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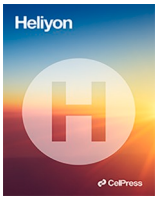

Research article

Development and validation of an ultra-performance liquid chromatography tandem mass spectrometry for target determination of bioactive compounds in *Dendrobium* spp.

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ABSTRACT

As a medicine-food homology herb, *Dendrobium* spp. has versatile applications in modern medicine and food industry. Herein, an ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) based method was established for simultaneous quantification of six active components, including gigantol, erianin, naringenin, quercetin, rutin, and *p*-coumaric acid in Dendrobium spp., on the basis of optimized sample preparation, mass spectrometry conditions, and chromatography conditions. Sample extraction was carried out using methanol at a temperature of 60 °C, followed by separation on a T3 C18 column utilizing a gradient eluting program. The results demonstrated excellent linearity ($r > 0.999$) for the six active components within a specified concentration range. The average recovery rates ranged from 84.7 % to 106.9 %, and the precision (RSD) was within 7.4 %. The detection and quantification limits of this method ranged from 0.34 to 4.17 ng mL^{-1} and 1.12–13.91 ng mL^{-1}, respectively. The established method demonstrates high accuracy and reliability and is applicable in practical sample detection. Different *Dendrobium* spp. exhibit specific variations in compound composition, with *D. fimbriatum* Hook. having a higher content of benzyl compounds and *D. crystallinum*. Rchb. f. having a higher content of flavonoids. This study provides experimental evidence for the quality and safety regulation of *Dendrobium* spp.

1. Introduction

Dendrobium, a member of the Orchidaceae family, is a rare and valuable traditional Chinese medicinal herb. Globally, it comprises between 1500 and 2000 species, predominantly found in the tropical and subtropical regions of Asia and northern Australia [1]. In China, *Dendrobium* spp. have been used for more than 2000 years and is believed to have the effects of balancing Yin and Yang,

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nourishing gastrointestinal tract, promoting body fluids, and relieving coughs [2]. Contemporary pharmacological research has revealed that Dendrobium species harbor numerous bioactive compounds, including alkaloids, polysaccharides, amino acids, and *p*-coumaric acids. These compounds exhibit significant medicinal properties, including antioxidant, anti-aging, anti-tumor, and hypoglycemic effects [3]. Additionally, *Dendrobium* spp. are also used as tea, salad and fermented food to satisfy people's needs for health and wellness [4]. Given the high nutritional and economic value of *Dendrobium* spp., there is a high demand for these herbs in the market place [5].

Quality evaluation of *Dendrobium* spp. has been a topic of significant concern in both industry and academia [6]. With the further exploration of its value, the artificial cultivation industry of *Dendrobium* spp. has experienced unprecedented development. However, it still fails to fully meet the demands of the *Dendrobium* products market. Some unscrupulous producers use non-authentic *Dendrobium* materials as substitutes for genuine ones, resulting in intermingling of the good and the bad, which seriously affects the efficacy of *Dendrobium* and the health of consumers [7]. Additionally, due to the extensive distribution of *Dendrobium* spp. and the influence of germplasm resources, geographical environment, climate, epiphytes, etc., it exhibits various morphological differences. Furthermore, the lack of cultivation standard and certification standard, as well as immature production techniques, have led to highly unstable quality of *Dendrobium* products [8]. Given the importance of medicinal *Dendrobium* in traditional Chinese medicine, it is imperative to develop a comprehensive set of verification methods for authenticating *Dendrobium*-derived components. This is crucial for ensuring the safety and clinical effectiveness of *Dendrobium*-based medicines during quality evaluation [9].

At present, quality detection methods for *Dendrobium* include morphological identification, DNA barcoding technology, and spectroscopic analysis techniques [10–12]. Traditionally, *Dendrobium* identification is mainly based on morphological and microscopic identification, which involves the recognition and classification of plants through the observation and comparison of their morphological characteristics. However, this method is highly subjective and requires a significant number of professional taxonomists, making it challenging to be widely adopted. DNA barcoding possesses advantages such as efficiency, accuracy, and automation, but its application is constrained by limitations in databases, genomic complexity, phylogenetic distance, and sample quality [13]. Spectroscopic analysis techniques encompass a diverse array of methodologies, including various chromatography techniques, mass spectrometry, and their intricate coupling strategies. Among these, UPLC-MS/MS stands out for its remarkable attributes such as unparalleled sensitivity, unparalleled stability, extensive detection capabilities, and swift analysis speed. These exceptional features enable UPLC-MS/MS to achieve exceptional qualitative and quantitative analysis of numerous components present in complex samples, thus making it a powerful tool in modern analytical chemistry [14–16]. Mu et al. successfully developed an efficient UPLC-MS/MS technique specifically tailored for the quantitative determination of phenolic compounds in D. officinale, which exhibited remarkable potential in exploring and analyzing bioactive compounds present in this herb [17,18]. Wu et al. utilized UPLC-MS/MS for profiling and differentiating D. huoshanense, which effectively demonstrates its applicability in ensuring the quality control of this herbal medicine and its derived products [19]. The selection of appropriate quality markers is critical for quality control of *Dendrobium* using UPLC-MS/MS. With the development and application of modern analytical chemistry techniques, the chemical components contained in *Dendrobium* are gradually becoming known, and polysaccharides, flavonoids, alkaloids, organic acids, amino acids, and trace elements have been identified as the pharmacologically active components of *Dendrobium* through modern pharmacological research. Previous studies have explored the quality of *Dendrobium* based on the characteristics of polysaccharides, but the complexity of chemical structure and the lack of specificity of polysaccharides in *Dendrobium* limit its usefulness as a quality marker [20]. Fingerprinting studies have shown that the metabolites with small molecular weight in *Dendrobium* are influenced by factors such as origin, variety, environment, and age, and these specific characteristics are potential markers for different *Dendrobium* spp [21].

In this study, flavonoids (naringenin, quercetin, rutin), bibenzyl compounds (gigantol and erianin), and phenolic acid (*p*-coumaric acid) were selected as quality markers because these compounds are relatively abundant in *Dendrobium* and their multiple beneficial effects have been approved in previous research [22]. A convenient and comprehensive *Dendrobium* spp. identification and evaluation method was established based on UPLC-MS/MS, which was used to detect and identify four different *Dendrobium* spp. The findings of this study provide experimental support and theoretical basis for the detection of bioactive components in *Dendrobium* spp.

2. Materials and methods

2.1. Materials and reagents

The chemical standards of gigantol (purity ≥98 %) and naringenin (purity ≥98 %) were bought from Weikeqi Biotechnology Co., Ltd. (Sichuan, China). The erianin (99.7 %) and rutin (purity ≥91.4 %) were provided by the China National Institute for Food and Drug Control. The quercetin (purity ≥97.1 %) and *p*-coumaric acid (purity ≥98.8 %) were provided by Anpel-Trace Standard Technical Services Co., Ltd. (Shanghai, China). The HPLC grade reagents, such as methanol, formic acid (FA), and ammonium formate (AF) were provided from Shanghai Aladdin Biochemical Technology Co., Ltd.

The stems of *D. officinale* Kimura et Migo, *D. Devonianum*, *D. catenatum* Lindl., *D. compactum* Rolfe ex W. Hackett, *D. fimbriatum* Hook., *D. chrysotoxum* Lindl., *D. crystallinum*. Rchb. f., *D. aphyllum* (Rohb.) C. E. Fishcher. were bought from local market and verified by Zhejiang Provincial Institute for Food and Drug Control (Hangzhou, China).

2.2. Instruments

The Shimadzu LC 30A UPLC system was equipped with a 8050 triple quadruple detector (Shimadzu, Japan). This system was complemented by a reciprocating shaker bath (Julabo Labortechnik GmbH, Germany), a KH-500DV ultrasonic cleaner (Kunshan

Hechuang Ultrasonic Instrument Co., Ltd., China), and a Multifuge X1R high-speed refrigerated centrifuge (Thermo Fisher Scientific, USA). Additionally, high-purity water with a resistivity of 18.2 MΩ cm at 25 °C was obtained from a Millipore Milli-Q water system (Bedford, MA, USA).

2.3. Sample preparation

The *Dendrobium* spp. was accurately weighed to 200 g, pulverized using a high-speed grinder, and placed in sample bags to obtain a powdered sample. Subsequently, 0.1 g of the sample was thoroughly combined with 10 mL of methanol. The mixture was agitated in a reciprocating shaker bath at 60 °C for 30 min, followed by cooling. Then, it was centrifuged at 6000 g for 5 min. The supernatant was carefully collected, while the precipitate underwent a second extraction process using 10 mL of methanol under identical conditions. The supernatants obtained were subsequently combined, and additional methanol was added to attain a final volume of 20 mL. Before analysis, the sample was filtered through a 0.45 μm organic membrane filter (Biosharp Co. Ltd., Hefei, China).

For the preparation of standard curves, 10 mg of reference standards, naringenin, quercetin, rutin, gigantol, erianin, and *p*-coumaric acid, were separately weighed and dissolved in methanol (HPLC grade) to a final volume of 20 mL to obtain standard stock solutions, which were then stored at −20 °C. Further dilutions of these six standard stock solutions were conducted with methanol to obtain five gradient levels of standard mixtures, including 2, 5, 20, 50, and 100 ng mL^{-1}.

2.4. Conditions of UPLC-MS/MS

A T3 C18 column (100 mm × 2.1 mm, 1.8 μm) was utilized for the separation of the target compounds. The mobile phases comprised of (A) deionized water with a mixture of 5 % FA and 5 mmol of AF, and (B) acetonitrile. The gradient elution was set as follows: from the initial point to 2 min, 5 % B; from 2 to 5 min, a linear increase from 5 % to 70 % B; from 5 to 8 min, maintained at 70 % B; and from 8 to 10 min, a linear decrease from 70 % to 5 % B. The additional parameters were set at a column temperature of 35 °C, a flow rate of 0.3 mL min^{-1}, and an injection volume of 4 μL.

During mass spectrometry, electrospray ionization was employed in both positive and negative ion modes. For the quantification of the target analytes, multiple reaction monitoring (MRM) was utilized. The capillary probe voltage was adjusted to 1 kV, while the cone gas flow consisted of nitrogen with a flow rate of 3 mL min^{-1}. The collision gas was set to high-purity argon at 270 kPa. Additionally, the interface temperature was set to 200 °C, the heating block temperature to 300 °C, and the desolvation temperature to 150 °C. The qualitative and quantitative ion pairs, as well as the optimized collision energy and other specific parameters for mass spectrometry detection can be found in Table 1.

2.5. Method validation

For method validation, the linearity, sensitivity, precision, accuracy, and stability were thoroughly evaluated. To assess linearity, a precise quantity of the mixed standard solution was measured accurately and diluted to create a series of standard curve solutions with concentrations spanning from 2 to 200 ng mL^{-1}, including 2, 5, 10, 20, 50, 100, and 200 ng mL^{-1}. A standard curve was constructed by plotting the peak area (y) of the analyte against its corresponding concentration (x, ng·mL^{-1}). The sensitivity of the method was tested by determining the limit of detection (LOD) and limit of quantitation (LOQ). A series of solutions derived from a 5 ng mL^{-1} mixed standard solution and its diluted versions were analyzed. The LOD was defined as the point where the signal-to-noise ratio (S/N) reached or exceeded 3, while the LOQ was set at an S/N ratio of 10 or higher. The intra-day precision and inter-day precision of the method were both evaluated by analyzing sample solutions spiked with varying concentrations of 2, 4, and 10 ng mL^{-1}. The samples were injected six times within 0, 2, 4, 6, 8, and 10 h on the same day to determine the compound concentrations and calculate the relative standard deviation (RSD) based on the standard curve, representing the intra-day precision. Additionally, the spiked samples were repeatedly injected for three consecutive days, and the RSD of the target compound concentrations within these three days was calculated, representing the inter-day precision. The accuracy was evaluated by recovery. A sample with known quantity of the target substances was taken and mixed with standard solutions at three concentration levels (2, 4, and 10 ng mL^{-1}). The extraction and detection were performed under optimized experimental conditions. Each set of samples was conducted in triplicate. The fortified concentrations were calculated based on the standard curve, and the mean recovery rate and deviation were calculated. The stability of the targets was further examined in triplicate at a concentration of 10 ng mL^{-1} under various conditions. These conditions

Table 1
The optimized parameters of UPLC-MS/MS for the determination of gigantol, erianin, naringenin, quercetin, rutin, and *p*-coumaric acid in *Dendrobium* spp.

Compounds	Ionization mode	Retention time/min	Precursor ion (*m/z*)	Product ion (*m/z*)	CE MS1/V	CE MS2/V
Gigantol	ESI+	5.614	275.1	137.0*, 151.0	21	13
Erianin	ESI+	5.938	319.2	151.0*, 195.1	13	13
Rutin	ESI-	4.447	609.1	299.9*, 270.9	37	60
Quercetin	ESI-	5.104	301.0	150.9*, 120.9	17	21
Naringenin	ESI-	5.330	271.1	119.0*, 150.9	25	13
p-Coumaric acid	ESI-	4.606	163.0	119.0*, 93.1	33	13

encompassed different storage periods at room temperature, spanning from 0 to 72 h in increments of 4, 8, 16, 24, 48, and 72 h.

3. Results and discussion

3.1. Optimization of sample extraction

The overall process of this study is shown in Fig. 1. At the first stage, ultrasound-assisted extraction and water bath extraction were the commonly used sample preparation methods for extracting the components of *Dendrobium*. The extraction solvents used are mainly methanol and methanol-water solutions, with differences in proportion and temperature. In this experiment, the influence of different extraction temperatures and ratios of methanol in water on extraction efficiency was tested using MeOH-water mixtures in ratios of 7:3 (v/v), 80 % MeOH-water (8:2, v/v), 90 % MeOH-water (9:1, v/v), and pure methanol under water bath conditions at 60, 70, and 80 °C, respectively. The concentrations of naringenin, quercetin, rutin, gigantol, erianin, and *p*-coumaric acid under different conditions were analyzed. The results (Table 2) demonstrate that solvent composition and temperature have differential impacts on the extraction efficiency of various compounds. For instance, the optimal extraction conditions for erianin, quercetin, naringenin, and *p*-coumaric acid were observed at 60 °C using methanol, yielding concentrations of 92.14, 0.68, 3.39, and 0.19 mg·100 g^{-1}, respectively. Gigantol and rutin showed optimal extraction at 60 °C using a MeOH-water (7:3, v/v) solution, with concentrations of 2.26 and 0.17 mg·100 g^{-1}, respectively. Furthermore, it was noted that gigantol and rutin also exhibited satisfactory extraction efficiency under methanol at 60 °C conditions, with concentrations of 2.17 and 0.17 mg·100 g^{-1}, respectively. Overall, methanol extraction at 60 °C was effective for most compounds, thus chosen as the pretreatment method.

3.2. Optimization of UPLC-MS/MS

The analysis of phenolic acids, flavonoids, and bibenzyl compounds in *Dendrobium* often utilizes Waters T3 C18 and BEH C18 columns [23–25]. In this experiment, the performance of these two columns were compared in separation efficiency (Fig. 2). The results indicated that the T3 C18 column performed better than the BEH C18 column, with good peak shape and stable response of all the compounds. Consequently, the T3 C18 column (100 mm × 2.1 mm, 1.8 μm) was chosen as the optimal option for the experiment.

The composition of the mobile phase plays a crucial role in the separation and detection of components. Therefore, the effect of varying mobile phase systems on separation efficiency was extensively studied, and relevant experiments were conducted using a mixture of aqueous and organic phases, where the organic phase was acetonitrile, and the aqueous phases were low-concentration FA and AF solution. Hence, in this experimental setup, the performance of three different combinations of mobile phase, water plus acetonitrile, water with 0.1 % FA mixed with acetonitrile, and water containing both 0.1 % FA and 5 mM AF along with acetonitrile, were evaluated for the detection of the *Dendrobium* sample, as depicted in Fig. 2. After a thorough analysis of the results, it was determined that employing water containing 0.1 % FA and 5 mM AF mixed with acetonitrile as the mobile phase yielded a stable chromatographic baseline, characterized by excellent peak shapes, stable retention times, and high separation efficiency. Therefore, this combination of water (0.1 % FA and 5 mM AF) + acetonitrile was adopted as the optimal mobile phase for this method.

Utilizing UPLC-MS/MS in both positive and negative ion modes, standard solutions of the six target compounds were analyzed. Through parameter optimization, it was observed that gigantol and erianin exhibited comparatively good ionization efficiency, albeit with a slight edge in positive ion mode. Consequently, the positive ion mode was chosen as the preferred method for detecting gigantol and erianin. On the contrary, good responses were observed for naringenin, quercetin, rutin, and *p*-coumaric acid in negative ion mode, thus the negative ion mode was selected for these compounds. Based on the response intensities, characteristic parent and product ions, optimal collision energies, and optimal cone voltages were determined to establish the best mass spectrometry conditions.

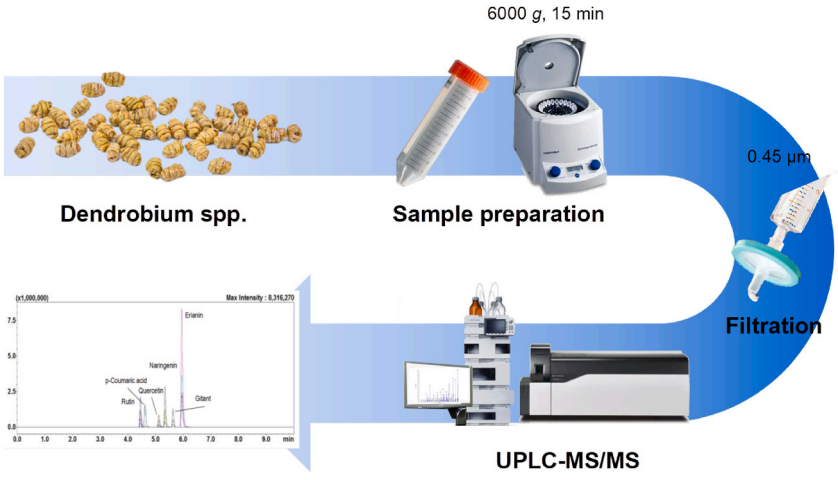

Fig. 1. The flow chart of UPLC-MS/MS based detection of gigantol, erianin, naringenin, quercetin, rutin, and *p*-coumaric acid in *Dendrobium* spp.

Table 2
The effect of solvent composition and temperature on the extraction performance of the target compounds.

	Gigantol	Erianin	Rutin	Quercetin	Naringenin	*p*-Coumaric acid
MeOH-water (7:3, v/v), 60 °C	**2.26**	90.04	**0.17**	0.56	2.91	0.15
MeOH-water (7:3, v/v), 70 °C	1.6	90.38	0.12	0.45	2.6	0.15
MeOH-water (7:3, v/v), 80 °C	1.31	88.34	0.13	0.53	3.14	0.18
MeOH-water (8:2, v/v), 60 °C	1.77	69.76	0.16	0.6	2.95	0.17
MeOH-water (8:2, v/v), 70 °C	1.66	121.1	0.14	0.63	2.72	0.17
MeOH-water (8:2, v/v), 80 °C	1.9	71.94	0.14	0.64	3.32	0.18
MeOH-water (9:1, v/v), 60 °C	1.52	82.83	0.13	0.67	3.01	0.16
MeOH-water (9:1, v/v), 70 °C	1.63	76.65	0.12	0.6	2.76	0.17
MeOH-water (9:1, v/v), 80 °C	1.6	85.4	0.14	0.63	3.14	0.17
MeOH-water, 60 °C	2.17	**92.14**	0.17	**0.68**	**3.39**	**0.19**
MeOH-water, 70 °C	1.73	84.43	0.13	0.61	3.07	0.18
MeOH-water, 80 °C	1.25	82.16	0.08	0.43	2.11	0.12

Note: *D. chrysotoxum* Lindl. was used for optimization the extraction conditions.

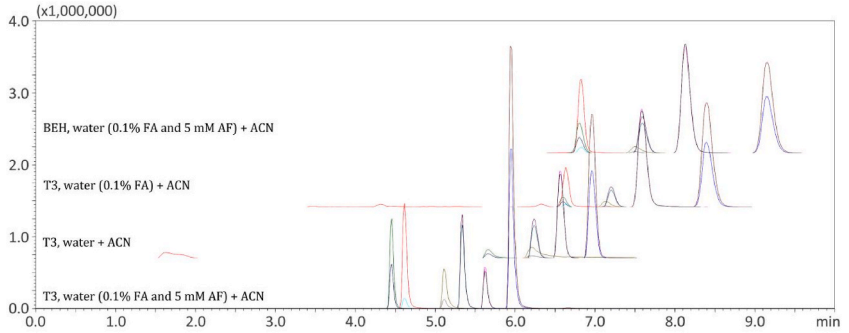

Fig. 2. The chromatogram of gigantol, erianin, naringenin, quercetin, rutin, and *p*-coumaric acid detected using a MRM mode based UPLC-MS/MS under different conditions of chromatographic columns and mobile phases.

3.3. Method performance

As evident from the validation results presented in Tables 3 and 4, all the tested compounds displayed remarkable linearity within the concentration range of 0.5–500 ng mL^{-1}, boasting correlation coefficients (R^2) exceeding 0.999. In terms of sensitivity, the method's LOD ranged from 0.34 to 4.17 ng mL^{-1}, while the LOQ ranged from 1.12 to 13.91 ng mL^{-1}. These results indicate that the method exhibits high sensitivity in detecting compounds in *Dendrobium* and is capable of detecting target substances with high sensitivity. The results of prevision demonstrated an intra-day precision of 5.2 % and an inter-day precision of 7.4 %. These results indicate that this method exhibits good precision and meets the requirements of the analytical determination. The accuracy of the method was assessed through recovery tests, revealing that the average recovery of the six target compounds ranged between 84.7 % and 106.9 %, with a deviation not exceeding 7.8 %. These experimental results indicate that the method possesses high recovery rates and excellent stability for the target compounds found in Dendrobium spp., thus fulfilling the experimental requirements. Furthermore, the stability test demonstrated that the samples remained stable, with a relative standard deviation (RSD) of ≤7.5 %, indicating no significant degradation of the target compounds during different storage periods.

3.4. Sample analysis

The collected samples were subjected to pre-treatment before being analyzed using the established UPLC-MS/MS method for the

Table 3
The linearity and sensitivity of UPLC-MS/MS for the determination of gigantol, erianin, naringenin, quercetin, rutin, and *p*-coumaric acid in *Dendrobium* spp.

Compounds	Linear range (ng·mL^{-1})	Regression equation	Correlation coefficient (R^2)	LOD (ng·mL^{-1})	LOQ (ng·mL^{-1})
Gigantol	0.5–500	y = 28540x+45180	0.9991	3.96	13.20
Erianin	0.5–500	y = 168060x+304545	0.9995	4.17	13.91
Rutin	0.5–500	y = 18650x+49260	0.9996	2.87	9.57
Quercetin	0.5–500	y = 19208x+44567	0.9993	1.22	4.05
Naringenin	0.5–500	y = 52800x+101428	0.9996	0.34	1.12
p-Coumaric acid	0.5–500	y = 53736x+110388	0.9994	1.43	4.76

Table 4
The precision and recovery of UPLC-MS/MS for the determination of gigantol, erianin, naringenin, quercetin, rutin, and *p*-coumaric acid in *Dendrobium* spp.

	Spiked level (ng·mL^{-1})	Intraday precision		Interday precision (RSD)	Recovery (%)	RSD
		Mean value	RSD			
Gigantol	2	2.07	4.1	4.5	103.4	5.7
	50	49.3	2.5	3.5	98.6	2.5
	200	201.4	3.2	3.7	100.7	4.1
Erianin	2	1.95	5.2	7.4	97.5	7.8
	50	50.75	1.8	2.2	101.5	3.1
	200	205.4	3.2	4.0	102.7	4.8
Rutin	2	1.69	2.8	3.5	84.7	2.5
	50	48.2	2.1	2.6	96.4	2.2
	200	194.8	4.4	5.1	97.4	4.4
Quercetin	2	2.14	1	1.1	106.9	1.9
	50	52.95	1.6	1.5	105.9	3.4
	200	206.4	2.5	2.5	103.2	4.5
Naringenin	2	1.73	4.9	6.8	86.4	5.8
	50	52.45	2.3	2.1	104.9	3.1
	200	198.8	4.8	6.1	99.4	4.1
p-Coumaric acid	2	1.76	2.2	2.9	88.1	0.3
	50	46.35	2.7	3.0	92.7	3.2
	200	202.8	3.3	4.4	101.4	4.5

detection of phytochemicals in *Dendrobium* spp. The results of the analysis of bioactive compounds in eight *Dendrobium* spp., including gigantol, erianin, naringenin, quercetin, rutin, and *p*-coumaric acid, are presented in Table 5. Among these species, phytochemicals such as gigantol were detected in all eight species, ranging in concentration from 0.24 to 15.12 mg·100 g^{-1}. Erianin was only detected in D. fimbriatum Hook., with high concentration of 92.14 mg·100 g^{-1}. Except for D. compactum Rolfe ex W. Hackett, rutin was detected in all species, exhibiting concentrations varying from 0.17 to 3.89 mg·100 g^{-1}. Similarly, quercetin was identified in all species, with concentrations ranging from 0.28 to 15.61 mg·100 g^{-1}. Among the eight Dendrobium spp., naringenin was present in concentrations spanning from 0.74 to 34.74 mg·100 g^{-1}. Furthermore, *p*-coumaric acid was detected in all species, displaying concentrations between 0.18 and 1.31 mg·100 g^{-1}. Overall, Den. chrysotoxum Lindl. exhibited higher levels of benzyl compounds, while D. crystallinum Rchb. f. had higher levels of flavonoids. These findings suggest that different *Dendrobium* spp. have distinct phytochemical compositions, contributing to their unique medicinal value. In conclusion, this method enables accurate and efficient simultaneous quantification of gigantol, erianin, naringenin, quercetin, rutin, and *p*-coumaric acid in *Dendrobium* samples.

4. Conclusions

The established UPLC-MS/MS method enables accurate and convenient detection of key components, such as gigantol, erianin, naringenin, quercetin, rutin, and *p*-coumaric acid, in *Dendrobium* spp. simultaneously. It provides a reliable scientific basis for quality control and safety regulation of *Dendrobium* spp. Different *Dendrobium* spp. exhibit specific variations in compound composition, with Den. fimbriatum Hook. having a higher content of benzyl compounds and Den. crystallinum. Rchb. f. having a higher content of flavonoids. These variations contribute to the unique medicinal value of each species. This study has fully utilized the advantages of UPLC-MS/MS technology, including its efficiency, accuracy, and sensitivity, and optimized factors such as sample pretreatment, UPLC conditions, and MS conditions, thereby improving the efficiency and precision of the analysis of *Dendrobium* spp. components. However, further research is needed to expand the range of sample size and sources, including samples from different geographical regions and under different ecological conditions, in order to reveal the diversity and variations more comprehensively. Moreover, exploring additional techniques and approaches is necessary to deeply investigate the active ingredients and mechanisms of action of *Dendrobium* spp., providing more comprehensive scientific support for its extensive application in medicine and healthcare.

Ethical approval

This article does not contain any studies involving human participants. All applicable guidelines for the use and care of animals were strictly adhered to, following institutional, national, and international standards.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Jingjing Liang: Writing – original draft, Methodology, Investigation. **Ruyan Chen:** Software, Investigation, Formal analysis.

Table 5
The contents of gigantol, erianin, naringenin, quercetin, rutin, and *p*-coumaric acid in different *Dendrobium* spp.

Dendrobium spp. (mg·100g^{-1})	Gigantol	Erianin	Rutin	Quercetin	Naringenin	*p*-Coumaric acid
D. officinale Kimura et Migo	0.24	n.d.	1.23	0.52	1.80	0.18
D. devonianum	1.40	n.d.	0.95	0.43	3.41	0.40
D. catenatum Lindl.	4.22	n.d.	3.89	1.03	0.78	0.56
D. compactum Rolfe ex W. Hackett	0.53	n.d.	0.00	0.31	1.61	0.16
D. fimbriatum Hook.	15.12	n.d	0.54	2.85	1.73	1.31
D. chrysotoxum Lindl.	2.26	92.14	0.17	0.68	3.39	0.19
D. crystallinum Rchb. f.	4.19	n.d.	0.50	15.61	34.74	0.41
D. aphyllum (Rohb.) C. E. Fishcher.	0.52	n.d.	0.26	0.28	3.59	0.28

Wenping Zhang: Resources, Data curation. **Lei Luo:** Validation, Methodology. **Yun Wang:** Visualization, Resources. **Qing Shen:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] B. Hou, J. Luo, Y. Zhang, Z. Niu, Q. Xue, X. Ding, Iteration expansion and regional evolution: phylogeography of Dendrobium officinale and four related taxa in southern China, Sci. Rep. 7 (1) (2017) 43525.
[2] J. Cheng, P.P. Dang, Z. Zhao, L.C. Yuan, Z.H. Zhou, D. Wolf, Y.B. Luo, An assessment of the Chinese medicinal Dendrobium industry: supply, demand and sustainability, J. Ethnopharmacol. 229 (2019) 81–88.
[3] Z.H.A.I. Denghui, L.I.N. Yanduan, N.I. Jun, L.I.N. Weilong, C.A.I. Jinyan, Z.H.A.O. Lin, Chemotaxonomic and pharmacological properties on Dendrobium chrysanthum Lindl, Journal of Holistic Integrative Pharmacy 2 (2) (2021) 112–122.
[4] A. Kirkwood, I. Fisk, C. Ayed, Y. Xu, N. Yang, A flavour perspective of Tiepishihu (Dendrobium officinale)-an emerging food ingredient from popular traditional Chinese medicinal plants: a review, Int. J. Food Sci. Technol. 58 (10) (2023) 4921–4930.
[5] W. Zhao, Y. Chen, Y. Tian, Y. Wang, J. Du, X. Ye, C. Sun, Dietary supplementation with Dendrobium officinale leaves improves growth, antioxidant capacity, immune function, and gut health in broiler chickens, Front. Microbiol. 14 (2023) 1255894.
[6] J. Xu, Q.B. Han, S.L. Li, X.J. Chen, X.N. Wang, Z.Z. Zhao, H.B. Chen, Chemistry, bioactivity and quality control of Dendrobium, a commonly used tonic herb in traditional Chinese medicine, Phytochemistry Rev. 12 (2013) 341–367.
[7] Y. Yuan, M. Yu, B. Zhang, X. Liu, J. Zhang, Comparative nutritional characteristics of the three major Chinese Dendrobium species with different growth years, PLoS One 14 (9) (2019) e0222666.
[8] S.M. Zuo, H.D. Yu, W. Zhang, Q. Zhong, W. Chen, W. Chen, H. Chen, Comparative metabolomic analysis of Dendrobium officinale under different cultivation substrates, Metabolites 10 (8) (2020) 325.
[9] M. Moudi, R. Go, C.Y.S. Yien, M.N. Saleh, A review on molecular systematic of the genus Dendrobium Sw, Acta Biol Malays 2 (2) (2013) 71–78.
[10] Y. Zhu, J. Yu, C. Jiao, J. Tong, L. Zhang, Y. Chang, Y. Cai, Optimization of quercetin extraction method in Dendrobium officinale by response surface methodology, Heliyon 5 (9) (2019) e02374.
[11] T. Lni, X.L. ChenN, G.W. Wu, L.J. Sha, J. Wang, Z.X. Hu, H.C. Liu, A simple method for distinguishing Dendrobium devonianum and Dendrobium officinale by ultra performance liquid chromatography-photo diode array detector, Food Sci. Technol. 43 (2023).
[12] C. Jiang, Y. Luo, Y. Yuan, X. Dong, Y. Zhao, L. Huang, Conventional octaplex PCR for the simultaneous identification of eight mainstream closely related Dendrobium species, Ind. Crop. Prod. 112 (2018) 569–576.
[13] W. Chen, X. Chen, J. Xu, J. Cai, X. Wang, Identification of Dendrobium officinale using DNA barcoding method combined with HRM and qPCR technology, Food Anal. Methods (2022) 1–11.
[14] L. Ge, H. Yang, W. Lu, Y. Cui, S. Jian, G. Song, Q. Shen, Identification and comparison of palmitoleic acid (C16: 1 n-7)-derived lipids in marine fish by-products by UHPLC-Q-exactive orbitrap mass spectrometry, J. Food Compos. Anal. 115 (2023) 104925.
[15] M. Zhang, P. Wang, D. Jin, S. Jian, J. Wu, M. Huang, Q. Shen, Chain-locked precursor ion scanning based HPLC–MS/MS for in-depth molecular analysis of lipase-catalyzed transesterification of structured phospholipids containing ω-3 fatty acyl chains, Food Chem. 399 (2023) 133982.
[16] Q. Shen, H. Wang, B. Quan, X. Sun, G. Wu, D. Huang, P. Luo, Rapid quantification of bioactive compounds in Salvia miltiorrhiza Bunge derived decoction pieces, dripping pill, injection, and tablets by polarity-switching UPLC-MS/MS, Front. Chem. 10 (2022) 964744.
[17] Y. Mu, L. Cheng, X. Gong, J. Ma, S. Zhang, Y. Mu, C. Zhao, Simultaneous determination of nine phenolic compounds in imitation wild Dendrobium officinale samples using ultrahigh-performance liquid chromatography–tandem mass spectrometry, Front. Nutr. 10 (2023) 1129953.
[18] J. Liu, Z. Li, M. Zeng, Y. Liang, B. Liang, Q. Ge, W. Zhou, Rapid comparison of antitumor chemical constituents and mechanisms between Dendrobium nobile and Dendrobium officinale by UPLC-IT-TOF, network pharmacology and experimental verification, Acta Chromatogr. 35 (2) (2023) 123–138.
[19] C. Wu, S. Gui, Y. Huang, Y. Dai, Q. Shun, K. Huang, G. Wei, Characteristic fingerprint analysis of Dendrobium huoshanense by ultra-high performance liquid chromatography-electrospray ionization-tandem mass spectrometry, Anal. Methods 8 (18) (2016) 3802–3808.
[20] X. Xing, S.W. Cui, S. Nie, G.O. Phillips, H.D. Goff, Q. Wang, A review of isolation process, structural characteristics, and bioactivities of water-soluble polysaccharides from Dendrobium plants, Bioactive Carbohydrates and Dietary Fibre 1 (2) (2013) 131–147.
[21] Y. Yuan, X. Tang, Z. Jia, C. Li, J. Ma, J. Zhang, The effects of ecological factors on the main medicinal components of Dendrobium officinale under different cultivation modes, Forests 11 (1) (2020) 94.

[22] T.B. Ng, J. Liu, J.H. Wong, X. Ye, S.C. Wing Sze, Y. Tong, K.Y. Zhang, Review of research on Dendrobium, a prized folk medicine, Appl. Microbiol. Biotechnol. 93 (2012) 1795–1803.
[23] X. Wu, H. Ding, Z. Zhang, M. Zheng, H. Ni, Z. Huang, D. Guo, An improved strategy for identification and annotation of easily in-sourced dissociation diterpene lactones from plant natural products: taking Andrographis paniculata (Burm. f.) as an example, Rapid Commun. Mass Spectrom. 37 (9) (2023) e9483.
[24] D. Rao, R. Zhao, Y. Hu, H. Li, Z. Chun, S. Zheng, Revealing of intracellular antioxidants in Dendrobium nobile by high performance liquid chromatography-tandem mass spectrometry, Metabolites 13 (6) (2023) 702.
[25] C. Jiao, C. Song, S. Zheng, Y. Zhu, Q. Jin, Y. Cai, Y. Lin, Metabolic profiling of Dendrobium officinale in response to precursors and methyl jasmonate, Int. J. Mol. Sci. 19 (3) (2018) 728.