Published By Guangdong Pharmaceutical University, Guangzhou

Journal of Holistic Integrative Pharmacy

2021,2(2): 112-122

EXPERIMENTAL RESEARCH

Chemotaxonomic and Pharmacological properties on *Dendrobium chrysanthum* Lindl.

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[Abstract] Many species of *Dendrobium* have been commonly used in traditional Chinese medicine. The aim of this study was to provide a critical and comprehensive evaluation of the phytochemistry and pharmacological properties of *Dendrobium chrysanthum* Lindl., with the hope to provide meaningful guidelines for future investigations. In our study, 105 compounds were tentatively identified by UPLC/Q-TOF-MS/MS comprehensively, novel polyphenylpropanoids were detected and characterized from the genus Dendrobium. 63 compounds were obtained by repeatedly chromatographs and recrystallization. Among them, 27 compounds were firstly reported from the species *D. chrysanthum*. Some valuable compounds like pseudo-spirostanols and novel polyphenylpropanoids were found and identified in *D. chrysanthum* for the first time, which might constitute the key characteristics and chemotaxonomic significance that distinguish *D. chrysanthum* from other Dendrobium species. The pharmacological activities of *D. chrysanthum* were summarized including the anti-inflammatory, antioxidant, anti-tumor and hypoglycemic activities of the extract and purified constituents.

[Key words] phytochemistry; pharmacological properties; chemotaxonomy; *Dendrobium chrysanthum* Lindl.

1 Introduction

The genus *Dendrobium*, which is widely distributed in tropical and subtropical regions in the world, comprises about 1 500 species. There are 76 species grow in China[1-2]. Many species of *Dendrobium* have been commonly used in folk medicine for the treatment of cataracts, hypoglycemia, antibacterial, anti-inflammatory, and diabetes[3].

Dendrobium chrysanthum Lindl., a perennial epiphytic herb belongs to the family Orchidaceae, is widely distributed in South Asia and parts of Southeast Asia like Guangxi, Guizhou, Yunnan and southeastern Tibet, growing on tree trunks or damp rocks in mountain forests or valleys at an altitude of 700-2 500 meters above sea level. Previous phytochemical investigations of *D. chrysanthum*

[Research Funding] This work was supported by the Special Innovation Project of Guangdong University (No. 2019KTSCX073) and Guangdong Natural Science Foundation (No. 2021A1515011510).

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These authors have no conflict of interest to declare.

led to the identification of diverse types of chemical constituents, including bibenzyls[4-6], phenanthrenes[6-8], anthraquinones[9], alkaloids[10], lignans[11], fluorenones and steroids[12-13].

In this study, we aim to provide a critical and comprehensive evaluation of the phytochemistry and pharmacological properties of *D. chrysanthum*, with the hope to provide a scientific basis for further development of *Dendrobium* and explore its effective components.

2 Phytochemistry of *D. chrysanthum*

2.1 Preparation of the *D. chrysanthum*

The fresh stems of *D. chrysanthum* were gathered from Wenshan Zhuang-Miao Autonomous Prefecture, Yunnan Province in 2013, and identified by Prof. Tie Zhang, Yunnan Wenshan college. A voucher specimen (SH-201307) has been deposited in the herbarium School of pharmacy, Guangdong Pharmaceutical University, Guangzhou, China.

2.2 Analysis of the crude extract of *D. chrysanthum*

The dried stems of *D. chrysanthum* (7.5 Kg) was cut into pieces, refluxed and extracted with 95% ethanol for 4 times, 3 hours each time, filtered and combined with filtrate. 550 g ethanol extract was recovered, and 1.0 g ethanol extract was dissolved by chromatographic methanol for UPLC/Q-TOF-MS/MS. The mobile phase A was deionized water while the mobile phase B was methanol, the flow rate was 1.0 mL/min, and the column temperature was 30 ℃, the injection volume was 20 μL. The mobile phase was consisted of deionized water (A) and methanol (B) and the elution condition was as follow: 0~30 min, 80%A; 30~50 min, 20%A; 50~70 min, 90%A. Mass spectrometry conditions: ESI source; data acquisition in positive ion mode; ion spray voltage: 1 500 V; ion source gas: 150 pis; ion source temperature: 550 ℃; collision voltage: 8 pis; mass scan range: *m/z* 100-1 200. Sample (5 μL) were subjected to HPLC-Q-TOF-MS analysis. The chemical constituents of *M. speciosa* were analyzed on the basis of MS^n results. The CAS, Molecular formula, Molecular weight and MOL file were collected by Chemspider and Scifinder. With the help of the Formula Finder function in Peakview software, the accurate mass-charge ratio (*m/z*) of compounds was obtained by TOF-MS first-order mass spectrometry. The MOL file, which is consistent with the deduced formula, is then imported into the Peakview software to verify the accuracy of the deductive compound by matching the information of the secondary fragments obtained at the lower end of the time.And 105 compounds was determined by combining the retention time, molecular ion peaks and structural fragments (Table 1), including 29 phenylpropanoids that have been reported[14].

2.3 Extraction and isolation of the extract of *D. chrysanthum*

Dry stems of *D. chrysanthum* were refluxed with 95% ethanol and the extract was suspended in water and then extracted successively with petroleum ether (PE), chloroform ($CHCl_3$), ethyl acetate (EtOAc) and n-butyl alcohol (n-BuOH). The $CHCl_3$, EtOAc and n-BuOH fractions were subjected to silica gel (200-300 mesh) column chromatography (CC) by petroleum ether/dichloromethane (PE/CH_2Cl_2) (*v/v*, 100∶1-1∶1) followed by dichloromethane/methanol (CH_2Cl_2/MeOH) (*v/v*, 100∶1-1∶1) to yield fractions. Then the fractions were repeatedly chromatographed over silica gel CC, sephadex LH-20 CC, and preparative thin layer chromatography (PTLC) for the next fractions. Compounds were obtained by HPLC method (Senshu pak PEGASIL ODS Al, 10 A 250 mm, MeOH∶H_2O (70∶30), flow rate 4 ml/min A1; UV detector at 254 nm) and repeatedly recrystallization. The main chemical compositions from *D. chrysanthum* were identified

Table 1 105 compounds of the crude extract of *D. chrysanthum*

No.	TR/min	Formula	[M-H]-	Error/ppm	Name	Classification
1	5.07	$C_{18}H_{13}O_4^*$	293.123 1		cyclop-hydroxyphenylacrylic acid-*p*-hydroxyphenylpropionate	phenylpropanoid
2	5.35	$C_{36}H_{27}O_8^*$	587.263 3		cyclodi-*p*-hydroxyphenylacrylic acid-*di*-*p*-hydroxyphenylpropionate	phenylpropanoid
3	8.97	$C_9H_7O_3^*$	163.038 4	–3.6	*p*-hydroxyphenylacrylic acid	phenylpropanoid
4	7.38	$C_9H_7O_4^*$	179.034 4	2.8	caffeic acid	phenylpropanoid
5	8.22	$C_{15}H_{19}O_8^*$	327.108 4	3.0	*p*-hydroxyphenylpropionic acid-hexaglycoside	phenylpropanoid
6	8.30	$C_{15}H_{17}O_8^*$	325.090 8	–3.2	*p*-hydroxyphenylacrylic acid-hexaglycoside	phenylpropanoid
7	8.32	$C_{21}H_{29}O_{12}^*$	473.162 1		phenylpropionic acid-hexose-hexaglycoside	phenylpropanoid
8	8.41	$C_9H_9O_3^*$	165.055 1	2.9	*p*-hydroxyphenylpropionic acid	phenylpropanoid
9	8.64	$C_8H_7O_3^*$	151.039 8		Methoxybenzoic acid	phenylpropanoid
10	8.90	$C_9H_6O_2^*$	147.044 0	–0.4	coumadin	phenylpropanoid
11	9.11	$C_9H_{10}NO_2^*$	164.044 7	2.5	phenylalanine	phenylpropanoid
12	10.14	$C_{36}H_{25}O_8^*$	585.206 9		cyclop-hydroxyphenylpropionic acid-*tri*-*p*-hydroxyphenyl acrylate	phenylpropanoid
13	10.87	$C_{15}H_{19}O_7^*$	311.113 2	2.2	hexaglycoside phenylpropionate	phenylpropanoid
14	13.76	$C_{27}H_{19}O_6^*$	439.117 7	0.2	cyclodi-*p*-hydroxyphenylacrylic acid-*p*-hydroxyphenylpropionate	phenylpropanoid
15	18.28	$C_{24}H_{27}O_{10}^*$	475.288 5		*di*-*p*-hydroxyphenylpropionic acid-hexaglycoside	phenylpropanoid
16	18.73	$C_{24}H_{25}O_{10}^*$	473.239 3		*p*-hydroxyphenylpropionic acid-*p*-hydroxyphenylacrylic acid-hexaglycoside	phenylpropanoid
17	22.33	$C_{27}H_{19}O_7^*$	455.242 8		*tri*-*p*-hydroxyphenyl acrylate	phenylpropanoid
18	24.55	$C_{18}H_{13}O_5^*$	309.206 3		*di*-*p*-hydroxyphenyl acrylate	phenylpropanoid
19	26.24	$C_{36}H_{27}O_9^*$	603.164 8	–0.3	*tri*-*p*-hydroxyphenylacrylic acid-*p*-hydroxyphenylpropionate	phenylpropanoid
20	27.46	$C_{18}H_{15}O_5^*$	311.223 3		*p*-hydroxyphenylacrylic acid-*p*-hydroxyphenylpropionate	phenylpropanoid
21	29.25	$C_{36}H_{29}O_9^*$	605.404 5		*di*-*p*-hydroxyphenylpropionic acid-*di*-*p*-hydroxyphenyl acrylate	phenylpropanoid
22	29.84	$C_{36}H_{29}O_8^*$	589.303 6		cyclop-hydroxyphenylacrylic acid-*tri*-*p*-hydroxyphenylpropionate	phenylpropanoid
23	31.26	$C_{18}H_{17}O_5^*$	313.237 7		*di*-*p*-hydroxyphenylpropionate	phenylpropanoid
24	31.80	$C_{18}H_{15}O_4^*$	295.229 9		cyclodihydroxyphenylpropionate	phenylpropanoid
25	31.83	$C_{36}H_{31}O_8^*$	591.313 6		cyclotetra-*p*-hydroxyphenylpropionate	phenylpropanoid
26	33.74	$C_{27}H_{21}O_6^*$	441.259 0		cyclop-hydroxyphenylacrylic acid-*p*-hydroxyphenylacrylic acid-*p*-hydroxyphenylacrylate	phenylpropanoid
27	36.17	$C_{27}H_{23}O_6^*$	443.258 1		cyclotrip-hydroxyphenylpropionate	phenylpropanoid
28	39.37	$C_{36}H_{31}O_9^*$	607.248 4		*tri*-*p*-hydroxyphenylpropionic acid-*p*-hydroxyphenyl acrylate	phenylpropanoid
29	41.91	$C_{27}H_{25}O_7^*$	461.257 3		*tri*-*p*-hydroxyphenylpropionate	phenylpropanoid
30	9.68	$C_{21}H_{19}O_{12}$	463.089 2	4.3	quercetin hexaglycoside	flavonoid
31	10.71	$C_{28}H_{35}O_{13}$	579.209 1	3.3	gardenia flavonoids	flavonoid
32	10.88	$C_{21}H_{19}O_{11}$	447.091 9	–0.6	kaempferol six-carbon glycoside	flavonoid
33	11.00	$C_{21}H_{19}O_{11}$	447.093 3	2.5	quercetin six-carbon deoxyglucoside	flavonoid
34	11.92	$C_{15}H_9O_7$	301.035 9	5.4	quercetin	flavonoid

Table 1 (Continued)

No.	T_R/min	Formula	[M-H]-	Error/ppm	Name	Classification
35	12.84	$C_{15}H_9O_6$	285.041 2	–0.9	kaempferol	flavonoid
36	12.93	$C_{15}H_9O_5$	269.044 4	–0.2	apigenin	flavonoid
37	15.08	$C_{15}H_{11}O_5$	271.060 4	1.1	naringenin	flavonoid
38	16.87	$C_{16}H_{11}O_7$	315.050 1	0.4	isorhamnetin	flavonoid
39	12.03	$C_{15}H_{13}O_4$	257.081 4	2.2	methoxy-trihydroxy phenanthrene	stilbene
40	12.65	$C_{15}H_{15}O_4$	259.096 4	–0.3	tristin	stilbene
41	14.08	$C_{16}H_{17}O_5$	289.107 5	1.4	trimethoxy-dihydroxy bibenzyl	stilbene
42	14.16	$C_{19}H_{23}O_6$	347.153 1	–3.5	pentamethoxy-hydroxy bibenzyl	stilbene
43	14.39	$C_{15}H_{15}O_4$	259.097 2	3.1	methoxy trihydroxy bibenzyl	stilbene
44	16.55	$C_{15}H_{13}O_3$	241.085 0	–3.8	methoxy-dihydroxy-dihydrophenanthrene	stilbene
45	16.76	$C_{17}H_{19}O_5$	303.123 5	2.6	dendrobium candidum	stilbene
46	16.88	$C_{15}H_{11}O_4$	255.065 5	1.3	methoxy-trihydroxy phenanthrene	stilbene
47	16.91	$C_{15}H_{11}O_4$	255.065 7	2.0	methoxy-hydroxy-9,10-dihydrophenanedione	stilbene
48	17.13	$C_{16}H_9O_6$	297.038 8	–1.8	dimethoxy phenanthrene tetraone	stilbene
49	17.27	$C_{16}H_{17}O_4$	273.113 2	3.9	gigantol	stilbene
50	17.28	$C_{16}H_{17}O_4$	273.113 3	4.3	dendrobium phenol	stilbene
51	17.30	$C_{15}H_{16}O_3$	243.102 5	3.7	batatasin-III	stilbene
52	19.86	$C_{17}H_{19}O_5$	303.121 3	–4.6	moscatilin	stilbene
53	19.89	$C_{17}H_{19}O_4$	287.126 6	–4.1	trimethoxy-hydroxy bibenzyl	stilbene
54	21.05	$C_{16}H_{13}O_4$	269.081 6	2.8	amoenumin	stilbene
55	22.60	$C_{15}H_{11}O_3$	239.070 2	–0.3	methoxy-dihydroxy phenanthrene	stilbene
56	17.92	$C_{16}H_{11}O_5$	283.060 5	1.6	dimethoxy-hydroxy-phenanthrene diketone	stilbene
57	18.11	$C_{15}H_{13}O_4$	257.081 2	1.4	methoxy-trihydroxy-dihydrobibenzyl	stilbene
58	18.96	$C_{16}H_{15}O_4$	271.062 1	7.4	trimethoxy-dihydroxy-9,10-dihydrophenanthrene	stilbene
59	19.15	$C_{15}H_9O_4$	253.049 9	1.4	methoxy-hydroxy-phenanthrene diketone	stilbene
60	19.87	$C_{17}H_{15}O_4$	283.096 3	–0.4	trimethoxy-hydroxyphenanthrene	stilbene
62	20.75	$C_{17}H_{13}O_6$	313.072 9	6.8	tetramethoxy-hydroxyphenanthrene	stilbene
62	20.77	$C_{17}H_{17}O_5$	301.099 5		trimethoxy-dihydroxy-9,10-dihydrophenanthrene	stilbene
63	22.62	$C_{17}H_{15}O_5$	299.091 9	1.7	trimethoxy-dihydroxy phenanthrene	stilbene
64	8.91	$C_{16}H_{26}NO_2$	264.194 9	–3.4	dendrobium alkaloid	alkaloid
65	9.04	$C_{19}H_{30}NO_3$	320.211 9		dendrobium paratine	alkaloid
66	9.57	$C_{16}H_{26}NO_3$	280.190 9	0.6	dendrobium aminophylline	alkaloid
67	7.49	$C_{16}H_{24}NO_4$	294.168 1	–4.4	3-OH-2-O-Dendrobium alkaloid	alkaloid
68	14.79	$C_{22}H_{34}NO_3$	360.251 8	–4.2	*n*-isopentene-6 murine OH-Dendrobium	alkaloid
69	8.56	$C_{17}H_{28}NO_2$	278.210 8	–2.4	*n*-methyl Dendrobium alkaloid	alkaloid
70	13.55	$C_{16}H_{24}NO_3$	278.174 4	–1.4	mubiroaines A	alkaloid
71	8.99	$C_{15}H_{24}NO_2$	250.179 6	–1.0	mubiroaines B	alkaloid
72	14.66	$C_{19}H_{32}NO_4$	338.232 5	–0.2	dendrowardine	alkaloid
73	4.54	$C_{12}H_{14}NO_2$	204.101 8	–0.7	dendrobium	alkaloid
74	9.16	$C_{21}H_{30}NO_3$	344.224 1	6.1	crepidine	alkaloid
75	14.36	$C_8H_{16}NO$	142.122 7	0.4	hygrine	alkaloid
76	54.12	$C_{29}H_{49}O$	413.375 8	–4.8	*β*-sitosterol	steroid
77	58.57	$C_{35}H_{59}O_4$	575.427 0	–1.1	daucosterol	steroid
78	39.96	$C_{27}H_{41}O_4$	429.296 0		*iso*-neutigenin	steroid

Table 1 (Continued)

No.	TR/min	Formula	[M-H]-	Error/ppm	Name	Classification
79	34.72	$C_{33}H_{51}O_9$	591.357 3		neutigenin glucoside	steroid
80	67.67	$C_9H_{11}O_3$	167.070 9		1, 3, 5-trimethoxybenzene	aromatics
81	61.31	$C_{16}H_{31}O_2$	255.231 3		hexadecanoic acid	aliphatic
82	17.95	$C_{16}H_{11}O_5$	283.060 3		emodin methyl ether	anthraquinone
83	16.88	$C_{15}H_{11}O_4$	255.065 7		chrysophanol	anthraquinone
84	15.79	$C_{14}H_9O_5$	257.045 4	3.7	trihydroxymethoxyfluorenone	fluorenone
85	11.77	$C_{14}H_9O_4$	241.050 8	5.5	dihydroxymethoxyfluorenone	fluorenone
86	18.81	$C_{15}H_{11}O_5$	271.062 1	7.4	dengibsinin	fluorenone
87	17.05	$C_{15}H_{11}O_6$	287.055 1	0.4	chrysotoxone	fluorenone
88	2.70	$C_{13}H_{15}O_{10}$	331.067 0	3.1	gal-glc	saccharide
89	2.60	$C_{12}H_{21}O_{11}$	341.107 7	−0.4	glc-glc	saccharide
90	4.13	$C_7H_5O_5$	169.012 9	−1.5	Gallic acid	phenolic acid
91	3.10	$C_{17}H_{29}O_{15}$	473.149 4	−1.5	glc-glc- five-carbon deoxy sugar	saccharide
92	9.02	C_8H_7O	119.050 3		hydroxy vinyl benzene	phenolic acid
93	7.47	$C_7H_5O_2$	121.030 5		hydroxybenzaldehyde	phenolic acid
94	10.09	C_8H_9O	121.067 8		methoxytoluene	aromatics
95	5.8	$C_7H_5O_3$	137.024 0	4.8	hydroxybenzoic acid	phenolic acid
96	8.67	$C_8H_7O_3$	151.039 7	4.8	methoxybenzoic acid/methyl hydroxybenzoate	phenolic acid
97	4.94	$C_7H_5O_4$	153.019 5	8.5	dihydroxybenzoic acid	phenolic acid
98	7.38	$C_8H_7O_4$	167.084 2	1.6	vanillic acid	phenolic acid
99	5.80	$C_8H_5O_5$	181.075 3	1.7	hydroxyphthalic acid	phenolic acid
100	9.20	$C_9H_9O_4$	181.050 2	3.7	clove aldehyde	aromatics
101	6.23	$C_8H_7O_5$	183.029 5	3.8	dihydroxymethoxybenzoic acid	phenolic acid
102	9.79	$C_{10}H_9O_4$	193.049 4	−0.3	dimethoxyphthalaldehyde	aromatics
103	7.40	$C_{10}H_{11}O_4$	195.065 0	−1.2	trimethoxybenzaldehyde	aromatics
104	11.94	$C_{13}H_7O_3$	211.040 7	8.1	hydroxy naphthoic acid	phenolic acid
105	7.55	$C_9H_9O_5$	197.044 0	−2.3	syringic acid	phenolic acid

*Reported before.

on the data of IR, MS, ^{1}H-NMR, ^{13}C-NMR, UV and physicochemical characters.

2.4 Structural elucidation of the isolated compounds

63 compounds were isolated including 11 steroids, 6 phenanthrenes, 4 fluorenones, 9 phenolic acids, 9 aliphatics, 3 anthraquinones, 7 phenylpropanoids, 5 flavones, 3 terpenoids, 2 alkaloids, 2 saccharides, 1 amino acid and 1 aromatic (Table 2). Among them, 27 compounds were first reported from *D. chrysanthum* including 6 steroids, 2 phenanthrenes, 4 phenolic acids, 1 aliphatic, 4 phenylpropanoids, 4 flavones, 3 terpenoids, 1 alkaloid, 1 amino acid and 1aromatic.

3 Chemotaxonomic significance

Dendrobium is characterized by the occurrence of steroids, stilbenoids, alkaloids, anthraquinones, et al. Besides these kinds of compounds, some valuable compounds like novel polyphenylpropanoid and pseudo-spirostanol had been identified at the same time, which might constitute the key characteristics that this medicinal material differentiated from other *Dendrobium* species on the basis of chemical substances, and then reveal the relationship between the unique secondary metabolic characteristics and medicinal value of *D. chrysanthum*.

Table 2 63 Compounds isolated from *D. chrysanthum*

No.	Compound	Classification	Fraction attribution
1	*β*-sitosterol	steroid	$CHCl_3$
2	daucosterol	steroid	$CHCl_3$
3	isonuatigenin	steroid	$CHCl_3$
4	26-*O*-*β*-*D*-glucopyranosylnuatigenin	steroid	$CHCl_3$
5	diosgenin-*3*-*O*-(*α*-L-rhamnopyranosyl-(1→2)-(*α*-L-rhamnopyranosyl-(1→3))-*β*-*D*-glucopyranoside)	steroid	*n*-BuOH
6	diosgenin3-*O*-(4-deoxygenated-*α*-L-rhamnopyranosyl-(1→2)-(*α*-L-glucose-(1→3))-*β*-*D*-glucopyraaanoside	steroid	*n*-BuOH
7	(*25R*)-26-*O*-(*β*-*D*-glucopyranosyl-furost-5-ene-3*β*, 22*α*, 26-trio-3-*O*-*β*-*D*-glucopyranoside-(1→2)-*α*-*L*-rhamnopyranosyl	steroid	*n*-BuOH
8	25*R*-spirost-5-ene-3*β*, 14*α*, 17*α*-triol-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-*D*-glucopyranoside	steroid	*n*-BuOH
9	stigmasterol	steroid	EtOAc
10	diosgenin	steroid	EtOAc
11	(*25R*)-26-*O*-(*α*-*D*-glucopyranosyl)-(1→2)-*α*-L-rhamnopyranosyl-furost-5-ene-3*β*, 22*α*, 26-triol-3-*O*-*α*-*D*-glucopyranoside)	steroid	*n*-BuOH
12	moscatin	phenanthrene	$CHCl_3$
13	2, 5-dihydroxy-4-methoxy-9, 10-dihydrophenanthrene	phenanthrene	$CHCl_3$
14	2, 4-dihydroxy-5-methoxy-9, 10-dihydrophenanthrene	phenanthrene	$CHCl_3$
15	loddigesiinol A	phenanthrene	$CHCl_3$
16	2, 4, 5-trihydroxy-9, 10-dihydrophenanthrene	phenanthrene	*n*-BuOH
17	dendrochrysanene	phenanthrene	EtOAc
18	2, 4, 7-trihydroxy-5-methoxy-9-fluorenone	fluorenone	$CHCl_3$
19	2, 7-dihydroxy-4-methoxy-9-fluorenone	fluorenone	$CHCl_3$
20	dengibsinin	fluorenone	$CHCl_3$
21	chrysotoxone	fluorenone	$CHCl_3$
22	1, 3, 5-trimethoxybenzene	phenolic acid	$CHCl_3$
23	syringic acid	phenolic acid	$CHCl_3$
24	syringaldazin	phenolic acid	$CHCl_3$
25	*p*-hydroxybenzaldehyde	phenolic acid	$CHCl_3$
26	vanillic acid	phenolic acid	$CHCl_3$
27	*p*-hydroxyphenyl propionic acid	phenolic acid	$CHCl_3$
28	erigeside C	phenolic acid	$CHCl_3$
29	isovanillic acid	phenolic acid	EtOAc
30	3, 4, 5-trihydroxybenzoic acid	phenolic acid	EtOAc
31	hentriacontanol alcohol	aliphatics	$CHCl_3$
32	palmitic acid	aliphatics	$CHCl_3$
33	dotriacontane	aliphatics	$CHCl_3$
34	dotriacontanol alcohol	aliphatics	$CHCl_3$
35	4-hydroxy-2-hexadecenoate	aliphatics	$CHCl_3$
36	2-methoxybenzenepropanoic acid	aliphatics	$CHCl_3$
37	2-methoxy-benzoic acid propyl ester	aliphatics	$CHCl_3$
38	myricetin	aliphatics	$CHCl_3$
39	physcion	anthraquinone	$CHCl_3$
40	chrysophanol	anthraquinone	$CHCl_3$
41	emodin	anthraquinone	EtOAc
42	defusine	phenylpropanoid	$CHCl_3$

Table 2 (Continued)

No.	Compound	Classification	Fraction attribution
43	coumarin	phenylpropanoid	$CHCl_3$
44	cyclo-di-p-hydroxyphenylpropionic acid-trans-p-hydroxyphenylacrylate lactone	phenylpropanoid	$CHCl_3$
45	*p*-hydroxy-cinnamic acid	phenylpropanoid	EtOAc
46	*p*-hydroxyphenyl propionic acid	phenylpropanoid	EtOAc
47	caffeic acid	phenylpropanoid	EtOAc
48	*trans*-cinnamic acid	phenylpropanoid	EtOAc
49	4, 5, 7-trihydroxyflavone	flavonoid	EtOAc
50	2-(3-methyl-2-ethoxyphenyl)-flavone	flavonoid	$CHCl_3$
51	4-hydroxy-2-hexadecenoate	aliphatics	$CHCl_3$
52	4', 5, 7-trihydroxyflavone	flavonoid	EtOAc
53	3, 5, 7, 3', 4' -pentahydroxyflavone	flavonoid	EtOAc
54	3, 4', 5, 7-tetrahydroxyflavone	flavonoid	EtOAc
55	3*β*-hydroxy-28-norurs-12, 17-dien	terpenoid	$CHCl_3$
56	oleana-11, 13(18)-dien-3-ol	terpenoid	$CHCl_3$
57	3, 12-dioxofriedelan-19-methoxyfriedelane	terpenoid	$CHCl_3$
58	(-)-*trans*-dendrochrysine	alkaloid	$CHCl_3$
59	3-methylpyrrole-dendrochrysine	alkaloid	$CHCl_3$
60	phenylalanine	amino acid	EtOAc
61	2, 2'- oxodibis (1,4-di-tert-butylbenzene)	aromatics	EtOAc
62	*D*-glucose	saccharide	*n*-BuOH
63	maltose	saccharide	*n*-BuOH

3.1 Polyphenylpropanoids

Phenylpropanoids refer to natural compounds with one or more C_6-C_3 units in the basic parent nucleus, including simple phenylpropanoids, coumarins, lignans and so on. In the process of separating and extracting the $CHCl_3$ part of *D. chrysanthum*, polyphenylpropanoid compounds were detected UPLC/Q-TOF-MS/MS and isolated. It was the first time for us to report a new phenylpropanoid compound, cyclo-di-p-hydroxyphenylpropionic acid-trans-p-hydroxyphenylacrylate lactone (Fig. 1) from *D. chrysanthum*. The new polyphenylpropanoids were found to have unique structures[14].

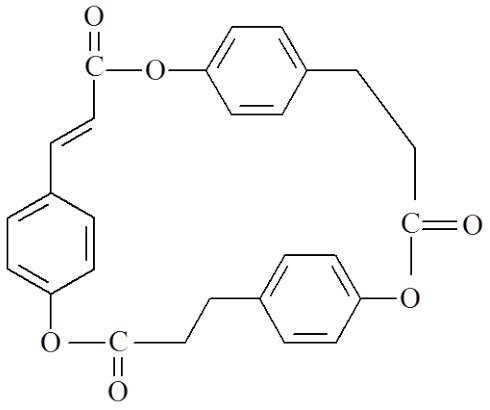

Fig. 1 Structure of the novel polyphenylpropanoid

3.2 Steroids

Pseudo-spirostanol, whose F ring was found to be a five-membered tetrahydrofuran ring, is rare in nature. Through the separation and extraction of $CHCl_3$ part, a pseudo-spirostanol, 26-*O*-*β*-*D*-glucopyranosyl nuatigenin was identified for the first time in *D. chrysanthum*. Steroids and their glycosides like *β*-sitosterol, daucosterol and isonuatigenin were also found in the $CHCl_3$ part of *D. chrysanthum* in our study. Isonuatigenin and *26-O-β-D*-glucopyranosyl nuatigenin were isolated for the first time from *Dendrobium* genus, especially the pseudo-spirostanol, which was distinct in only a few origins such as Lilium[15]. And (*25R*)-*26-O*-(*α-D*-glucopyranosyl)-(1→2)-*α*-

L-rhamnopyranosyl-furost-5-ene-3β, 22α, 26-triol-3-*O*-α-*D*-glucopyranoside, *(25R)*-26-*O*-(β-*D*-glucopyranosy-furost-5-ene-3β, 22α, 26-triol-3-*O*-β-*D*-glucopyranoside-(1→2)- -L-rhamnopyranosyl, diosgenin 3-*O*-(α-L-rhamnopyranosyl-(1→2)-(α-L-rhamnopyranosyl-(1→3))-β-*D*-glucopyranoside were reported in the genus *Dendrobium*[9,16].

3.3 Stilbenoids

Stilbenoid is a kind of characteristic components spread in epiphytic orchid plants, which has a wide range of biological activities and good application prospects. But its synthesis is tedious, and the source of natural products is very limited. The contents of stilbenoids, including phenanthrenes and bibenzyls in 18 species of *Dendrobium* were determined by reversed-phase high performance liquid chromatography (RP-HPLC) in previous study and the results showed that there were 1-2 or even 3 components in different *Dendrobium* species. Phenanthrenes and dihydrophenanthrene were substituted by polyhydroxyl and methoxy groups in *Dendrobium*, and only a small amount of phenanthrenes were dimer and phenanthrenequinone. Bibenzyl compounds were the characteristic active components isolated from *Dendrobium* in recent years. In our study, 6 stilbenes were found in *D. chrysanthum* including moscatin, loddigesiinol A, dendrochrysanene, 2, 4, 5-trihydroxy-9, 10-dihydrophenanthrene, 2, 4-dihydroxy-5-methoxy-9,10-dihydrophenanthrene and 2, 5-dihydroxy-4-methoxy-9,10-dihydrophenanthrene[8]. Moscatin was previously isolated from the *Dendrobium aphyllum* and loddigesiinol A from *Dendrobium loddigesii*[17]. And it is the first report of 2, 4-dihydroxy-5-methoxy-9, 10-dihydrophenanthrene in the genus *Dendrobium*[8].

3.4 Flavonoids

Flavonoids have a wide range of biological activities and have been applied in the development and utilization of food, health products and drugs. It has been reported that flavonoids have the effect of anti-cardiovascular and cerebrovascular diseases, anti-oxidation, anti-aging, analgesia and anti-virus. Previous studies have reported that flavonoids are the main components of antioxidant activity of *Dendrobium candidum*. A large number of flavonoid glycosides were also found in the flowers, leaves and stems of *D. candidum*. In the course of our study on the components of *D. chrysanthum*, we identified 5 flavonoids including 4,5,7-trihydroxyflavone, 2-(3-methyl-2-ethoxyphenyl)-flavone, 4',5,7-trihydroxyflavone, 3,5,7,3',4'-pentahydroxyflavone and 3,4',5,7-tetrahydroxyflavone in *D. chrysanthum*.

3.5 Alkaloids

Alkaloid is a kind of main characteristic component of *Dendrobium*. There are differences in the quantity and content of alkaloids in different species of *Dendrobium*. The alkaloids in *D. candidum* are mainly amides, while in *D. chrysanthum*, we identified 2 alkaloids, (-)-trans-dendrochrysine and 3-methylpyrrole-dendrochrysine. It was the first time to find 3-methylpyrrole-dendrochrysine from *D. chrysanthum*.

3.6 Fluorenones

The isolation of fluorenones from *D. chrysanthum* might expand our understanding of the chemical composition of the family Orchidaceae. In our study, four fluorenones, 2, 4, 7-trihydroxy-5-methoxy-9-fluorenone, 2, 7-dihydroxy-4-methoxy-9-fluorenone, dengibsinin and chrysotoxone were found in the dry stem of *D. chrysanthum*.

3.7 Others

In our study, 9 phenolic acids, 9 aliphatics, 3 anthraquinones, 3 terpenoids, 2 saccharides, 1 amino acid and 1 aromatic were isloated from the dry stem of *D. chrysanthum*. Although these compounds do not seem to have systematic

significance as they have been reported from other families, like Ranunculaceae[18], Cruciferae[19-20], Caprifoliaceae[21], Scrophulariaceae[22], Leguminosae sp[23] and Polygonaceae[24]. The occurrence of these compounds in the genus *Dendrobium* is worthy of further study. Compounds like 1, 3, 5-trimethoxybenzene, syringic acid, syringaldehyde, p-hydroxybenzaldehyde, vanillic acid and palmitic acid were widespread in family of Orchidaceae. Defuscin was isolated as a new natural product from the genus *Dendrobium*[25].

4 Pharmacological properties

Many species of *Dendrobium* have been commonly used in folk medicine for the treatment of inflammatory, hypoglycemia, tumor, oxidation, immunomodulatory. The studies on the activity of chemical constituents of *Dendrobium* were mainly focused on polysaccharides, but few on small molecules. In order to further explore the pharmacological activities of *D. chrysanthum*, this experiment focuses on the anti-inflammatory, antioxidant, anti-tumor and hypoglycemic activities of the chemical constituents of *D. chrysanthum*.

4.1 Anti-inflammatory

It had been reported that a new phenanthrene with a spirolactone from *D. chrysanthum* might presents anti-inflammatory activities[26]. The studies on the anti-inflammatory activities of chemical constituents of *Dendrobium* were mainly focused on polysaccharides. In order to further explore the pharmacological activity of *D. chrysanthum*, the inhibitory effects of different fractions and related active components of *D. chrysanthum* on the production of nitric oxide (NO) in BV2 cells were observed through the mouse microglia BV2 inflammation model induced by lipopolysaccharide (LPS). The results showed that the active components of *D. chrysanthum* could significantly inhibit the release of NO from BV2 cells induced by LPS in a dose-dependent manner[27].

4.2 Antioxidation

With the in-depth study of the chemical constituents of *Dendrobium*, more and more attention has been paid to the antioxidant activity of other plants of *Dendrobium*. In the previous study, EtOAc fraction was selected as the antioxidant active part of *D. chrysanthum* by using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) assay. In our study, total antioxidant capacity assay kits with ABTS method (ABTS) radical scavenging ability and hydroxyl radical scavenging ability were used to investigate the antioxidant capacity of *D. chrysanthum* extract and its different parts. The results showed that the total extract of *D. chrysanthum* and its fractions had a certain scavenging effect on ABTS free radicals, and the scavenging ability was dose-dependent, among which EtOAc fraction had the strongest scavenging ability on ABTS free radicals. The hydroxyl radical scavenging ability of n-BuOH fraction was the strongest.

4.3 Antitumor

The characteristics of multi-level, multi-link and multi-target of traditional Chinese medicine (TCM) making it useful in the prevention and treatment of tumor. A large body of literature has reported that *Dendrobium* has antitumor effects in various tumor cells. In our study, human cervical cancer cell line (Hela), human prostate cancer cell lines (PC3 and LNCaP), human lung adenocarcinoma cell line (SPC-A1) and human breast cancer cell line (MCF-7) were used to study the antitumor activity of compounds isolated from *D. chrysanthum*. The results showed that compounds isolated from *D. chrysanthum* had obvious inhibitory effect on SPC-A1, PC3, LNCaP and MCF-7 cancer cell lines, and the inhibitory effect on SPC-A1 was stronger than that of MCF-7[27-28].

4.4 Hypoglycemia activity

In recent years, as a main component of some prescriptions for diabetes, the pharmacology and

mechanism of *Dendrobium* for diabetes attracted many researchers. In our study, the hypoglycemic activity of the extract from *D. chrysanthum* was screened by *in vivo* and *in vitro* experiment. The $CHCl_3$ fraction had the best hypoglycemic activity with less cytotoxicity. At the same time, the crude extract of *D. chrysanthum* was used to investigate the effect on type 2 diabetic rats induced by high-glucose and high-fat diet combined with low-dose Streptozotocin (STZ) injection. It had significant hypoglycemic effect and could comprehensively improve the dyslipidemia and insulin resistance of type 2 diabetic rats, and enhance the glucose tolerance of type 2 diabetic rats[12,29-31].

5 Conclusion

Dendrobium has a long history of medicinal use of "nourishing Yin and invigorating Qi"and beneficial medical and healthy functions. It is characterized by the occurrence of steroids, alkaloids, stilbenoids and anthraquinones in previous study[33]. The present phytochemical investigation has further enriched our knowledge about the chemistry of the family Orchidaceae and the *Dendrobium* genus, and has identified polyphenylpropanoids and pseudo-spirostanol as the potential chemotaxonomic markers for the species. Additionally, compounds like *(25R)-26-O-(β-D*-glucopyranosyl-furost-5-ene-*3β*, *22α*, 26-triol-*3-O-β-D*-glucopyranoside-(1→2)-*α*-L-rhamnopyranosyl, dip-hydroxyphenylpropionic acidic p-coumaric acid lactone, diosgenin, and 3-*O*-(*α*-L-rhamnopyranosyl-1→2)-(*α*-L-*r*hamnopyranosyl-(1→3))-*β-D*-glucopyranoside were firstly reported from this plant. These identified compounds could be potential chemotaxonomic markers for the species. The pharmacological activities of *D. chrysanthum* were summarized including the anti-inflammatory, antioxidant, anti-tumor and hypoglycemic activities of the ethanol extract and identified constituents.

The study of *Dendrobium* is a work of excavating the essence of TCM, which has broadened research prospects and application value. And it is also necessary to domesticate wild *Dendrobium* to achieve large-scale artificial planting to solve the problem of shortage of *Dendrobium* resources.

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