating each single factor and determining the optimal combination of extraction parameters could improve production efficiency and enable the successful application of *P. praeruptorum* volatile oil.

The main advantage of RSM is that it reduces the number of experimental trials required to evaluate multiple parameters and their interactions. This makes it a labour-efficient and time-saving method for optimizing processes [10,11]. Headspace solid-phase microextraction-gas chromatography/mass spectrometry (HS-SPME-GC/MS) is commonly employed for the analysis of volatile compounds and noted for its straightforward and rapid extraction process [12,13]. It provides benefits over previous techniques by eliminating the need for solvents or heating, thereby maintaining the genuine aroma and preventing artifacts generated by the heating process in steam distillation [14]. Odour functions as a significant sensory indicator in traditional Chinese medicine, closely linked to its quality and efficacy. In current studies on traditional Chinese medicine, sensory evaluation has gained considerable attention and is extensively used for the identification and processing of medicinal materials, the optimisation of preparation processes, and the control of drug quality [15]. Partial least squares discriminant analysis (PLS-DA) is a supervised multivariate statistical method used to identify and quantify differences between sample groups by making mathematical comparisons of mass spectrometry data [16]. Variable importance in projection (VIP) can be used to assess the weight of variables in the PLS-DA model. Its magnitude reflects each variable's contribution to the model's overall fitting and classification ability [17,18].

In summary, this study focused on *P. praeruptorum* volatile oil as the research object. To address the industrial issue of the lack of unified standards have been established for the extraction parameters of its volatile oil, the RSM was employed to optimize and determine the optimal extraction parameters. This study is the first to combine HS-SPME-GC-MS component analysis, multivariate statistical discrimination, and sensory evaluation were progressively combined, overcoming the limitations of previous studies on plant volatile oils which either overemphasised compositional analysis while neglecting sensory attributes, or relied too heavily on subjective sensory evaluation. This study aims to develop a comprehensive system that encompasses process optimization, compositional analysis, differential screening and sensory evaluation. It also presents novel ideas and methodologies for the extraction of volatile oils derived from Apiaceae

plants. Specifically, the response surface methodology was adopted to optimize the extraction process; the chemical composition of the volatile oil obtained under the optimal process was comprehensively analyzed via HS-SPME-GC-MS; key marker components were identified through PLS-DA statistical analysis; and sensory evaluation was performed to verify the correlation between the chemical composition and the aromatic characteristics. Collectively, this study provides a complete scientific basis for the industrial production and quality control of *P. praeruptorum* volatile oil.

2 Materials and Methods

2.1 Instruments and Reagents

The parameter settings of HS-SPME-GC/MS are attached in Table 1. Anhydrous sodium sulfate (purity: ≥99.0%), α-pinene (purity: ≥95%), α-ionone (purity: ≥90%), *Cis*-3-hexene-1-ol (purity: ≥98%), and 1-octen-3-alcohol capsaicin (purity: ≥95% were purchased from Shanghai Maclean's Biochemical Technology Co., Ltd.

Table 1: Parameters of HS-SPME-GC-MS.

Project	Parameter
Incubate Temperature	60°C
Preheat Time	15 min
Incubate Time	30 min
Desorption Time	4 min
Front Inlet Mode	Splitless Mode
Front Inlet Septum Purge Flow	3 mL/min
Carrier Gas	Helium
Column	DB-Wax (30 m × 250 μm × 0.25 μm)
Column Flow	1 mL/min
Oven Temperature Ramp	40°C hold on 4 min, raised to 245°C at a rate of 5°C/min, hold on 5 min
Front Injection Temperature	250°C
Transfer Line Temperature	250°C
Ion Source Temperature	230°C
Quad Temperature	150°C
Electron Energy	−70 eV
Mass Range	m/z: 20–400
Scan Mode	Scan
Solvent Delay	2.30 min

2.2 Plant Sample Collection

Plants were selected based on their strong growth, lack of pests and diseases, and similar sizes to ensure the uniformity of the experimental materials. We purchased plants directly from growers and The authenticated standard reference materials are stored at the West Anhui University Museum of Traditional Chinese Medicine (catalog No. 300511-250101). After the fresh *P. praeruptorum* root system is rinsed with clean water to remove the surface sediment, it is placed in a ventilated and cool place to dry in the shade for 24 h, during which it is turned regularly to avoid mildew caused by sample accumulation. A total of 11 batches of *P. praeruptorum* samples from differet origins were collected, with details regarding collection time and origin provided (Table 2).

Table 2: *P. praeruptorum* sample collection and relevant information.

Sample Number	Material Parts	Source of Origin	Collection Time
S1-G S1-Y	Underground root Above-ground stems and leaves	Changsha, Hunan (28°13′41″N, 112°56′20″E)	2024.12
S2-G S2-Y	Underground root Above-ground stems and leaves	Guiyang, Guizhou (26°38′55″N, 106°37′39″E)	2024.12
S3-G S3-Y	Underground root Above-ground stems and leaves	Bijie, Guizhou (27°16′58″N, 105°17′30″E)	2024.12
S4-G S4-Y	Underground root Above-ground stems and leaves	Chun'an, Zhejiang (29°36′18″N, 119°02′18″E)	2024.12
S5-G S5-G	Underground root Above-ground stems and leaves	Zhaotong, Yunnan (27°20′18″N, 103°43′03″E)	2024.12
S6-G S6-Y	Underground root Above-ground stems and leaves	, Baoshan, Yunnan (25°06′44″N, 99°09′42″E)	2024.12
S7-G S7-Y	Underground root Above-ground stems and leaves	Wanzhou, Chongqing (30°50′02″N, 108°24′32″E)	2024.12
S8-G S8-Y	Underground root above-ground stems and leaves	Ningguo, Anhui 30°38′02″N, 118°58′59″E	2024.11
S9-G S9-Y	Underground root Above-ground stems and leaves	Ningguo, Anhui (30°38′02″N, 118°58′59″E)	2024.12 (early this month)
S10-G S10-Y	Underground root Above-ground stems and leaves	Ningguo, Anhui (30°38′02″N, 118°58′59″E)	2024.12 (end this month)
S11-G S11-Y	Underground root Above-ground stems and leaves	Ningguo, Anhui (30°38′02″N, 118°58′59″E)	2025.01

2.3 Extraction Method of Volatile Oil in P. praeruptorum

Following the 'Volatile Oil Determination Method A' outlined in the China Pharmacopoeia (General Principles 2204), freshly chopped *P. praeruptorum* was placed in a 5 L round-bottom flask and covered with the correct amount of water to allow it to soak. The flask was then placed on an electric heating mantle, connected to a liquid separator and a reflux condenser, and heated. The mixture was heated to the boiling point and kept at this temperature for a specified period. Heating was then stopped and the mixture was left to stand for 30 min. The combined volatile oil and water mixture was collected in the separator, treated with the correct amount of anhydrous sodium sulphate and centrifuged to produce pure *P. praeruptorum* volatile oil. This was then stored in a refrigerator at 4°C for later use.

$$Volatile\ oil\ yield\ (\%) = volatile\ oil\ mass\ (\mathrm{g}) \div Input\ in\ sample\ quality\ (\mathrm{g}) \times 100\% \quad (1)$$

2.4 Experimental Design of Single Factor and Response Surface

A single-factor test was conducted at an extraction temperature of 110°C, with an extraction time of 4 h, a liquid-to-solid ratio of 8, and a soaking time of 30 min, to investigate the effect of these four factors on the yield of volatile oil. The volatile oil yield (Y) was set as the response value using RSM, and the independent variables were extraction time (A), soaking time (B), liquid-to-material ratio (C), and extraction temperature (D). A four-factor, three-level response surface test was then carried out (Table 3).

Table 3: Box–Behnken experimental design.

Level	Factors			
	A Extraction Time/h	B Soaking Time/h	C Liquid-to-Material Ratio/(mL/g)	D Extraction Temperature/°C
−1	3	1.5	10	120
0	4	2	12	130
1	5	2.5	14	140

2.5 HS-SPME-GC/MS Analysis of Volatile Oil from Different Parts of P. praeruptorum in Different Origins

The sample was diluted 1000-fold with n-hexane, and 5 μL of the diluted solution was transferred to a 20 mL headspace vial, together with 10 μL of a 10 mg/L 2-octanol internal standard solution in H_2O. Separately, 10 μL of a mixed n-alkane solution (10 mg/L) was added to another headspace vial. GC-MS analysis was performed using a TG-5SilMS quartz capillary column (30 m × 0.25 mm × 0.25 μm). The temperature program was set as follows: an initial temperature of 50°C was held for 5 min, then increased to 160°C at a rate of 5°C/minute, and finally raised to 290°C at a rate of 10°C/min, after which it was held for 3 min. The injection volume was 1000 μL with helium as the carrier gas at a flow rate of 1.2 mL/min.

2.6 Sensory Evaluation of Volatile Oil in P. praeruptorum

The sensory evaluation of volatile oil has been reviewed by the Ethics Committee of West Anhui University (review number: 2024-E(r)-012), and strictly adhered to the "Ethical Review Measures for Life Science and Medical Research Involving Humans" (2023 edition). Due to the condition of the evaluators, ten health assessors (seven females and three males, aged 22–45 years) with a normal sense of smell and no smoking, allergy or olfactory disorders were screened. Three rounds of systematic training were conducted in accordance with GB/T 16291.1-2012, 'General Guidelines for the Selection, Training and Management of Evaluators in Sensory Analysis', to unify the evaluation criteria and descriptors. A sensory evaluation panel was established by selecting one hundred volunteers aged 22–26 to assess the volatile oil at different times. Prior to evaluating odor attributes and intensity, the panelists underwent systematic training, which included repeated sniffing and discussion of samples, to ensure a consistent understanding of and approach to odor descriptors. The panel collectively identified and defined five sensory descriptors for *P. praeruptorum* volatile oil: grassy, woody, spicy, earthy, and pine resin. To evaluate this, scent strips were dipped 1 cm into the volatile oil, and the excess liquid was wiped off at the bottle's mouth. Sensory analysis was then performed by sniffing the strips at a distance of 2 cm from the nose. Each sample was tested three times. Sensory evaluation was conducted using a 10-point descriptive analysis method. Odor intensity was scored on a scale from 0 to 10, with the intensity gradually increasing as the score increased. Specifically, a score of 0 indicated no detectable odor, a score of 5 indicated moderate odor intensity, and a score of 10 indicated an extremely strong odor intensity. Once all panelists completed their scoring, the highest and lowest scores were removed, and the arithmetic mean of the remaining scores was calculated to determine the final odor intensity score for each sample. A higher score indicated greater aroma intensity, and the final score for each aroma attribute was calculated as the average of the ratings from all ten panelists. This value was then used to construct a radar fingerprint chart [19]. A quantitative descriptive analysis of the literature was performed (Table 4).

Table 4: Odor characteristics and reference sample standards for sensory description analysis.

Sensory Properties	Smell Description	Compare Samples	Points
Resin flavor	The resinous taste of pine oozing	α-pinene	5
Woody taste	The wood fragrance of trees	α-ionone	5
Grass flavor	The smell of grass or young leaves	*Cis*-3-hexene-1-ol	5
Earthy flavor	The smell of soil after plowing	1-octen-3-ol	5
Spicy flavor	The warm, pungent aroma of dried chili [20]	Capsaicin	5

2.7 Data Processing and Softwares Applied

All the data were organized and analyzed using Office Excel 2019. IBM SPSS Statistics 27.0 was used to test for significant differences. The response surface test was designed using Design-Expert 8.0.6. GraphPad Prism 5 and Origin 2021 were used to analyze and plot the data. Peak extraction, baseline correction, deconvolution, peak integration, peak alignment, and mass spectrometry matching were performed on the mass spectrometry data using Chroma TOF software (version 4.3x, LECO) and the NIST 23 library. PCA and PLS-DA analyses were conducted using SIMCA 14.1.

3 Results

3.1 Optimization of Essential Oil Extraction Based on RSM

The yield of volatile oil from *P. praeruptorum* increased initially and then decreased with longer extraction times (Fig. 1A). The highest yield of 0.1435% was achieved after 4 h. Therefore, extraction times of 4, 5, and 6 h were selected for the response surface experimental design. When the soaking time was 2 h, the highest yield was 0.1373%. Therefore, soaking times of 1.5, 2, and 2.5 h were selected for the test design (Fig. 1B). The results also showed that the yield of volatile oil first increased and then decreased with an increase in the amount of solvent, reaching a maximum of 0.1033% at a liquid-to-solid ratio of 12 mL/g (Fig. 1C). Accordingly, ratios of 10, 12, and 14 mL/g were selected. Similarly, the volatile oil yield initially increased and then decreased with a higher extraction temperature, peaking at 0.1321% at 130°C. Thus, temperatures of 120, 130, and 140°C were selected for the response surface experimental design (Fig. 1D). A Box–Behnken experimental design based on factor and level tables was carried out with a total of 29 combinations to determine the effect of each factor on the yield of volatile oil in *P. praeruptorum* (Table 5). The results indicated that under factor combination No. 26, the extraction rate of volatile oils reached its highest level at 0.1281%.

Table 5: Box-Behnken experimental design and results for *P. praeruptorum* volatile oil.

Serial Number	A Extraction Time/h	B Soaking Time/h	C Extraction Temperature/°C	D Liquid-to-Material Ratio/(mL/g)	Y Volatile Oil Yield/%
1	3	1.5	130	12	0.0592
2	5	1.5	130	12	0.0831
3	3	2.5	130	12	0.0897
4	5	2.5	130	12	0.0726
5	4	2	120	14	0.0084
6	4	2	140	14	0.0449
7	4	2	120	10	0.0352
8	4	2	140	10	0.0625
9	3	2	130	14	0.0191
10	5	2	130	14	0.0536

Table 5: *Cont.*

Serial Number	A Extraction Time/h	B Soaking Time/h	C Extraction Temperature/°C	D Liquid-to-Material Ratio/(mL/g)	Y Volatile Oil Yield/%
11	3	2	130	10	0.0478
12	5	2	130	10	0.0502
13	4	1.5	120	12	0.0334
14	4	2.5	120	12	0.0537
15	4	1.5	140	12	0.0785
16	4	2.5	140	12	0.0709
17	3	2	120	12	0.0022
18	5	2	120	12	0.0541
19	3	2	140	12	0.0803
20	5	2	140	12	0.0352
21	4	1.5	130	14	0.0685
22	4	2.5	130	14	0.0573
23	4	1.5	130	10	0.0602
24	4	2.5	130	10	0.0792
25	4	2	130	12	0.1216
26	4	2	130	12	0.1281
27	4	2	130	12	0.1249
28	4	2	130	12	0.1271
29	4	2	130	12	0.1186

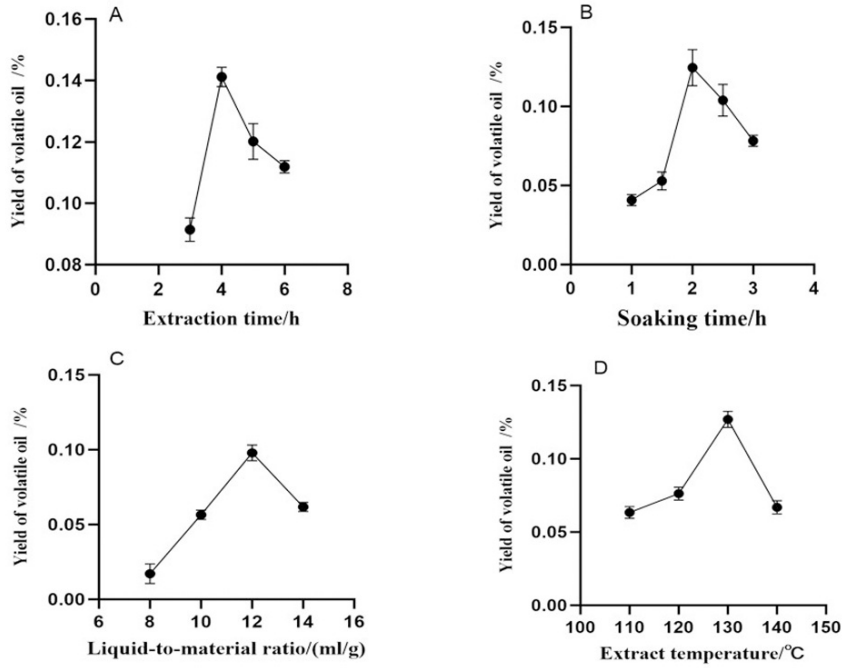

Figure 1: Effect of the single factor on volatile oil extraction rate. (**A**) Effect of extraction time on volatile oil extraction rate; (**B**) Effect of soaking time on volatile oil extraction rate; (**C**) Effect of liquid-to-material ratio on volatile oil extraction rate; (**D**) Effect of extraction temperature on volatile oil extraction rate.

3.2 Establishment of Regression Model and Analysis of Variance

The results of the analysis of variance for the response surface of volatile oil extraction in *P. praeruptorum* are shown in Table 6. The quadratic regression equation model with volatile oil yield (Y) as the objective function was obtained using the software and is given by Y = 0.12 + 4.208E − 003A + 3.375E − 003B + 0.015C

+ 6.942E − 003D − 0.010AB − 0.024AC − 8.025E − 003AD − 6.975E − 003BC + 7.550E − 003BD − 2.300E − 003CD − $0.035A^2$ − $0.015B^2$ − $0.046C^2$ − $0.043D^2$. The ANOVA results indicated that the model is significant ($p < 0.001$), indicating that the regression model is statistically significant and that the experiment based on this model is highly feasible. The lack-of-fit term shows $p = 0.1624 > 0.05$, suggesting that the model is suitable for subsequent analysis and prediction. The adjusted R^2 value of 0.9690 means that the model explains 96.9% of the variation in the response variable when the effect of the number of independent variables on the goodness of fit is taken into account. This indicates that the model fits the data perfectly.

Table 6: Box-behnken experimental design and results of *P. praeruptorum* volatile oil.

Sources	Sum of Squares	Degree of Freedom	Mean Square	F-Number	*p*-Value
Model	0.032	14	2.29E−03	63.54	<0.0001
A—Extraction time	2.13E−04	1	2.13E−04	5.9	0.0292
B—Soaking time	1.37E−04	1	1.37E−04	3.79	0.0718
C—Extraction temperature	2.86E−03	1	2.86E−03	79.38	<0.0001
D—Liquid-to-material ratio	5.78E−04	1	5.78E−04	16.04	0.0013
AB	4.20E−04	1	4.20E−04	11.66	0.0042
AC	2.35E−03	1	2.35E−03	65.26	<0.0001
AD	2.58E−04	1	2.58E−04	7.15	0.0182
BC	1.95E−04	1	1.95E−04	5.4	0.0357
BD	2.28E−04	1	2.28E−04	6.33	0.0247
CD	2.12E−05	1	2.12E−05	0.59	0.4563
A^2	8.08E−03	1	8.08E−03	224.26	<0.0001
B^2	1.54E−03	1	1.54E−03	42.68	<0.0001
C^2	0.014	1	0.014	385.38	<0.0001
D^2	0.012	1	0.012	330.06	<0.0001
Residuals	5.05E−04	14	3.60E−05		
Missing item	4.43E−04	10	4.43E−05	2.85	0.1624
Pure error	6.21E−05	4	1.55E−05		
Sum	0.033	28			

3.3 Response Surface Analysis and Extraction Process Validation

According to the results of the Box–Behnken optimization test and the fitted regression equation, the optimal parameters for the volatile oil extraction were an extraction time of 3.98 h, a soaking time of 2.05 h, a liquid-to-solid ratio of 12.18 (mL/g), and an extraction temperature of 131.63°C, as determined using Design-Expert 8 software. Surface and contour plots were created for each factor to demonstrate the effect of each factor on the response value and to present the optimal values for each factor under ideal conditions (Fig. 2). The interaction between extraction temperature and liquid-to-solid ratio had the most significant impact on extraction efficiency. The greater the concavity or convexity of the response surface, the stronger the influence of the interaction between the two factors on the response value. When the contour line is circular, the result indicates that the interaction between the two factors is insignificant. When the contour line is elliptical or saddle-shaped, the result indicates a significant interaction. The response surfaces of the interaction terms AB, AC, AD, BC, and BD are curved with elliptical contours, proving that their interactions are significant (Fig. 2). In contrast, the response surface of CD is flatter with a circular contour, indicating that its interaction is not significant (Table 6). The combined effect of factors A and B and other factors had a great impact on the extraction rate of volatile oil in *P. praeruptorum* while the main effect of factors C and D existed, but the interaction effect between the two was low and the influence was small. The optimal process conditions were determined according to the optimal extraction conditions obtained by the response surface test, combined with the experimental conditions and actual operation: extraction

time 4 h, soaking time 2 h, liquid-to-material ratio 12 mL/g, and extraction temperature 132°C. Under these conditions, the extraction volumes of volatile oil were 0.1278, 0.1288, and 0.1254 mL, respectively, with an average value of 0.1215 mL. This was a very small deviation from the predicted model value of 0.1281 mL.

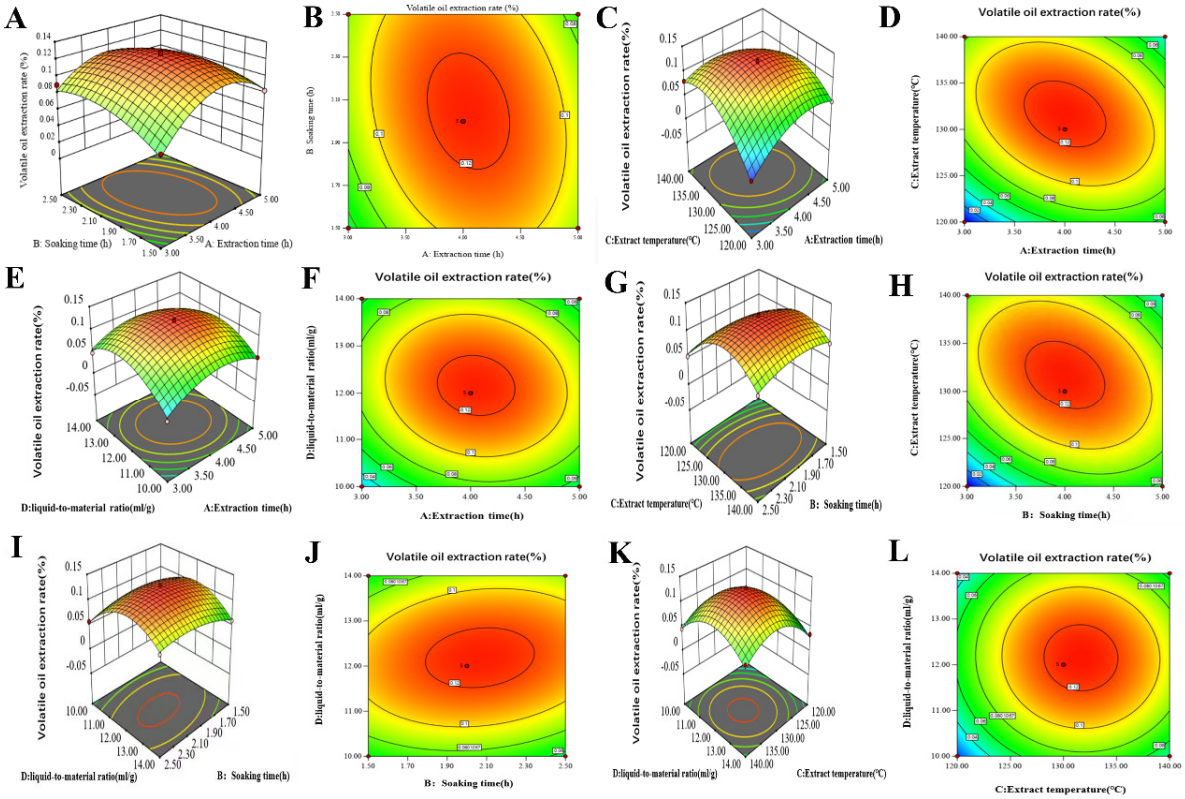

Figure 2: The contour plot of response surface analysis and the extraction rate of volatile oil among factors. (**A**,**B**) Extraction time and soaking time; (**C**,**D**) Extraction time and extraction temperature; (**E**,**F**) Extraction temperature and liquid-to-material ratio; (**G**,**H**) Soaking time and extraction temperature; (**I**,**J**) Soaking time and liquid-to-material ratio; (**K**,**L**) Liquid-to-material ratio and extraction temperature.

3.4 Volatile Oil Composition Analysis of P. praeruptorum from Different Origins and Periods

The volatile oil of *P. praeruptorum* from various origins and periods was analyzed using an Agilent 7890A-5977B GC-MS. The peak area normalization method was employed for quantitative analysis to determine the relative percentage contents of each volatile component. The specific names of the compounds, their respective retention times, and the relative content of the volatile oil components are represented in Tables S1–S3. The total ion chromatogram (TIC) baseline of the sample is generally stable with no significant interference from noise peaks, indicating stable instrument operation and the absence of contamination during sample preparation. In terms of peak intensity distribution, the main components are found at retention times of 5–12 and 20–25 min. In the 5–12 min range, the main peaks correspond to low-molecular-weight, highly volatile monoterpene compounds such as α-pinene, camphene, β-myrcene, and D-limonene (Fig. 3). The peaks in this range are sharp and dense, reflecting the abundance of these light components in the sample. In the 20–25 min range, the main peaks represent oxygenated terpenes and higher-molecular-weight, high-boiling sesquiterpenes such as dipterocarpol acetate, α-caryophyllene, caryophyllene, and snakegourd ketone. The chromatographic peaks in this range are typically broader,

indicating stronger retention of these compounds. The remaining minor components have small peak areas, with good resolution and no overlapping peak interference, ensuring the accuracy of subsequent qualitative and quantitative analyses.

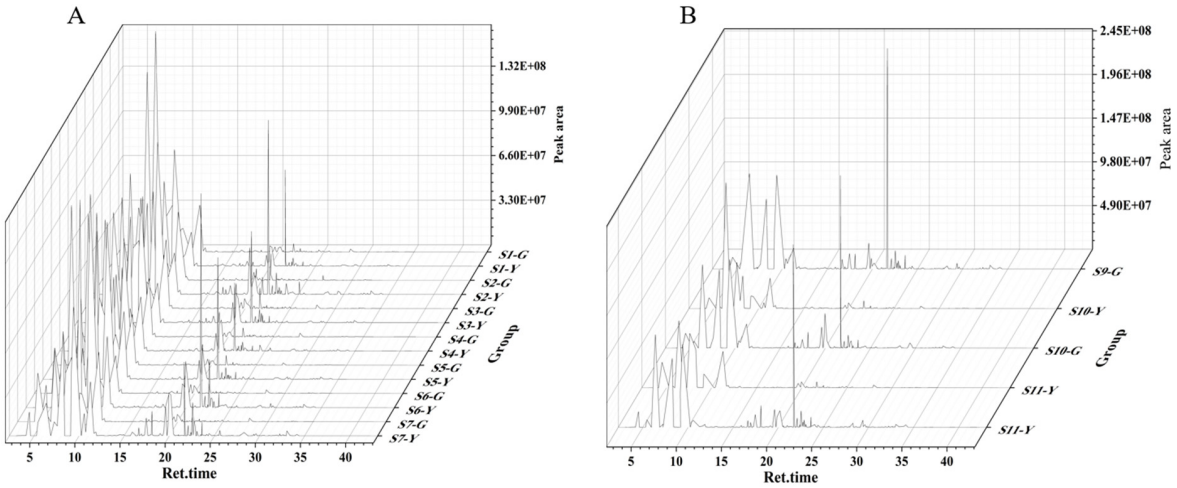

Figure 3: The total ion chromatographic graphs of the volatile oils in *P. praeruptorum*. (**A**) TIC of volatile oil components of *P. praeruptorum* in different origins; (**B**) TIC of volatile oil components of *P. praeruptorum* in different periods.

3.5 Volatile Oil Components in the Aboveground and Underground Parts of P. praeruptorum in Different Origins

Principal component analysis (PCA) was performed on the volatile flavor substances of *P. praeruptorum* volatile oil from different producing areas, and the analytical results of the established PLS-DA model are shown in the figure. The contribution rates of PCA1 and PCA2 in Fig. 4A were 29.4% and 27.7%, respectively. Regarding the model validation parameters in Fig. 4B, the prediction ability parameter $Q^2 = 0.57$ (>0.5) indicated that the model was acceptable and stable. The permutation test results in Fig. 4C showed $Q^2 = -0.695$; this negative value confirmed that there was no overfitting in the model. The contribution rates of PCA1 and PCA2 in Fig. 4D were 37.7% and 33%, respectively. In terms of the model validation parameters shown in Fig. 4E, the prediction ability parameter $Q^2 = 0.707$ (>0.5) indicates that the model is both acceptable and stable. The permutation test result in Fig. 4F is $Q^2 = -0.389$; this negative value confirms that there is no overfitting in the model. These results demonstrate that the proposed PLS-DA model is reliable and suitable for the discriminant analysis of the volatile oil found in the aboveground part of *P. praeruptorum* from different origins. The findings provide a reliable mathematical model basis for further studying the differences in the chemical characteristics of different *P. praeruptorum* volatile oils.

The screening threshold for differential biomarkers was set at VIP > 1 and $p < 0.05$. A total of 39 key differentiated components were identified in the aboveground parts of plants of different origins, including α-pinene, camphene, (-)-β-pinene, 3-carene, D-limonene, and (-)-α-phellandrene (Fig. 4C). Twenty differentiated components were identified in the underground parts from different origins, including α-pinene, camphene, β-myrcene, D-limonene, β-phellandrene, γ-elemene, *cis*-β-farnesene, and juniperene (Fig. 4F). The following differential components were common to both parts: α-pinene, camphene, β-pinene, 3-carene, β-myrcene, α-phellandrene, D-limonene, β-phellandrene, o-cymene, trans-β-ocimene, γ-terpinene, (-)-β-bourbonene, dipterocarpol acetate, spatulenol, and caryophyllene. At different times,

volatile oil components were present in the aboveground and underground parts of *P. praeruptorum*. PLS-DA was conducted on the volatile flavor compounds of *P. praeruptorum* volatile oil from various time intervals.

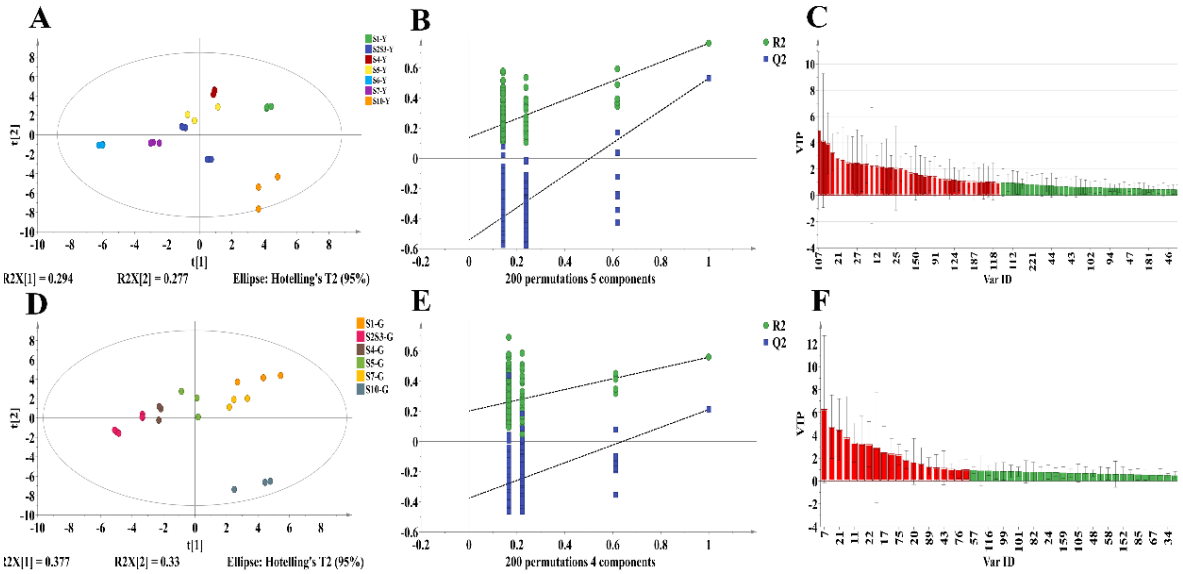

Figure 4: PLS-DA model of volatile oil components from different origins. (**A**) Score map of volatile oil in the aboveground part of different origins; (**B**) Substitution test of PLS-DA model of aboveground volatile oil in different production areas; (**C**) VIP value of volatile oil in the underground part of different origins; (**D**) Score map of volatile oil in the underground part of different origins; (**E**) Substitution test of PLS-DA model of underground volatile oil in different origins; (**F**) VIP value of volatile oil in the underground part of different origins.

Principal component analysis was performed on the volatile flavor substances of *P. praeruptorum* volatile oils at different growth periods. The results of the PLS-DA model are presented in Fig. 5. In terms of model validation parameters, the prediction ability parameter Q^2 = 0.618 (>0.5) indicates that the model is acceptable and has good stability. Regarding model reliability verification, the permutation test showed Q^2 = −0.542; this negative value confirms that there is no overfitting in the model, indicating that the PLS-DA model is reliable and suitable for the discriminant analysis of volatile oil found in the aboveground part of *P. praeruptorum* at different periods (Fig. 5A,B). These results provide a reliable mathematical model basis for further studying the differences in chemical characteristics among different volatile oils of *P. praeruptorum*. A total of 98 differentiated components were identified among the aboveground and underground parts across different periods. The relative contents of the aboveground and underground parts from different origins and periods are compared (Fig. 5C).

The volatile oil components with a relative content exceeding 1% in both the aboveground and underground parts of *P. praeruptorum* from various origins included α-pinene, (-)-β-pinene, 3-carene, β-myrcene, D-limonene, β-phellandrene, o-cymene, isobornyl acetate, and α-humulene (Fig. 6). In both the aboveground and underground parts, the components with a relative content greater than 1% consisted of α-pinene, (-)-β-pinene, D-limonene, o-cymene, and isobornyl acetate. Among these, (-)-β-pinene constituted the predominant component in the majority of samples designated as “Y” or “G”. α-Pinene, (-)-β-pinene, D-limonene, o-cymene, and isobornyl acetate were detected in the volatile oil of *P. praeruptorum* from all origins and periods.

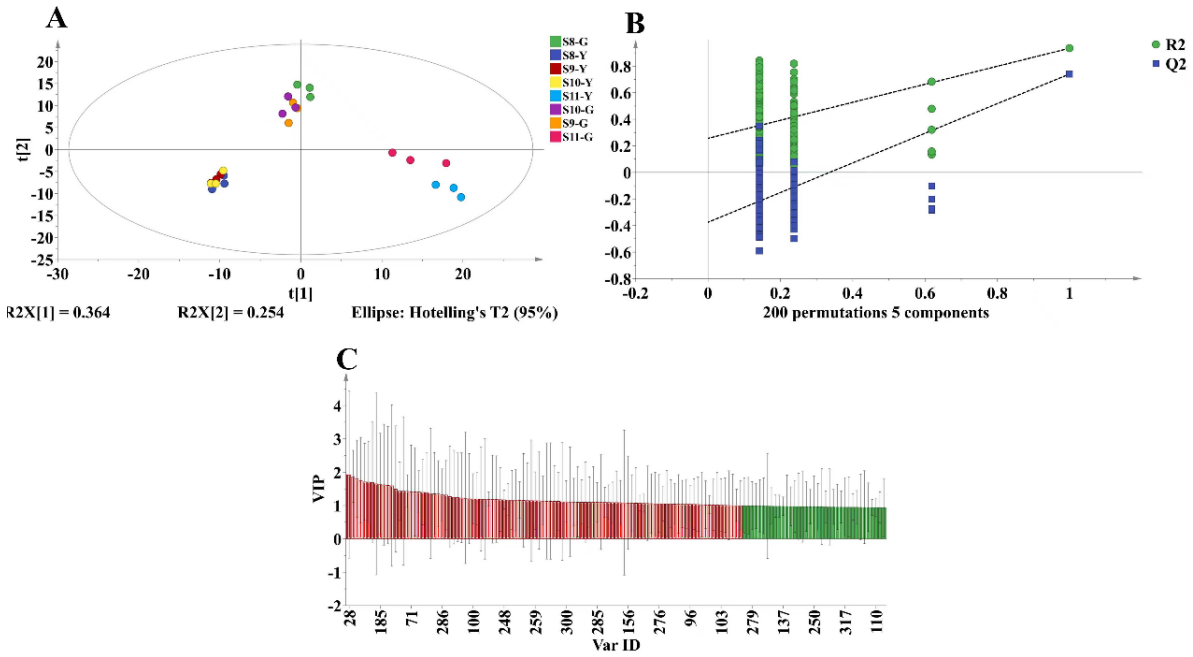

Figure 5: PLS-DA model of volatile oil components in different periods. (**A**) Volatile oil score chart in different periods; (**B**) Substitution test of volatile oil PLS-DA model in different periods; (**C**) VIP value of volatile oil in different periods.

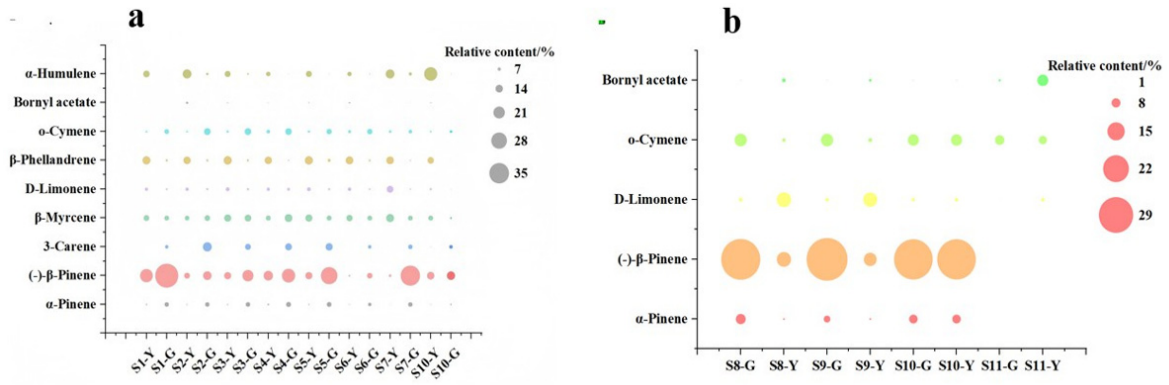

Figure 6: Bubble diagram of the volatile oil contents in *P. praeruptorum*. (**a**) Common composition of volatile oils from different origins; (**b**) Common components of volatile oils in different periods.

3.6 Sensory Evaluation of Volatile Oils in the Aboveground and Underground Parts of P. praeruptorum in Different Periods

The sensory evaluation panel assessing the overall odor acceptance of 11 kinds of volatile oils was compiled and visualized in radar charts (Fig. 7). The intensity of related attributes and overall odor acceptance were determined. The figures demonstrate that the performance of *P. praeruptorum* volatile oil varies across different odor types, origins, and periods. The aroma of *P. praeruptorum* volatile oil has been described as having characteristics reminiscent of grass, wood, spice, earth, and pine resin. The pungency of the *P. praeruptorum* volatile oil consistently received higher scores, suggesting that the pungent note is more prominent in its overall sensory characteristics. Conversely, the attributes of grass, pine resin, earth, and wood received comparatively lower scores, indicating that these scents contribute to a lesser extent to the overall aroma profile.

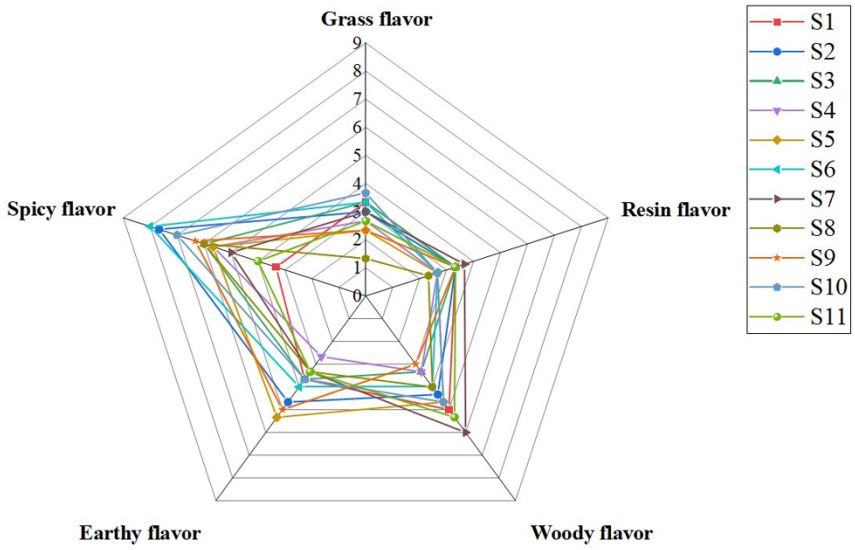

Figure 7: Sensory evaluation diagram of volatile oils from different regions of *P. praeruptorum*.

4 Discussion

This study utilized the Box-Behnken RSM to optimize the extraction process. The results demonstrated that the interplay between extraction temperature and liquid-to-material ratio had the most significant impact on extraction efficiency in the single-factor experiments. When the extraction temperature surpasses 132°C, some volatile oil components may experience thermal degradation. Heat-sensitive terpene derivatives are especially susceptible to thermal decomposition, resulting in the loss of active constituents and a decrease in volatile oil yield [21,22]. The liquid-to-material ratio was established at 12 mL/g. This ratio guaranteed complete solvent penetration of the fibrous tissue in the *P. praeruptorum* root, facilitating the dissolution of its constituents. It also circumvented elevated expenses in later separation and purification resulting from excessive solvent utilization. Compared to traditional steam distillation methods, the optimized technique demonstrated a significant decrease in extraction time from 8 h to 4 h, thereby greatly improving production efficiency. The 2-h soaking duration demonstrated a strong correlation with the structural properties of the medicinal material. The fibrous composition of the *P. praeruptorum* root obstructs the rapid infiltration of solvents. Extending the soaking period beyond two hours increases the dissolution of water-soluble impurities, thereby reducing the purity of the volatile oil. The current extraction process has only been validated at the laboratory scale, and its feasibility at pilot and industrial production scales requires further evaluation. Variations in the active ingredient content of *P. praeruptorum* caused by differences in geographical origin, variety, harvest season, and storage conditions may affect the efficiency of extraction and consistency of product quality. Future research should therefore establish quality control standard for raw material s and optimize a robust manufacturing process.

HS-SPME-GC/MS was employed to analyze the chemical composition of volatile oil from *P. praeruptorum* across different origins and periods. The PLS-DA model was then applied to the volatile oil composition, revealing distinct differences between the aboveground and underground parts of *P. praeruptorum* from various origins and periods. By screening for VIP > 1 and $p < 0.05$, thirty-nine differential components were identified in the aboveground parts, a number significantly higher than that in the underground parts. This indicates that the volatile oil composition of the aboveground parts is more sensitive to environmental changes in the growing region. This may be attributed to the direct exposure of aboveground tissues to environmental factors such as light, temperature, and humidity, whereas

the underground parts develop in a comparatively stable environment, leading to reduced compositional diversity [23,24]. Fourteen volatile oil components were consistently found in both the aboveground and underground parts of *P. praeruptorum* across different origins. *P. praeruptorum* is rich in active compounds such as (-)-β-pinene, α-pinene, D-limonene, o-umbelliferone, and didymetin acetate. Among these, (-)-β-pinene exhibits an inhibitory effect on *Staphylococcus aureus* by targeting inflammation-related pathways to reduce the inflammatory response, and regulates the monoaminergic system to exert sedative and antidepressant effects [25]. D-limonene reduces inflammatory infiltration, scavenges free radicals and reduces lipid peroxidation, anti-tumor and digestive regulation [26]. α-Pinene has broad-spectrum antibacterial properties, disrupts microbial cell membranes and inhibits biofilm formation. It is particularly effective against *Candida albicans*, while also exerts anti-allergic and anti-inflammatory effects [27]. There is currently no direct literature supporting the antibacterial and anti-inflammatory effects of o-cymene or bornyl acetate alone. However, when these compounds are present in the volatile oils of plants, such as *Origanum compactum Rhynchanthus beesianus*, and *Alpinia*, they exhibit antibacterial and anti-inflammatory activities through synergistic interactions [28–30]. Based on this, we speculated that these components may interact with multiple targets in this volatile oil, giving it comprehensive pharmacological potential. As there are few pharmacological studies on *P. praeruptorum* volatile oil, this hypothesis offers a preliminary theoretical basis for its development as an anti-inflammatory sleep aid. Nevertheless, the existence of the synergistic effects and their mechanisms remain unresolved.

The significant variations in the proportion of volatile oil components in *P. praeruptorum* across diverse production periods are presumably associated with environmental factors, including climate, soil composition, and altitude of the cultivation region [31,32]. The chemical composition of sage essential oil, a significant cash crop in the Mediterranean region, is predominantly influenced by soil salinity. Concurrently, elevated temperatures have been observed to impact the ratio of monoterpenes to diterpenes in the essential oil of its subspecies [33]. Numerous studies have shown that changes in the essential oil composition of Apiaceae plants are significantly influenced by annual climatic variations, highlighting the crucial role of precipitation and temperature in essential oil quality [34]. Some studies reported that altitude had little effect on terpene content in lavender, while monoterpene content increased with altitude in frankincense leaves and juniper needles [35]. The analysis of volatile oil components between the aboveground (Y) and underground (G) parts of *P. praeruptorum* from the same origin reveals metabolic differences across its different growing tissues. The underground part is of significant importance for nutrient absorption and secondary metabolism in plants. It may possess unique advantages in synthesizing and storing terpenoids. In contrast, the aboveground part may adjust its synthesis and distribution of components due to differences in functional emphasis. This study provides a direction for further exploration of the synthesis sites, transport, and accumulation of volatile oils in *P. praeruptorum*. α-Pinene and (-)-β-pinene are representative monoterpenes found in the essential oils of many plants.

The volatile oil in the underground part contains a higher relative content of α-pinene, camphene, β-pinene, 3-carene, o-cymene, and trans-β-ocimene compared to the aboveground part. Conversely, the aboveground part has higher relative contents of isobornyl acetate, α-humulene, and caryophyllene than the underground part. Monoterpene hydrocarbons, such as α-pinene and β-pinene, which are more concentrated underground, act as broad-spectrum defense compounds in plants against bacteria and insects [36,37]. Its preferential accumulation in the roots forms the initial chemical barrier against soil pathogens and pest infestations, which is essential for maintaining the survival and function of underground organs. Spatulenol, which is enriched in the aboveground part, is a known insect pheromone and antibacterial component, often specifically induced when plants are mechanically damaged or

infested [38,39]. This difference reflects the organ-specific nature of plant secondary metabolites, wherein different parts perform distinct physiological and ecological functions, resulting in adaptive differentiation in the types and amounts of secondary metabolites they produce [40]. This finding elucidates the volatile material basis of the medicinal parts of *P. praeruptorum* and provides a scientific basis for the comprehensive utilization of its aboveground resources. This finding elucidates the volatile material basis of the medicinal parts of *P. praeruptorum* and provides a scientific basis for the comprehensive utilization of its aboveground resources. The chemical composition of *P. praeruptorum* volatile oil can be affected by factors such as origin, harvest period, processing method and genetics, but how these factors coharently regulate its biological activity has not yet been addressed.

The sensory evaluation of the volatile oil indicated that its aroma was characterized by a dominant spicy note, accompanied by grassy, woody, earthy, and pine resin tones. This unique aroma profile clearly corresponds with the chemical components identified by HS-SPME-GC/MS analysis. Combined with the data and sensory evaluation, the spicy attributes of the three components—(-)-β-pinene, β-phellandrene, and o-cymene—directly support the "spicy flavor" of the volatile oil. α-Pinene and 3-carene contribute a "pine resin aroma"; however, the low score for pine resin in the sensory evaluation suggests that this note is masked by the more prominent spicy perception. It was indicated that only β-phellandrene carries a "grassy, herbal feel", yet its main aromatic profile is described as "citrusy and peppery spicy", with grassiness being a secondary attribute. The "woody" intensity of (-)-β-pinene and o-cymene is rated as "medium", while that of α-humulene is "weak", resulting in an overall insufficient contribution to the woody note. The data does not include any components that are designated as "earthy", which corresponds with the low rating for earthiness in the sensory evaluation. The combination of these components constitutes the fundamental aroma profile of *P. praeruptorum* volatile oil, thereby establishing the foundational elements that give rise to its characteristic attributes, namely the sensation of grass, wood, and pine resin [41,42]. The ratio and interaction of these ingredients collectively shape the unique aromatic profile of *P. praeruptorum* volatile oil.

The "spicy" note in sensory evaluation is now explicitly associated with specific chemical compounds: (-)-β-pinene, β-phellandrene, and o-cymene. A direct correlation has been established between a specific sensory attribute (spiciness) and particular chemical compounds, with explanations provided for sensory variations among plant parts [43]. Since one of the key contributors to spiciness, "o-cymene", is more abundant in the underground part, this provides a scientific chemical explanation for why the traditional perception holds that the underground roots of *P. praeruptorum* usually have a stronger, spicier aroma—thereby verifying and quantifying traditional experience with modern science. This study establishes a crucial scientific basis and explicit developmental trajectory for the quality evaluation of *P. praeruptorum* medicinal materials, the extensive utilization of plant resources, particularly the value identification of traditionally non-medicinal components, and the further investigation of its metabolic biology. Based on the chemical and sensory characterization of this study, *P. praeruptorum* volatile oil has the following potential applications. In the food industry, it can be used as a natural flavoring agent or preservative. In the personal care products industry, it can be used to develop functional fragrances or aromatic products. In the pharmaceutical field, after further activity validation, it is expected to be developed into a natural drug for relieving coughs, reducing phlegm, and providing anti-inflammatory effects. However, the realization of these application prospects still requires systematic biological activity evaluation, safety research, and process scale-up verification.

5 Conclusion

Here the Box-Behnken response surface method was employed to optimize the extraction process across multiple parameters. This method was ultimately used to determine the optimal conditions of the volatile oils. The chemical composition of volatile oil from *P. praeruptorum* at different origins and periods was analyzed using HS-SPME-GC/MS, and the PLS-DA model used identified key differentiated components. The sensory evaluation of *P. praeruptorum* volatile oil aligned with the composition analysis, identifying three key components—(-)-β-pinene, β-phellandrene, and o-cymene—as the primary contributors to the prominent spicy note.

Acknowledgement: Not applicable.

Funding Statement: This work was supported by Traditional Chinese Medicine Inheritance and Innovation Research Project of Anhui Province (2025CCCX033), Demonstration Experiment Training Center of Anhui Provincial Department of Education (2022sysx033), National Key R&D Program of China (2023YFC3503804) and Quality Engineering Project of West Anhui University (wxxy2024011).

Author Contributions: Yuxian Liu: Writing—review & editing, Writing—original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. Jinzhuo Yao: Visualization, Investigation, Writing—review & editing. Bangxing Han: Supervision, Funding acquisition. Cheng Song: Conceptualization, Supervision, Funding acquisition, Writing—original draft, Writing—review and editing. All authors reviewed and approved the final version of the manuscript.

Availability of Data and Materials: Not applicable.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

Supplementary Materials: The supplementary material is available online at https://www.techscience.com/doi/10.32604/phyton.2026.082999/s1.

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