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Identification of the Genus *Pleione* Based on Fingerprinting and Determination of the Content of their Key Components

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Abstract: The genus *pleione* (Orchidaceae) is famous for its ornamental value because of the special color of plants, and it is also a traditional medicinal plant in southeast Asia. The genus comprises of 24 species and nine natural hybrids, with 16 species found in China, primarily in the southwestern, central, and eastern regions. The diverse range of variants and transitional forms observed within *Pleione* poses challenges in its classification system, making it intricate to discern conventional morphological taxonomic traits. Hence, the present work employed chemical taxonomy as a method to classify *Pleione* for the initial time. A total of 13 species of *Pleione* were collected to construct high-performance liquid chromatography (HPLC) fingerprints, and similarity evaluation and cluster and principal component analyses were conducted. Moreover, the distribution of chromatographic peaks in the pseudobulbs of *Pleione* was determined. The developed HPLC fingerprinting approach for *Pleione* exhibits stability, feasibility, and reliability, thereby offering a valuable reference for taxonomic investigations in *Pleione*. The primary objective of this study was to improve the precision and accuracy in developing and applying the resources associated with *Pleione.*

Keywords: *Pleione*; fingerprint chromatograms; content determination. © 2023 ACG Publications. All rights reserved.

1. Introduction

The genus *pleione* (Orchidaceae) is highly valued for its ornamental and medicinal properties. The genus has a diverse range of species and contains approximately 33 species (including nine natural hybrids), with 16 of them found in China. This includes five natural hybrids, which makes it the center of distribution for the genus worldwide [1,2]. Traditional taxonomy has generally

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concentrated on the anatomy of the labellum, particularly the presence or absence of markings, the morphology of its appendages, and the shape and size of *Pleione* pseudobulbs from its inception [3]. However, due to convergent adaptation, the interaction of local adaptations, and the complex field habitats, *Pleione* exhibits rich variation and transitional types. Moreover, identifying complex morphological types in some regions is extremely difficult [4,5]. As a result, identifying and categorizing *Pleione* species only by morphological studies has significant challenges. Gravendeel et al. [6] conducted molecular systematic research using nuclear and chloroplast genes, but their study did not obtain convincing support rates owing to the unclear origin of the samples used in their study and the limited number of molecular markers. Hunt and Vosa [7] proposed combining the *P. bulbocodioides* complex into *P. bulbocodioides*, drawing upon cytological investigations. Additionally, they recommended considering distinct chromosome ploidy types as subspecies. Nevertheless, this categorization has not been acknowledged and implemented.

Owing to their evolutionary relationships, plants within the same genus often contain similar chemical components [8,9]. Currently, compounds isolated from species of the *Pleione* primarily include phenanthrenes, bibenzyls and derivatives of glucosyloxybenzyl succinate [10]. The research group had previously performed plant sampling, chemical component separation, and experimental procedures to ascertain the plant identities. This study represents the first application of chemical taxonomy in investigating *Pleione* species. A total of 13 species were collected to construct a fingerprint using high-performance liquid chromatography (HPLC), and similarity evaluation and cluster and principal component analyses were conducted. Determining the distribution of chromatographic peak contents in the pseudobulbs of *Pleione* was also conducted. This study offers a theoretical framework for the chemical classification of the *Pleione* genus and establishes a scientific foundation for future advancement and exploitation.

2. Materials and Methods

2.1. Plant Materials

Thirteen species of *Pleione* were used as experimental materials. Detailed introduction information can be found in Table 1. These plants were introduced in the winter of 2017 and managed at the highland base of Quanzhou Jingpu Biotechnology Co., Ltd. located in Yongchun County, Quanzhou City. After cultivation, they were used for experiments and deposited in Key Laboratory of Orchid Plant Conservation and Utilization, Fujian Agriculture and Forestry University. And identified by Associate Professor Shasha Wu (School of Landscape Architecture, Fujian Agriculture and Forestry University).

Table 1. The locality information of *Pleione* species

No	Flowering	Latin name	Voucher specimen	Locality
S1	Sect. Humiles	*P. grandiflora*	Wss062	Guizhou
S2		*P. yunnanensis*	Zh070	Yunnan
S3		*P. humilis*	Wzx059	Yunnan
S4		*P. bulbocodioides*	Wsso63	Yunnan
S5		*P. forrestii*	Wzx061	Yunnan
S6		*P. pleionoides*	Wss066	Chongqing
S7		*P. formosana*	Wzx060	Taiwan
S8		*P. aurita*	Wzx058	Yunnan
S9	Sect. Pleione	*P. limprichtii*	Wss064	Sichuan
S10		*P. scopulorum*	Zh068	Yunnan
S11		*P. maculata*	Wss065	Yunnan
S12		*P. saxicola*	Zh069	Sichuan
S13		*P. praecox*	Zh067	Yunnan

2.2. Standards and Solvents

The 15 compounds isolated from *P. grandiflora* were used as reference standards (see Figure 2). The 15 compounds were previously isolated in our laboratory, with purities exceeding 98%. For specific nuclear magnetic resonance (NMR) data, please refer to the literature [10,11]. The reagents utilized in this experimental procedure consisted of methanol, acetonitrile, and acetic acid, all of which were of analytical grade and were supplied by China National Pharmaceutical Group Chemical Reagent Corporation. Additionally, HPLC-grade methanol and acetonitrile were procured from Merck. A solution of 10% sulfuric acid, ethanol, and ultrapure water was prepared in the laboratory.

2.3. Sample Preparation

2.3. Preparation of Standard Solutions

Accurately weigh 1.5 mg of each of the 15 compounds and dissolve them in 1.5 mL of chromatographic grade methanol to prepare a 1 mg/mL reference stock solution. Take 0.1 mL of each of the above reference solutions, put them in a 1.5 mL liquid phase vial, and shake well to prepare a mixed reference solution.

2.3.2. Preparation of Test Solutions

Based on the results of previous experiments, accurately weigh 3 g of dried pseudobulbs powder of 13 species of *Pleione*, add them to 30 mL of 80% ethanol (volume ratio of 10:1), and reflux for 150 minutes at 80 ℃. Repeat this process twice, combine the filtrate, and concentrate under reduced pressure. Add methanol to make up to 5 mL and dissolve using ultrasound. Take 1.5 mL of the solution, filter it, and store it in a liquid phase vial as the test sample for later use.

2.4. Chromatographic Conditions

Analyses were performed on the Waters series liquid chromatographer. The chromatography was carried out on a Dikma Diamonsil C18 column (5 μm, 400 × 4.6 nm) at a column temperature of 30 °C and flow rate of 1 $mL \cdot min^{-1}$ using acetonitrile (A) and 0.1% formic acid in water (B) as mobile phase, chromatographic separation was performed under the following linear gradient conditions: 0-20 minutes (10% A →22% A), 20-120 minutes (22% A → 50% A).

2.5. Methodology Investigation

2.5.1 HPLC Fingerprint

The sample solution of S1 was subjected to five injections, as outlined in Section 2.3. Determining the retention duration and peak area for each common peak was conducted and afterward utilized in calculating the relative standard deviation (RSD). The results showed that the RSD values of the relative retention time for each common peak < 0.1% (n=5), and the RSD values of the relative peak area are < 2% (n=5), indicating that the experimental instrument and injections were highly precise.

Five portions of the sample solution of S1 were injected separately as described in Section 2.3. The retention time and peak area of each common peak were determined, and the RSD was calculated. The results showed that when the RSD values of the relative retention time for each common peak < 0.1% (n=5), and the RSD values of the relative peak area were< 2% (n=5), the method of preparation was highly repeatable.

Take five portions of the sample solution of S1 and inject them separately at 0, 2, 4, 12, and 24 hours as described in Section 2.3. The retention time and peak area of each common peak were determined, and the RSD was calculated. The findings indicate that the solution can be considered relatively stable within 24 hours for detection purposes, provided that the RSD values of the relative retention time for each common peak are less than 0.1% (n=5) and the RSD values of the relative peak area are less than 2% (n=5).

2.5.2 Content Determination

The sample solution of S1 was subjected to six injections, as outlined in Section 2.3. Compound 13 was used as the reference peak (with the maximum peak area and stable retention time). The relative standard deviation (RSD) values for the relative retention time and relative peak area of nine compounds were calculated. The results showed that the average RSD values for relative retention time and relative peak area were 0.08% and 2.20%, respectively, indicating excellent precision performance of the instrument.

Six portions of the sample solution of S1 were injected separately as described in Section 2.3. Compound 13 was used as the reference peak. The relative standard deviation (RSD) values for the relative retention time and relative peak area of nine compounds were calculated. The results showed that the average RSD values for relative retention time and relative peak area were 0.07% and 3.17%, respectively, indicating good repeatability of the method.

Take six portions of the sample solution of S1 and inject them separately at 0, 3, 6, 9, 12, and 24 hours as described in Section 2.3. Compound 13 was used as the reference peak. The relative standard deviation (RSD) values for the relative retention time and relative peak area of nine compounds were calculated. The results showed that the average RSD values for relative retention time and relative peak area were 0.08% and 3.77%, respectively, indicating that the test solution remained stable within 24 hours and could be used for analysis.

3. Results and Discussion

3.1. Elucidation of the Structures

The test solutions for each of the 13 types of specimens were produced according to the methodology outlined in Section 2.2. The samples were next subjected to analysis through injection into the HPLC system, following the methodology outlined in Section 2.3. The resulting chromatogram was then recorded and imported into the "Chinese Herbal Medicine Chromatographic Fingerprint Similarity Evaluation System" (2012 edition) for fingerprint analysis. A "time window" width of 0.3 min was selected, and the median method was used. Sample S1 was used as the reference chromatographic peak R. Multipoint calibration was used to establish fingerprint maps from the HPLC, which were superimposed from *Pleione*. This produced a reference fingerprint map (R), as described in Figure 1.

To compare the similarity more accurately between different species of the *Pleione* genus, 46 common chromatographic peaks were determined based on their retention times and peak areas (S1). Among them, peak 37 (tR=65.072) exhibited a relatively large peak area and stable retention time in the chromatograms of various samples. Therefore, it was selected as the reference chromatographic peak, with its peak area set to 1. The similarity between the 13 species of the *Pleione* genus was then calculated (S2). The relative retention times and relative peak areas of each chromatographic peak were calculated as the ratio to the retention time and peak area of peak 37, respectively, and the corresponding RSD values were determined (S3 and S4, supplementary 1 table in supporting information)

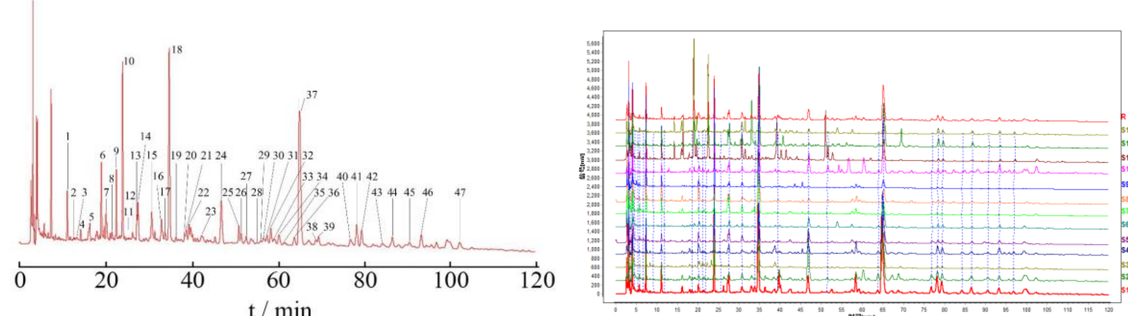

Figure 1. Compared fingerprints and HPLC fingerprint overlay chart of 13 species of *Pleione*

A set of 15 compounds was employed as reference standards to establish an HPLC fingerprint under uniform chromatographic circumstances. The retention periods and UV absorption values were utilized as thresholds for efficient liquid phase analysis. A total of 15 compounds were identified (Figure 2). Peak 13 was compound **7**, and peak 19 contained compounds **8** and **10**. Peak 20 was compound **11**, and peak 23 was compound **12**. Peak 27 was compound **1**, and peak 33 was compound **2**. Peak 34 was compound **14**, and peak 37 was compound **13**. Peak 40 was compound **3**. Peak 41 contained compounds **4**, **9**, and **15**. peak 42 was compound **6**, and peak 46 was compound **5**.

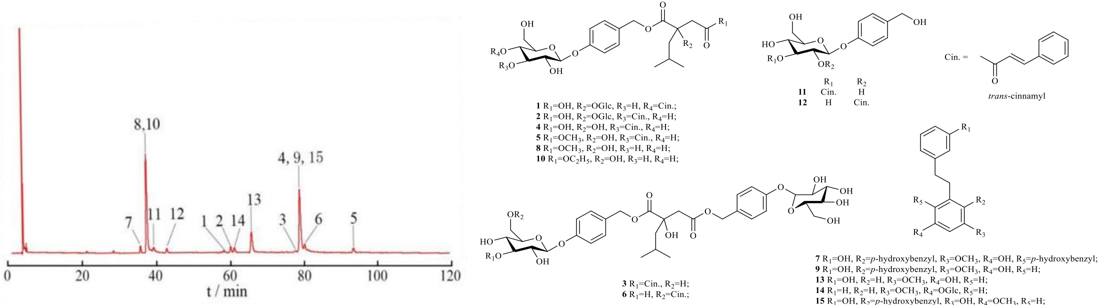

Figure 2. Reference substance chromatogram and compound structure

1-6 : Pleioneside A-F; **7** : 5-O-Methylshanciguo; **8** : 1-(4-β-D-Glucopyranosyloxybenzyl)4-methyl (2R)-2-isobutylmalate; **9** : 3', 5-dihydroxy-2-(4-hydroxybenzyl)-3-methoxybibenzyl; **10** : 1-(4-β-D-Glucopyranosyloxybenzyl)4-ethyl (2R)-2-isobutylmalate; **11** : 1-O-(4-hydroxymethylphenoxy)-3-O-trans-cinnamoyl-β-D-glucoside; **12** : 1-O-(4-hydroxymethylphenoxy)-2-O-trans-cinnamoyl-β-D-glucoside; **13** : Batatasin III; **14** : Shancigusin F; **15** : 3, 3'-dihydroxy-2-(*p*-hydroxybenzyl)-5-methoxybibenzyl.

3.2. Similarity Analysis

A total of 13 species of *Pleione* were evaluated for their similarity using the "Chinese Herbal Medicine Chromatographic Fingerprint Similarity Evaluation System" (2012 edition). The results showed that the RSD values of the peak relative retention times ranged from 0.0% to 0.01%, indicating high stability and similarity of the HPLC fingerprint established in this study. The RSD values of the relative areas of the peaks ranged from 50.46% to 279.61%, indicating that there were many common chemical components among the 13 species of plants, but there were still significant differences in the composition and content of chemical components among the different species. The similarity of samples S1 to S13 to the control fingerprint were 0.932, 0.864, 0.894, 0.921, 0.906, 0.826, 0.756, 0.793, 0.752, 0.924, 0.504, 0.897, and 0.646. The similarities of S1, S4, S5, and S10 > 0.900, while S11 and S13 were only 0.505 and 0.646, indicating a high similarity between S1, S4, S5, and S10. Nevertheless, the degree of resemblance between S11 and S13 and the remaining *Pleione* species in terms of chemical makeup was low (see supplementary 2 table in supporting information). The HPLC fingerprint profiles of the 13 species of the *Pleione* exhibited an overall similarity ranging from 0.231 to 0.945, suggesting notable variations in the chemical composition of their pseudobulbs.

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3.3. Clustering Analysis

SPSS 22.0 (IBM, Inc., Armonk, NY, USA) was used with the inter-group average linkage method to perform a systematic cluster analysis using the primary common chromatographic peak area of 13 batches of samples as variables [12]. The results are shown in Figure 3. When the Euclidean distance was 20, the 13 different *Pleione* plants were divided into two major categories. S11 and S13, both Sect. *Pleione* from Yunnan Province, clustered into one category, while the other 11 plants (except for S12, also in Sect. *Humiles*) clustered into another category, indicating significant differences in chemical composition between Sect. *Humiles* and Sect. *Pleione*. S12 clustered with Sect. *Humiles* could be due to the similarity of its pseudobulbs to those of Sect. *Humiles*, while the morphology of its pseudobulbs was distinctly different from that of Sect. *Pleione*. This observation indicated a strong association between the chemical content and form of the pseudobulbs. When the Euclidean distance equaled 15, the 13 plants were partitioned into three groups. Specifically, S11 and S13 were observed to cluster together in the first category, while S1, S2, S4, and S10 formed a cluster in the second category. Lastly, S3, S5-S9, and S12 were observed to cluster together in the third category. The limits mentioned above followed the outcomes of the similarity assessment conducted on the fingerprint spectra. When the Euclidean distance is less than 2, it can be observed that S3, S5, and S6 remain clustered together, suggesting a significant level of similarity in the HPLC fingerprint spectra across these three plant types (supplementary 3 table in supporting information)

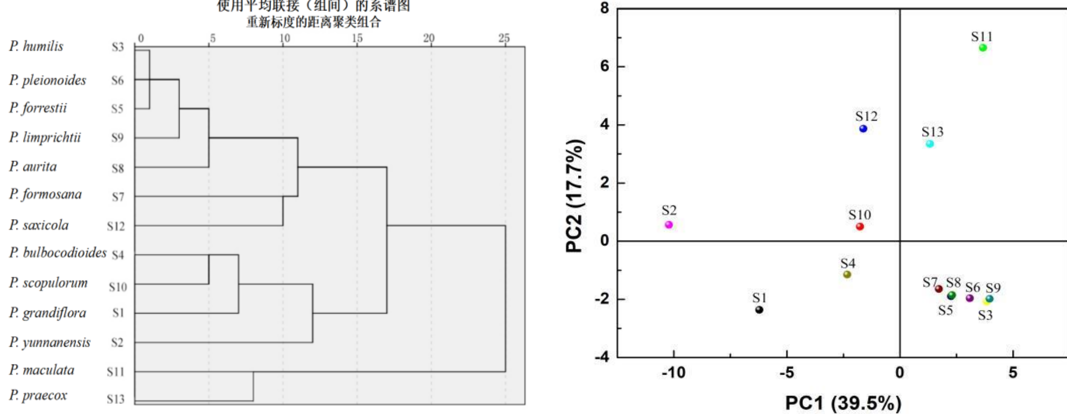

Figure 3. Dendrogram and PCA scores of 13 species of *Pleione*

3.4. Principal Component Analysis (PCA)

The common peak data of the HPLC fingerprints of 13 samples were imported into SPSS 23.0 statistical software for a Principal Component Analysis (PCA), and comprehensive scores were calculated for the samples. The two primary principal components obtained from the analysis, Principal Component 1 and Principal Component 2, were used as the x and y axes to create a scatter plot (Figure 3). The scatter plot analysis revealed that the 13 species of *Pleione* exhibited a dispersed distribution across four distinct zones, suggesting notable variations in chemical composition across the various species. Section *Pleione* S11-S13 showed a clustering pattern, while S11, S13, and S12 were scattered throughout distinct locations. This observation suggests that S11, S13, and S12 possess comparable chemical compositions but with modest variations. From an alternative perspective, it can be observed that plants S3 and S5-S9 exhibit a close grouping, indicating a potential similarity in their chemical compositions. The data points S1, S2, S4, and S10 had a certain degree of dispersion, although they tended to aggregate and were found to align with the outcomes of similarity and clustering studies (supplementary 4 table in supporting information).

3.5. Determination of the Content of Key Components

The identification and quantification of the content of nine common peaks among the 47 chromatographic peaks in the HPLC chromatograms of pseudobulbs from 13 species of the *Pleione* were conducted based on the findings of earlier investigations. Preparing the test sample and standard solutions followed the procedure outlined in Section 2.2. The chromatographic conditions used were identical to those detailed in Section 2.3.

Nine pre-prepared control samples were separately injected as 2, 4, 6, 8, 10, and 12 µL and detected and analyzed by HPLC. The chromatographic peak area of the control solution was taken as the x-axis, and the mass concentration of the control solution was taken as the y-axis to draw standard curves for the nine compounds. Table 2 displays the linear regression formulae. The findings indicated a strong linear correlation between the peak area and the mass concentration of the control solutions across all nine compounds examined.

Table 2. The linear regression equation of nine compounds

Compound number	Compound name	Regression equation	R^2
1	Pleioneside A	*y = 4×10-6x - 0.0131*	1.0000
2	Pleioneside B	*y = 2×10-6x - 0.0154*	1.0000
3	Pleioneside C	*y = 5×10-6x - 0.0239*	1.0000
5	Pleioneside E	*y = 2×10-6x + 0.0194*	0.9998
6	Pleioneside F	*y = 3×10-6x + 0.0016*	0.9901
11	1-O-(4-hydroxymethylphenoxy)-3-O-trans-cinnamoyl-*β*-D-glucoside	*y = 4×10-6x + 0.1865*	0.9986
12	1-O-(4-hydroxymethylphenoxy)-2-O-trans-cinnamoyl-*β*-D-glucoside	*y = 5×10-6x + 0.0713*	0.9991
13	Batatasin III	*y = 6×10-7x + 0.242*	0.9930
14	Shancigusin F	*y = 2×10-6x + 0.2001*	0.9960

The chromatograms of 13 samples were evaluated in Section 2.5.2 to record and analyze the peak regions of seven compounds of butanedioic acid and two benzyl compounds. The analysis involved determining the content of nine compounds in the 13 species of *Pleione* using the regression equations provided in Table 3. Additionally, the link between the chemical content and the clustering of the 13 *Pleione* species was examined, as depicted in Figure 4.

Table 3. Content of nine compounds in 13 species of *Pleione* (µg/g)

	1	**2**	**3**	**5**	**6**	**11**	**12**	**13**	**14**
S1	4.413	1.967	7.632	3.366	8.894	3.780	3.687	12.505	0.902
S2	5.805	2.665	13.465	3.439	9.029	/	10.920	9.685	7.194
S3	0.381	/	0.594	0.932	1.451	3.777	/	4.667	0.304
S4	3.593	1.456	2.277	3.391	4.883	9.243	4.858	10.215	0.435
S5	3.054	/	3.798	1.679	2.332	/	3.459	3.727	/
S6	2.408	0.236	1.907	1.403	2.037	1.402	/	3.688	/
S7	2.754	0.894	1.082	0.546	0.804	1.864	2.906	1.642	0.446
S8	3.210	/	0.989	0.775	2.314	2.224	2.348	2.114	/
S9	0.964	0.721	0.326	0.285	0.371	1.015	/	1.113	0.189
S10	/	0.494	2.007	4.352	1.841	3.459	/	7.647	/
S11	0.698	/	0.544	0.848	1.110	/	4.520	2.519	/
S12	1.591	0.173	0.874	0.918	3.815	5.084	3.732	5.458	/
S13	2.536	/	1.245	1.688	2.694	5.027	5.913	3.930	/

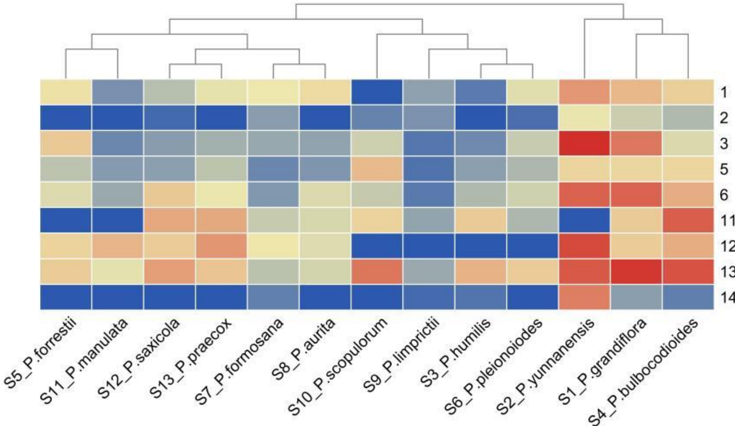

Figure 4. Heatmap and cluster analysis of nine compounds in 13 species of *Pleione*

4. Conclusion

4.1. Fingerprinting and Multivariate Statistical Analysis

Fingerprinting encompasses quantifying information, specificity, and quantity of chromatographic components possessing established chemical structures [13]. HPLC fingerprinting is useful for inter-species classification and the identification of plants [14,15]. The systematic classification of the *Pleione* plants has primarily focused on morphology [16], cytology[17,18], molecular biology [19], and other aspects of research. Nevertheless, typical morphological and cytological techniques are inadequate in reliably distinguishing them due to the diverse range and intermediate forms of the *Pleione* species and the challenges associated with the morphological identification of complexes in specific geographical areas [20]. Therefore, chemical taxonomical research is required to identify the species of *Pleione*. The inter-species relationship was explored by constructing HPLC fingerprinting of the 13 species of *Pleione*. A total of 47 chromatographic peaks were selected for similarity, cluster, and principal component analyses, and 21 were common to all the species of *Pleione*. Regarding the specificity of the chromatographic peaks, peaks 10, 18, and 37 among the 47 peaks had larger peak areas than the other peaks and are the primary chemical components of the pseudobulbs of the 13 species of *Pleione* . Peak 4 was only detected in Sect. *Pleione* can be used as a characteristic compound for Sect. *Pleione*. Peaks 6 and 9 had significantly higher peak areas and heights in S11 and are the primary components of the pseudobulbs of S11. Peaks 31 and 35 have significantly larger peak areas and heights in S10 than in other species and can be used as characteristic chromatographic peaks to identify S10 (see supporting information).

The clustering analysis results indicated that when the Euclidean distance was 20, the 13 species of *Pleione* could be divided into two categories, with S11 and S13 clustered into one category and the other 11 species clustered into another category (see supporting information). This result is consistent with the clustering results of the phylogenetic tree constructed by Li T Y [21] using chloroplast genomes. The *P. bulbocodioides* complex has always been a difficult problem for taxonomists [22]. The retrieval classification approach, as provided by Cribb and Butterfield [23,24], does not provide an apparent distinction for the *P. bulbocodioides* complex. In the obtained clustering outcome, it can be observed that S4 did not form a cluster with S6, S7, and S9. When comparing the chromatographic peaks of S6 with those of S7 and S9, it was observed that S6 had distinct peaks at positions 24 and 32. The chromatograms obtained from samples S7 and S9 exhibited high similarity (see supporting information). However, it was observed that the peak regions corresponding to peaks 13 and 14 in S7 were notably greater in magnitude when compared to those in S9 (see supporting information). This methodology can differentiate and classify the four plants within the *P. bulbocodioides* complex.

4.2. Analysis of the Content of Key Components

Factors such as germplasm and ecological environment determine the content of chemical components in plants. In this experiment, by establishing standard curves, the content of seven glucosyloxybenzyl and two bibenzyls in the pseudobulbs of 13 species of *Pleione* was measured for the first time.

The distribution of seven glucosyloxybenzyl compounds ranged from 3.682 to 45.324 μg/g with the highest concentration found in S1, S2, and S4, and the lowest in S3, S8, and S9. Compounds **3**, **5**, and **6** were detected in the pseudobulbs of all 13 species, while compounds **1**, **2**, **11**, and **12** were only found in some species. The distribution of two bibenzyl compounds ranged from 10.650 to 16.880 μg/g with the highest concentration in S1, S2, and S4 and the lowest in S7, S8, S9, and S11. Compound **13** was detected in the pseudobulbs of all 13 species, while compound **14** was only found in some species (see supporting information).

In conclusion, this work effectively implemented HPLC fingerprinting of the *Pleione* and employed chemical pattern recognition analysis to ascertain the presence of nine key components. From a chemical perspective, this enables the differentiation and identification of different species within the *Pleione*, which promotes the progress of taxonomic research on *Pleione* and enables more accurate and comprehensive development and utilization of *Pleione* resources.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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References

[1] W. Hua (2019). Orchidaceae plants—*Pleione bulbocodioides*, *Lan. Arc. Aca. J.* **09**, 63-67.

[2] P. Komínek, S. Massart, K. Pham, P. V. Leeuwen and M. Komínková (2019). Characterisation of a novel virus infecting orchids of the genus *Pleione*, *Virus Res.* **261**, 56-59.

[3] X. Q. Chen, P. J. Cribb and S. W. Gale (2009). Orchidaceae: flora of China: vol. 25. Beijing and Missouri Botanical Garden and Science Press, Beijing.

[4] X. Q. Chen and Z. J. Liu (2009). Flora of China. Science Press, Beijing.

[5] S. S. Wu, L. Chen, Y. M. Zhao, Z. X. Zheng, H. Zhang and J. W. Zhai (2020). Review of the germplasm resources and crossbreeding of *Pleione*, *J. Plant Genet. Resour.* **21**, 785-793.

[6] B. Gravendeel, C. M. E. Marcel, V. D. B. Cassio and J. C. Phillip (2004). Phylogeny of *Pleione* (Orchidaceae) and parentage analysis of its wild hybrids based on plastid and nuclear ribosomal ITS sequences and morphological data, *Syst. Bot*. **29**, 50-63.

[7] P. F. Hunt and C. G. Vosa (2016). The cytology and taxonomy of the genus *Pleione* D. Don (Orchidaceae), *Kew. Bull*. **25**, 423-432.

[8] Y. P. Li, X. L. Chen, S. X. Yuan, Y. J. Chen and R. P. Zhang (2023). Chemical compounds isolated from *Pleione maculata* and their bioactive activity, *J. Kunming. Med. Uni*. **44**, 1-6.

[9] X. X. Liu, H. D. Liu, L. L. Pan, Y. F. Wu, N. Wan, X. Zhou and B. Li (2019). Research progress on chemical constituents and bioactivities of plants of *Pleione Bulbocodi-oides*(*Franch*.) Rolfe, *J. Jiangxi. Uni. Chin. Med*. **31**, 106-111.

[10] M. T. Wu, X. Q. Wu, L. J. Zheng, L. J. Zhang, J. R. Fu, X. Q. Zhang, S. S. Wu and L. Ni (2021). Discovery of glucosyloxybenzyl 2-hydroxy-2-isobutylsuccinates with anti-inflammatory activities from *Pleione grandiflora*, *Fitoterapia* **155**, 105062.

[11] W. Li, J. R. Fu, L. J. Zheng, L. Nin, J. Q. Liu, J. W. Zhai, Z. Zhou and S. S. Wu (2022). Two new bibenzyls from *Pleione* grandiflora (Rolfe) Rolfe and their antioxidant activity, *Nat. Prod. Res*. **37**, 15.

[12] M. Zhang, L. Chen, H. Zhu, L. Li, H. F. Da, L. Long and R. T. He (2019). Establishment of HPLC fingerprint of *Pholidota chinensis* and its cluster analysis, *China Pharm*. **30**, 1792-1795.

[13] L. L. Zhang, Y. Q. Liang, P. Zhang, L. B. Yang, X. F. Li, X. P. Wang and H. M. Wu (2019). Identification of flowers of Musa nana Lour., flowers of Musa basjoo Sieb. et Zucc and flowers of Musa balbisiana Colla by HPLC fingerprinting, *IOP Conf. Ser.: Earth Environ. Sci*. **267**, 032078.

[14] X. H. Zhang and J. M. Chen (2003). Application of hierarchical cluster with high-performance liquid chromatography in the classification of genus *Clinopodium chinensis*, *China. J. Mat. Med*. **28**, 812-816.

[15] X. Ma, H. M. Zou, Y. B. Pan, J. Su, Y. J. Qiu and M. F. Qiu (2019). Quality assessment of pollen typhae by high-performance liquid chromatography fingerprint, hierarchical cluster analysis, and principal component analysis, *Pha. Mag*. **15**, 177-182.

[16] Y. Zhang, S. F. Li and B. Li (2010). The research status of *Pleione bulbocodioides*, *Nor. Hor.* **10**, 232-234.

[17] K. K. Stergianou (1989). Habit differentiation and chromosome evolution in *Pleione*, *Plant Syst. Evol*. **166**, 253-264.

[18] K. K. Stergianou and F. L. S. Harberd (1989). A cytotaxonomic study of some species of *Pleione* D. Don (Orchidaceae), *Bot. J. Linn. Soc*. **101**, 213-228.

[19] Z. X. Wang, H. Zhang, L. Chen, Y. M. Zhao, J. W. Zhai, S. P. Chen and S. S. Wu (2020). Leaf epidermal micro-morphology and taxonomic significance of 15 species of *Pleione*, *Acta Bot. Bor-Occid. Sin*. **40,** 1527-1538.

[20] Y. Y. Zhang, X. Q. Wu, M. X. Cao, L. M. Shen, J. W. Zhai and S. S. Wu (2018). Identification of classification relationship between *Pleione barbarae* and *P. grandiflora*, *Agric. For. Univ*. **47**, 661-666.

[21] T. Y. Li (2022). Molecular phylogeny of the genus *Pleione* (Orchidaceae). Northwest University.

[22] X. Y. Dai, J. M. Tiang, W. Li, Y. M. Zhao, L. Chen, J. W. Zhai, D. H. Peng and S. S. Wu (2020). The taxonomic revision of *Pleione formosana* (arethuseae, epidendroideae, orchidaceae) based on molecular, morphological and cytological analyses, *Phytotaxa* 4**77**, 60-70.

[23] P. Cribb and I. Butterfield (1999). The Genus *Pleione*, second edition. Royal Botanic Gardens, Kew.

[24] P. Cribb and I. Butterfield (2017). The enigma of *Pleione* 'Frank Kingdon Ward', *Curtis's Bot. Mag*. **34**, 133-138.

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