**First systematic analysis for chemical composition by
HPLC-ESI-MSⁿ and antioxidant activity comparison of
Dendrobium huoshanense and *Dendrobium officinale***

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1 Abstract

2 **Background:** *Dendrobium huoshanense* C. Z. Tang et S. J. Cheng (*D. huoshanense*) is a
3 precious Dao-di herb with high quality, excellent efficacy and edible value of China,
4 *Dendrobium officinale* Kimura et Migo (*D. officinale*) is widely utilized and commonly
5 used as a substitute for *D. huoshanense*. But few studies have been conducted to sys-
6 tematically analyze chemical composition and bioactivities of the two.

7 **Methods:** HPLC-ESI-MSⁿ was used to identify the compounds in *D. huoshanense*
8 and *D. officinale* grown in Huoshan from methanol extracts and different polar frac-
9 tions of water extracts, including petroleum ether, ethyl acetate and n-butanol; The *in*
10 *vitro* antioxidant activity were evaluated by the scavenging effects on DPPH and ·OH
11 radicals, total reduction ability and total antioxidant ability.

12 **Results:** 61 compounds were identified in *D. huoshanense* and 49 compounds were
13 identified in *D. officinale* and with 43 shared compounds; Among them, 44 and 7
14 compounds were respectively identified from the two for the first time. Oxyresvera-
15 trol, quercetin-3-O-glucoside, quercetin-3-O-arabinoside, naringenin-7-O-glucoside,
16 baicalin, hesperetin, vitexin and apigenin-6-C-pentosyl-8-C-(2''-O-quinoyl)-glucoside
17 were first identified in *Dendrobium*. In addition, 5 compounds were unique in *D. hu-*
18 *oshanense* compared with other species of *Dendrobium*. The results of *in vitro* antioxidant
19 activity showed that n-BuOH-soluble fraction of *D. huoshanense* and *D. officinale*
20 both revealed the strongest antioxidant activities compared with other fractions, fur-
21 thermore, the antioxidant activities of different fractions of *D. huoshanense* were sep-
22 arately superior to that of *D. officinale*.

23 **Conclusion:** These results may provide a reference for the substances study about *D.*
24 *huoshanense*'s high-quality efficacy, and also provide a scientific basis for its devel-
25 opment and utilization.

Keywords: Chemical composition; *Dendrobium huoshanense*; *Dendrobium officinale*; HPLC-ESI-MSⁿ; Antioxidant activity.

Background

Dendrobium is a large genus of *Orchidaceae*, and grows on cliffs and tree trunks covered with mosses throughout the tropical and subtropical Asia and eastern Australia, and is well known over the world for its high commercial value and excellent medicinal effects [1]. In China, there are 74 species and 2 varieties of *Dendrobium* [2], and it has been utilized as traditional Chinese medicine and food for centuries with the tonic ability for body building and life prolonging, nourishing stomach and promoting saliva secretion, nourish Yin and clear Heat [3]. *Dendrobium* is documented as early as in “Shen Nong's Herbal Classic” of Qin and Han Dynasties [4], according to other ancient Chinese medicine classics records, *Dendrobium* grows on the mountains and nearing the river in valley, and *Dendrobium huoshanense* C. Z. Tang et S. J. Cheng (*D. huoshanense*) grown in Huoshan County is regarded as the “Dao-di herb” with best quality and curative effects [5-7]. Now, *D. huoshanense* is known as national first-level protected plant and Chinese National Geographic Indication product, and it was officially included in the 2020 edition of the Chinese Pharmacopoeia.

D. huoshanense has a variety of pharmacological effects such as anti-oxidation activity, liver protection, immunomodulatory activity, anti-cataract activity, promotion of saliva production and building of immunity [8-11]. Due to their unique habitat, the slow growth rate and over-harvesting, *D. huoshanense* is on the verge of extinction and expensive. *Dendrobium officinale* Kimura et Migo (*D. officinale*) is commonly used as a substitute for *D. huoshanense* in the market, because of their similar morphological characteristics in processed products [12], but their efficacies are quite different. The antioxidant ability of polysaccharides from *D. huoshanense* is higher than

51 *D. officinale* *in vitro* free radicals scavenging assays [13]. The protective effect of *D.*
52 *huoshanense* was the strongest among *D. huoshanense*, *D. moniliforme*, *D. henanense*,
53 *D. officinale* (Yunnan) and *D. officinale* (Huoshan) in CCl₄-induced liver injury mice
54 [14].

55 In this study, we simultaneously qualitative analyzed the chemical composition of
56 *D. huoshanense* and *D. officinale* grown in Huoshan County using HPLC-ESI-MSⁿ,
57 and compared the *in vitro* antioxidant activities of different polar fractions from water
58 extracts by scavenging effects on DPPH and ·OH radicals, total reduction ability and
59 total antioxidant ability, hoping to clarify the material basis of the difference in effi-
60 cacy of *D. huoshanense* and *D. officinale*, and provide the basis for their development
61 and utilization.

62 **Materials and methods**

63 **Plant Materials, Chemicals and Reagents**

64 The stems of *D. huoshanense* (1605A01HS02) and *D. officinale* grown in the Hu-
65 oshan area (1605A01HS01) (Fengdou) were collected from Lu'an, Huoshan County
66 (Anhui, China), which were 3 years old. All the plant samples were authenticated by
67 Prof. Zhen Ouyang of Jiangsu University (Zhenjiang, China).

68 HPLC-grade methanol and acetonitrile were purchased from Merck (Darmstadt,
69 Germany). Methanol, acetic acid, n-Butanol, EtOAc and PET were of analytical purity.
70 Naringenin was purchased from Shanghai Yueteng Biotechnology Co., Ltd. (Shanghai,
71 China). Gigantol was purchased from Jining Tianzhi Blue Biotechnology Co., Ltd.
72 (Shandong, China). Rutin was purchased from China National Institute for the Control
73 of Pharmaceutical and Biological Products. Syringic acid was purchased from Nan-
74 jing Jinzhu Bio-Technology Co., Ltd. (Nanjing, China). 1,1-diphenyl-2-picrylhydrazyl
75 (DPPH) and ascorbic acid (V_C) were purchased from Thermo Fisher Scientific (Wal-

tham, Mass, USA), other analytical reagents were purchased from Sinopac Chemical Reagents Co. LTD (Beijing, China).

Analysis of methanol extracts by HPLC-ESI-MSⁿ

Sample preparation

Samples were ground into fine powder (0.5g) and extracted with methanol two times (25 mL) by reflux extraction at 60 °C for 2 h. The extracts were filtered and concentrated under reduced pressure (BuchiR-200, Buchi, Germany), and freeze-drying for 12 hours by a vacuum freeze dryer (ALPHA 1-2 LD, Marin Christ, Germany). The residue was dissolved in 1.0 mL of methanol and filtered through 0.45 µm membranes as sample solutions for HPLC-ESI-MSⁿ analysis. The extraction procedure was performed triplicate (n=3).

The references standards, including naringenin, gigantol, rutin and syringic acid, were accurately weighed and dissolved in methanol to obtain a stock standard solution at a concentration of 1 mg·mL⁻¹ and filtered through 0.45 µm membranes as sample solutions for HPLC analysis.

HPLC and MS conditions

Chromatographic separations were achieved using gradient elution on Elite Sino-Chrom ODS-AP (4.6 mm×250 mm, 5 µm) column at 25 °C, and the detection wavelength was 270 nm. The mobile phase consisted of 100% (v/v) acetonitrile (A) and 0.2% (v/v) aqueous acetic acid solution (B) with a 1.0 mL·min⁻¹ flow rate. The gradient elution program was as follows: 0-13 min, 10% A; 13-15 min, 10%-15% A; 15-35 min, 15%-20% A; 35-40 min, 20%-24% A; 40-60 min, 24%-35% A; 60-82 min, 35%-48% A; 82-87 min, 48%-52% A; 87-97 min, 52%-54% A.

HPLC-ESI-MSⁿ analysis was performed by Liquid Chromatography-Ion Trap Mass Spectrometry (FinniganTM LXQTM, Thermofisher, USA) and used a negative mode.

The source voltage and capillary voltage were set at 3.5 kV and -30 V. The capillary temperature was maintained at 315°C. Nitrogen was used as sheath gas and aux gas at a flow rate of 36 a.u. and 10 a.u.. Full-scan MS spectra were monitored within the range of m/z 50-1000. The collision energy was adjusted to 50%. Data were processed using Xcalibur software, version 2.0.7.

Analysis of different polarity extracts by HPLC-ESI-MSⁿ

Sample preparation

The powder of all samples (100 g) were extracted with distilled water (1500 mL× 3) for 1 h at 100 °C, the extraction was repeated thrice with fresh solvent. The filtrate was combined and concentrated to 2000 mL using a rotary evaporator at 60 °C. The concentrated solution (Total water extracts, D_{TW}) was successively extracted with petroleum ether, EtOAc and n-BuOH to afford petroleum ether-soluble fraction (D_{PE}), EtOA-soluble fraction (D_{EA}), n-BuOH-soluble fraction (D_{BU}) and residual water-soluble fraction (D_W). Finally, 0.62 g of D_{PE}, 1.02 g of D_{EA}, 1.15 g of D_{BU} and 32.85 g of D_W were obtained after freeze-drying. The D_{BU}, D_{EA} and D_{PE} fractions (10 mg) were dissolved in 1 mL of water and filtered through 0.45 µm membranes as sample solutions for HPLC-ESI-MSⁿ analysis. The extraction procedure was performed triplicate (n=3).

HPLC and MS conditions of D_{PE} analysis

Chromatographic separations were achieved using gradient elution on Elite Si-no-Chrom ODS-AP (4.6 mm×250 mm, 5 µm) column at 25 °C, and the detection wave-length was 270 nm. The mobile phase consisted of 100% (v/v) acetonitrile (B) and 0.2% (v/v) aqueous acetic acid solution (C) at a flow rate of 0.8 mL·min⁻¹. The gradient elution program was as follows: 0-20 min, 15%-31% C; 20-30 min, 31%-39% C; 30-40 min, 39% C; 40-45 min, 39%-40% C; 45-50 min, 40%-45% C; 50-60 min,

126 45%-50% C.

127 MS conditions were same as the conditions of “2.2.3 HPLC conditions and MS
128 conditions of Methanol Extracts”.

129 ***HPLC and MS conditions of D_{EA} analysis***

130 The mobile phase consisted of 100% (v/v) acetonitrile (A) and 0.2% (v/v) aqueous
131 acetic acid solution (B) at a flow rate of 1.0 mL·min⁻¹. The gradient elution program
132 was as follows: 0-13 min, 10%-12% A; 13-20 min, 12%-15% A; 20-22 min, 15% A;
133 22-30 min, 15%-17% A; 30-35 min, 17%-20% A; 35-40 min, 20%-24% A; 40-45 min,
134 24%-25% A; 45-50 min, 25%-26% A; 50-55 min, 26%-30% A; 55-60 min, 30%-33%
135 A; 60-65 min, 33%-35% A; 65-70 min, 35%-42% A. Other conditions were same as
136 above.

137 MS conditions were same as the conditions of “2.2.3 HPLC conditions and MS
138 conditions of Methanol Extracts”.

139 ***HPLC and MS conditions of D_{PE} analysis***

140 The mobile phase consisted of 100% (v/v) acetonitrile (B) and 0.2% (v/v) aqueous
141 acetic acid solution (C) at a flow rate of 0.8 mL·min⁻¹. The gradient elution program
142 was as follows: 0-20 min, 15%-25% C; 20-30 min, 25%-40% C; 30-40 min, 40%-60%
143 C; 40-50 min, 60%-80% C; 50-60min, 80%-85% C. Other conditions were same as
144 above.

145 HPLC-ESI-MS analysis used a positive and negative mode. The source voltage and
146 capillary voltage were set at ±3.5 kV and ±30 V. The capillary temperature was main-
147 tained at 325°C. Nitrogen was used as sheath gas and aux gas at a flow rate of 37 a.u.
148 and 9 a.u.. Full-scan MS spectra were monitored within the range of *m/z* 120-1000.
149 The collision energy was adjusted to 50%.

150 ***Antioxidant activity of water extracts and different polar fractions *in vitro****

Accurately weigh 25 mg D_{TW}, D_{PE}, D_{EA}, D_{BU} and D_W of *D. huoshanense* and *D. officinale* which prepared as “4.3 Analysis of water extracts by HPLC-ESI-MSⁿ”, and dilute to 25 mL with distilled water to obtain 1 mg/mL of the test solution.

DPPH scavenging assay

The extracts capacity (0-0.8mg/mL) for scavenging DPPH was evaluated following the procedure previously described by YL Zhu *et al* [15]. Ascorbic acid (V_C) was used as positive control, and distilled water was served as blank control.

•OH radical scavenging assay

The extracts capacity (0-0.8mg/mL) for scavenging •OH radical assay was applied by the salicylic acid method [16]. The extracts and V_C (1.0 mL, 0-0.8 mg/mL) were respectively mixed with FeSO₄ solution (1.0 mL, 6 mmol/L) and salicylic acid-ethanol solution (1.0 mL, 6 mmol/L) and H₂O₂ solution (1.0 mL, 6 mmol/L) and then allowed to react in a water bath at 37 °C for 1 h. V_C was used as a positive control drug. The absorbance of samples (A_i) was measured at 510 nm. The •OH radical scavenging rate was calculated as follows:

$$Scavenging\ rate(\%) = (1 - \frac{A_i - A_j}{A_0}) \times 100 \quad (1)$$

Where A₀ was the absorbance at 510 nm of the reagent mixture with 1 mL distilled water, and A_j was the absorbance at 510 nm of 1.0 mL sample with distilled water instead of the reagent mixture.

Total reduction ability assay

Total reduction ability was carried out according the previous report with some changes. The extracts and V_c (1 mL, 0-0.8 mg/mL) were respectively mixed with phosphate buffer (2.5 mL, pH 6.6) and K₃Fe(CN)₆ (2.5 mL, 1%, w/v), and then allowed to react at 50 °C for 20 min. The reaction was terminated by adding trichloroa-

cetic acid (2.0 mL, 10%, w/v), followed by mixing the supernatant (2.5 mL) with anhydrous ethanol (2.5 mL) and FeCl₃ solution (0.5 mL, 0.1%, w/v) after centrifugation, then the absorbance was measured at 700 nm with distilled water as blank control.

Total antioxidant ability assay

The mixed solution was prepared according to the procedure described by YL Zhu *et al* [15]. Then extracts and V_C (1.0 mL, 0-0.8 mg/mL) were respectively mixed with 3.0 mL of mixed solution, and kept in a water bath at 95 °C for 90 min. The absorbance was measured at 695 nm. V_C was used as a positive control, and distilled water as the blank control.

Results

Identification of compounds in methanol extracts by HPLC-ESI-MSⁿ

HPLC-ESI-MSⁿ technique was used to qualitatively analyze the chemical constituents of *D. huoshanense* and *D. officinale*, the compounds structures were confirmed according to the molecular weight, multistage mass fragmentation, retention time and UV characteristics, and combined with literature data, natural product databases such as Sci Finder, Chemistry Database, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), Mass Bank and TCM.

Most compounds of the methanol extracts respond in positive and negative ion mode, but more sensitive in negative mode (Fig. 1, Table 1). 43 compounds were elucidated in methanol extracts, including 16 flavonoids, 13 bibenzyls, 5 phenanthrenes, 3 phenolic acids, 2 amides, 1 steroid and 3 other compounds. Among them, ox-yresveratrol (9), quercetin-3-O-glucoside (17), naringenin-7-O-glucoside (20) and quercetin-3-O-arabinoside (21) were identified for the first time from *Dendrobium*. A total of 40 compounds were identified from the methanol extracts of *D. huoshanense*, in which 31 compounds were identified for the first time. 32 compounds were identi-

200 fied from *D. officinale* and compound 9, 17, 20, 21, 28, 44 and 57 were identified for
201 the first time from *D. officinale*.

Fig. 1 The HPLC and base peak intensity (BPI) chromatogram of methanol extracts of *D. huoshanense* (A) and *D. officinale* (B)
 (A₁) HPLC of *D. huoshanense*; (A₂) BPI chromatogram of *D. huoshanense*; (B₁) HPLC of *D. officinale*; (B₂) BPI chromatogram of *D. officinale*.

Table 1 Compounds characterized in methanol extracts of *D. huoshanense* (A) and *D. officinale* (B)

No.	tr/min	[M-H] ⁺ /m/z	Fragment ion /m/z	Formula	Identification	Classification	Found	Reference
1	8.88	379.26	321.61, 319.35, 285.16, 268.47, 225.17, 203.17, 191.05, 169.01	-	#	-	B	
2	15.98	197.22	153.23, 182.10	C ₉ H ₁₀ O ₅	Syringic acid ^s	Phenolic acid	A, B	[17]
3	19.64	575.21	553.53, 481.32, 379.26, 285.40, 191.28	-	#	-	B	
4	21.98	613.34	594.58, 535.57, 519.29, 425.34, 363.12	-	#	-	B	
5	22.29	583.48	547.64, 447.54, 421.44, 147.1	C ₃₉ H ₆₈ O ₃	Defuscin ^a	Other	A	[18]
6	24.68	563.66	545.48, 503.51, 473.48, 443.44, 425.49, 383.41, 353.39, 259.39, 178.72	C ₂₆ H ₂₈ O ₁₄	Apig-enin-6-C-β-D-xyloside-8-C-β-D-glucoside ^a	Flavonoid	B	[19]
7	26.30	563.40	545.58, 503.60, 473.55, 443.61, 383.38, 353.50	C ₂₆ H ₂₈ O ₁₄	Isoschaftoside	Flavonoid	A, B	[20]
8	26.65	593.63	533.73, 503.36, 473.48, 455.52, 413.59, 383.38	C ₂₆ H ₂₈ O ₁₄	Apigenin-6, 8-di-C-β-D-glucopyranoside	Flavonoid	A	[19]
9	26.72	243.05	228.05, 224.98, 217.00, 200.95, 181.03, 185.00, 157.00	C ₁₄ H ₁₂ O ₄	Oxyresveratrol ^{abc}	Bibenzyl	A, B	[21]
10	28.49	311.41	261.43, 253.50, 235.39, 195.22, 151.31, 94.78	C ₁₈ H ₁₈ O ₄	2,3,4,7-tetramethoxyphenanthrene	Phenanthrene	A	[22]
11	29.87	593.62	500.26, 473.56, 413.50, 335.67, 293.28, 265.63	C ₂₇ H ₃₀ O ₁₅	Vitexin-2''-O-β-D-glucopyranoside ^a	Flavonoid	B	[23]
12	30.48	709.59	649.42, 619.61, 589.69, 545.66, 499.56, 485.66, 413.50, 337.51, 98.21	C ₃₂ H ₃₉ O ₁₈	6-C-(α-Arabinopyranosyl)-1-C-[(2-O-α-rhamnopyranosyl)-β-galactopyranosyl]-apigenin	Flavonoid	A	[24]
13	31.94	363.29	335.35, 319.57, 269.23, 220.13, 205.16, 183.26	-	#	-	A, B	

14	32.72	533.61	515.53, 473.54, 443.50, 383.49, 353.51	C ₂₅ H ₂₆ O ₁₃	Apig-enin-6-C-β-D-xylose-8-C-α-L-arabinoside ^a	Flavonoid	A	[23]
15	33.05	609.40	447.36, 343.37, 301.35, 271.28	C ₂₇ H ₃₀ O ₁₆	Rutin ^s	Flavonoid	A, B	[19]
16	34.02	563.61	545.49, 503.58, 473.63, 425.28, 413.54, 383.53	C ₂₆ H ₂₈ O ₁₄	Schaftoside	Flavonoid	A	[20]
17	35.40	463.45	433.76, 381.10, 343.44, 301.44, 285.44, 243.19, 227.64, 189.48, 179.33, 151.08	C ₂₁ H ₂₀ O ₁₂	Quercetin-3-O-glucoside ^{abc}	Flavonoid	A, B	[17]
18	37.54	533.66	515.62, 473.42, 443.46	C ₂₅ H ₂₆ O ₁₃	Apigenin-6,8-di-C-α-L-pyranosin	Flavonoid	A, B	[20]
19	38.95	433.44	417.60, 375.46, 351.44, 313.14, 301.43, 227.64, 209.40	-	#	-	A	
20	40.54	433.58	415.60, 373.23, 343.35, 273.62, 227.38, 179.33, 150.93	C ₂₁ H ₂₂ O ₁₀	Naringenin-7-O-glucoside ^{abc}	Flavonoid	A, B	[17]
21	41.97	433.46	415.58, 373.23, 343.59, 301.39, 283.62, 217.25, 179.21, 151.14	C ₂₁ H ₂₂ O ₁₀	Quercetin-3-O-arabinoside ^{abc}	Flavonoid	A, B	[17]
22	42.81	493.35	457.55, 435.85, 353.37, 291.38	-	#	-	A, B	
23	46.84	227.34	209.34, 198.46, 183.35, 181.26	C ₁₄ H ₁₂ O ₃	2,4,7-trihydroxy-9,10-dihydrophenanthrene ^a	Phenanthrene	A, B	[18]
24	47.91	417.55	387.51, 255.54, 237.48, 179.09, 151.17, 125.29	C ₂₂ H ₂₆ O ₈	Syringaresinol ^a	Bibenzyl	A, B	[19]
25	49.68	282.46	268.35, 224.32, 162.11, 145.04, 134.17, 119.00	C ₁₇ H ₁₇ NO ₃	N-P-coumaryl tyramine ^a	Amide	A, B	[25]
26	50.34	229.42	214.40, 186.34, 147.22, 123.08	C ₁₄ H ₁₄ O ₃	Dihydroresveratrol ^a	Bibenzyl	A, B	[26]
27	51.24	312.48	297.57, 270.46, 252.59, 218.20, 192.42, 178.13, 149.18, 135.09, 123.20	C ₁₈ H ₁₉ NO ₄	Trans-N-(4-hydroxyphenethyl) ferulic acid amide ^a	Amide	A, B	[25]

28	52.01	259.30	244.17, 199.43, 177.06, 137.09, 121.06, 92.81	C ₁₅ H ₁₆ O ₄	Dendrosinen B ^{ab}	Bibenzyl	A, B	[27]
29	54.01	287.49	269.53, 229.42, 192.91, 161.26, 151.11, 134.98	C ₁₅ H ₁₂ O ₆	3',5,5',7-tetrahydroxydihydroketone ^a	Flavonoid	A, B	[26]
30	55.03	329.35	313.37, 287.41, 269.34, 235.23, 195.64, 171.21, 165.08	C ₁₉ H ₂₂ O ₅	Dendrocandin R ^a	Other	A	[18]
31	55.35	363.26	319.17, 281.33, 269.21, 252.32, 219.88, 199.10, 128.94	-	#	-	B	
32	56.31	353.37	338.46, 311.20, 290.69, 273.44, 271.25, 216.28, 196.14	C ₁₆ H ₁₈ O ₉	Chlorogenic acid ^a	Phenolic acid	A	[28]
33	56.61	259.37	259.14, 244.36, 227.70, 191.47, 177.17, 174.95, 138.15, 120.92, 97.07	C ₁₅ H ₁₆ O ₄	Tristin ^a	Bibenzyl	A, B	[29]
34	57.71	289.48	288.53, 274.39, 245.44, 231.03, 195.26, 138.14,	C ₁₆ H ₁₈ O ₅	Dendrocandin C ^a	Bibenzyl	A, B	[18]
35	58.01	317.56	299.59, 272.58, 255.40, 215.24, 133.26	C ₁₈ H ₂₂ O ₅	Erianin ^a	Bibenzyl	A	[30]
36	58.44	453.67	436.45, 408.34, 391.71, 368.12, 267.38, 225.30, 197.21, 143.29	-	#	-	B	
37	58.93	323.42	309.27, 244.35, 243.38, 227.45, 148.29	-	#	-	B	
38	59.44	399.46	385.65, 384.46, 371.48, 355.56, 313.47, 279.29, 239.11, 211.34	-	#	-	A	
39	63.12	271.33	256.27, 177.19, 165.04, 151.10, 125.25, 93.06	C ₁₅ H ₁₂ O ₅	Naringenin ^s	Flavonoid	A, B	[19]
40	63.92	574.39	541.59, 538.67, 514.53, 481.35, 409.30, 379.34	C ₃₅ H ₆₀ O ₆	Daucosterol ^a	Steroid	A, B	[26]
41	64.62	437.45	407.62, 345.65, 331.47, 273.34, 269.49, 255.31, 239.54, 181.24, 107.21	C ₂₅ H ₂₆ O ₇	Dendrocandin N ^a	Bibenzyl	A, B	[18]

42	67.05	329.62	311.59, 293.60, 285.49, 229.41, 211.31, 183.16, 165.13, 122.03	C ₁₉ H ₂₂ O ₅	Dihydroconiferyl dihydro-p-coumarate ^a	Phenolic acid	A, B	[19]
43	67.93	303.49	289.65, 288.40, 220.37, 193.38, 152.38, 106.81	C ₂₃ H ₂₉ O ₉	(<i>R</i>)-3, 4-dihydroxy-5,4', α-trimethoxybibenzyl ^a	Bibenzyl	A, B	[18]
44	68.17	315.23	299.59, 283.41, 239.40, 224.42	C ₁₈ H ₁₈ O ₅	Chrisotoxene ^{ab}	Phenanthrene	A, B	[30]
45	69.07	241.44	227.27, 226.29, 211.26, 197.54	C ₁₄ H ₁₀ O ₄	4-methoxy-9,10-dihydrophenanthrene-2,5-diol ^a	Phenanthrene	A	[18]
46	69.68	243.42	137.92, 137.05, 122.16, 105.89	C ₉ H ₁₂ N ₂ O ₆	Uridine ^a	Other	A, B	[18]
47	70.72	273.42	259.39, 249.03, 199.14, 180.15, 137.02, 122.09	C ₁₆ H ₁₈ O ₄	Gigantol ^s	Bibenzyl	A, B	[30]
48	71.63	243.38	227.32, 201.47, 184.30, 157.21, 136.32, 122.23, 105.88, 80.84	C ₁₅ H ₁₆ O ₃	3,4'-dihydroxy-5-methoxybibenzyl ^a	Bibenzyl	A	[31]
49	73.42	337.44	351.77, 322.48, 257.33, 217.01, 195.22	-	#	-	A, B	
50	74.49	307.33	292.37, 243.44, 228.33, 227.34, 212.22, 123.11	-	#	-	A, B	
51	75.35	497.52	467.53, 415.15, 390.36, 357.42, 301.27, 287.32, 209.22, 151.16	-	#	-	B	
52	76.36	353.44	338.28, 321.40, 273.37, 196.14	-	#	-	B	
53	78.19	273.38	258.36, 247.22, 179.11, 137.24	C ₁₆ H ₁₈ O ₄	3,4-dihydroxy-5,4'-dimethoxybibenzyl ^a	Bibenzyl	A, B	[32]
54	78.71	497.55	499.00, 482.52, 465.48, 414.74, 226.35	C ₂₇ H ₃₀ O ₉	Dendrocandin T ^a	Bibenzyl	A	[33]
55	79.45	531.55	513.66, 449.42, 379.26, 270.46, 224.34	-	#	-	B	
56	84.60	283.42	269.46, 268.21, 255.43, 239.41, 224.28, 211.34, 191.05	C ₁₆ H ₁₅ O ₂	Denbinobin ^a	Phenanthrene	A, B	[22]
57	86.31	271.51	271.56, 256.23, 195.24, 177.18, 151.24, 93.06	C ₁₅ H ₁₂ O ₅	Dihydroapigenin ^{ab}	Flavonoid	A, B	[18]

58	88.61	431.76	413.79, 411.78, 377.84, 332.01, 273.98, 243.44, 213.33, 155.23	C ₂₁ H ₂₀ O ₁₀	Isovitexin	Flavonoid	B	[34]
59	96.78	311.59	293.76, 275.55, 227.56, 211.43, 185.16, 129.27	-	#	-	A	

206 Note: #, not identified; -, none; A and B were identified from *D. huoshanense* and *D. officinale*, respectively; a, b and c were reported for the first time in *D. huoshanense*, *D.*
207 *officinale* and *Dendrobium*, respectively; s was identified by standard; \ represented that reference to the identification of the compound was described above.

Identification of compounds in D_{BU} by HPLC-ESI-MSⁿ

The D_{BU} fraction of *D. huoshanense* and *D. officinale* showed sensitive in positive and negative mode (Fig. 2, Table 2). A total of 27 compounds of structures were elucidated in D_{BU}, including 17 flavonoids, 8 bibenzyls, 2 phenanthrenes compounds. Among them, naringenin-7-O-glucoside (13) was identified for the first time from *Dendrobium*. 21 compounds were identified from *D. huoshanense*, in which 11 compounds were identified in *D. huoshanense* for the first time. 16 compounds were identified from *D. officinale* grown in Huoshan country and compounds 1, 2, 3, 13 and 30 were identified for the first time from *D. officinale*.

Identification of compounds in D_{EA} by HPLC-ESI-MSⁿ

The D_{EA} fraction of *D. huoshanense* and *D. officinale* in negative mode showed more sensitive than that in positive mode (Fig. 3, Table 3). A total of 31 compounds of structures were elucidated in D_{EA}, including 13 flavonoids, 9 bibenzyls, 5 phenanthrenes, 2 amides, 1 phenolic acid and 1 alkanoic acid compounds. Among them, baicalin (4), hesperetin (6) and vitexin (34) were identified for the first time from *Dendrobium*. A total of 26 compounds were identified from *D. huoshanense*, in which 20 compounds were identified for the first time. 17 compounds were identified from *D. officinale* and compounds 4, 6, 7, 28 and 34 were identified for the first time from *D. officinale*.

Identification of compounds in D_{PE} by HPLC-ESI-MSⁿ

The D_{PE} fraction of *D. huoshanense* and *D. officinale* in negative mode also showed more sensitive than that in positive mode (Fig. 4, Table 4). A total of 7 compounds of structures were elucidated, including 3 bibenzyls, 2 flavonoids, 1 phenolic acid and 1 other compounds. Apigenin-6-C-pentosyl-8-C-(2''-O-quinoyl)-glucoside (6) were identified in *Dendrobium* for the first time. 7 compounds were identified from *D. hu-*

233 *oshanense*, in which compound 2, 6, 8, 9 were identified in *D. huoshanense* for the
234 first time. And 4 compounds were identified from *D. officinale*.

Fig. 2 The HPLC and BPI chromatogram of DBU of *D. huoshanense* (A) and *D. officinale* (B)

(A₁) HPLC of *D. huoshanense*; (A₂) BPI chromatogram of *D. huoshanense*; (B₁) HPLC of *D. officinale*; (B₂) BPI chromatogram of *D. officinale*.

Table 2 Compounds characterized in D_{BU} of *D. huoshanense* (A) and *D. officinale* (B)

No.	t _R /min	[M+H] ⁺ /m/z	Fragment ion /m/z	[M-H] ⁻ /m/z	Fragment ion /m/z	Formula	Identification	Classification	Found	Reference
1	7.31	-	-	301.09	213.12, 180.72, 161.12, 159.10, 143.21, 139.13	C ₁₇ H ₁₈ O ₅	4,7-dihydroxy-2,3,6-trimethoxy-9,10-dihydrophenanthrene ^{ab}	Phenanthrene	A, B	[30]
2	9.46	-	-	331.19	169.07, 160.93, 152.29	C ₁₉ H ₂₄ O ₅	Chrysotobibenzyl ^{ab}	Bibenzyl	A, B	[30]
3	11.81	275.20	-	272.56	259.39, 249.03, 199.14, 180.15, 137.02, 122.09	C ₁₆ H ₁₈ O ₄	Gigantol ^s	Bibenzyl	B	\
4	12.83	-	-	431.18	387.33, 372.32, 357.33, 342.58, 311.37, 205.23	C ₂₁ H ₂₀ O ₁₀	Isovitexin	Flavonoid	B	\
5	15.67	305.38	305.38, 287.33, 163.04, 139.60	-	-	C ₁₇ H ₂₀ O ₅	Dendrocandin A ^a	Bibenzyl	A, B	[18]
6	16.24	-	-	473.11	-	-	#	-	A, B	
7	17.99	305.38	305.35, 287.25, 153.20, 139.57	-	-	C ₁₇ H ₂₀ O ₅	Moscatilin ^a	Bibenzyl	A, B	[29]
8	19.33	-	-	657.07	521.38, 385.68, 363.02, 249.58	-	#	-	A, B	
9	21.42	-	-	418.16	161.68, 159.60, 129.38, 123.46	C ₂₂ H ₂₆ O ₈	Syringaresinol	Bibenzyl	B	\
10	22.10	-	-	505.98	486.94, 445.98, 428.18, 341.58, 283.89, 155.44	-	#	-	A	
11	23.27	261.85	260.90, 255.14, 243.48, 205.75, 169.73, 163.66, 149.67	-	-	C ₁₅ H ₁₆ O ₄	Tristin ^a	Bibenzyl	A	\
12	24.88	299.71	281.70, 271.91, 263.70, 239.78, 97.38	-	-	C ₁₈ H ₁₈ O ₄	2,3,4,7-tetramethoxyphenanthrene ^a	Phenanthrene	A	\
13	27.29	433.46	415.18, 373.23, 343.65, 301.15, 273.32, 227.18, 179.33, 150.63	-	-	C ₂₁ H ₂₂ O ₁₀	Naringenin-7- <i>O</i> -glucoside ^{abc}	Flavonoid	A	
14	28.49	595.28	475.41, 457.41, 355.33	593.26	503.20, 473.30, 383.25, 353.14	C ₂₇ H ₃₀ O ₁₅	Apigenin-6,8-di- <i>C</i> - β - <i>D</i> -glucopyranoside	Flavonoid	A, B	\
15	31.42	-	-	381.33	249.25, 217.34, 159.25	-	#	-	A, B	\
16	32.06	565.34	475.40, 547.34, 445.35, 353.21	563.29	473.31, 443.09, 383.15, 353.21	C ₂₆ H ₂₈ O ₁₄	Isoschaftoside	Flavonoid	A, B	\

17	32.82	565.40	475.44,445.30,427.32,355.38,	563.26	586.7, 545.22, 503.17, 473.15, 443.14, 383.11, 353.20	C ₂₆ H ₂₈ O ₁₄	Schaftoside	Flavonoid	A, B	\
18	34.54	-	-	581.07	419.12, 389.46, 341.32, 329.29, 311.39	-	#	-	B	
19	34.87	525.54	525.55,482.11,287.68,181.82,171.70,156.64	-	-	C ₃₀ H ₂₂ O ₉	Dendrocandin H	Bibenzyl	B	[18]
20	35.53	595.89	577.89,565.94,541.99,529.90,457.97,439.96,355.33	593.95	575.90, 563.02, 474.06, 413.82, 383.90, 352.75	C ₂₇ H ₃₀ O ₁₅	Vitexin-4''-O-glucoside ^a	Flavonoid	A	[35]
21	36.01	565.19	547.95,504.13,459.83,354.13,	563.85	503.97, 473.95, 455.98, 443.91, 353.94	C ₂₆ H ₂₈ O ₁₄	Apig-enin-6-C-β-D-xyloside-8-C-β-D-glucoside	Flavonoid	A	\
22	36.92	711.37	645.77,615.94,565.80,547.83,499.56,445.91,413.50,338.06,98.21	710.01	649.42, 619.61, 589.69, 545.66, 455.89, 437.99, 425.88, 413.50, 335.79, 307.62	C ₃₂ H ₃₉ O ₁₈	6-C-(β-Xylopyranosyl)-1-C-[(2-O-α-rhamnopyranosyl)-β-golactopyranosyl]-apigenin	Flavonoid	A	[32]
23	37.81	595.26,	475.41,433.38,415.35,295.35	593.26	473.27, 413.12, 293.05	C ₂₇ H ₃₀ O ₁₅	Vitex-in-2''-O-β-D-glucopyranoside ^a	Flavonoid	A, B	\
24	38.70	711.28	675.89,573.94,547.91,529.83,511.88,427.96,409.84,289.21	709.91	649.99, 619.86, 545.97, 425.88, 335.79	C ₃₂ H ₃₉ O ₁₈	6-C-[(2-O-α-Rhamnopyranosyl)-β-glucopyranosyl]-1-C-(α-arabinopyranosyl)-apigenin	Flavonoid	A	[32]
25	40.99	-	-	579.87	460.08, 417.94, 181.69	C ₂₇ H ₃₂ O ₂₄	Naringin	Flavonoid	A	[20]
26	45.12	711.78	565.80,547.83,445.91,428.04,397.81,379.87,338.06	709.95	709.95, 620.08, 546.03, 456.03, 425.87, 383.98, 336.05	C ₃₂ H ₃₉ O ₁₈	6-C-(α-Arabinopyranosyl)-1-C-[(2-O-α-rhamnopyranosyl)-β-golactopyranosyl]-apigenin	Flavonoid	A	\
27	46.08	-	-	609.24	301.14	C ₂₇ H ₃₀ O ₁₆	Rutin ^s	Flavonoid	B	\
28	47.89	535.93	517.90,481.93,469.91,451.92,433.91,415.93,397.94	533.96	515.90, 473.90, 444.00, 413.93, 383.84, 353.84	C ₂₅ H ₂₆ O ₁₃	Apigenin-6,8-di-C-α-L-arabinoside	Flavonoid	A	\
29	48.12	565.78	547.87,529.96,511.93,501.08, 481.99,457.93	564.02	545.88, 503.94, 474.01, 455.93, 413.94, 384.03	C ₂₆ H ₂₈ O ₁₄	Apigenin-6-C-β-D- glucoside -8-C-β-D- xyloside	Flavonoid	A	[36]

30	48.74	459.52	255.61,183.15,155.36	457.20,	253.11, 181.05, 153.06	C ₂₂ H ₂₆ O ₉ N ^a	(7S,8S,8R)-4,4-Dihydroxy-3,3,5,5-tetramethoxy-7,9-epoxylignan-9-ol-7-one ^{ab}	Bibenzyl	A, B	[30]
31	49.80	614.30	596.29,570.14,508.09,437.96,332.35,305.67	-	-	-	#	-	A	
32	50.09	-	-	459.27	163.18, 148.10	-	#	-	A, B	
33	54.99	535.42	519.40, 499.32,481.40,445.42.	533.20	517.21, 443.26, 473.30, 353.33	C ₂₅ H ₂₆ O ₁₃	Apig-enin-6-C- α -L-arabinoside-8-C- β -D-xyloside ^a	Flavonoid	A, B	[36]
34	55.75	-	-	805.06	665.45, 643.40, 625.22	-	#	-	A, B	
35	56.49	565.37	475.40,403.34,385.34,295.32	563.32	473.45, 383.05, 293.07	C ₂₆ H ₂₈ O ₁₄	Apig-enin-6-C-(2''-O- β -D-glucopyranoside)- α -L-arabinoside	Flavonoid	B	[36]

239 Note: #, not identified; -, none; A and B were identified from *D. huoshanense* and *D. officinale*, respectively; a, b and c were reported for the first time in *D. huoshanense*, *D.*
240 *officinale* and *Dendrobium*, respectively; s was identified by standard; \ represented that reference to the identification of the compound was described above.

Fig. 3 The HPLC and BPI chromatogram of D_{EA} of *D. huoshanense* (A) and *D. officinale* grown in the Huoshan County (B)
(A₁) HPLC of *D. huoshanense*; (A₂) BPI chromatogram of *D. huoshanense*; (B₁) HPLC of *D. officinale*; (B₂) BPI chromatogram of *D. officinale*.

Table 3 Compounds characterized in D_{EA} of *D. huoshanense* (A) and *D. officinale* (B)

No.	t _R /min	[M-H] ⁺ /m/z	Fragment ion /m/z	Formula	Identification	Classification	Found	Reference
1	5.17	437.65	419.62, 409.57, 393.81, 364.86, 323.61	C ₂₅ H ₂₆ O ₇	Dendrocandin N ^a	Bibenzyl	A	\
2	6.98	255.45	241.16, 212.11, 155.17	C ₁₆ H ₃₂ O ₂	Hexadecanoic acid ^a	Other	A, B	\
3	8.11	273.38	258.31, 180.14, 137.02, 122.02	C ₁₆ H ₁₈ O ₄	Gigantol ^s	Bibenzyl	A, B	\
4	8.77	445.31	384.17, 363.21, 346.33, 333.26, 269.04, 241.32, 223.28, 197.03	C ₂₁ H ₁₈ O ₁₁	Baicalin ^{abc}	Flavonoid	A, B	[37]
5	10.44	241.10	227.27, 211.04, 197.54, 154.94	C ₁₅ H ₁₄ O ₃	4-methoxy-9,10-dihydrophenanthrene-2,5-diol ^a	Phenanthrene	A, B	\
6	13.62	301.32	286.32, 257.11, 94.18	C ₁₆ H ₁₄ O ₆	Hesperetin ^{abc}	Flavonoid	A, B	[37]
7	16.27	271.43	256.26, 241.19	C ₁₆ H ₁₆ O ₄	Erianthridin ^{ab}	Phenanthrene	A, B	\
8	19.80	521.06	439.52, 379.14, 356.15	-	#	-	A	
9	21.37	515.07	258.31, 107.21	C ₃₀ H ₂₇ O ₈	Dendrocandin P ^a	Bibenzyl	A	[18]
10	23.29	249.39	205.19, 167.02, 153.05, 133.03	-	#	-	A, B	
11	26.12	165.21	147.02, 129.23, 118.04	-	#	-	A	
12	30.27	197.22	181.98, 152.95, 138.05	C ₉ H ₁₀ O ₅	Syringic acid ^s	Phenolic acid	B	\
13	30.71	297.53	261.13, 252.97, 235.29, 195.11, 151.31, 94.78	C ₁₈ H ₁₈ O ₄	2,3,4,7-tetramethoxyphenanthrene ^a	Phenanthrene	A	\
14	32.10	311.23	293.76, 275.55, 227.56, 218.10, 211.43, 152.78, 129.27	-	#	-	A	
15	35.57	533.31	515.13, 473.54, 443.44, 383.41, 353.31	C ₂₅ H ₂₆ O ₁₃	Apigenin-6-C-β-D-xyloside-8-C-β-D-arabinoside	Flavonoid	A	\
16	36.05	309.11	307.50, 292.37, 277.33, 243.44, 228.33, 123.11	-	#	-	A	
17	37.20	533.58	515.90, 473.90, 444.00, 413.93, 383.84, 353.84	C ₂₅ H ₂₆ O ₁₃	Apigenin-6,8-di-C-α-L- arabinoside ^a	Flavonoid	A	\
18	37.92	533.47	515.36, 443.26, 473.30, 353.33	C ₂₅ H ₂₆ O ₁₃	Apigenin-6-C-α-L-arabinoside-8-C-β-D-xyloside	Flavonoid	A	\
19	40.13	563.27	503.43, 473.39, 443.39, 353.31	C ₂₆ H ₂₈ O ₁₄	Apigenin-6-C-β-D-xyloside-8-C-β-D-glucoside	Flavonoid	A, B	\
20	41.83	453.54	393.42, 213.27, 163.33, 107.21	C ₂₅ H ₂₅ O ₈	Dendrocandin O ^a	Bibenzyl	A	[18]
21	41.86	577.37	559.46, 503.33, 473.34, 457.41, 383.36, 353.33	C ₂₇ H ₃₀ O ₁₄	Isoviolanthin	Flavonoid	B	\
22	41.93	579.80	417.11	C ₂₇ H ₃₂ O ₂₄	Naringin	Flavonoid	A	\

23	42.52	417.45	402.44, 371.38, 181.12, 166.04	C ₂₂ H ₂₆ O ₈	Syringaresinol ^a	Bibenzyl	A	\
24	46.84	459.38	327.25, 175.03, 165.13	-	#	-	A	
25	47.61	609.51	301.20, 286.17, 242.26, 173.17, 125.05	C ₂₇ H ₃₀ O ₁₆	Rutin ^s	Flavonoid	B	\
26	47.75	282.46	268.35, 224.32, 188.13, 162.11, 145.04, 134.17, 119.00	C ₁₇ H ₁₇ NO ₃	N-P-coumaryl tyramine ^a	Amide	A	\
27	48.31	227.34	207.41, 197.04, 183.35, 181.26, 155.13	C ₁₄ H ₁₂ O ₃	2,4,7-trihydroxy-9,10-dihydrophenanthrene ^a	Phenanthrene	A	\
28	48.46	457.36	253.31, 181.25, 153.06	C ₂₂ H ₂₆ O ₉ Na	(7S,8S,8R)-4,4-Dihydroxy-3,3,5,5-tetramethoxy-7,9-epoxylignan-9-ol-7-one ^{ab}	Bibenzyl	A, B	\
29	50.95	433.01	418.45, 403.39, 373.32, 223.19	-	#	-	A, B	\
30	51.84	710.82	649.29, 619.46, 545.17, 425.88, 335.09	C ₃₂ H ₃₉ O ₁₈	6-C-[(2-O- α -Rhamnopyranosyl)- β -glucopyranosyl]-1-C-(α -arabinopyranosyl)-apigenin	Flavonoid	A	\
31	53.18	289.12	288.03, 274.39, 245.64, 231.43, 195.06, 138.34, 121.32	C ₁₆ H ₁₈ O ₅	Dendrocandine ^a	Bibenzyl	A	\
32	56.87	312.36	297.57, 270.46, 252.59, 218.20, 192.42, 178.13, 149.18, 135.09, 123.20	C ₁₈ H ₁₉ NO ₄	Trans-N-(4-hydroxyphenethyl) ferulic acid amide ^a	Amide	A, B	\
33	58.32	417.38	402.44, 324.26, 181.16, 166.18	C ₂₂ H ₂₆ O ₈	Syringaresinol ^a	Bibenzyl	A, B	\
34	60.29	431.44	413.24, 341.45, 311.36, 270.22, 259.14, 244.36, 227.70, 191.47,	C ₂₁ H ₂₀ O ₁₀	Vitexin ^{bc}	Flavonoid	B	[37]
35	60.52	259.27	177.17, 174.95, 138.15, 120.92, 97.07	C ₁₅ H ₁₆ O ₄	3,4',5-trihydroxy-3'-methoxybibenzyl ^a	Bibenzyl	A	\
36	62.59	431.40	342.48, 311.37	C ₂₁ H ₂₀ O ₁₀	Isovitexin	Flavonoid	B	\
37	65.40	283.53	265.39, 255.43, 239.23, 201.20, 171.21	C ₁₆ H ₁₅ O ₂	Denbinobin ^a	Phenanthrene	A, B	\
38	66.75	271.21	177.08, 151.00	C ₁₅ H ₁₂ O ₅	Naringenin ^s	Flavonoid	A, B	\

246 Note: #, not identified; -, none; A and B were identified from *D. huoshanense* and *D. officinale*, respectively; a, b and c were reported for the first time in *D. huoshanense*, *D.*
247 *officinale* and *Dendrobium*, respectively; s was identified by standard; \ represented that reference to the identification of the compound was described above.

Fig. 4 The HPLC and BPI chromatogram of D_{PE} *D. huoshanense* (A) and *D. officinale* (B)

(A₁) HPLC of *D. huoshanense*; (A₂) BPI chromatogram of *D. huoshanense*; (B₁) HPLC of *D. officinale*; (B₂) BPI chromatogram of *D. officinale*.

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Table 4 Compounds characterized in D_{PE} of *D. huoshanense* (A) and *D. officinale* (B)

No.	t _R /min	[M-H] ⁻ /m/z	Fragment ion /m/z	Formula	Identification	Classification	Found	Refer- ence
1	4.19	197.91	153.82, 101.86	C ₉ H ₁₀ O ₅	Syringic acid ^s	Phenolic acid	A, B	\
2	31.62	498.81	362.91, 268.94	C ₂₇ H ₃₀ O ₉	Dendrocandin T ^a	Bibenzyl	A, B	\
3	33.50	514.68	431.72, 378.88, 284.85	-	#	-	A, B	
4	38.29	248.89	204.70, 152.80, 112.81, 56.74	-	#	-	A, B	
5	39.83	319.17	304.09, 301.11, 285.99, 180.76	-	#	-	A	
6	42.96	737.36	677.49, 564.46, 473.21, 443.03, 383.26	C ₃₂ H ₃₄ O ₂₀	Apig-enin-6-C-pentosyl-8-C-(2''-O-quinoyl)-g lucoside ^{ac}	Flavonoid	A	[38]
7	44.03	520.76	384.74, 204.75, 152.92	-	#	-	A	
8	45.38	449.08	368.14, 359.21, 283.66	C ₃₃ H ₅₆ O ₃	Ttracosyl- 3-(4-hydroxyphenyl)acrylate ^a	Other	A	\
9	48.39	303.14	288.07, 151.92, 122.21,	C ₂₃ H ₂₉ O ₉	Dendrocandin D ^a	Bibenzyl	A	\
10	51.48	273.15	258.10, 179.85	C ₁₆ H ₁₈ O ₄	Gigantol ^s	Bibenzyl	A	\
11	54.07	271.07	256.06, 177.97, 151.02	C ₁₅ H ₁₂ O ₅	Naringenin ^s	Flavonoid	A, B	\

252 Note: #, not identified; -, none; A and B were identified in *D. huoshanense* and *D. officinale*, respectively; a, b and c were reported for the first time in *D. huoshanense*, *D.*

253 *officinale* and *Dendrobium*, respectively; s was identified by standard; \ represented that reference to the identification of the compound was described above.

Structural identification of different types of compounds

The specific resolution of mass spectrometry was investigated and discussed in the following section.

Flavonoids compounds

UV spectroscopy showed that flavonoids had two similar absorption bands. The maximum absorption wavelength of band I and band II were 335 and 271 nm.³⁹ The position and shape of the absorbent bands were consistent with the UV absorption characteristics of the flavonoids (Fig. 5).

Fig. 5 The representative UV, ESI-MS/MS profiles and proposed fragmentation pathways of compound 7 (A), compound 33 (B), compound 23 (C)

In the analysis of methanol extracts, compounds 6, 7, 8, 11, 12, 14, 16, 18 and 58 showed the [M-H]⁻ ion at m/z 563.66, 563.40, 593.63, 593.12, 709.59, 533.61, 563.61, 533.43 and 431.76, respectively. The MS² spectra of 9 flavonoid C-glycosides exhibited ions of m/z [(M-H)-60]⁻, m/z [(M-H)-90]⁻ and m/z [(M-H)-120]⁻, which were

demonstrated as characteristic ions of flavonoid C-glycosides and belonged to sugar ring opening reaction (Fig. 5A). These compounds were tentatively identified as apigenin-6-C- β -D-xyloside-8-C- β -D-glucoside, isoschaftoside, apigenin-6, 8-di-C- β -D-glucopyranoside, vitexin-2''-O- β -D-glucopyranoside, 6-C-(α -Arabinopyranosyl)-1-C-[(2-O- α -rhamnopyranosyl)- β -galactopyranosyl]-apigenin, apigenin-6-C- β -D-xylose-8-C- α -L-arabinoside, schaftoside, apigenin-6,8-di-C- α -L-pyranosin and isovitexin according to the literatures, respectively. Compounds 17 and 21 produced the characteristic fragment ion at m/z 301, 229, 179, 151, which were classified the aglycone quercetin, combined with literature and Chemical Database, they were assigned as quercetin-3-O-glucoside and quercetin-3-O-arabinoside (Fig. 6). Compound 20 gave the characteristic fragment ion at m/z 271, 227, 177, 151, which were classified the aglycone naringenin. Combined with literature and Chemistry Database, thus, it was assigned as naringenin-7-O-glucoside (Fig. S1). Compounds 15 and 39 were identified as rutin and naringenin by their mass spectra and reference standards, supported in literatures.

Fig 6. The proposed fragmentation pathways of compound 17 and 21 in methanol extracts

Fig. S1 The proposed fragmentation pathways of compound 20 in methanol extracts

Bibenzyls

UV spectroscopy showed that bibenzyls have 2 absorption bands, generally appearing at 200-230 nm, 270-290 nm; strong absorption at 200-220 nm, and weak absorption at

270-290 nm. The position and shape of the absorbent bands were consistent with the UV absorption characteristics of the bibenzyls (Fig. 5B) [36].

In the analysis of methanol extracts, compounds 26, 28, 33, 34, 41, 43, 48, 53 and 54 showed the $[M-H]^-$ ion at m/z 229.27, 259.23, 259.33, 289.40, 437.45, 303.47, 243.38, 273.36 and 497.55, respectively. The MS^2 spectra of 9 simple bibenzyls exhibited ions at m/z 123 and 139, which were indicated that there were two and three hydroxyl groups attached to the benzene ring; ions at m/z 121, 151 and 181, indicating that one, two and three methoxy groups were attached to the benzene ring.

The primary mass spectrum of compound 9 showed strong ion peaks $[M-H]^-$ ion at m/z 243.05, and the secondary mass spectrum $[M-H]^-$ ion generated fragment ions $[M-H-OH]^-$ at m/z 224.98, $[M-H-CO-CH_2]^-$ at m/z 200.95 and $[M-H-CO-CH_2-OH]^-$ at m/z 185.00, combined with literature, it was speculated as oxyresveratrol (Fig. 7). Compound 47 was identified as gigantol by their mass spectra and reference standards, supported in literatures.

Fig. 7 The proposed fragmentation pathways of compound 9 in methanol extracts

Phenanthrenes

UV spectra showed that four compounds had two absorption bands (K band and B band), K band appeared at 210 nm or 270 nm, 270-290 nm, B band at about 270 -280 nm. The position and shape of the absorbent bands were consistent with the UV absorption characteristics of the phenanthrenes (Fig. 5C) [40].

In the analysis of methanol extracts, compounds 9, 23, 44, 45 and 56 showed the $[M-H]^-$ ion at m/z 297.53, 227.34, 315.23, 241.44 and 283.42, respectively. A strong fragment peak and a base peak was observed in the MS^2 spectra of phenanthrenes, when the molecular ion was most readily broken off $-CH_3$. These compounds were tentatively identified as 2,3,4,7-tetramethoxyphenanthrene, 2,4,7-trihydroxy-9,10-dihydrophenanthrene, chrisotoxene, 4-methoxy-9,10-dihydrophenanthrene-2,5-diol and denbinobin according to the literatures and Chemistry Database, respectively.

Phenolic acids

In the analysis of methanol extracts, compound 2 produced the characteristic ion $[M-H]^-$ at m/z 197.22; the product ions of the $[M-H]^-$ ion gave the fragment ions $[M-H-COOH]^-$ at m/z 153.23 and $[M-H-OH]^-$ at m/z 182.10, which was assigned as Syringic acid by reference standards and literatures. Compounds 32 and 42 produced the characteristic ion $[M-H]^-$ at m/z 353.37 and 329.62, being similar to the way of breaking of compound 2, and were tentatively identified as chlorogenic acid and dihydroconiferyl dihydro-p-coumarate by literatures and databases (Fig. 8).

Fig. 8 The proposed fragmentation pathways of phenolic acid

The chemical structure of all identification is shown in Fig. S1.

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Fig. S2 Chemical structures of the compounds identified in *D. huoshanense* and *D. officinale*

Antioxidant activity analysis of water extracts and different polar fractions *in vitro*

The fractions of D_{TW} , D_{PE} , D_{EA} , D_{BU} and D_W of *D. huoshanense* and *D. officinale* all had varying degrees ability to scavenge DPPH (Fig. 9) and $\bullet OH$ radicals (Fig.10) within the concentration range, as well as total reduction ability (Fig. 11) and total antioxidant ability (Fig. 12), and showed dose-dependent manner. Compared with other fractions, D_{BU} of *D. huoshanense* and *D. officinale* both showed the strongest antioxidant activity. Comprehensive analysis of scavenging effects on DPPH and $\bullet OH$ radicals, total reduction ability and total antioxidant ability *in vitro*, the antioxidant activity of D_{TW} , D_{BU} , D_{EA} , D_{PE} and D_W of *D. huoshanense* were separately superior to the fractions of *D. officinale*.

Fig. 9 Scavenging effects of *D. huoshanense* (A) and *D. officinale* (B) on DPPH radical

Fig. 10 Scavenging effects of *D. huoshanense* (A) and *D. officinale* (B) on $\bullet OH$ radical

Fig. 11 Total reduction abilities of *D. huoshanense* (A) and *D. officinale* (B)

Fig. 12 Total antioxidant abilities of *D. huoshanense* (A) and *D. officinale* (B)

Discussion

A total of 66 compounds were identified from the two herbs (Fig. A1), of which 61 compounds were identified from *D. huoshanense* and 49 compounds were identified from *D. officinale*, 44 compounds common to both, including 16 flavonoids, 15 bibenzyls, 6 phenanthrene, 2 amides, 2 phenolic acids, 1 alkanoic acid, 1 steroid and 1 others (Table 1, 2, 3 and 4). In addition, 17 compounds were only present in *D. huoshanense*, one of chlorogenic acid has a protective effect on the digestive system and anti-oxidation effect [41]; Bibenzyl compounds such as erianin and dendrocandin O show good anti-oxidation and anti-aging effects as well flavonoid compounds [42]; Naringin inhibits the gastric mucosal ulcer by increasing the glycoprotein content in the stomach and the viscosity of the gastric mucosa [43]. The pharmacological activity of these compounds consistent with ancient books recordation “*Dendrobium* can enhance the function of stomach and intestines, make the physical body to become

more alive and prolong life”.

The result of antioxidant activity *in vitro* showed that D_{BU} of *D. huoshanense* and *D. officinale* both had the strongest antioxidant activity, moreover, the antioxidant activity of D_{TW}, D_{BU}, D_{EA}, D_{PE} and D_W of *D. huoshanense* were separately superior to the fractions of *D. officinale*. Combined with HPLC-ESI-MSⁿ results, the peak area of syringic acid, oxyresveratrol, naringenin, dihydroresveratrol, dihydroapigenin, denbinobin, baicalin, isophoroside, apigenin-6,8-di-C- α -L-arabinoside, apigenin-6-C- α -L-arabinoside-8-C- β -D-xyloside, 2,4,7-trihydroxy-9,10-dihydrophenanthrene, (7S,8S,8R)-4,4-dihydroxy-3,3,5,5-tetramethoxy-7,9-epoxylignan-9-ol-7-one in *D. huoshanense*, some of the shared compounds, were larger than that in *D. officinale*, and the contents of compounds in D_{BU} were higher than other fractions, these results were consistent with previous reports, what's more, the authors found the antioxidant activity of crude polysaccharide fraction was not as good as different polarity fraction [44], and most of those compounds were reported to have antioxidant effects [45-48]. These results suggested that the high-quality efficacy of *Dendrobium* is closely related to their low molecular weight compounds.

Conclusions

In this study, we simultaneously qualitative analyzed the chemical composition of *D. huoshanense* and *D. officinale* grown in Huoshan County using HPLC-ESI-MSⁿ, 61 and 49 compounds were respectively identified from *D. huoshanense* and *D. officinale*, and with 22 compounds variance, of which 17 compounds were identified from *D. huoshanense*. The composition of small molecular compounds in *D. huoshanense* was more varied and higher in content. The result of antioxidant activity *in vitro* showed that n-butyl alcohol-soluble fraction of *D. huoshanense* and *D. officinale* both

had the strongest antioxidant activity, and the antioxidant activity of different polarity extractions of *D. huoshanense* were separately superior to the fractions of *D. officinale*. These results may provide a reference for the substances research about *D. huoshanense*'s high-quality efficacy, and also lay a foundation for its clinical application as food and medicine.

Abbreviations

HPLC-ESI-MSⁿ: High-performance liquid chromatography electrospray ionization mass spectrometry; TCMSP: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; BPI: base peak intensity; DPPH: 1,1-diphenyl-2-picrylhydrazyl; D_{TW}: Total water extracts of *Dendrobium*; D_{PE}: petroleum ether-soluble fraction of *Dendrobium*; D_{EA}: EtOAc-soluble fraction of *Dendrobium*; D_{BU}: n-BuOH -soluble fraction of *Dendrobium*; D_W: Residual water-soluble fraction of *Dendrobium*.

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Authors' contributions

W-JQ carried out the experiment, drafted the manuscript and validated the data; G-XH and C-SR carried out the literature search and analyzed the data; W-CW, WY and K-LD contributed to the interpretation of the research and revision of the manuscript; H-BX and OY-Z proposed the conception and designed the structure of the manuscript. All authors read and approved the final manuscript

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Availability of data and materials

The research data generated from this study is included within the article.

Ethics approval and consent to participate

Not applicable

Consent for publication

The manuscript is approved by all authors for publication.

Competing interests

The authors declare that they have no competing interests.

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