

Chemical fingerprinting and simultaneous determination of main compounds combined with multivariate analysis of the *Syringa pubescens* Turcz. leaves from different origins

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Abstract: Background: *Syringa pubescens* Turcz. is a folk medicinal plant in Henan Province, China and its leaves have been used for medicinal purposes. However, the bioactive compound content varies with geographical origin and quality control methods remain limited. **Objectives:** This aim is to develop a reliable HPLC method for the simultaneous determination of seven bioactive compounds in *S. pubescens* leaves from different origins and to evaluate sample differences using chemometric analysis. **Methods:** Thirty-three batches of leaves from six main regions in China were analyzed using HPLC. Hierarchical cluster analysis (HCA), principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were employed to the quantitative data. **Results:** All seven components showed good linearity ($R^2 > 0.999$). Recovery ranged from 98.23% to 100.12% and RSD values for precision and stability were below 1.84%. The contents of echinacoside, verbascoside and oleuropein were higher than those of other ingredients. HCA and PCA classified the 33 batches into three groups according to their geographical origin. OPLS-DA further differentiated the samples, with oleuropein, echinacoside, verbascoside and syringin identified as major discriminating markers. **Conclusion:** These findings could support the resource development and utilization and provide a reference for the quality control of SPLs.

Keywords: Chemical fingerprint; Chemometrics; Geographical origin; HPLC; *Syringa pubescens* Turcz. Leaves

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INTRODUCTION

Syringa pubescens Turcz., a deciduous shrub of the Oleaceae family, is native to northern China, growing at altitudes of 900–2100 m in mountainous regions, valleys and riparian zones across Henan, Shaanxi, Liaoning and other provinces (Deng *et al.*, 2010). The flowers of this plant have commonly been used as a spice (Zhonghua Bencao Edit Committee, 1999) and the leaves of *S. pubescens* (SPLs) have been used to treat tonsillitis (Wang *et al.*, 2019). This difference between historical documentation and modern research priorities highlights a critical gap in the literature. Current studies mainly focus on the flowers, assessing their bioactive compounds for their anti-inflammatory, antioxidant and α -glucosidase inhibitory properties (Zhang *et al.*, 2024; Wang *et al.*, 2023; Wu *et al.*, 2022). In contrast, SPLs are explicitly identified in authoritative sources, such as the Flora of Henan (Wang *et al.*, 2019) and Zhonghuabencao (Zhonghua Bencao Edit Committee, 1999), as the principal medicinal part. However, SPLs remain underexplored in scientific research. Therefore, there is a need for in-depth investigations into the development and application of SPLs.

HPLC is widely used in the analysis of natural compounds (Xiao *et al.*, 2025). Chemometric analysis, including principal component analysis (PCA), hierarchical clustering (HCA) and orthogonal partial least squares method-discriminant analysis (OPLS-DA), was commonly

used for analysis and evaluation of traditional Chinese medicine (TCM) (Li *et al.*, 2020a; Liu *et al.*, 2023). The PCA and OPLS-DA can enable the transformation of complex, high-dimensional data into simpler, low-dimensional structures and it is easier to reflect the interrelation among data. The HPLC combined with chemometric analysis has been widely employed in the complex components of TCM, making data analysis simpler, more accurate and faster (Gaiao Calixto *et al.*, 2023).

In this study, HPLC fingerprints of 33 batches of SPL samples from 6 main geographical regions were established and the differences and similarities among these samples were evaluated. Main components, including salidroside, syringin, echinacoside, forsythoside B, verbascoside, isoacteoside and oleuropein of SPLs were simultaneously determined. Chemometric analysis methods such as HCA, PCA and OPLS-DA were employed to elucidate the relationship among samples of different origins. The proposed quantitative technique could be employed for future quality assessment of SPLs. These findings from this study would establish a detailed, scientific framework for the utilization and development of SPL resources.

MATERIALS AND METHODS

Chemicals and material

Methanol and acetonitrile were sourced from Tianjin Kemiou Reagent Co., Ltd (Tianjin, China). Ethanol absolute and acetic acid (A.R.) were supplied from Tianjin

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Deen Reagent Co., Ltd (Tianjin, China). The double-distilled water was prepared using double-distilled water equipment (Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China). The salidroside (C1), syringin (C2), echinacoside (C3), forsythoside B (C4), verbascoside (C5), isoacteoside (C6) and oleuropein (C7) were purchased from Chengdu PufeiDe Biotech Co., Ltd (Chengdu, China). The 33 batches of SPLs were collected from different geographical origins of China (Table 1). The samples were authenticated in situ using morphological criteria by Prof. Yanfang Wu, who used the diagnostic traits outlined in Flora of China. All samples were promptly air-dried in the shade without delay and crushed into powder (60 mesh).

Preparation of sample solution

Each sample powder was accurately weighed to 1.0 g and mixed with 10 mL of 70% ethanol, then extracted for 45 min using an ultrasonic extractor (Nanjing YMNL Instrument and Equipment Co., Ltd., Nanjing, China) at 40 °C. The extract was centrifuged for 15 min (13,000 rpm) and subjected to filtration with a 0.22 µm membrane filter before HPLC analysis.

Preparation of standard solution

Seven compounds containing C1-C7 were selected as the quality standards of the samples in this experiment. Standards were accurately weighed and added 1mL of methanol, forming mixed standard stock solution. The standard stock solution was suitably diluted to construct standard curves.

Chromatographic parameters and method validation

The analysis was conducted using an EClassical® 3100 HPLC System equipped with a UV detector, an auto-sampler and a temperature controller for the column (Elite, China). The Eclipse XDB-C18 column (250 mm, 4.6 mm, 5 µm; Agilent, USA) was employed to separate the sample and the column temperature was kept at 30°C. A mobile phase containing acetonitrile (A) and 0.5% v/v acetic acid water (B) was utilized for gradient elution. The optimized elution conditions were as follows: 0-5min, 10% A; 5-18min, 10-17%A; 18-35min, 17-20%A; 35-45min, 20-23%A. The flow rate was 1.0 mL/min. The initial wavelength was 276nm for C1 and the duration was 0-10min, the next was 332nm for C2-C6 and the duration was 10-29 min and the last wavelength was 285nm for C7 and the duration was 29-45min. The injection volume was 5 mL (Xu *et al.*, 2025).

The method was validated for linearity by plotting analyte peak area vs concentration. Sensitivity was assessed via limits of detection (LOD) and quantification (LOQ), defined as signal-to-noise ratios (S/N) of 3 and 10, respectively. Precision was evaluated through intra-day (six replicate injections within one day) and inter-day (six replicates across three consecutive days) analyses. Repeatability was confirmed using six replications of the

same sample. The accuracy was confirmed by recovery tests. The method stability was determined by repeating injections of the same sample solution within 24 h, while selectivity was verified by injecting blank solvent to confirm absence of interfering peaks (Wang *et al.*, 2013).

Chemometric analysis

The similarity of 33 batch samples was analyzed using the chromatographic fingerprint similarity evaluation system for Traditional Chinese Medicine (Version 2012.1) (Luo *et al.*, 2024). The content of seven compounds was calculated using the standard curve.

HCA

Cluster analysis is a kind of analysis method commonly used in data analysis, which belongs to a branch of statistics (Serra-Burriel and Ames, 2022). There are two types of cluster analysis: bottom-up clustering, which categorizes each value in turn. The other is to split from the top down, which means classifying all the values in order. The corresponding result is presented in a tree diagram. HCA is one method of cluster analysis. In this paper, based on the contents of the seven characteristic glycosides of SPLs, SIMCA software (Umetrics, Umea, Sweden) was used to perform HCA on the data.

PCA

PCA is a widely recognized unsupervised multivariate data reduction method (Qing, *et al.*, 2025). It operates via orthogonal linear transformations that map correlated variables into a new orthogonal basis. By maximizing variance retention via covariance matrix decomposition, PCA generates principal components (PCs) ordered by explained variance. This process eliminates noise and low-variance components while retaining essential information from high-dimensional data. The orthogonality of PCs ensures minimal information loss while enabling comprehensive data analysis. Each original variable has a loading value for each principal component. These values can be used to evaluate sample clustering similarities and help understand the relationships between loading variables and samples.

OPLS-DA

OPLS-DA is a technique for building a linear regression model. It achieves this by mapping predictive and observable variables into a new space (Zhang, *et al.*, 2025). Variable importance (VIP) is a metric derived from OPLS-DA and used to assess the overall contribution of individual variables to the model. VIP values were computed using equation (1).

$$VIP = \sqrt{K \times \left(\frac{\sum_{xi=1}^X W_{xi}^2 \times SSY_{comp,xi}}{SSY_{cum}} \right)} \quad (1)$$

$SSY_{comp,xi}$ is the sum of squares explained by compound xi, X is the total number of compounds and W_{xi} is the squared OPLS weight.

RESULTS

Simultaneous determination of seven glycosides from SPLs

Table 2 demonstrates the results of HPLC method validation. It was observed that each compound exhibited good linearity over the tested range ($R^2 > 0.999$). The RSDs of stability were less than 1.84%. The RSDs of intra-day and inter-day precision were less than 1.04% and 1.66%, respectively. The recovery values ranged from 98.23% to 100.12%. The HPLC chromatograms were presented Fig. 1. The contents of seven compounds were evaluated using the developed method and the results were displayed in Table 1.

HPLC fingerprint and similarity analysis

Thirty-three batches of SPLs fingerprints were evaluated using the traditional Chinese medicine fingerprint similarity analysis system. The results are shown in Fig. 1. The similarity results for SPLs are listed in Table 3.

Chemometrics analysis

In this study, the contents of 7 glycosides were analyzed using HCA, PCA and OPLS-DA to assess the similarities and differences of SPLs from various sources. The results were illustrated Fig. 2-4. As shown in Fig. 3, the 33 batches of SPLs were divided into three groups based on their source. S1-S6 and S8-S20 from North China were classified as a group. S23 from Henan Province was a group. And other samples from Henan, Shaanxi and Liaoning Provinces were categorized together. It was clear that the PCA results were consistent with those of HCA. Likewise, the 33 batches of SPLs were grouped into three categories by origin. The S23 from Henan Province was classified into a group whose scores differed considerably from those of the other samples. The samples (except S7) from North China were grouped into a category. Other samples belonged to a category that was primarily concentrated in the positive region of the score plot. The samples from Hebei Province, Shanxi Province and Beijing City, all in the North China region, were fairly similar. While the samples from Henan Province exhibited considerable variability.

The OPLS-DA and VIP diagrams of the samples were presented in Fig. 4. And the R^2X and R^2Y of the model were 0.921 and 0.247, respectively and Q^2 was -0.118, meeting the conditions of $R^2 > 0$ and $Q^2 < 0$, which proved that the model was not overfitted. In VIP, C7, C3, C5 and C2 contributed the most to the model. In OPLS-DA, the SPLs from North China were similar to each other. S33 from Liaoning Province was a group. However, the samples from Henan Province exhibited significant differences, which might be due to the different harvesting time (Table 1). It was observed that S23 and S24 differed significantly from other samples from Henan Province.

DISCUSSION

In this study, the seven glycosides, including C1-C7 from

SPLs, were simultaneously determined for the first time. These results in table 2 indicate that the established HPLC method was validated and found to be precise, accurate and practical for the quantitative analysis of seven glycosides from SPLs.

From Table 1, an obvious difference was observed in the content of the same compound of the SPLs from different geographical regions. In all the samples, the contents of echinacoside, verbascoside and oleuropein were high, the contents of salidroside and syringin were low. The contents of 7 components in the SPLs from North China, including Beijing City, Hebei and Shanxi Provinces, were relatively low and no active components were detected in some samples. However, it was clear that the glycoside content of S7 was higher than that of other samples from North China. This might be because the harvesting time for S7 differed from that of other samples from North China (Table 1). The contents of 7 glycosides of SPLs from Henan, Shaanxi and Liaoning provinces were relatively high. It was worth noting that oleuropein was the most abundant of all the glycosides in the SPLs from Henan Province. The data indicated that the contents of glycosides exhibited noticeable diversity among different samples, which could be attributed to different producing areas, environmental factors and other factors (Wang *et al.*, 2024). Hence, it was initially determined that considerable differences in active component content were present in SPLs from different geographical regions.

It could be found that the SPLs from North China (except S7) possessed a high similarity and the value was more than 0.9 (Table 2). The SPLs from other origins exhibited low similarity, with a value of about 0.7. The similarity of all SPLs was about 0.4, which further confirmed that the levels of 7 glycosides in SPLs from different locations displayed considerable variation.

The results HCA and PCA confirmed that the developmental and environmental factors are essential influences on the variations of secondary metabolites from plant (Li *et al.*, 2020b). In addition, S7 from Hebei Province was found to be close to samples from Henan Province. The OPLS-DA models could be employed to determine the geographic origin of samples from North China and Liaoning Province. Meanwhile, it could be concluded that the active compounds of the SPLs fluctuated with environmental conditions (Qaderi *et al.*, 2023).

CONCLUSION

In this study, HPLC fingerprints of the SPLs were developed and the 7 bioactive compounds were identified. An approach for the simultaneous determination of 7 characteristic compounds, including salidroside, syringin, echinacoside, forsythoside B, verbascoside, isoacteoside and oleuropein, from SPLs was proposed.

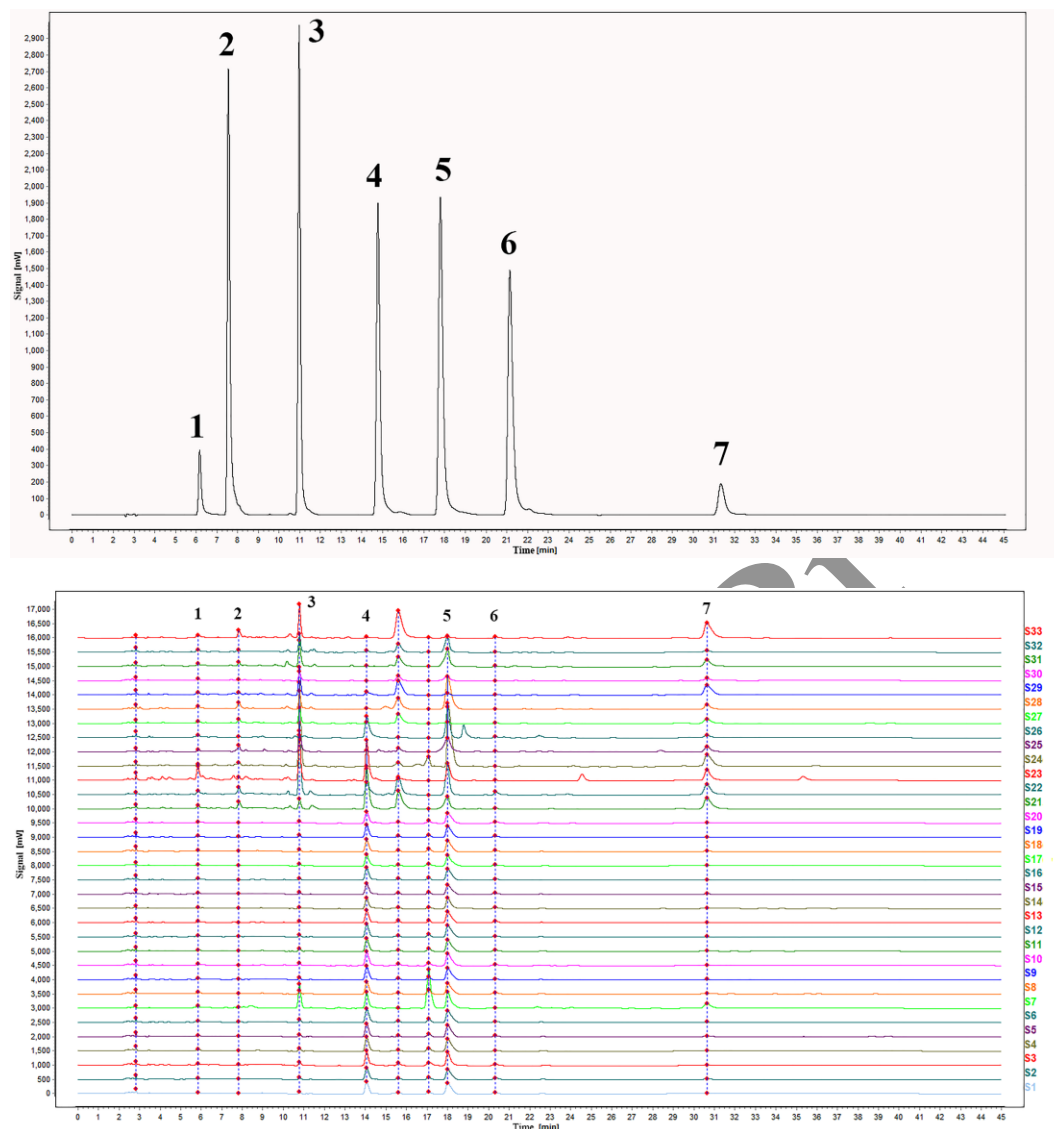


Fig. 1: The HPLC chromatograms of (A) mixed standard substances and (B) SPLs.

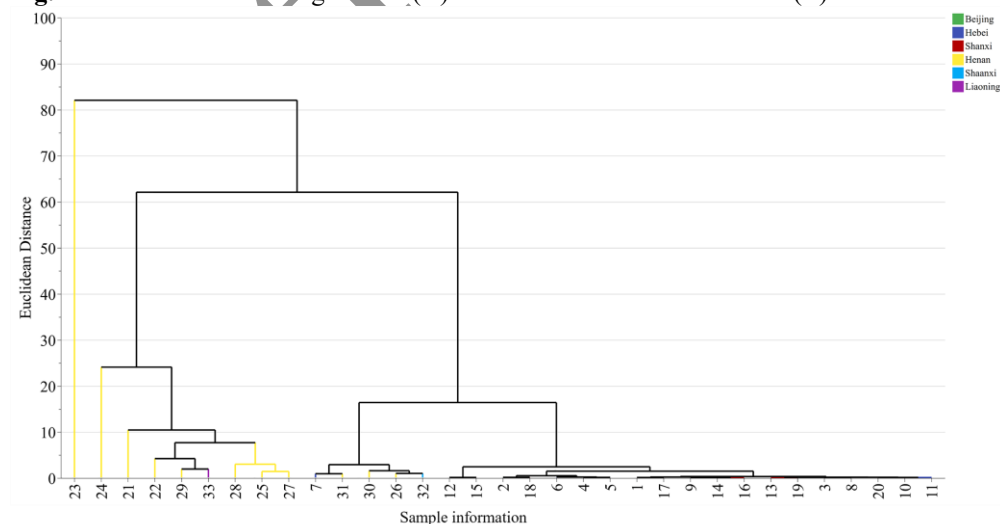


Fig. 2: The result of HCA.

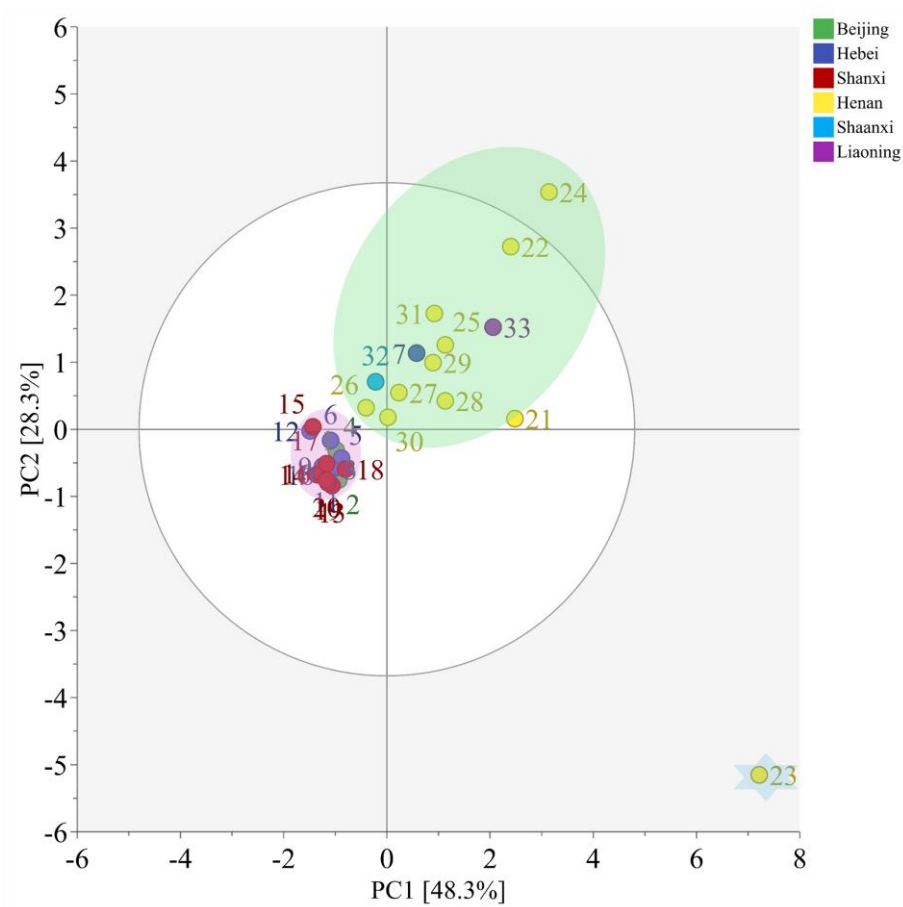


Fig. 3: The score plot of the PCA for the 33 batches.

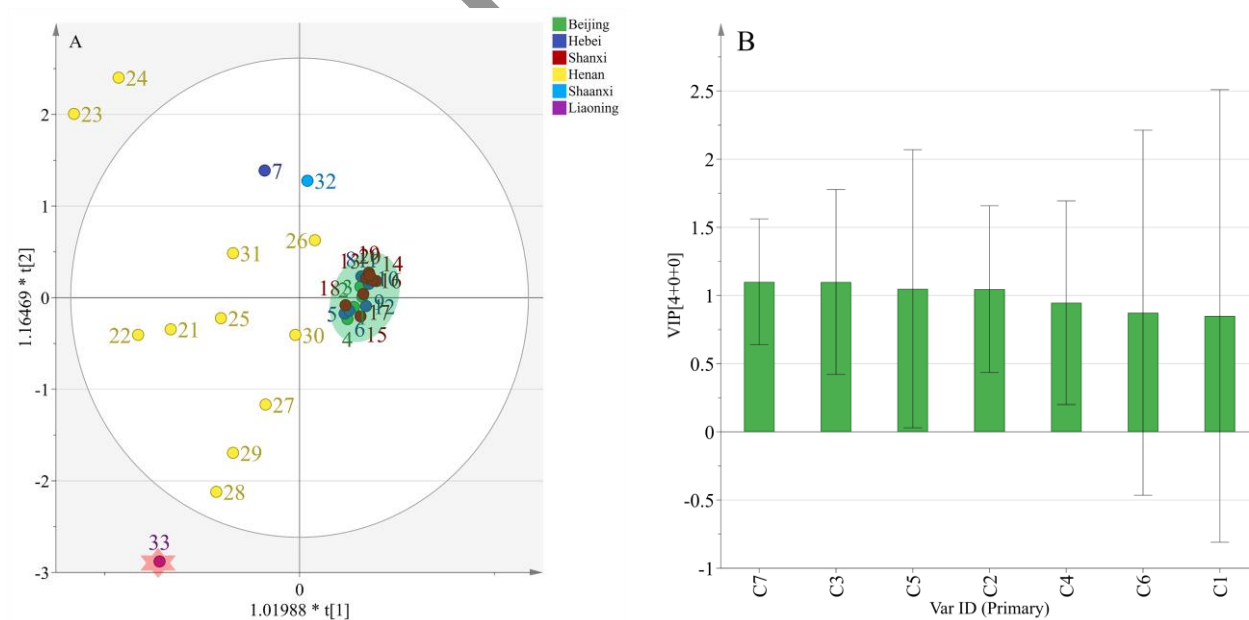


Fig. 4: The results of OPLS-DA (A) and VIP (B).

Table 1: Sample information, contents of 7 compounds (mg/g) of 33 batches of *S. pubescens* leaves from different geographical origins

No	Origin	Harvest time	C1	C2	C3	C4	C5	C6	C7
1	Beijing	2020.5.4	0.377±0.097 ^{de}	0.184±0.078 ^{ghij}	0.053±0.002 ^{no}	0.031±0.051 ^f	0.424±0.003 ⁱ	0.532±0.002 ^{bcdef}	4.843±0.880 ^j
2	Beijing	2020.5.4	0.531±0.015 ^{de}	0.290±0.020 ^{efghi}	0.064±0.003 ^{mmo}	ND	0.197±0.539 ⁱ	0.690±0.000 ^b	4.221±1.452 ^j
3	Beijing	2020.5.5	0.644±0.018 ^{de}	0.156±0.065 ^{ghij}	0.196±0.002 ^{lmmo}	ND	0.791±0.003 ⁱ	0.572±0.002 ^{bed}	5.176±0.516 ^{ij}
4	Beijing	2020.5.5	0.622±0.008 ^{de}	0.311±0.003 ^{efghi}	0.144±0.002 ^{lmmo}	ND	0.622±0.003 ⁱ	0.436±0.001 ^{defgh}	5.537±0.026 ^{ij}
5	Hebei	2020.5.5	0.596±0.012 ^{de}	0.331±0.004 ^{efg}	0.145±0.001 ^{lmmo}	0.139±0.001 ^f	0.694±0.001 ⁱ	0.529±0.000 ^{bcdef}	5.352±0.077 ^{ij}
6	Hebei	2020.5.5	0.604±0.094 ^{de}	0.301±0.016 ^{efghij}	0.252±0.005 ^{lm}	ND	0.855±0.007 ⁱ	0.335±0.368 ^{gh}	4.436±0.586 ^j
7	Hebei	2021.6.5	0.942±0.085 ^{de}	0.293±0.016 ^{efghi}	3.863±0.003 ^h	0.077±0.000 ^f	9.924±0.014 ^b	0.457±0.003 ^{defg}	13.541±0.068 ^{ef}
8	Hebei	2020.5.6	0.297±0.081 ^{de}	0.161±0.013 ^{ghij}	0.341±0.000 ^l	ND	0.991±0.003 ^{lm}	0.613±0.003	4.190±0.977 ^j
9	Hebei	2020.5.6	0.767±0.033 ^{de}	0.125±0.017 ^{ghij}	0.060±0.003 ^{mmo}	ND	0.581±0.007 ⁱ	0.460±0.028 ^{defg}	3.606±0.050 ^j
10	Hebei	2020.5.6	0.714±0.005 ^{de}	0.109±0.000 ^{hij}	0.105±0.003 ^{mmo}	0.020±0.000 ^f	0.578±0.005 ⁱ	0.549±0.004 ^{bode}	3.712±0.047 ^j
11	Hebei	2020.5.6	0.667±0.001 ^{de}	0.118±0.001 ^{ghij}	0.130±0.002 ^{mmo}	ND	0.637±0.004 ⁱ	0.570±0.002 ^{bed}	3.964±0.092 ^j
12	Hebei	2020.5.6	0.647±0.043 ^{de}	0.193±0.090 ^{ghij}	0.025±0.003 ^{no}	ND	0.498±0.004 ⁱ	0.111±0.001 ^{ij}	3.203±1.256 ^j
13	Shanxi	2020.5.7	0.619±0.001 ^{de}	0.157±0.000 ^{ghij}	0.066±0.000 ^{mmo}	0.150±0.000 ^f	0.471±0.000 ⁱ	0.673±0.000 ^b	3.485±0.000 ^j
14	Shanxi	2020.5.7	0.649±0.394 ^{de}	0.085±0.036 ⁱ	ND	ND	0.224±0.155 ⁱ	0.495±0.001 ^{defg}	3.071±2.645 ^j
15	Shanxi	2020.5.7	0.582±0.140 ^{de}	0.230±0.020 ^{efghi}	ND	ND	0.479±0.003 ⁱ	0.102±0.000 ^{ij}	4.081±0.240 ^j
16	Shanxi	2020.5.8	0.527±0.021 ^{de}	0.102±0.001 ^{ij}	0.061±0.002 ^{mmo}	ND	0.440±0.003 ⁱ	0.525±0.003 ^{bcdef}	3.488±0.028 ^j
17	Shanxi	2020.5.8	0.498±0.003 ^{de}	0.173±0.005 ^{ghij}	0.096±0.002 ^{mmo}	ND	0.494±0.002 ⁱ	0.492±0.004 ^{defg}	4.428±0.467 ^j
18	Shanxi	2020.5.9	0.821±0.005 ^{de}	0.302±0.009 ^{ghij}	0.158±0.002 ^{lmmo}	0.196±0.011 ^f	0.716±0.006 ⁱ	0.616±0.002 ^{bc}	5.458±0.413 ^{ij}
19	Shanxi	2020.5.9	0.555±0.285 ^{de}	0.148±0.029 ^{ghij}	0.093±0.002 ^{mmo}	0.140±0.071 ^f	0.576±0.000 ⁱ	0.630±0.001 ^{bc}	2.549±2.161
20	Shanxi	2020.5.9	0.472±0.155 ^{de}	0.113±0.013 ^{hij}	0.059±0.006 ^{mmo}	0.091±0.001 ^f	0.540±0.001 ⁱ	0.613±0.001 ^{bc}	3.691±0.057
21	Henan	2019.5.4	0.687±0.694 ^{de}	0.842±0.329 ^b	1.274±0.076 ^k	0.941±0.276 ^b	3.008±0.131 ^g	0.373±0.212 ^{fgh}	29.809±8.173 ^b
22	Henan	2019.5.1	ND	0.724±0.008 ^{bc}	9.968±0.336 ^b	2.830±0.729 ^c	7.311±0.698 ^{cd}	0.074±0.000 ^j	32.188±3.476 ^b
23	Henan	2020.4.21	19.579±4.743 ^a	0.567±0.243 ^{cd}	8.049±0.041 ^c	12.984±0.077 ^a	4.937±0.024 ^f	2.490±0.022 ^a	25.796±0.020 ^c
24	Henan	2020.4.26	ND	0.286±0.001 ^{efghij}	14.499±0.005 ^a	0.804±0.028 ^{de}	17.584±0.020 ^a	0.437±0.062 ^{deigh}	30.854±0.197 ^b
25	Henan	2020.4.26	0.615±0.058 ^{de}	0.756±0.008 ^{bc}	5.185±0.012 ^e	0.789±0.010 ^e	5.636±0.002 ^{ef}	0.433±0.002 ^{deigh}	16.627±0.317 ^{de}
26	Henan	2019.5.20	1.162±0.120 ^c	0.318±0.001 ^{efgh}	0.214±0.004 ^{lmmo}	ND	5.840±2.913 ^{ef}	0.390±0.036 ^{efgh}	8.930±1.451 ^{gh}
27	Henan	2019.5.11	1.610±0.316 ^{bc}	0.753±0.390 ^{bc}	3.624±0.001 ⁱ	0.521±0.000 ^e	0.370±0.001 ⁱ	0.272±0.004 ^{lm}	11.166±0.003 ^{fg}
28	Henan	2020.5.2	1.894±0.770 ^{bed}	1.250±0.044 ^a	2.631±0.093 ^j	0.576±0.026 ^e	ND	0.571±0.074 ^{bed}	15.485±0.353 ^{de}
29	Henan	2021.4.16	0.562±0.130 ^{de}	0.482±0.040 ^{de}	4.593±0.018 ^f	1.090±0.003 ^d	0.425±0.001	0.343±0.005 ^{gh}	30.717±0.026 ^b
30	Henan	2021.5.6	2.912±0.265 ^{bc}	0.266±0.025 ^{hij}	2.824±0.002 ^j	0.110±0.078 ^f	2.064±0.004 ^{gh}	0.336±0.003 ^{gh}	15.364±2.432 ^{de}
31	Henan	2021.5.6	ND	0.423±0.005 ^{def}	6.776±0.341 ^d	0.163±0.265 ^c	7.641±0.648 ^c	0.340±0.188 ^{gh}	18.570±0.122 ^d
32	Shaanxi	2021.5.9	ND	0.203±0.069 ^{ghij}	4.285±0.109 ^g	0.546±0.086 ^e	6.219±0.105 ^{de}	0.375±0.009 ^{fgh}	5.231±2.047 ^{ij}
33	Liaoning	2019.5.20	3.006±0.139 ^b	0.895±0.015 ^b	6.771±0.197 ^d	0.050±0.035	0.411±0.290 ⁱ	0.324±0.113 ^{gh}	38.254±1.120 ^a

Note: C1: Salidroside; C2: Syringin; C3: Echinacoside; C4: Forsythoside B; C5: Verbascoside; C6: Isoacteoside; C7: Oleuropein. ND: not detected. Values in the same column with different letters are significantly different ($p < 0.05$).

Table 2: The results of the validation of HPLC method.

Analyte	Calibration curves	R ²	Stability RSD (%)	LOD (µg/mL)	LOQ (µg/mL)	Recovery (%)
C1	y=266.4x+211.04	0.9998	0.74	0.54	1.80	98.23
C2	y=2405.4x+34.741	0.9997	0.35	0.06	0.20	100.09
C3	y=1658.7x+507.55	0.9994	0.44	0.12	0.41	99.72
C4	y=1542.2x+339.62	0.9996	0.63	0.07	0.24	99.62
C5	y=2010.3x+262.57	0.9998	1.84	0.41	1.38	100.12
C6	y=2025.8x-150.23	0.9997	1.37	0.20	0.67	99.06
C7	y=326.4x+33.067	0.9995	0.35	0.29	0.96	98.68

Table 3: Similarity results of 33 batches of SPLs.

No.	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	
S1	1	1.00	1.00	1.00	1.00	0.99	0.66	0.99	1.00	1.00	1.00	0.99	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.81	0.70	0.79	0.70	0.69	0.73	0.33	0.25	0.36	0.55	0.69	0.70	0.27	
S2		1	1.00	1.00	1.00	1.00	0.68	0.99	0.99	1.00	1.00	0.99	1.00	0.99	1.00	0.99	1.00	1.00	1.00	1.00	0.82	0.70	0.79	0.68	0.69	0.75	0.34	0.29	0.37	0.55	0.69	0.69	0.30	
S3			1	1.00	1.00	1.00	0.72	1.00	1.00	1.00	1.00	0.99	1.00	0.99	1.00	0.99	1.00	1.00	1.00	1.00	0.81	0.70	0.80	0.69	0.69	0.78	0.35	0.33	0.37	0.55	0.69	0.70	0.32	
S4				1	1.00	1.00	0.67	0.99	1.00	1.00	1.00	0.99	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.81	0.68	0.79	0.68	0.68	0.75	0.31	0.26	0.34	0.53	0.68	0.69	0.27	
S5					1	1.00	0.69	0.99	1.00	1.00	1.00	0.99	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	0.81	0.69	0.79	0.68	0.69	0.76	0.32	0.29	0.35	0.54	0.68	0.69	0.29	
S6						1	0.70	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	0.81	0.68	0.79	0.67	0.66	0.77	0.32	0.30	0.34	0.51	0.67	0.68	0.28		
S7							1	0.74	0.67	0.67	0.69	0.66	0.66	0.63	0.68	0.65	0.67	0.71	0.68	0.68	0.48	0.54	0.58	0.52	0.54	0.95	0.32	0.82	0.34	0.44	0.51	0.50	0.76	
S8								1	0.99	0.99	0.99	0.99	0.99	0.98	0.99	0.99	0.99	0.99	0.99	0.99	0.80	0.68	0.79	0.67	0.66	0.80	0.33	0.35	0.35	0.52	0.67	0.68	0.32	
S9									1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	0.80	0.66	0.78	0.66	0.65	0.75	0.29	0.25	0.32	0.50	0.66	0.67	0.24
S10										1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	0.79	0.67	0.77	0.68	0.66	0.74	0.29	0.24	0.32	0.50	0.67	0.69	0.24
S11											1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.79	0.68	0.78	0.68	0.67	0.75	0.30	0.27	0.33	0.51	0.67	0.69	0.26		
S12												1	1.00	1.00	0.99	1.00	1.00	0.99	1.00	1.00	0.77	0.66	0.75	0.68	0.65	0.73	0.28	0.23	0.29	0.48	0.67	0.69	0.21	
S13													1	1.00	1.00	1.00	1.00	1.00	1.00	0.80	0.69	0.77	0.68	0.67	0.73	0.32	0.25	0.34	0.52	0.69	0.70	0.24		
S14														1	1.00	1.00	0.99	1.00	1.00	0.78	0.65	0.76	0.67	0.64	0.71	0.25	0.18	0.28	0.47	0.65	0.67	0.18		
S15															1	0.99	1.00	1.00	1.00	1.00	0.81	0.71	0.79	0.70	0.70	0.74	0.36	0.29	0.38	0.56	0.71	0.72	0.29	
S16																1	1.00	0.99	1.00	1.00	0.80	0.66	0.78	0.67	0.65	0.73	0.28	0.22	0.31	0.49	0.66	0.67	0.22	
S17																	1	1.00	1.00	1.00	0.79	0.68	0.79	0.69	0.68	0.74	0.30	0.24	0.33	0.52	0.68	0.69	0.26	
S18																		1	1.00	1.00	0.80	0.70	0.79	0.70	0.70	0.77	0.35	0.31	0.37	0.56	0.70	0.71	0.32	
S19																			1	1.00	0.79	0.68	0.77	0.69	0.67	0.74	0.30	0.26	0.33	0.52	0.68	0.70	0.26	
S20																				1	0.79	0.68	0.77	0.69	0.68	0.75	0.30	0.26	0.33	0.52	0.68	0.70	0.26	
S21																					1	0.69	0.85	0.48	0.61	0.55	0.65	0.32	0.69	0.69	0.61	0.56	0.37	
S22																						1	0.82	0.92	0.97	0.40	0.81	0.37	0.80	0.92	0.97	0.93	0.54	
S23																							1	0.67	0.77	0.56	0.62	0.31	0.68	0.73	0.71	0.64	0.51	
S24																								1	0.95	0.37	0.57	0.24	0.56	0.77	0.96	0.96	0.39	
S25																									1	0.39	0.73	0.35	0.72	0.89	0.98	0.94	0.54	
S26																										1	0.18	0.73	0.22	0.31	0.37	0.37	0.61	
S27																											1	0.50	0.98	0.91	0.76	0.68	0.60	
S28																												1	0.48	0.44	0.34	0.31	0.86	
S29																													1	0.94	0.73	0.64	0.65	
S30																														1	0.90	0.83	0.64	
S31																															1	0.99	0.46	
S32																																1	0.37	
S33																																	1	

The variation of each glycoside was analyzed, providing data to support the quality evaluation of SPLs. The 33 batches of SPLs were divided into three categories according to their origins. The OPLS-DA analysis basically differentiated SPLs from different origins. This study provided a reliable method for evaluating the quality of SPLs and laid the groundwork for their integrated use and evolution.

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Author's contributions

Xinyi Guan: Methodology and writing original draft; Suyu Zhang: Resource; Yitong Shi and Ting Zhang: Data curation; Yanfang Wu and Xinsheng Wang: Project administration, review and editing manuscript.

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Ethical approval

Not applicable.

Data availability statement

The data are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare no conflict of interest.

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