Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.110122

A simple method for distinguishing *Dendrobium devonianum* and *Dendrobium officinale* by ultra performance liquid chromatography-photo diode array detector

Tao LIN[1], Xing-Lian CHEN[1], Guang-Wei WU[2], Ling-Jie SHA[1], Jing WANG[3], Zheng-Xu HU[3], Hong-Cheng LIU[1]*

Abstract

Using UPLC and PDA detector, the fingerprints of *Dendrobium devonianum* and *Dendrobium officinale* produced in the Longling area of Yunnan Province, China were obtained quickly and efficiently, and 26 common peaks in *Dendrobium devonianum* and *Dendrobium officinale* samples were obtained by automatic peak matching through the "Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System". The relative peak areas of the common peaks were analyzed by PCA using R, and the results showed that *Dendrobium devonianum* and *Dendrobium officinale* could be well distinguished by PCA, which was consistent with the analysis results of OPLS-DA. The results fully demonstrate that *Dendrobium devonianum* and *Dendrobium officinale* grown in the same area can be effectively distinguished by using the method of common peaks combined with stoichiometry. The VIP value of the 13 common peaks is greater than 1.0, which has a significant contribution to the difference and it was differential marker in *Dendrobium devonianum* and *Dendrobium officinale*. At the same time, normal-phase silica gel column chromatography was used to separate and purify the compounds that are related to the common peaks, and a total of 7 compounds were identified, among which eupatolide and isoschaftoside belong to differential markers and characteristic compounds in *Dendrobium devonianum* and *Dendrobium officinale*, which have good biological health care activity. The successful identification of *Dendrobium devonianum* and *Dendrobium officinale* in Longling area of Yunnan Province and the discovery of related differential markers play an important role in the further research and development of *Dendrobium devonianum* as a new food raw material and its products.

Keywords: distinguishing; *Dendrobium devonianum*; *Dendrobium officinale*; ultra performance liquid chromatography; PCA; OPLS-DA.

Practical Application: *Dendrobium devonianum* and *Dendrobium officinale* could be better separated by PCA and OPLS-DA analysis. The VIP value of 13 common peaks ≥ 1.0 has a significant contribution to the difference, which was a differential marker in *Dendrobium devonianum* and *Dendrobium officinale*. Two of differential markers were eupatolide and isoschaftoside, and their better biological activity was of great significance for the quality control of *Dendrobium devonianum* and its wide application in the food field.

1 Introduction

Dendrobium devonianum Paxt., as a characteristic species of *Dendrobium* in Longling area of Yunnan Province, China, has good biological health effects (Deng et al., 2018; Wu et al., 2019; Fan et al., 2022; Wang et al., 2022). In China, like *Dendrobium officinale* Kimura et Migo (Wang et al., 2022; Li et al., 2021), *Dendrobium devonianum* is also a medicinal and food homologous plant, which was a Chinese medicinal material, and can also be used as a local specialty food or as a raw material for making other foods (Heussner & Bingle, 2015; Luo et al., 2021; Dong et al., 2016). The distribution of *Dendrobium devonianum* was mainly concentrated in the southeast to west of Yunnan, northwest Guangxi, southwest Guizhou (Wu et al., 2020), while the quality produced in Longling area was higher than that of *Dendrobium officinale* produced locally. Because the quality of Chinese medicinal materials was closely related to the environment of their origin (Liu et al., 2021; Xu et al., 2013; Zhang et al., 2022), the origin of *Dendrobium officinale* was not in the Longling area, but was an imported variety from other origins (Yan et al., 2020).

Dendrobium devonianum and *Dendrobium officinale* were both the main cultivars in Longling area. In view of the superior biological activity of *Dendrobium devonianum*, *Dendrobium ffficinale* produced in Longling area was often sold as *Dendrobium devonianum*, especially when processed into *Dendrobium devonianum* tea, *Dendrobium devonianum* superfine powder and other foods, it was difficult to distinguish the counterfeit food of *Dendrobium officinale* (Lin et al., 2022), and a quick and

Received 30 Sept., 2022
Accepted 14 Nov., 2022
[1] *Quality Standards and Testing Technology Research Institute, Yunnan Academy of Agricultural Sciences, Kunming, China*
[2] *College of Chemical Engineering, Nanjing Forestry University, Nanjing, China*
[3] *Longling Agricultural Environmental Protection Monitoring Station, Longling, China*
*Corresponding author: lhc@yaas.org.cn

easy method for distinguishing *Dendrobium devonianum* and *Dendrobium officinale* was urgently needed.

At present, the most commonly used method to distinguish different species of *Dendrobium* was high-resolution mass spectrometry combined with chemometric methods (Lin et al., 2022; Ma et al., 2018; Yang et al., 2020; Fang et al., 2020), although high-resolution mass spectrometry can effectively identify different species of *Dendrobium*, but the high-resolution mass spectrometry used was expensive, and most of the ordinary laboratory conditions were difficult to achieve, especially for the original areas in China where *Dendrobium devonianum* and *Dendrobium officinale* were harvested and processed, there was almost no high-resolution mass spectrometry in the laboratory. Therefore, it was very important to establish method of fast and simple instrument needed for distinguishing *Dendrobium devonianum* and *Dendrobium officinale*, which was important for the authenticity identification and quality control in the process of raw material acquisition, processing and sales in the production area of *Dendrobium devonianum*.

The fingerprint method was a more traditional identification method in China, but the similarity was used in the fingerprint method, which has certain limitations. For example, after the powder of *Dendrobium officinale* was added in a fixed proportion to the powder of *Dendrobium devonianum*, the similarity remains unchanged, but the relative content of *Dendrobium devonianum* in it was reduced and the content of relevant active ingredients was also reduced. Inspired by the traceability method of high-resolution mass spectrometry, UV spectroscopy combined with chemometric analysis can be effectively applied to related food fields (Tahir et al., 2022; Núñez et al., 2020; Campmajó et al., 2019). In this study, UPLC and PDA detector were used to quickly collect the fingerprints of *Dendrobium devonianum* and *Dendrobium officinale* produced in Longling, Yunnan Province, China. PCA, OPLS-DA analysis and modern separation and purification techniques as well as NMR spectroscopic were utilized in order to successfully identify *Dendrobium devonianum* and *Dendrobium officinale*, and to find the differential markers that can effectively identify *Dendrobium devonianum* and *Dendrobium officinale*. The research results will provide a simple and fast method for the identification and origin of *Dendrobium devonianum*, which is a characteristic *Dendrobium* resource in Yunnan Province, and will be of great significance for the quality control, quality improvement and wide application of *Dendrobium devonianum* as a new food raw material.

2 Materials and methods

2.1 Sample collection and preparation

In the Longling area of Yunnan Province, China, local specialized farmers' cooperatives and companies that grow *Dendrobium* were selected. Fresh stems (length about 15-20 cm) of 13 samples of *Dendrobium devonianum* and 13 samples of *Dendrobium officinale* with a growth period of more than three years were collected, dried at 60 °C, pulverized, sieved by 0.28 μm sample sieve, and then stored at 4 °C in the dark.

2.2 Chemicals and reagents

Ammonium acetate (≥ 99.995%) and formic acid of HPLC grade were obtained from Millipore Sigma Company (St Louis, MO, USA). Highly purified water was prepared by a centralized laboratory water systems from Elga (Wycombe, United Kingdom). Methanol of HPLC grade were purchased from Merck KGaA (Darmstadt, Germany).

2.3 Sample preparation and analysis

Sample preparation method

2.0 g of the sample was weighed and put into a 50 mL centrifuge tube, 20 mL methanol-water solution (V:V = 90:10) was added, vortexed for 1 min and then sonicated for 30 min. After centrifugation at 5000 r/min for 5 min, 5 mL of the supernatant was taken and evaporated to dryness under reduced pressure on a rotary evaporator at 40 °C, and then reconstituted with 1 mL of methanol-water solution (V:V = 90:10), filtered through a 0.22 μm membrane and analyzed by UPLC.

Instrumental analysis method

Waters ACQUITY UPLC I-Class was used for data acquisition along with Diode Array Detector (Waters, MA, USA) and Waters ACQUITY UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm, Waters, MA, USA), the column temperature maintained at 35 °C. Solvents A was ultrapure water with 0.01% formic acid and B was methanol. The flow rate for UPLC was 0.2 mL/min with the following gradient: 5% B ~ 15% B (0~5.0 min), 15% B ~ 55% B (5.0~20.0 min), 55% B ~ 95% B (20.0~30.0 min), 95% B (30.0~35.0 min), 95% B ~ 5% B (35.0~35.5 min), 5% B (35.1~40.0 min). The injection volume was 2 μL and the range of UV spectral scans was 210-400 nm.

Data processing and differential marker identification

The chromatogram obtained by UV absorption at 360 nm was selected, and the obtained chromatograms of 26 Dendrobium samples were imported into the "Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System (Chinese Pharmacopoeia Commission, 2012)" in CDF format. The chromatograms of the samples with relatively large, stable, and well-resolved chromatographic peaks were selected as reference chromatogram. Multi-point correction was performed using the median method with a time window width of 0.1, automatic peak matching was performed, and a common pattern was established to identify common peaks in *Dendrobium devonianum* and *Dendrobium officinale* samples. Principal component analysis was performed on the common peaks using R, and the 26 samples were analyzed by OPLS-DA using SIMCA-P 14.1 software, and the VIP values and differential markers of each common peak were obtained.

3 Results and discussion

3.1 Optimum selection of chromatographic conditions

Compared with traditional HPLC, the UPLC used in this study has better separation effect, faster analysis speed, and the

analysis time of a single sample was only 40 minutes, while the analysis time of HPLC was about 70 minutes (Ye et al., 2017), which greatly saves analysis time and less mobile phase volume required to reduce environmental pollution. On the other hand, compared with HPLC, the injection volume was generally 10 μL, while the injection volume of UPLC was smaller. In this experiment, the injection volume was only 2 μL, but a similar or even better chromatographic separation effect has been achieved, compared with HPLC.

In this study, the separation effects of different elution gradients on the sample were compared. As shown in Figure 1, there were many chromatographic peaks in the sample, especially between 5 min and 20 min, when the elution gradient during this period was 15% ~ 75% methanol, many chromatographic peaks cannot be completely separated, and as the proportion of organic phase was continuously reduced, the trend of chromatographic separation gradually improves, but if the proportion of organic phase was lower, the retention time of some compounds was increased. On the whole, the gradient between 5 min and 20 min was selected as 15%~ 55% methanol. Under this chromatographic condition, the compounds could be effectively separated and a better chromatographic separation effect was achieved.

3.2 Selection of detection wavelength

The responses of different compounds in the sample at 220, 290, 360 and 395 nm were compared respectively. As shown in Figure 2, at the absorption wavelengths of 220 and 290 nm, the response of each compound was lower and the chromatographic peaks were less. When the absorption wavelength was 360 nm, more chromatographic peaks appear within 6-20 minutes, because the main components of *Dendrobium* (including flavonoids, polyphenols and bibenzyl compounds) have an ultraviolet absorption wavelength around 360 nm (Liang et al., 2019; Phechrmeekha et al., 2012; Zhang et al., 2019; Ma et al., 2019; Sritularak & Likhitwitayawuid, 2009). When it was 395 nm, the response of compounds was low within 6-20 min, so the absorption wavelength of 360 nm was selected as a suitable one.

3.3 Repeatability and stability

The quality control samples (QC) were prepared by mixing 50 μL of each of the 26 samples in this experiment to investigate the repeatability and stability of the method. Under optimized and stable instrument conditions, QC was injected continuously for 6 times on the same day, and the samples were injected at the same time (every morning at 10 am) on day 0, 0.5, 1, 2, 4 and 6, respectively, the peak area of each common peak's RSD was calculated. The results show that the peak area of the common peak, the RSD range of the same day was 0.19-1.28%, at different times was 0.78-2.69%, the stability and repeatability of injections on the same day and different days were good.

3.4 PCA analysis of Dendrobium devonianum and Dendrobium officinale

The chromatographic peaks (common peaks) that in all samples were pointed out by "Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System (2012 Edition)" through the peak alignment, but the common peaks may not be the main peaks in chromatograms due to the different varieties, sources, etc. In this study, after automatic peak matching through the

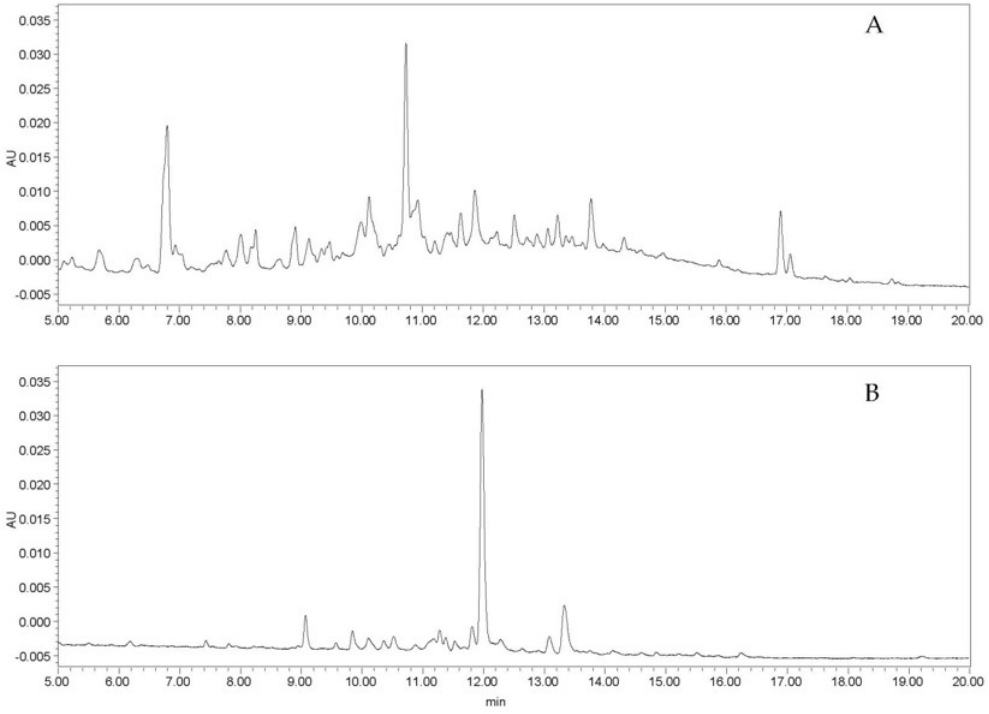


Figure 1. The separation effects of different elution gradients in 360 nm (A: 5-20 min with the elution gradient of 15% to 75% methanol, B: 5-20 min with the elution gradient of 15% to 55% methanol).

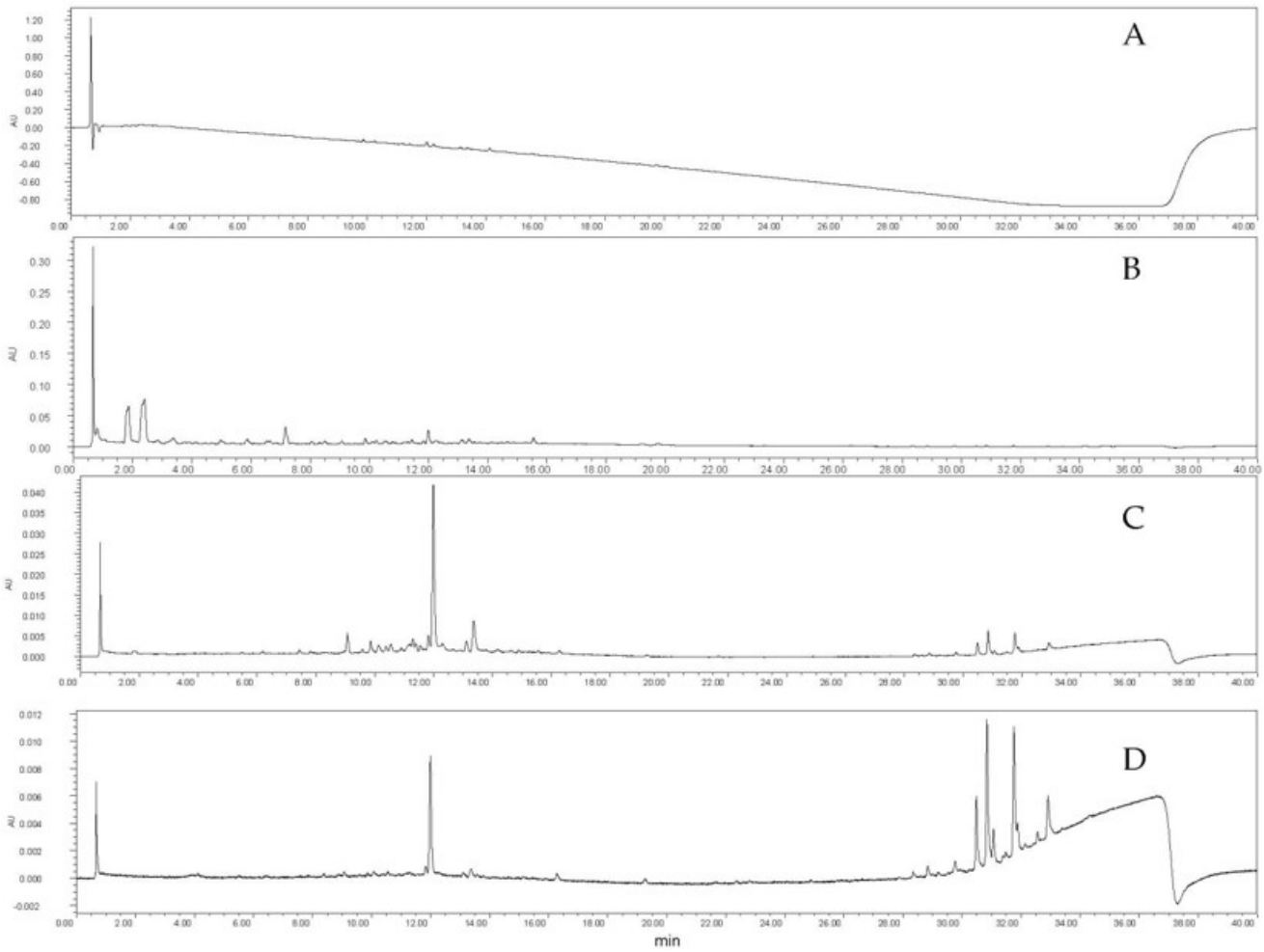


Figure 2. Compound response at different UV absorption wavelengths (A: 220 nm, B: 290 nm, C: 360 nm, D: 395 nm).

"Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System (2012 Edition)", 26 common peaks in the samples of *Dendrobium devonianum* and *Dendrobium officinale* were found. The relative peak areas of the common peaks were used as different risks in the principal component analysis using R. The results were shown in Figure 3, *Dendrobium devonianum* and *Dendrobium officinale* can be well distinguished by PCA, indicating that the use of common peaks combined with PCA can effectively distinguish *Dendrobium devonianum* and *Dendrobium officinale* grown in the same area.

3.5 OPLS-DA analysis of Dendrobium devonianum and Dendrobium officinale

The relative peak areas of the common peaks of the 26 samples were analyzed by OPLS-DA using SIMCA-P 14.1 software, as shown in Figure 4, it can be seen that Dendrobium devonianum and Dendrobium officinale produced in Longling area can be clearly divided into two groups by OPLS-DA, which were consistent with the PCA analysis results.

Further, each common peak was subjected to VIP analysis, and the differential marker was screened by the VIP value. When the VIP value is less than 0.5, it was considered as an irrelevant variable (Galindo-Prieto et al., 2014; Paban et al., 2014), and when the VIP value was greater than or equal to 0.5, it indicates that there was a difference and can be used as a differential marker (Yang et al., 2017), and when the VIP value was greater than or equal to 1.0, it indicates that the contribution of the difference was the most significant (Zhou et al., 2022; Jang et al., 2018; Morvan & Demidem, 2018). The VIP values of the common peaks were shown in Figure 5. The VIP values of the total 13 peaks of S18, S4, S5, S20, S25, S21, S22, S17, S23, S7, S8, S14 and S6 were ≥ 1.0, which contributions were significant, so these peaks were selected as differential markers in *Dendrobium devonianum* and *Dendrobium officinale*. The VIP rankings and retention times of the 26 common peaks were shown in Table 1.

3.6 Separation and purification of differential marker from Dendrobium devonianum

Dendrobium devonianum was used as the raw material, the compounds that are related to the common peaks were separated and purified by normal-phase silica gel column chromatography, and their structures were elucidated by ^{1}H-NMR and ^{13}C-NMR spectroscopic comparison with literature data. Unfortunately, due to the low content of some compounds

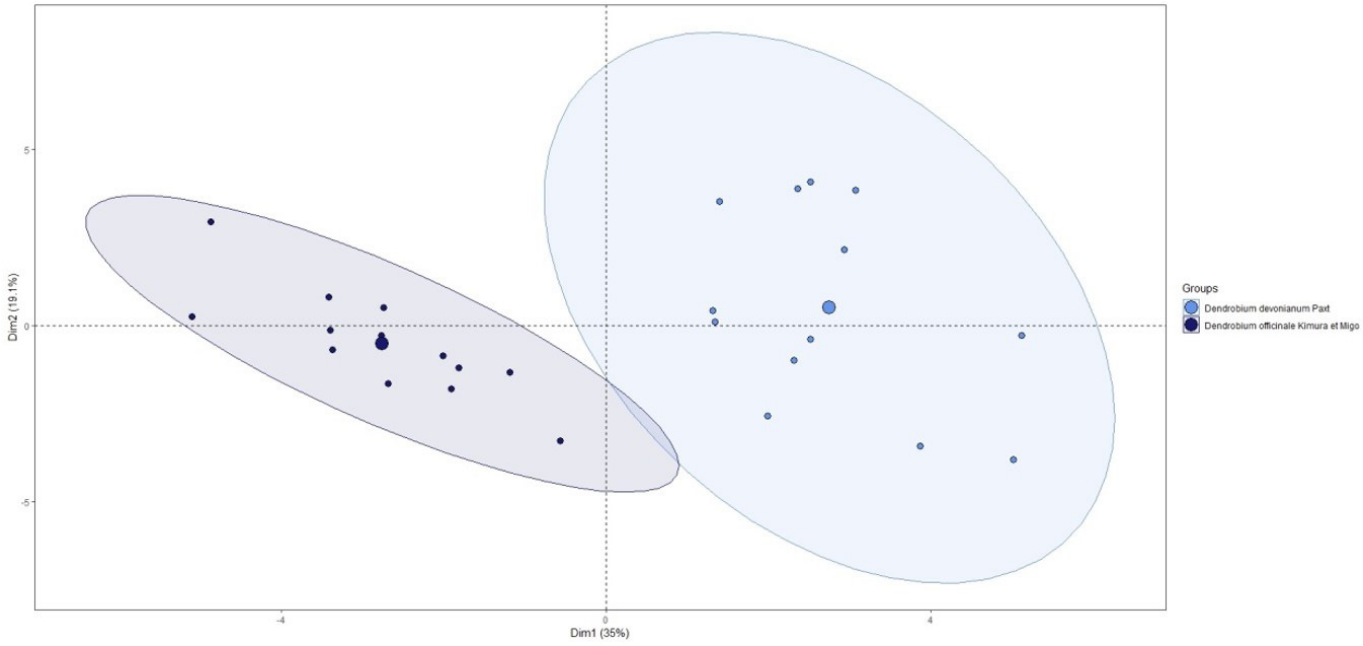


Figure 3. PCA - Principal Component Analysis of *Dendrobium devonianum* and *Dendrobium officinale* by R.

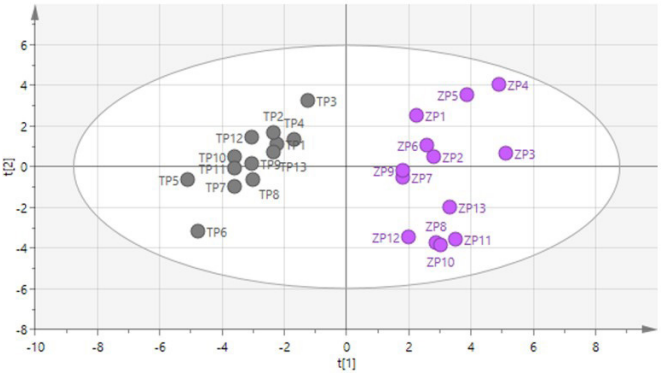


Figure 4. PCA of *Dendrobium devonianum* and *Dendrobium officinale* by OPLS-DA (ZP: *Dendrobium devonianum*, TP: *Dendrobium officinale*).

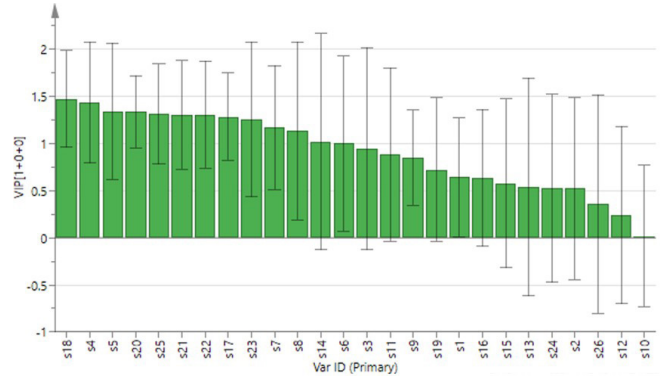


Figure 5. VIP value of common peak.

Table 1. The VIP - Variable Influence on Projection rankings and retention times of the 26 common peaks.

Ranking of VIP Value	Common peaks	Retention time (min)
1	S18	28.812
2	S4	9.877
3	S5	10.149
4	S20	30.408
5	S25	32.440
6	S21	30.773
7	S22	30.938
8	S17	28.320
9	S23	31.673
10	S7	10.576
11	S8	11.350
12	S14	15.790
13	S6	10.400
14	S3	9.636
15	S11	14.233
16	S9	11.955
17	S19	29.820
18	S1	8.898
19	S16	21.664
20	S15	16.256
21	S13	15.532
22	S24	31.842
23	S2	9.100
24	S26	32.766
25	S12	14.996
26	S10	13.408

in *Dendrobium devonianum*, all the common peaks were not successfully separated and purified, and only 7 compounds were identified, as shown in Table 2, one of which was dendrophenol, and its VIP value < 0.5, indicating that it was an irrelevant variable in *Dendrobium devonianum* and *Dendrobium officinale*, but was a characteristic component in other *Dendrobium* species

Table 2. NMR Nuclear Magnetic Resonance data and VIP values of 7 common peaks.

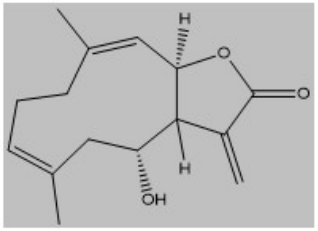
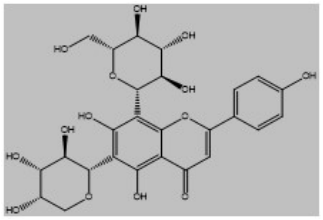
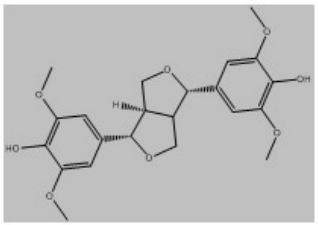
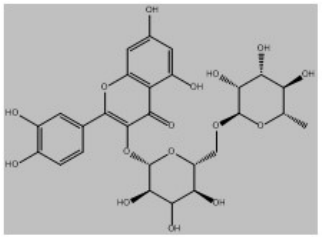
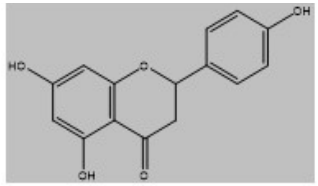
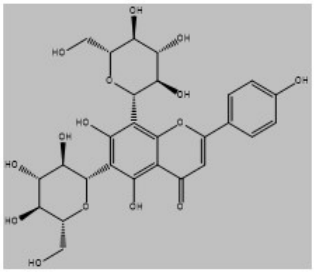
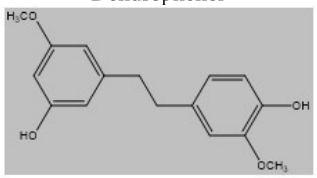
Compound and Structure	NMR	VIP Value	Peak number (retention time/min)	References
Eupatolide 	^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 1.55 (3H, br s, H-15a), 1.57 (3H, br s, H-4), 2.07 (1H, m, H-2b), 2.05 (1H, m, H-3a), 2.18 (1H, br d, 12.6, H-8b), 2.25 (1H, m, H-3b), 2.27 (1H, m, H-2a), 2.48 (1H, dd, 12.5, 4.0, H-9a), 2.85 (1H, dd, 7.8, 4.0, H-7), 4.47 (1H, m, H-8), 4.74 (1H, dd, 11.0, 1.7, H-1a), 4.76 (1H, d, 9.5, H-5a), 5.11 (1H, dd, 7.8, 4.0, H-6), 6.13 (1H, d, 3.0, H-13a), 5.62 (H, d, 3.0, H-13b). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 136.0 (C-1), 25.6 (C-2), 38.7 (C-3), 140.4 (C-4), 128.0 (C-5), 74.8 (C-6), 52.7 (C-7), 70.3 (C-8), 47.1 (C-9), 127.7 (C-10), 138.8 (C-11), 169.9 (C-12), 120.1 (C-13), 16.8 (C-14), 19.5 (C-15)	VIP Value < 1.5	S7 (10.576)	Lee et al. (1972), Zhou et al. (1994)
Isoschaftoside 	^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 6.69 (1H, br s, H-3), 6.72 (2H, d, J=8.7 Hz, H-3', 5'), 7.99 (2H, d, J=8.4 Hz, H-2', 6'), 13.67 (1H, br s, OH-5); 6-*C*-α-Ara: 3.44 (1H, m, H-3"), 3.78 (1H, m, H-4"), 3.81, 3.59 (2H, m, 5"-CH_2), 4.04 (1H, br m, H-2"), 4.64 (1H, d, J=9.4 Hz, H-1"); 8-*C*-β-Glc: 3.28 (1H, m, H-5'''), 3.32 (1H, m, H-3'''), 3.37 (1H, m, H-4'''), 3.75, 3.56 (2H, m, 6'''-CH_2), 3.92 (1H, m, H-2'''), 4.83 (1H, d, J=9.9 Hz, H-1'''). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 128.8 (C-2', 6'), 115.9 (C-3', 5'), 102.2 (C-3); 6-*C*-α-Ara: 74.1 (C-1"), 69.2 (C-2"), 74.1 (C-3"), 68.6 (C-4"), 69.9 (C-5"); 8-*C*-β-Glc: 73.9 (C-1'''), 71.2 (C-2'''), 78.9 (C-3'''), 70.5 (C-4'''), 81.7 (C-5'''), 61.2 (C-6''')	VIP Value < 1.5	S8 (11.350)	Fernando et al. (2019), Xie et al. (2003)
(+)-Syringaresinol 	^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 3.05 (2H, br s, H-8, 8'), 3.87 (2H, m, H-9, 9'), 3.88 (12H, s, OMe), 4.27 (2H, m, H-9, 9"), 4.70 (2H, s, H-7, 7'), 5.49 (2H, s, OH), 6.55 (4H, s, H-2, 6, 2', 6'). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 147.6 (C-3, 5, 3', 5'), 134.6 (C-4, 4'), 132.6 (C-1, 1'), 103.2 (C-2, 6, 2', 6'), 86.7 (C-7, 7'), 72.3 (C-9, 9'), 56.8 (OMe), 54.8 (C-8, 8')	0.5 < VIP Value < 1.0	S11 (14.233)	Park et al. (2003), El-Hassan et al. (2003)
Rutin 	^{1}H-NMR (MeOH-d_4, 500 MHz) δ: 1.15 (3H, d, J = 6.1 Hz, rha-H-6), 4.54 (1H, d, J = 1.5 Hz, rha-H-1), 5.13 (1H, d, J = 7.7 Hz, glc-H-1), 6.22 (1H, d, J = 2.2 Hz, H-6), 6.43 (1H, d, J = 2.2 Hz, H-8), 6.88 (H, d, J = 8.3 Hz, H- 5'), 7.64 (1H, dd, J = 8.6, 2.2 Hz, H-6'), 7.68 (1H, d, J = 2.5 Hz, H-2'). ^{13}C-NMR (MeOH-d_4, 125 MHz) δ: 159.6 (C-2), 135.7 (s, C-3), 179.6 (s, C-4), 163.2 (s, C-5), 100.1 (d, C-6), 166.3 (s, C-7), 95.1 (d, C-8), 158.7 (s, C-9), 105.9 (s, C-10), 123.4 (s, C-1'), 116.3 (d, C-2'), 146.1 (s, C-3'), 150.2 (s, C-4'), 117.8 (d, C-5'), 123.8 (d, C-6'), 104.9 (d, glc-C-1), 76.0 (d, glc-C-2), 78.3 (d, glc-C-3), 71.6 (d, glc-C-4), 77.5 (d, glc-C-5), 68.9 (t, glc-C-6), 102.5 (d, rha-C-1), 72.4 (d, rha-C-2), 72.2 (d, rha-C-3), 74.2 (d, rha-C-4), 69.9 (d, rha-C-5), 18.1 (q, rha-C-6).	0.5 < VIP Value < 1.0	S9 (11.955)	Li et al. (2008), Meng et al. (2009)

Table 2. Continued...

Compound and Structure	NMR	VIP Value	Peak number (retention time/min)	References
Naringeni  n	^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 2.71 (1H, dd, J=2.5, 16.0 Hz, H-3a), 3.15 (1H, dd, J=13.3, 16.0 Hz, H-3b), 5.43 (1H, dd, J=2.5, 13.3 Hz, H-2), 5.98 (2H, s, H-6, 8), 6.88 (2H, d, J=8.0 Hz, H-3', 5'), 7.37 (2H, d, J=8.0 Hz, H-2', 6'), 12.18 (1H, s, OH-5). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 41.84 (C-3), 78.35 (C-2), 94.30 (C-8), 95.25 (C-6), 101.64 (C-10), 114.65 (C-3', C-5'), 127.50 (C-2', C-6'), 129.24 (C-1'), 157.18 (C-4'), 162.90 (C-9), 163.82 (C-5), 165.83 (C-7), 195.82 (C-4)	0.5 < VIP Value < 1.0	S13 (10.400)	Jeon et al. (2008), Singh et al. (2018)
Vicenin II 	^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 6.43 (1H, s, H-3), 6.86 (2H, d, J=8.8 Hz, H-3', 5'), 7.92 (2H, d, J=8.8 Hz, H-2', 6'), 13.69 (1H, s, OH-5); 6-C-β-Glc: 3.17 (1H, m, H-5"), 3.22 (2H, m, H-3", 4"), 3.64, 3.49 (2H, m, 6"-CH_2), 4.07 (1H, m, H-2"), 4.63 (1H, d, J=9.8 Hz, H-1"); 8-C-β-Glc: 3.29 (1H, m, H-3'"), 3.34 (1H, m, H-4'"), 3.26 (1H, m, H-5'"), 3.74, 3.53 (2H, m, 6'"-CH_2), 3.86 (1H, m, H-2'"), 4.83 (1H, d, J=9.8 Hz, H-1'"). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ: 127.8 (C-2', 6'), 115.3 (C-3', 5'), 101.0 (C-3); 6-C-β-Glc: 74.2 (C-1"), 70.6 (C-2"), 78.6 (C-3'"), 70.3 (C-4'"), 80.4 (C-5"), 60.9 (C-6"); 8-C-β-Glc: 74.3 (C-1'"), 71.6 (C-2'"), 78.9 (C-3'"), 70.6 (C-4'"), 81.2 (C-5'"), 61.2 (C-6'")	0.5 < VIP Value < 1.0	S2 (9.100)	Xie et al. (2003), Siciliano et al. (2004)
Dendrophenol 	^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 2.73 (2H, m, H-7'), 2.77 (2H, m, H-7), 3.69 (3H, s, 3′-OCH_3), 3.77 (3H, s, 5-OCH_3), 6.18 (1H, t, J=2.2 Hz, H-4), 6.20 (1H, t, J=2.0 Hz, H-2), 6.22 (1H, t, J=1.6 Hz, H-6), 6.59 (1H, dd, J=8.0, 1.9 Hz, H-6'), 6.65 (1H, d, J=1.9 Hz, H-2'), 6.68 d (1H, d, J=8.0 Hz, H-5'). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 38.5 (C-7′), 121.9 (C-6′), 116.0 (C-5′), 145.6 (C-4′), 56.3 (3′-OCH_3), 148.7 (C-3′), 113.4 (C-2′), 134.8 (C-1′), 39.6 (C-7), 106.7 (C-6), 55.6 (5-OCH_3), 162.2 (C-5), 99.9 (C-4), 159.4 (C-3), 109.2 (C-2), 145.6 (C-1)	VIP Value < 0.5	S26 (32.766)	Leong et al. (1997), Shimizu et al. (1988)

(Min et al., 1987; Li et al., 1991). In addition, the identified compounds such as (+)-Syringaresinol, rutin, naringenin and vicenin II had a value of 0.5 < VIP Value < 1.0, indicating that these compounds are different in *Dendrobium devonianum* and *Dendrobium officinale*, while eupatolide and isoschaftoside had VIP value ≥ 1.0, was identified as a differential marker, and also belongs to the characteristic components of *Dendrobium devonianum* and *Dendrobium officinale* (Tao et al., 2021; Wu et al., 2016; Tao et al., 2015; Liao et al., 2018).

Since eupatolide and isoschaftoside were differential markers in *Dendrobium devonianum* and *Dendrobium officinale*, and eupatolide had good anti-cancer, anti-inflammatory, anti-proliferative and anti-migratory effects (Avila-Carrasco et al., 2019; Ma et al., 2021; Boldbaatar et al., 2017), isoschaftoside was reported to have SARS-CoV-2 3CLpro inhibitory activity, antioxidant activity, anti-inflammatory activity (Cattaneo et al., 2016; Liao et al., 2021). In particular, isoxafutoside, which was first reported to have SARS-CoV-2 3CLpro inhibitory activity, was considered a new alternative to functional food or food supplement formulations. These two active compounds play an important role in the further development of *Dendrobium devonianum* products.

4 Conclusion

In this study, UPLC and PDA detector were used to collect the fingerprints of *Dendrobium devonianum* and *Dendrobium officinale*. Through the "Chinese medicine chromatographic fingerprint similarity evaluation system (2012 edition)", a total of 26 common peaks in the samples of *Dendrobium devonianum* and *Dendrobium officinale* were found, after automatic peak matching. The relative peak areas of the common peaks were used as different risks for principal component analysis by R, the results showed that *Dendrobium devonianum* and *Dendrobium officinale* could be better distinguished by PCA. On the other hand, the relative peak areas of the common peaks were combined by OPLS-DA, *Dendrobium devonianum* and *Dendrobium officinale* produced in Longling area can be clearly divided into two categories, which were consistent with the results of PCA analysis, indicating that common peaks combined with stoichiometry can effectively distinguish *Dendrobium devonianum* and *Dendrobium officinale* grown in the same growth area. The VIP value of 13 common peaks ≥ 1.0 has a significant contribution to the difference, which was a differential marker in *Dendrobium devonianum* and *Dendrobium officinale*. The compounds that are related to the common peaks were separated and purified by normal-phase silica gel column chromatography, and a total of 7 compounds were identified, of which eupatolide and isoschaftoside were differential markers, and their better biological activity was of great significance for the quality control of *Dendrobium devonianum* and its wide application in the food field. In this study, common UPLC instrument was used. Compared with large instrument such as high-resolution mass spectrometer, UPLC is relatively cheap, and the waste generated is mainly liquid, which is easier to collect, and is more environmentally friendly than the high-temperature gasification gas generated by high-resolution mass spectrometer. Therefore, the method of this experiment was economical and less polluting to the environment, and has a good application prospect.

Conflict of interest

The authors declare no conflict of interest.

Availability of data and material

Data are contained within the article.

Funding

This study was funded by Yunnan Fundamental Research Projects, grant number 202001AT070017, Yunnan Key Research and Development, grant number 202102AE090021, 202002AE3200053 and 2019ZG001.

Author contributions

Conceptualization, T.L. and H.-C.L.; methodology, T.L. and X.-L.C.; software, J.W.; validation, Z.-X.H.; formal analysis, X.-L.C.; investigation, L.-J.S.; resources, G.-W.W., J.W. and Z.-X.H.; data curation, T.L.; writing—original draft preparation, T.L.; writing—review and editing, H.-C.L.; supervision, H.-C.L.; funding acquisition. All authors have read and agreed to the published version of the manuscript.

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