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Identification of Yunnan Guangnan *Dendrobium officinale* With Different Growth Years Using UHPLC–Orbitrap–MS

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ABSTRACT

Dendrobium officinale from Guangnan was screened nontargeted using UHPLC–Orbitrap–MS for different growth years. OPLS–DA effectively differentiated *D. officinale* based on growth years, identifying 70 different compounds. Eight indole diketopiperazine alkaloids did not differ considerably among the growth years. The differential compounds of *D. officinale* with 4 years of growth were more distinct from other years, with 10 compounds showing greater variability. Pathways in cancer, cancer metabolic pathways, metabolic pathways, chemical carcinogenesis—receptor activation were more enriched. This suggests that *D. officinale* functional compounds have pharmacological activities in these areas and can regulate the metabolic processes of lipids and atherosclerosis.

1 | Introduction

Guangnan *Dendrobium officinale*, grown in Guangnan County, Yunnan Province, China, is a national geographic indication product of China. Guangnan *D. officinale* possesses good antitumor, antioxidant, and other bio-health functional activities because it grows in the low-latitude, high-altitude plateau area of Yunnan Province, China. In November 2023, Guangnan *D. officinale* was included in China's list of substances that are traditionally used as food and Chinese medicine, which means that Guangnan *D. officinale* can be used as a food ingredient to fully utilize its better health benefits.

Flavonoids are one of the main active ingredients in *Dendrobium*, with functions including antitumor (Liu et al. 2023; Zhao

et al. 2004, 2021), hypoglycemic (Lv et al. 2023; Peng et al. 2023), anti-inflammatory (Wang 2021), antioxidant (Luo et al. 2023), and immune regulation properties (Liang et al. 2019). Flavonoids have gradually become significant small-molecule compounds in *Dendrobium* research, second only to polysaccharides. Guangnan *D. officinale*, a characteristic *Dendrobium* species from Yunnan Province, China, contains rutin, vicienin II, vicienin I, schaftoside, isoschaftoside, violanthin, and isoviolanthin (Luo et al. 2023; Zhang et al. 2019; Zhou et al. 2018). However, the accumulation, metabolism, and change patterns of other related components, such as secondary metabolites like alkaloids and other characteristic compounds, have hardly been reported.

Dendrobium, as a type of traditional Chinese medicine, has efficacy components closely related to the region and

Jiao Zhang and Yanping Ye contributed equally to this study.

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environment where it grows (Li et al. 2020; Liu et al. 2017; Pant et al. 2021). The same species in different areas can have varying types and contents of efficacy components (LingShang et al. 2009; Zhou et al. 2021), and even the same area can produce different efficacy components in different years (Hu et al. 2021; Li et al. 2023). The content change and accumulation patterns of related active compounds in different growth stages of Guanganan *D. officinale* are not clear. Therefore, to deeply investigate the characteristic compounds in Guanganan *D. officinale* and to study their accumulation and change patterns over different growth years, this study was conducted using UHPLC–Orbitrap–MS and stoichiometry (Cui et al. 2015; Ju et al. 2025; Zeng et al. 2017). The results suggested that the characteristic compounds in Guanganan *D. officinale* varied with the growing year, providing a reference for quality control and scientific cultivation.

2 | Materials and Methods

2.1 | Sample Collection and Preparation

Guanganan *D. officinale* grown for 1, 2, 3, 4, and 5 years were collected from Guangan County, Wenshan City, China, and 20

samples of *D. officinale* from each growth year were collected. The samples were collected in December 2023 and all samples were collected at a local wholesale market, with a representative five stems of approximately 15 cm in length selected for each sample, dried at 60°C, pulverized, passed through a sample sieve of 0.28 μm, and stored at 4°C away from light.

2.2 | Chemicals and Reagents

HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). HPLC-grade ammonium acetate and formic acid were purchased from DiKMA Technologies (Beijing, China). Ultrapure water was prepared from Elga's water system (Wycombe, UK).

2.3 | Sample Preparation and Analysis

2.3.1 | Sample Preparation Method

Sample pretreatment steps: Briefly, 1.0 g of *D. officinale* powder was precisely weighed. Subsequently, 5.0 mL of 60% methanol solution was added, extracted using 80 kHz ultrasonic waves at

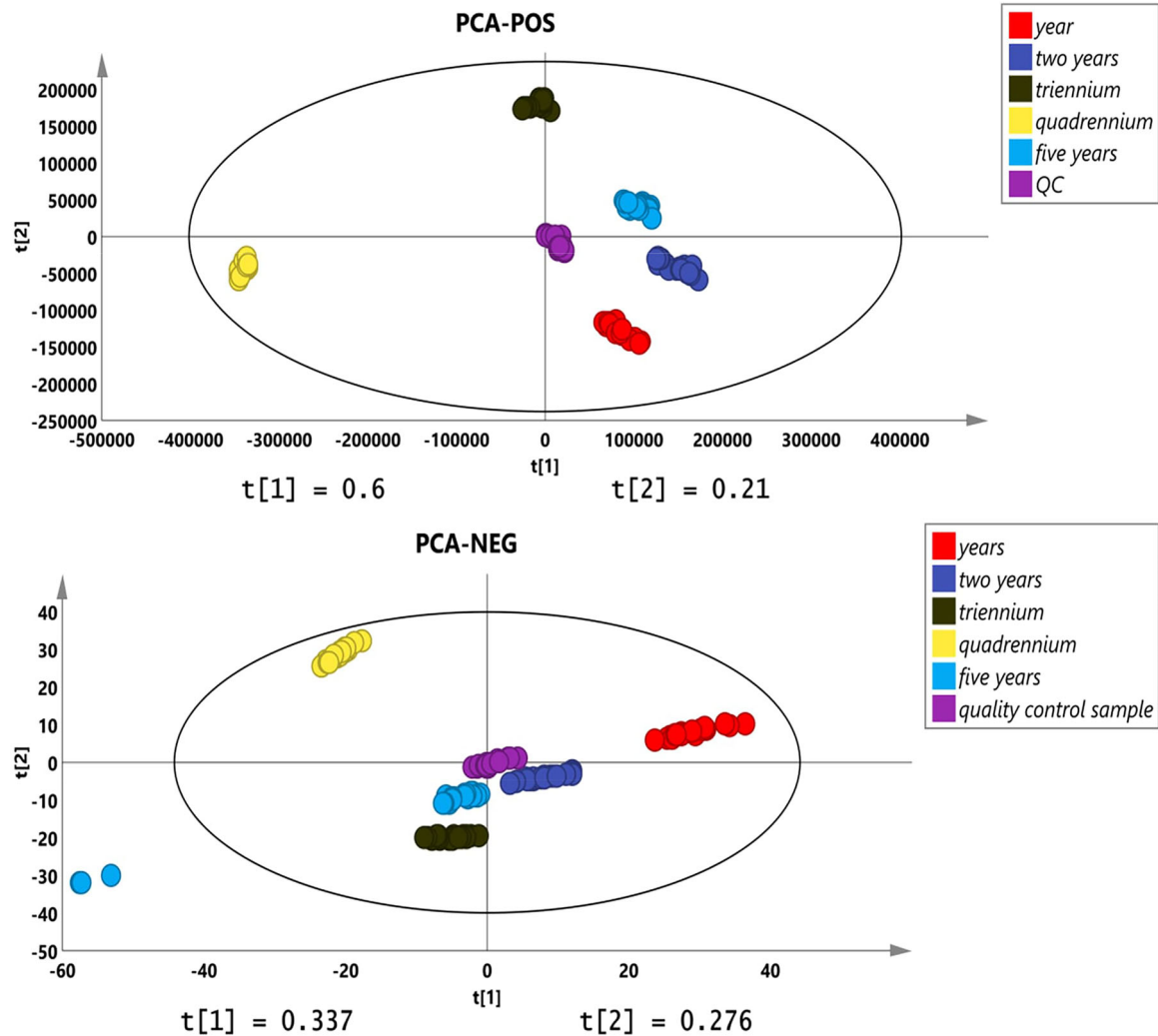


FIGURE 1 | PCA score plot in positive and negative modes.

25°C (ice bath) for 30 min, and passed through a 0.22- μ m membrane (0.22 μ m) for determination.

Sample preparation process for nontargeted screening experiments: (a) Blank sample preparation: Briefly, 5 mL of 60% methanol solution was measured into a 50 mL centrifuge tube according to the sample pretreatment protocol. (b) QC sample preparation: Here, 0.25 g of each sample was weighed and mixed well to obtain QC sample powder. Subsequently, 1.0 g of QC sample powder was weighed and prepared according to the sample pretreatment protocol (repeat to make five QC samples). (c) Actual sample preparation: Briefly, 1.0 g of sample powder was weighed and extracted according to the sample pretreatment.

2.3.2 | Instrumental Analysis Method

The Ultra High Performance Liquid Chromatography–Q Exactive High Resolution Mass Spectrometry (Thermo Fisher Scientific, Rockford, IL, USA) system (Framingham, MA, USA) equipped with HPG-3400RS ultrahigh performance liquid chromatography (Thermo Fisher Scientific) and

ACQUITY UPLC HSS T3 (100 $\times$ 2.1 mm, 1.8 μ m; Waters, Milford, MA, USA) was used. Liquid chromatography was performed in a gradient elution mode, with mobile phase A as 0.1% formic acid in water and mobile phase B as acetonitrile. The elution program was as follows: 0–5.5 min, 2%–8% A, 98%–92% B; 18–20 min, 60%–90% A, 40%–10% B; and 20.5–25 min, 2% A, 98% B. The injection volume was 1 μ L, the flow rate was 0.2 mL/min, and the temperature was 35°C. Mass spectrometry data were acquired in positive and negative ESI modes (spray voltage: 3.5 kV, capillary temperature: 325°C, probe heater temperature: 350°C, and SLens: 50 V). The full MS/dd-MS2 mode was used. In full MS, the scan range was m/z 80–1200; mass resolution at 35,000 FWHM; and AGC target and maximum IT at 3.0 e6 and 100 ms, respectively. For dd-MS2, the relevant parameters were set as follows: mass resolution: 17,500 FWHM; AGC target: 1 e5; maximum IT: 50 ms; loop count: 5; MSX count: 1; isolation window: 4.0 m/z ; and stepped normalized collision energy: 20%, 40%, and 60%. The spray voltage in positive and negative modes was set as 3.5 and 3.0 kV, respectively. The flow rate of sheath gas and aux gas was 45 and 10 (in arbitrary units), respectively. TraceFinder 4.1 EFS and Compound Discoverer 3.3 were used.

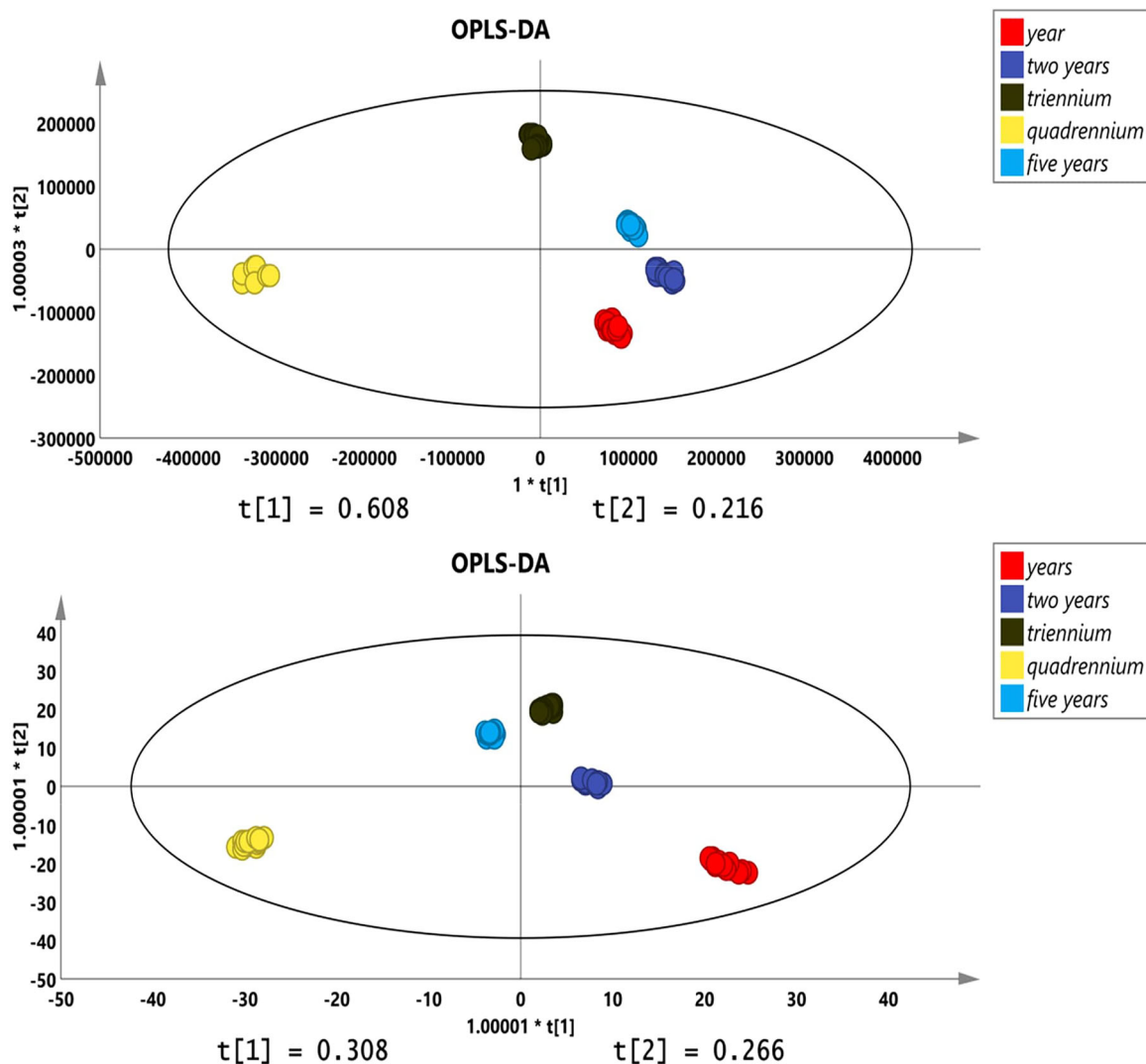


FIGURE 2 | OPLS-DA score chart and model diagram in positive and negative modes.

2.3.3 | Data Processing and Compound Structure Identification

The raw data files were imported into Compound Discoverer 3.3 software for peak alignment, retention time correction, and peak area extraction. The data extracted by CD3.3 were first subjected to metabolite structure identification and data pre-processing, then to experimental data quality evaluation, and finally to data analysis.

Data preprocessing included the following steps: (1) preliminary screening of the identified structures (retaining metabolites with a relative standard deviation of < 30% of the peak area of the QC samples) and (2) obtaining retained metabolites in the database and de-emphasizing them, including positive and negative ionic modes, in the priority order: MzVault > MzCloud > MassLists > ChemSpider. When metabolite levels are the same, the one with the higher score is selected as the identification result. For the deduplication process between positive and negative ions, the higher priority results were preferentially retained, and if the results were the same, the pattern result with the highest expression was selected.

The data analysis included heatmaps, volcano maps, orthogonal partial least squares discrimination analysis (OPLS-DA), and differential metabolite screening. The data were normalized and grouped for heatmap analysis after column clustering and row clustering. Volcano plots were analyzed by using Fold Change and *p* value as horizontal and vertical coordinates, respectively. The predictor variable matrix and response variables were confirmed, and the number of principal components or latent variables to be used was determined and after orthogonal signal correction, OPLS-DA analysis was performed. The variable importance in projection (VIP) obtained from the OPLS-DA model can be used to measure the intensity of the influence of the expression pattern of each metabolite on the classification of each group of samples and to identify biologically significant differential metabolite molecules. In the current study, the screening criteria for differential metabolites were set as follows: VIP > 1 and *p* < 0.05 by the OPLS-DA model.

2.3.4 | Pathway Enrichment Analysis

The targets of the compounds were predicted using SwissTargetPrediction software, and the target functions were analyzed

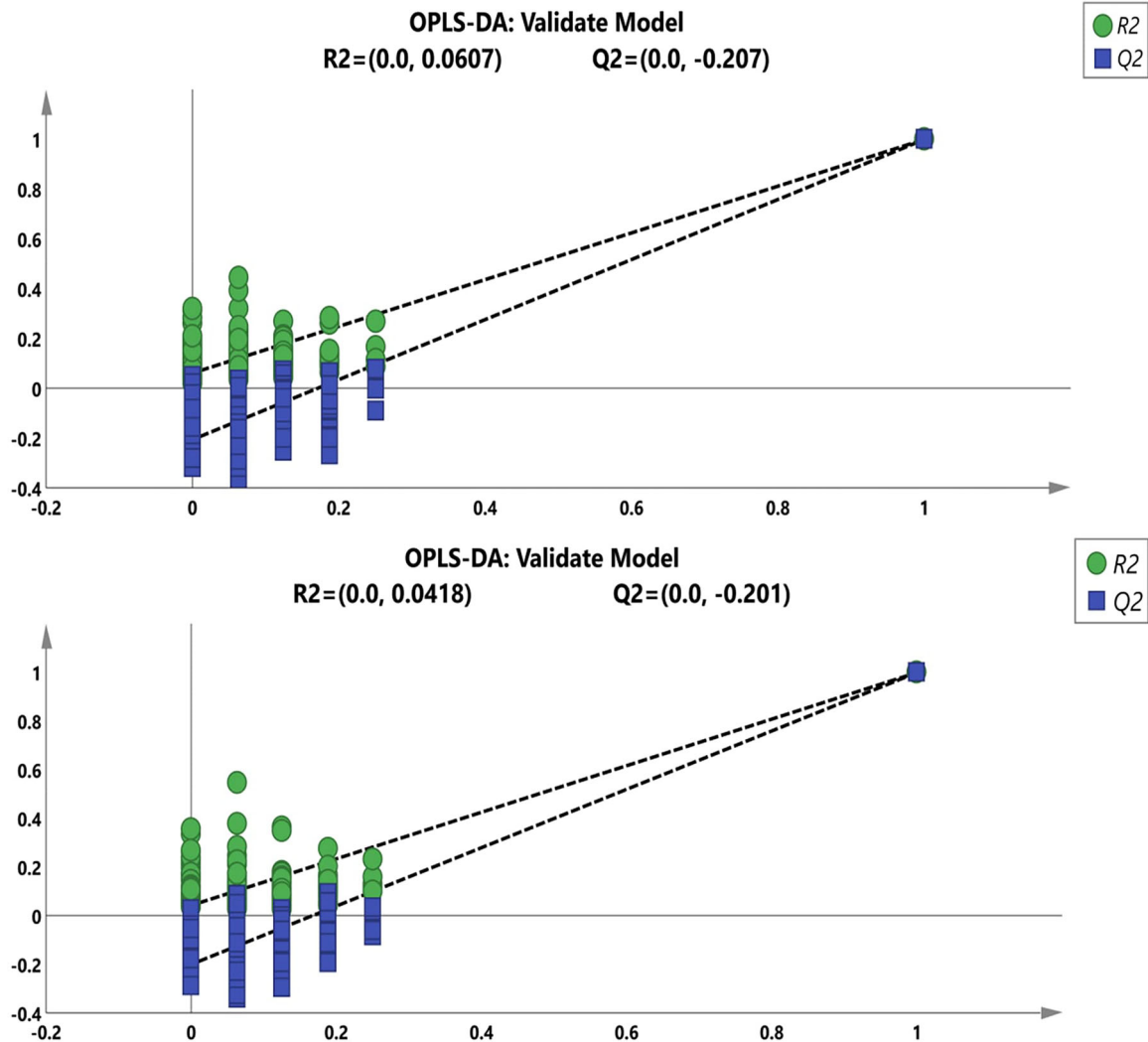


FIGURE 3 | OPLS-DA cross-validation details in positive and negative modes.

TABLE 1 | Different marker compounds in Guangan *Dendrobium officinale*.

Peak	Retention time/min	Component name	Formula	m/z	Annot. DeltaMass [ppm]	Adduct/charge	References
1	1.226	Hydantocidin	C ₇ H ₁₀ N ₂ O ₆	217.0475	4.23	[M – H] [–]	Haruyama et al. (1991)
2	1.848	Allopurinol	C ₅ H ₄ N ₄ O	137.0458	0.38	[M + H] ⁺	Peng et al. (2016)
3	2.145	Leptazoline D	C ₁₆ H ₂₂ N ₂ O ₇	355.1496	–0.99	[M + H] ⁺	Bhandari Neupane et al. (2019)
4	2.168	Acetyl-L-carnitine	C ₉ H ₁₇ NO ₄	202.1077	–3.96	[M – H] [–]	Nie et al. (2021)
5	3.105	Leucyl-4-hydroxyproline	C ₁₁ H ₂₀ N ₂ O ₄	245.1496	0	[M + H] ⁺	Hernández et al. (2004)
6	3.117	Vochysiamide B	C ₁₁ H ₁₇ NO ₄	245.1495	–0.06	[M + NH ₄] ⁺	She et al. (2022)
7	9.067	3-(Dimethylamino)-4-hydroxybutyl dimethylcarbamate	C ₉ H ₂₀ N ₂ O ₃	205.1546	–0.19	[M + H] ⁺	—
8	9.441	Ac-Glu(OtBu)-OH	C ₁₁ H ₁₉ NO ₅	246.1336	0.19	[M + H] ⁺	Zhang et al. (2017)
9	9.516	3-(<i>tert</i> -Butoxycarbonyl)-2,2-dimethyl-1,3-oxazolane-4-carboxylic acid	C ₁₁ H ₁₉ NO ₅	244.1188	–0.87	[M – H] [–]	Chattopadhyay et al. (2006)
10	9.734	2-[2-{2-(<i>N-tert</i> -butoxycarbonyl)aminoethoxy}ethoxy]ethoxyacetic acid	C ₁₃ H ₂₅ NO ₇	308.1717	4.3	[M + H] ⁺	Adamczyk et al. (2002)
11	9.883	Isobutyl-4-[4-(benzyloxy)phenyl]-2-methyl-5-oxo-7-phenyl-1,4,5,6,7,8-hexahydro-3-quinolinecarboxylate	C ₃₄ H ₃₅ NO ₄	520.2511	3.39	[M – H] [–]	—
12	10.049	Ethyl <i>N</i> -[(1-acetamidocyclopentyl)carbonyl]serinate	C ₁₃ H ₂₂ N ₂ O ₅	287.16	–0.52	[M + H] ⁺	—
13	10.091	(4 <i>R</i>)-4-[3-oxo-3-(1-piperidinyl)propyl]-L-glutamic acid	C ₁₃ H ₂₂ N ₂ O ₅	285.1457	0.18	[M – H] [–]	Assaf et al. (2013)
14	10.337	<i>N</i> -(3-hydroxyhexanoyl)-homoserine lactone	C ₁₀ H ₁₇ NO ₄	216.1229	–0.49	[M + H] ⁺	Ortori et al. (2011)
15	10.884	L-leucyl-L-prolinamide	C ₁₁ H ₂₁ N ₃ O ₂	228.1705	–0.56	[M + H] ⁺	Brunetti et al. (2013)
16	11.272	Phomolide D	C ₁₂ H ₂₀ O ₅	243.1236	–0.76	[M – H] [–]	Li et al. (2010)
17	11.653	1- <i>O</i> -sinapoyl-beta-D-glucose	C ₁₇ H ₂₂ O ₁₀	385.1141	0.19	[M – H] [–]	Offor et al. (2022)
18	12.394	3,3-Dimethylhexahydroimidazo[1,5- <i>a</i>]pyrazine-1(5H)-one	C ₈ H ₁₅ N ₃ O	170.1286	–1.23	[M + H] ⁺	—
19	12.422	Chlortolide	C ₁₂ H ₁₈ O ₄	225.1128	–2.11	[M – H] [–]	Ostermeier and Schobert (2014)
20	12.734	Quercetin 3-robinobioside	C ₂₇ H ₃₀ O ₁₆	611.1602	–0.81	[M + H] ⁺	Jaramillo et al. (2011)
21	12.812	2,2'-(Phenylimino)diethanol	C ₁₀ H ₁₅ NO ₂	182.1174	–0.8	[M + H] ⁺	Moon et al. (1996)
22	12.949	Gercalcin D	C ₁₅ H ₂₂ N ₂ O ₆	327.1547	–1.09	[M + H] ⁺	Le Goff et al. (2013)
23	12.986	Perennipin B	C ₂₁ H ₂₆ O ₉	421.1507	0.8	[M – H] [–]	Kim et al. (2019)
24	13.127	Afalanine	C ₁₁ H ₁₃ NO ₃	206.0815	–3.91	[M – H] [–]	Tawfike et al. (2018)

(Continues)

TABLE 1 | (Continued)

Peak	Retention time/min	Component name	Formula	m/z	Annot. DeltaMass [ppm]	Adduct/charge	References
25	13.275	2-{2-[(2-Ethylhexyl)oxy]-2-oxoethyl}-2-hydroxysuccinic acid	C ₁₄ H ₂₄ O ₇	303.145	0.15	[M – H] [–]	—
26	13.595	Rhoifolin	C ₂₇ H ₃₀ O ₁₄	579.1704	–0.76	[M + H] ⁺	Refaat et al. (2015)
27	13.999	Lotaustralin	C ₁₁ H ₁₉ NO ₆	260.1138	–0.62	[M – H] [–]	Opala et al. (2013)
28	14.334	2-[(E)-(hydroxyimino)methyl]-3-methyl-1-(2-nitroethyl)-1H-imidazol-3-ium	C ₇ H ₁₁ N ₄ O ₃	198.0761	1.35	[M – H] [–]	—
29	14.339	Swertisin 6''-O-feruloyl 2''-O-glucoside	C ₂₉ H ₃₂ O ₁₅	619.1646	–3.58	[M – H] [–]	Becchi and Fraisse (1989)
30	14.833	1,7-Hydroxy-3-methylxanthone	C ₁₄ H ₁₀ O ₃	227.07	–1.25	[M + H] ⁺	Fujimoto et al. (1998)
31	14.921	3-Cyclohexylpropyl 1-[methoxy(oxo)acetyl]-2-piperidinecarboxylate	C ₁₈ H ₂₉ NO ₅	338.1974	0.18	[M – H] [–]	—
32	14.926	Isomucronulatol 7-O-glucoside	C ₂₃ H ₂₈ O ₁₀	463.161	0.13	[M – H] [–]	Yoo et al. (2021)
33	15.083	Flutimide	C ₁₂ H ₁₈ N ₂ O ₃	237.124	–2.11	[M – H] [–]	Hensens et al. (1995)
34	15.601	Endophenazine E	C ₁₉ H ₁₈ N ₂ O ₆	369.1091	–0.38	[M – H] [–]	Wu et al. (2016)
35	15.677	Phomoxydiene C	C ₁₄ H ₁₄ O ₃	229.0864	–2.75	[M – H] [–]	Zhang et al. (2021)
36	15.925	Variecolorin C	C ₂₄ H ₂₉ N ₃ O ₃	408.2275	–1.59	[M + H] ⁺	Wang et al. (2007)
37	16.069	Nigirpexin D	C ₁₅ H ₂₀ O ₆	295.1182	–1.67	[M – H] [–]	Zhou et al. (2018)
38	16.09	(2S,3 R,5S)-(–)-2,3-dihydroxytetradecan-5-olide	C ₁₄ H ₂₆ O ₄	257.1756	–0.8	[M – H] [–]	Toshima et al. (1999)
39	16.109	1-(2,4-Dihydroxyphenyl)-3-hydroxy-3-(4'-hydroxyphenyl)-1-propanone	C ₁₅ H ₁₄ O ₅	273.0768	–0.08	[M – H] [–]	Rafi et al. (2002)
40	16.111	UCS1025B	C ₂₀ H ₂₅ NO ₆	374.1607	–0.47	[M – H] [–]	Agatsuma et al. (2002)
41	16.221	(E)-2-methoxy-1,4 naphthoquinone-1-oxime	C ₁₁ H ₉ NO ₃	202.0501	–4.36	[M – H] [–]	Zhang et al. (2013)
42	16.401	YF-0200R-B	C ₁₂ H ₂₀ O ₅	244.1300	–2.38	[M – H] [–]	Wang et al. (2016)
43	16.407	1,2-Dihydro-5-hydroxy-2-(1-hydroxy-1-methylethyl)-4-(isobutyl)-6-phenylfuran [2,3-h] [1]	C ₂₄ H ₂₄ O ₆	407.1498	–0.43	[M – H] [–]	Zhou et al. (2022)
44	16.422	benzopyran-8-one					
45	16.823	Variecolorin O	C ₁₉ H ₂₁ N ₃ O ₃	340.1649	–1.85	[M + H] ⁺	Chen et al. (2023)
46	16.851	Dichotosin	C ₂₃ H ₂₈ O ₉	447.1659	–0.39	[M – H] [–]	Ghosal et al. (1985)
47	16.879	Diorcinol H	C ₁₅ H ₁₄ O ₄	257.0817	–1.03	[M – H] [–]	Li et al. (2015)
48	16.911	LMA-P1	C ₁₄ H ₂₄ O ₄	255.1599	–1.02	[M – H] [–]	Adelin et al. (2011)
		Trimethyl 1,2,8-octanetricarboxylate	C ₁₄ H ₂₄ O ₆	287.1499	–0.33	[M – H] [–]	Ballini and Bosica (1996)

(Continues)

TABLE 1 | (Continued)

Peak	Retention time/min	Component name	Formula	m/z	Annot. DeltaMass [ppm]	Adduct/charge	References
49	17.294	Aspertetranone D	C ₂₂ H ₂₈ O ₉	435.1661	0.07	[M - H] ⁻	Wang et al. (2015)
50	17.315	Dihydrodroneochinulin B	C ₁₉ H ₂₁ N ₃ O ₂	324.17	-1.99	[M + H] ⁺	Gao et al. (2013)
51	17.315	2-(5-Nitro-2-pyridinyl)-1,2,3,4-tetrahydroisoquinoline	C ₁₄ H ₁₃ N ₃ O ₂	256.1075	-2.16	[M + H] ⁺	Andrew Hedley and Stanforth (1992)
52	17.315	Neoechinulin B	C ₁₉ H ₁₉ N ₃ O ₂	322.1546	-1.17	[M + H] ⁺	Wohlgemuth et al. (2017)
53	17.573	Okanin 3,4,3'-trimethyl ether 4'-glucoside	C ₂₄ H ₂₈ O ₁₁	491.1559	-0.06	[M - H] ⁻	Piispanen et al. (2023)
54	17.659	2-[Methyl-(3-phenylpropionyl)amino]-benzoic acid	C ₁₇ H ₁₇ NO ₃	282.1134	-0.52	[M - H] ⁻	Farooq Biabani et al. (1998)
55	18.189	4-(2,5-Dimethoxyphenyl)-1-(4-ethylphenyl)-4,7-dihydrofuro[3,4-b]pyridine-2,5(1H,3H)-dione	C ₂₃ H ₂₃ NO ₅	392.1503	-0.07	[M - H] ⁻	—
56	18.218	UCS1025A	C ₂₀ H ₂₅ NO ₅	358.1659	-0.26	[M - H] ⁻	Nakai et al. (2000)
57	18.408	Citreoviripyrone B	C ₁₄ H ₁₆ O ₃	231.1021	-2.4	[M - H] ⁻	Asai et al. (2013)
58	20.246	Talathermophilin D	C ₂₄ H ₂₉ N ₃ O ₃	408.2278	-0.99	[M + H] ⁺	Guo et al. (2011)
59	20.565	4-Hydroxyphenethyl 2-(4-hydroxyphenyl)acetate	C ₁₆ H ₁₆ O ₄	271.0975	-0.23	[M - H] ⁻	Wang et al. (2009)
60	20.693	Citreopyrone D	C ₁₅ H ₁₆ O ₄	259.0974	-0.71	[M - H] ⁻	Kosemura et al. (1997)
61	21.075	Asperglaucide	C ₂₇ H ₂₈ N ₂ O ₄	445.2118	-0.89	[M + H] ⁺	Jaderson and Park (2020)
62	21.091	Cladoniamide C	C ₂₂ H ₁₇ N ₃ O ₅	404.1238	-0.79	[M + H] ⁺	Williams et al. (2008)
63	21.095	Neoechinulin D	C ₂₄ H ₂₉ N ₃ O ₂	392.2328	-1.1	[M + H] ⁺	Marchelli et al. (1977)
64	21.702	Dehydrovariecolorin L	C ₂₉ H ₃₇ N ₃ O ₂	460.2956	-0.67	[M + H] ⁺	Li et al. (2008)
65	21.778	(9E,11Z)-13-oxo-9,11-octadecadienoic acid	C ₁₈ H ₃₀ O ₃	293.2123	0.26	[M - H] ⁻	Cantrell et al. (2008)
66	21.963	2-(2-Oxo-2H-chromen-3-yl)-5H-chromeno[4,3-b]pyridin-5-one	C ₂₁ H ₁₁ NO ₄	342.0767	1.73	[M + H] ⁺	Patel et al. (2013)
67	22.16	Arestrictin A	C ₂₉ H ₃₉ N ₃ O ₃	478.3061	-0.71	[M + H] ⁺	Itabashi et al. (2006)
68	22.273	Radianspene J	C ₂₀ H ₂₇ NO ₄	344.1865	-0.58	[M - H] ⁻	Ou et al. (2012)
69	22.281	4,4'-Dihydroxy-5,5'-diisopropyl-7,7'-dimethyl-3H,3'-H-8,8'-binaphtho[1,8-bc]furan-3,3'-dione	C ₃₀ H ₂₆ O ₆	481.1655	-0.28	[M - H] ⁻	Jaroszewski et al. (1992)
70	22.484	Dehydrodroneochinulin	C ₂₉ H ₃₇ N ₃ O ₂	460.2953	-1.24	[M + H] ⁺	Li et al. (2008)

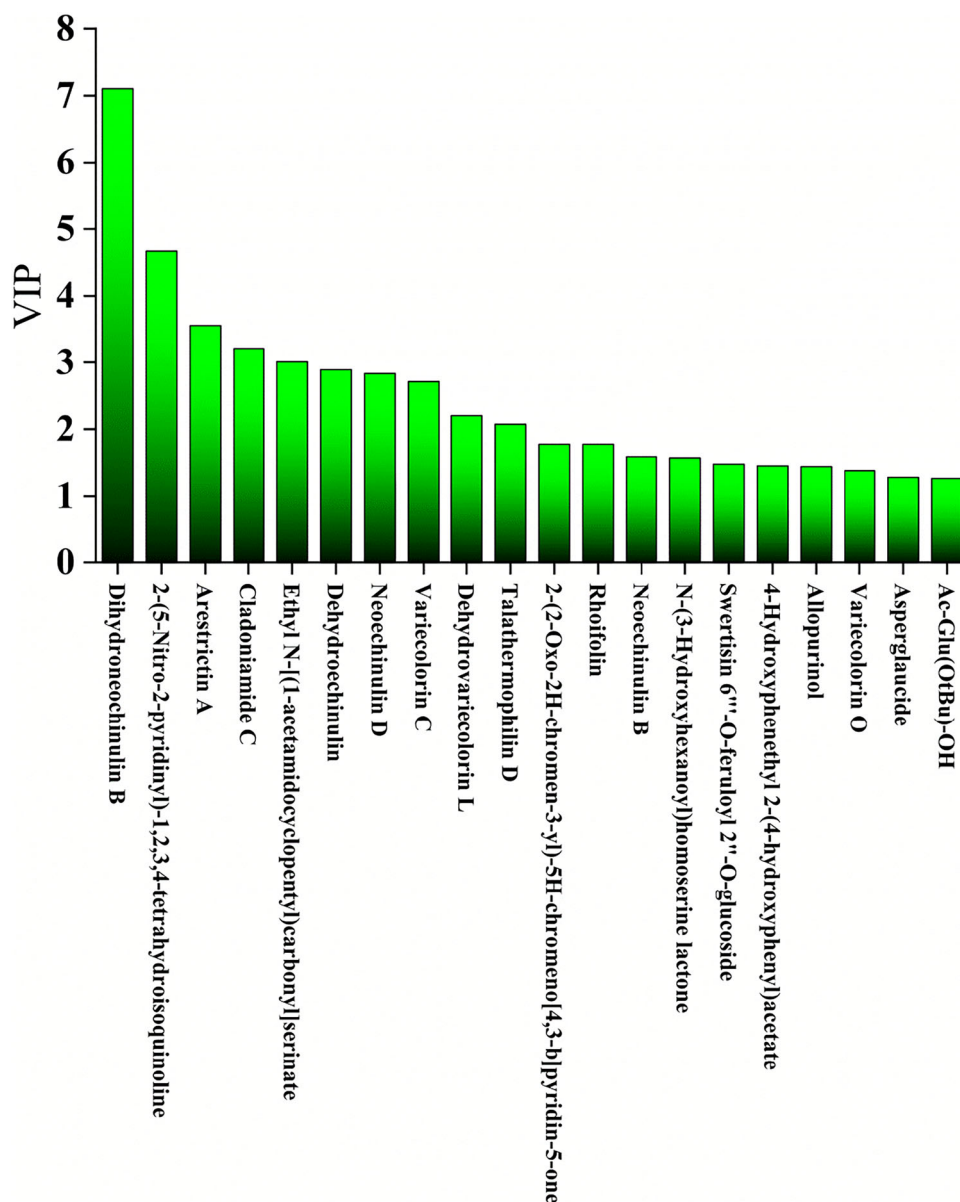


FIGURE 4 | VIP scores of Guangan *Dendrobium officinale*.

by Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway enrichment analysis using clusterProfiler. The results obtained were screened and analyzed using $p < 0.05$ as an indicator.

2.3.5 | Molecular Docking

The SDF format of the core target corresponding to the active ingredient obtained from the PubChem database was imported into ChemDraw 3D. Energy minimization was performed using the MM2 module to obtain the dominant conformation with the lowest energy, saved as mol2. This conformation was then hydrogenated by AutoDockTools 1.5.7, charged, the root of the ligand was detected, rotatable bonds were searched for and defined, and saved as a pdbqt file. The 3D structure of the core target protein was obtained from the PDB database. The protein was dewatered, and small molecules were removed using PyMOL software. Hydrogen atoms were then added, and

Gasteiger charges were calculated by AutoDockTools 1.5.7 software. Finally, the 3D structure was defined as a receptor and saved as a pdbqt file. The ligand was docked to the receptor using AutoDockTools 1.5.7 software, and the binding pattern was analyzed and visualized using PyMOL software.

3 | Results

3.1 | Identification of *D. officinale* Grown in Different Years

In positive and negative ion modes, 2372 and 1087 compound signals were detected, respectively, of which 2214 and 933 compounds were recognized as validly detected after initial screening and de-emphasis by QC samples, respectively. For the 2214 and 933 compounds detected, OPLS-DA and PCA were conducted, and the results are shown in Figure 1. When PCA was used, samples with different growth years could be

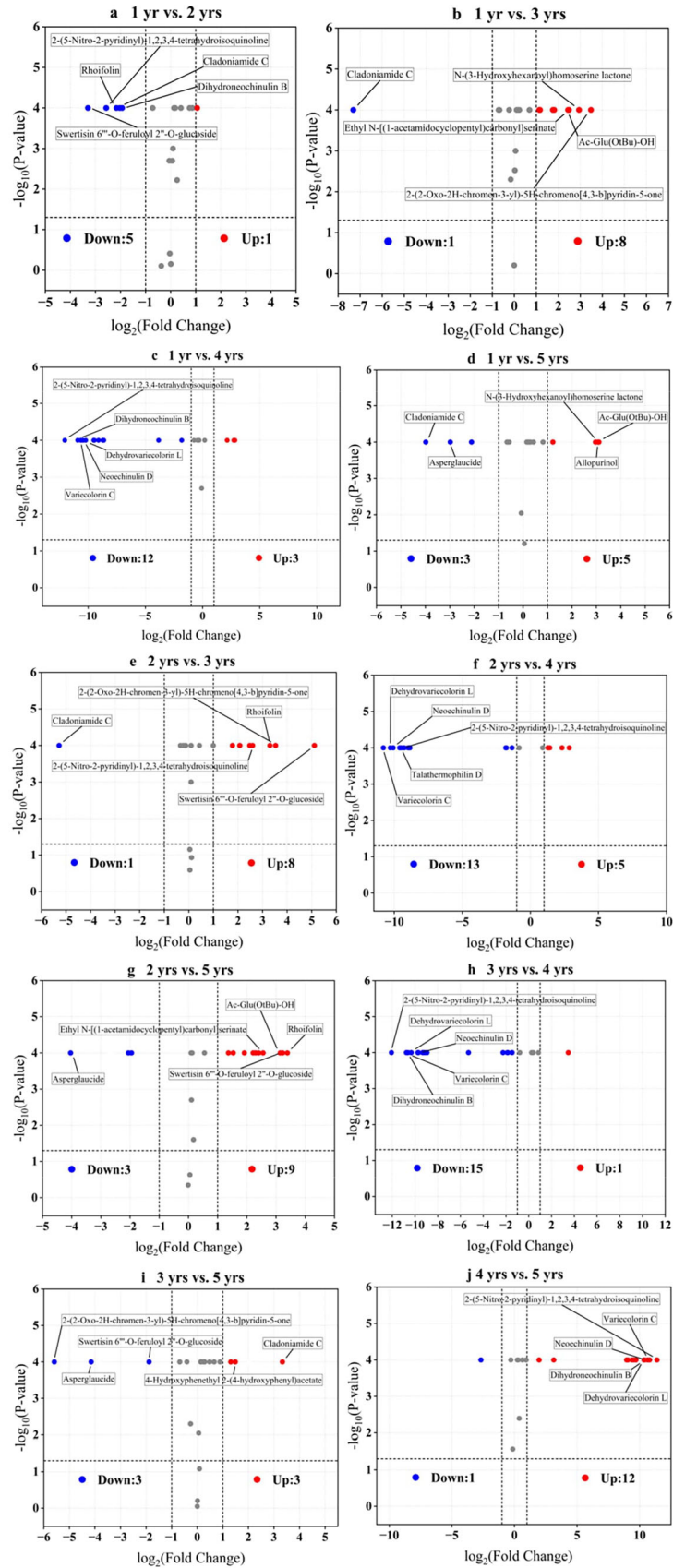


FIGURE 5 | Volcano plot of Guangan *Dendrobium officinale* with different growth years.

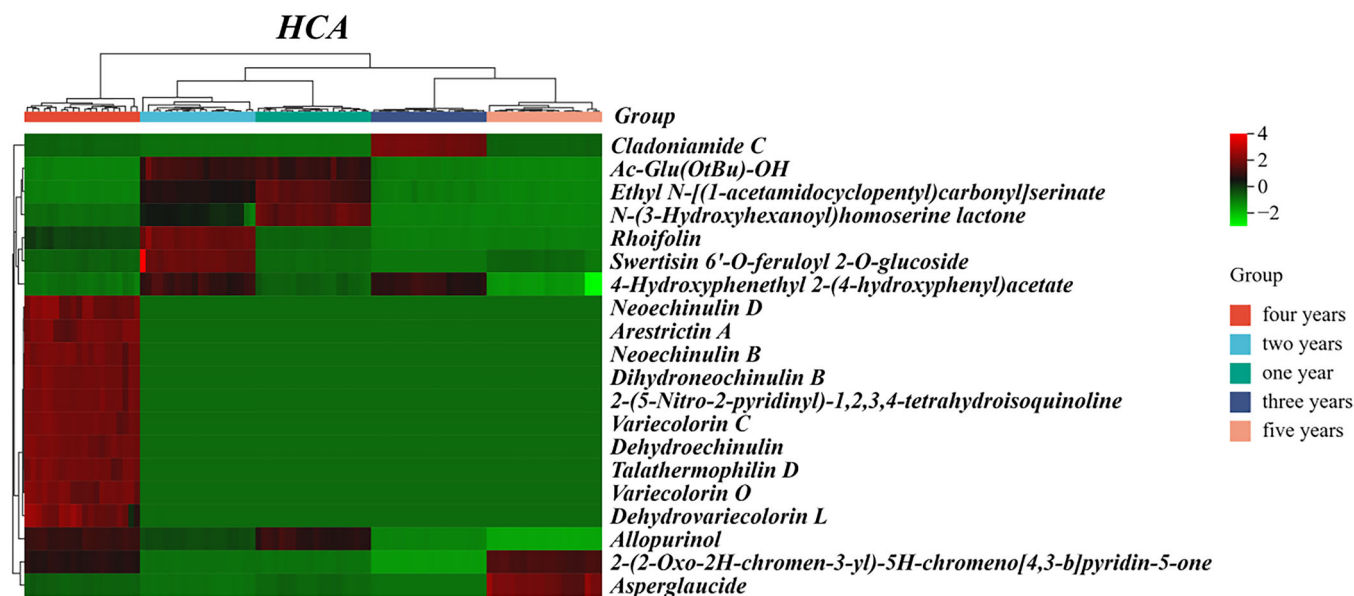


FIGURE 6 | Heatmap of Guangan *Dendrobium officinale* with different growth years.

effectively distinguished in the positive ion mode. However, for the compounds obtained in the negative ion mode, samples of 2, 3, and 5 years showed a more aggregated phenomenon, and some of the samples in the fifth year showed a serious offset phenomenon. Since PCA is an unsupervised dimensionality reduction method and is insensitive to variables with small correlations, it may not effectively differentiate the samples in this experiment, as they are all from the same origin and species, differing only in growth years. Some variables are not significantly different from each other. However, OPLS-DA combines orthogonal signals and PLS-DA to screen the differing variables, effectively addressing the issue of small correlations and difficulty in distinguishing.

In this experiment, the use of OPLS-DA can effectively differentiate *D. officinale* with different growth years, as shown in Figure 2. In positive and negative ion modes, *D. officinale* samples with different growth years can be better distinguished. *D. officinale* with 4 years of growth shows greater differences compared with other years. The permutation test was used to verify the reliability of the model, using R2Y and Q2Y as the basis for judgment. If the intercept of R2Y was < 0.3–0.4, the intercept of Q2Y was < 0.05, and the closer the Q2Y was to 1, then the better the model was. In this experiment, the results of the permutation test based on 200 randomized tests of this OPLS-DA are shown in Figure 3, where the intercept of R2Y was < 0.3, the intercept of Q2Y was < 0.05, and Q2Y was 1, thus suggesting that the model was not overfitted and that the model was highly predictable and reliable.

3.2 | Screening of Different Marker Compounds

In the current study, after a database search and comparison and following the requirement of mass accuracy ≤ 5 ppm in SANTE/11312/2021 (Diallo et al. 2023; Maldonado-Reina et al. 2022), a total of 2040 compounds were retrieved in the positive ion mode and 826 in the negative ion mode using

CD 3.3 screening. However, their structural information needs further corroboration. The OPLS-DA model identified compounds with $VIP > 1$ and $p < 0.05$, screening 29 different marker compounds in the positive ion mode and 41 in the negative ion mode, as shown in Table 1.

To further investigate the variation of difference marker compounds in *D. officinale* with different growth years, the top 20 compounds with $VIP > 1$ and $p < 0.05$ were selected for the analysis of VIP scores (Figure 4), volcano plots (Figure 5), and heatmaps (Figure 6). The volcano plots presented the up- and downregulation of the related compounds between different growth years. Arestrictin A, neoechinulin B, neoechinulin D, variecolorin O, variecolorin C, dehydrovariecolorin L, dehydroechinulin, talathermophilin D, and eight other indole diketopiperazine alkaloid compounds showed no significant difference between different growth years. All of these compounds belong to the secondary metabolites of *D. officinale*, primarily synthesized via the amino acid pathway. Normally, once biosynthesized, secondary metabolites in plants generally do not change drastically with the change of growth years (Verma and Shukla 2015). The *D. officinale* samples selected in this experiment were all of the same species and growth environment, differing only in growth years. Therefore, the contents of these compounds in *D. officinale* did not differ significantly with the change of growth years. The interconversions between indole diketopiperazine alkaloids were postulated, as shown in Figure 7. Preechinulin is the key secondary metabolite for the biosynthesis of indole diketopiperazine alkaloids. These alkaloids are subsequently synthesized through isopentenylation, dehydration, oxygenation, and cyclization reactions and are catalyzed by EchPT2 and EchP450 enzymes, respectively, to generate different series of indole diketopiperazine alkaloids (Nies and Li 2020).

From the heatmap, the difference marker compounds of *D. officinale* with 4 years of growth were more distinct compared with

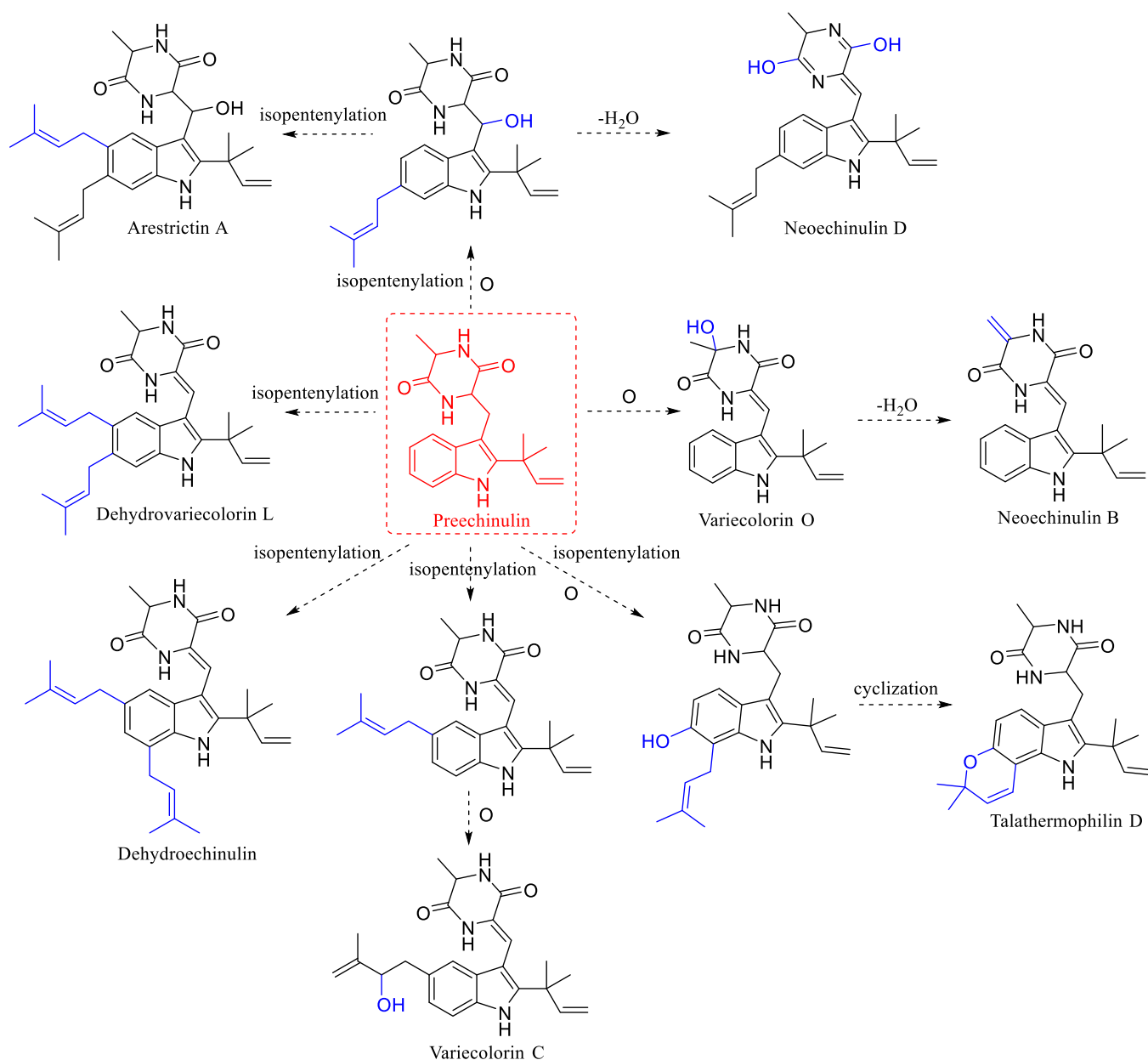


FIGURE 7 | Biotransformation relationship of indole diketopiperazine alkaloids in Guangnan *Dendrobium officinale*.

other years. This was consistent with the results of the OPLS-DA analysis, where 10 compounds of *D. officinale* with 4 years of growth, such as neoechinulin D, variecolorin C, and dehydrovariecolorin L, showed greater differences. The peak areas of these 10 compounds across different years are shown in line graphs in Figure 8. The content of these compounds increases with growth, reaching a maximum in the fourth year, then decreases afterward. This suggests that during the early growth stages, *D. officinale* primarily accumulates secondary metabolites, peaking at the 4th year (Jin et al. 2016; Yuan et al. 2022). Therefore, based on the differences in compounds from different years in the heatmap, the differentially characterized compounds in *D. officinale* from other growth years can be further inferred. A 1-year growth includes the following: Ac-Glu(OtBu)-OH, ethyl *N*-[(1-acetamidocyclopentyl)carbonyl]serinate, *N*-(3-hydroxyhexanoyl) homoserine lactone, and allopurinol. Additionally, a 2-year growth includes the following: rhoifolin and swertisin

6'''-*O*-feruloyl 2''-*O*-glucoside. A 3-year growth includes the following: cladoniamide C, 4-hydroxyphenethyl 2-(4-hydroxyphenyl)acetate. Finally, a 5-year growth includes the following: 2-(2-oxo-2H-chromen-3-yl)-5H-chromeno[4,3-*b*]pyridin-5-one, asperglaucide, but these conclusions still need to be further verified by expanding the number of samples further.

3.3 | Screening of Potential Targets for Difference Marker Compounds

Using SwissTargetPrediction, STITCH, and PharmMapper databases with parameters set to probability ≥ 0.9 , score ≥ 0.7 , and norm fit score ≥ 0.9 , respectively, a total of 48 potent compounds (Table 2) were searched to obtain 6, 36, and 96 potential targets, and after removing duplicate entries, 133 potential targets of action were finally obtained (Figure 9).

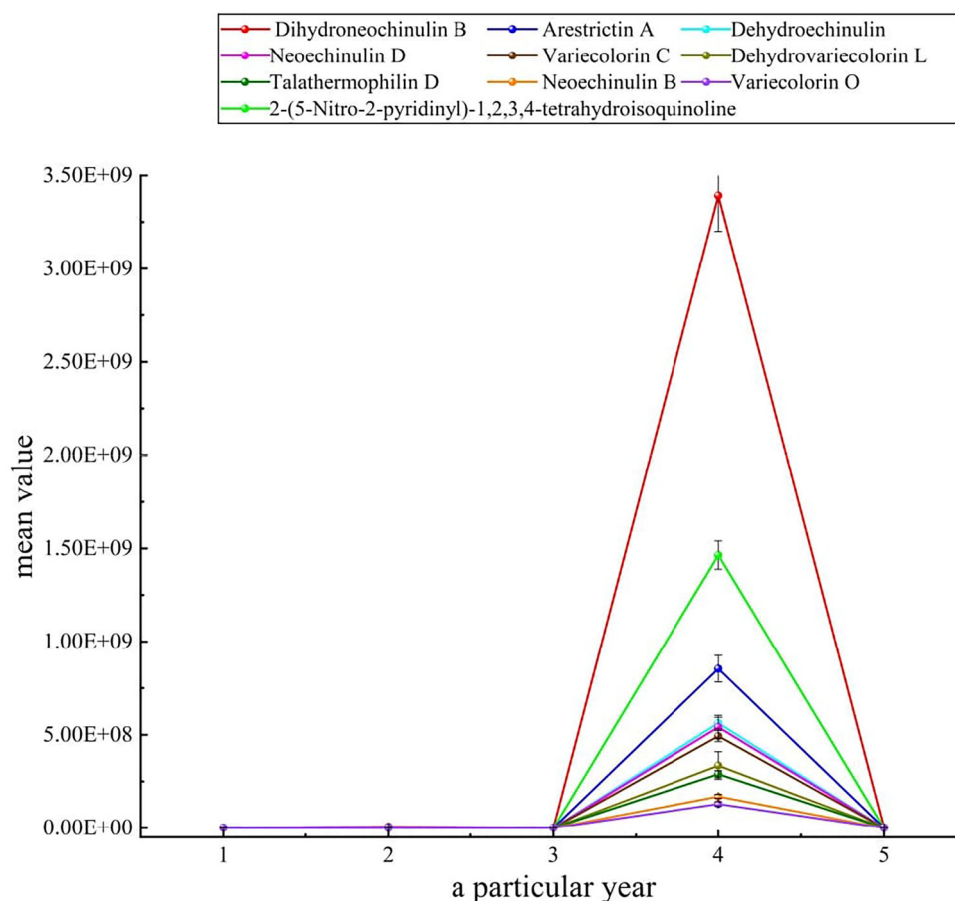


FIGURE 8 | Cumulative changes of difference marker compounds in Guangan *Dendrobium officinale* at different growth years.

3.4 | GO Function and KEGG Signaling Pathway Enrichment Analysis of Key Action Targets of *D. officinale* Functional Compounds

Using the 24 key action targets of the functional components of *D. officinale* screened in Section 3.3, which were obtained by searching the GeneCards database for relevant efficacy (relevance score: > 10), 60 GO functional pathways were enriched, as shown in Figure 10 ($p < 0.05$). The functional compounds in *D. officinale* may mainly exert pharmacological activities by participating in these pathways. Among these pathways, 40 are biological processes (BPs) including signal transduction, positive regulation of DNA-templated transcription, glutathione metabolic process, cellular response to cadmium ion, and cellular response to estradiol stimulus. There are 15 molecular functions (MFs), covering identical protein binding, enzyme binding, electron transfer activity, DNA binding, and glutathione transferase activity. Additionally, five cellular components (CCs) are included: cytosol, nucleus, cytoplasm, extracellular exosome, mitochondrial exosome, and cellular response to estradiol stimulus.

The key targets of action of the 24 *D. officinale* functional compounds obtained were enriched to a total of 67 KEGG signaling pathways, as shown in Figure 10. Among these pathways, cancer pathways, metabolic pathways, chemical carcinogenesis—receptor activation, chemical carcinogenesis—reactive oxygen species, platinum drug resistance, and lipid and atherosclerosis signaling pathways were more enriched, suggesting that *D. officinale*

differentially functional compounds have pharmacological activities in these areas.

The first 30 KEGG signaling pathways included lipid and atherosclerosis, fluid shear stress, and atherosclerosis, suggesting that *D. officinale* can regulate lipid and atherosclerosis metabolic processes, consistent with literature reports (Lin et al. 2023). Additionally, the finding suggests that *D. officinale* may have active functions in regulating the endocrine system, such as antiviral and anti-inflammatory effects.

4 | Conclusions

In the present study, UHPLC–Orbitrap-MS was used for non-targeted screening of *D. officinale* from Guangan growing 1, 2, 3, 4, and 5 years. In the positive and negative ion modes, 2214 and 933 compounds were effectively detected, respectively. OPLS-DA was able to effectively differentiate *D. officinale* from those grown in different years, and compounds with $VIP > 1$ and $p < 0.05$ were obtained by using the OPLS-DA model, with a total of 29 different compounds screened in the positive ion mode and 41 in the negative ion mode.

The volcano map indicated that arestrictin A, neoechinulin B, neoechinulin D, variecolorin O, variecolorin C, dehydrovariecolorin L, dehydroechinulin, talathermophilin D, and eight other indole diketopiperazine alkaloid compounds showed no significant differences between different growth years. The

TABLE 2 | Screening of the functional compounds of Guangan *Dendrobium officinale*.

Number	Component name	Formula	<i>m/z</i>	Number of targets
1	Hydantocidin	C ₇ H ₁₀ N ₂ O ₆	217.0475	2
2	Allopurinol	C ₅ H ₄ N ₄ O	137.0458	10
3	Leptazoline D	C ₁₆ H ₂₂ N ₂ O ₇	355.1496	2
4	Acetyl-L-carnitine	C ₉ H ₁₇ NO ₄	202.1077	10
5	Leucyl-4-hydroxyproline	C ₁₁ H ₂₀ N ₂ O ₄	245.1496	5
6	Vochysiamide B	C ₁₁ H ₁₇ NO ₄	245.1495	1
7	3-(Dimethylamino)-4-hydroxybutyl dimethylcarbamate	C ₉ H ₂₀ N ₂ O ₃	205.1546	1
8	Ac-Glu(OtBu)-OH	C ₁₁ H ₁₉ NO ₅	246.1336	3
9	<i>N</i> -(3-hydroxyhexanoyl)-homoserine lactone	C ₁₀ H ₁₇ NO ₄	216.1229	3
10	L-Leucyl-L-prolinamide	C ₁₁ H ₂₁ N ₃ O ₂	228.1705	7
11	Phomolide D	C ₁₂ H ₂₀ O ₅	243.1236	1
12	1- <i>O</i> -sinapoyl-beta-D-glucose	C ₁₇ H ₂₂ O ₁₀	385.1141	48
13	Quercetin 3-robinobioside	C ₂₇ H ₃₀ O ₁₆	611.1602	17
14	Geralcin D	C ₁₅ H ₂₂ N ₂ O ₆	327.1547	11
15	Perennipin B	C ₂₁ H ₂₆ O ₉	421.1507	8
16	Afalanine	C ₁₁ H ₁₃ NO ₃	206.0815	7
17	Lotaustralin	C ₁₁ H ₁₉ NO ₆	260.1138	2
18	Swertisin 6'''- <i>O</i> -feruloyl 2''- <i>O</i> -glucoside	C ₂₉ H ₃₂ O ₁₅	619.1646	2
19	1,7-Hydroxy-3-methylxanthone	C ₁₄ H ₁₀ O ₃	227.07	4
20	Isomucronulatol 7- <i>O</i> -glucoside	C ₂₃ H ₂₈ O ₁₀	463.161	7
21	Flutimide	C ₁₂ H ₁₈ N ₂ O ₃	237.124	1
22	Endophenazine E	C ₁₉ H ₁₈ N ₂ O ₆	369.1091	16
23	Phomoxydiene C	C ₁₄ H ₁₄ O ₃	229.0864	7
24	1-(2,4-Dihydroxyphenyl)-3-hydroxy-3-(4'-hydroxyphenyl)-1-propanone	C ₁₅ H ₁₄ O ₅	273.0768	9
25	UCS1025B	C ₂₀ H ₂₅ NO ₆	374.1607	8
26	(<i>E</i>)-2-methoxy-1,4 naphthoquinone-1-oxime	C ₁₁ H ₉ NO ₃	202.0501	1
27	YF-0200R-B	C ₁₂ H ₂₀ O ₅	244.13	2
28	Variecolorin O	C ₁₉ H ₂₁ N ₃ O ₃	340.1649	16
29	Dichotosin	C ₂₃ H ₂₈ O ₉	447.1659	8
30	Diorcinol H	C ₁₅ H ₁₄ O ₄	257.0817	7
31	LMA-P1	C ₁₄ H ₂₄ O ₄	255.1599	4
32	Trimethyl 1,2,8-octanetricarboxylate	C ₁₄ H ₂₄ O ₆	287.1499	16
33	Aspertetranone D	C ₂₂ H ₂₈ O ₉	435.1661	4
34	Dihydronoeochinulin B	C ₁₉ H ₂₁ N ₃ O ₂	324.17	22
35	Okanin 3,4,3'-trimethyl ether 4'-glucoside	C ₂₄ H ₂₈ O ₁₁	491.1559	1
36	UCS1025A	C ₂₀ H ₂₅ NO ₅	358.1659	8
37	Citreoviripyron B	C ₁₄ H ₁₆ O ₃	231.1021	12
38	Talathermophilin D	C ₂₄ H ₂₉ N ₃ O ₃	408.2278	16
39	4-Hydroxyphenethyl 2-(4-hydroxyphenyl)acetate	C ₁₆ H ₁₆ O ₄	271.0975	24
40	Citreopyron D	C ₁₅ H ₁₆ O ₄	259.0974	6
41	Asperglaucide	C ₂₇ H ₂₈ N ₂ O ₄	445.2118	22

(Continues)

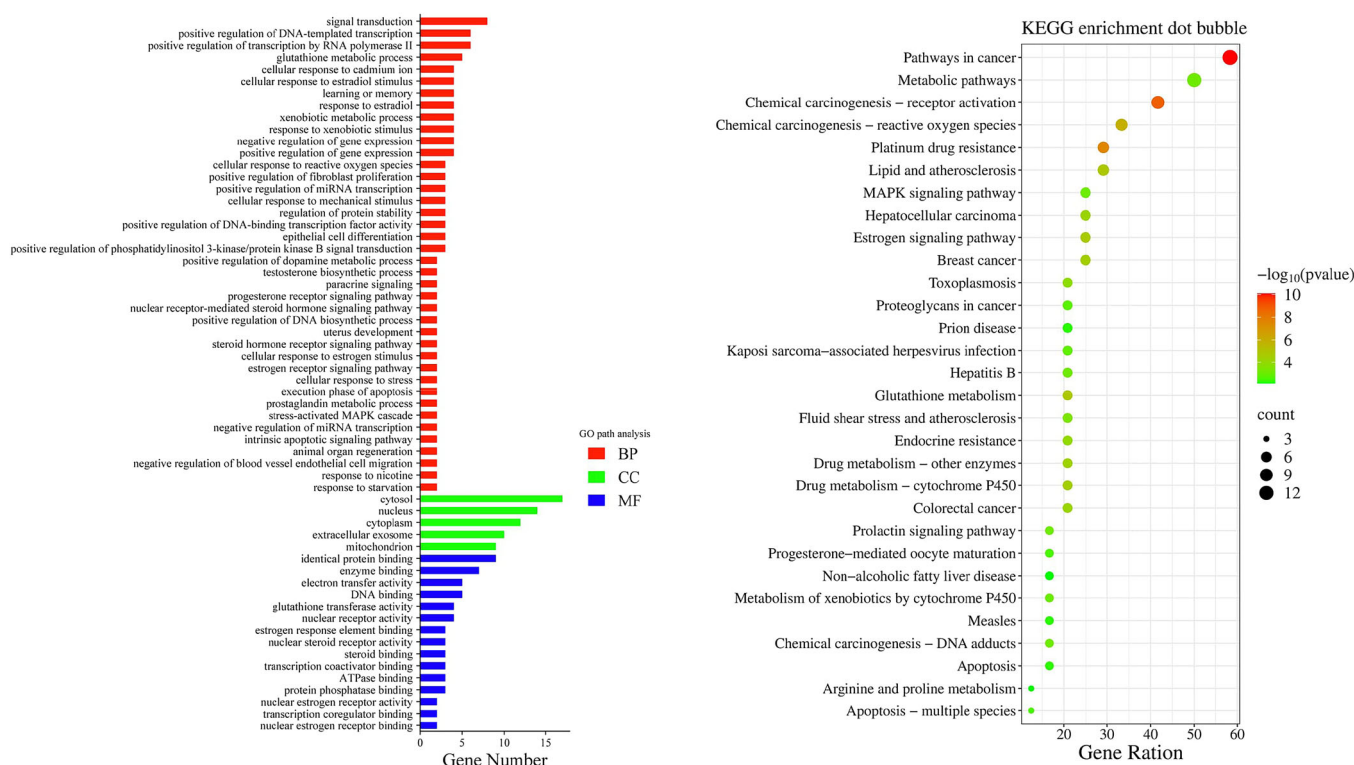


FIGURE 10 | GO functional enrichment and KEGG signal pathway enrichment of key targets of bioactive components in *Dendrobium officinale*.

interconversion relationship between indole diketopiperazine alkaloids was hypothesized, with preechinulin being the key secondary metabolite in their biosynthesis. Different indole diketopiperazine alkaloids are synthesized through isopentenylation, dehydration, oxygenation, cyclization, and catalysis by EchPT2 and EchP450 enzymes, respectively.

The heatmap showed that the compounds in *D. officinale* with 4 years of growth differed more significantly from those of other years. Ten compounds, such as neoechinulin D, variegolorin C, and dehydrovariegolorin L, showed greater differences in 4-year-old *D. officinale*. The content of these compounds increased with years of growth, reaching a maximum in the fourth year, and then decreased. The differential characteristic compounds in 1-year growth were Ac-Glu(OtBu)-OH, ethyl *N*-[(1-acetamidocyclopentyl)carbonyl]serinate, *N*-(3-hydroxyhexanoyl) homoserine lactone, and allopurinol. For 2-year growth, the compounds were rhoifolin and swertisin 6'''-*O*-feruloyl 2''-*O*-glucoside. Additionally, for 3-year growth, the compounds were cladoniamide C and 4-hydroxyphenethyl 2-(4-hydroxyphenyl) acetate. For 5-year growth, the compounds were 2-(2-oxo-2H-chromen-3-yl)-5H-chromeno[4,3-*b*]pyridin-5-one and asperglaucide.

A total of 6, 36, and 96 potential targets of 48 efficacy compounds were obtained using SwissTargetPrediction, STITCH, and PharmMapper databases. After removing duplicates, 133 potential targets were identified. The 24 key action targets were enriched to obtain 60 GO functional pathways, comprising 40 BPs, 15 MFs, and 5 CCs. Additionally, a total of 67 KEGG signaling pathways were enriched, with cancer pathways, metabolic pathways, chemical carcinogenesis—receptor activation, chemical carcinogenesis—reactive oxygen species, platinum

drug resistance, and lipid and atherosclerosis signaling pathways being more enriched. This suggests that *D. officinale* differential efficacy compounds are pharmacologically active in these areas. Furthermore, this finding demonstrates the efficacy of *D. officinale* in regulating metabolic processes of lipids and atherosclerosis, as well as having active functions in the regulation of the endocrine system and antiviral and anti-inflammatory activities.

Author Contributions

Jiao Zhang: data curation, writing – original draft. **Yanping Ye:** software. **Yan Ding:** resources. **Heng Tian:** methodology. **Xinglian Chen:** methodology. **Kaixi Li:** resources. **Hongcheng Liu:** supervision. **Tao Lin:** writing – review and editing.

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Ethics Statement

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be made available on request.

References

- Adamczyk, M., J. R. Fishpaugh, and M. Thiruvazhi. 2002. "Concise Synthesis of N-Protected Carboxyalkyl Ether Amines." *Organic Preparations and Procedures International* 34, no. 3: 326–331.
- Adelin, E., C. Servy, S. Cortial, et al. 2011. "Biotransformation of Natural Compounds. Oxido-Reduction of Sch-642305 by *Aspergillus ochraceus* ATCC 1009." *Bioorganic & Medicinal Chemistry Letters* 21, no. 8: 2456–2459.
- Agatsuma, T., T. Akama, S. Nara, et al. 2002. "UCS1025A and B, New Antitumor Antibiotics From the Fungus *Acremonium* Species." *Organic Letters* 4, no. 25: 4387–4390.
- Andrew Hedley, K., and S. P. Stanforth. 1992. "Ring-Opening Reactions of N-Aryl-, 1, 2, 3, 4-Tetrahydroisoquinoline Derivatives." *Tetrahedron* 48, no. 4: 743–750.
- Asai, T., D. Luo, K. Yamashita, and Y. Oshima. 2013. "Structures and Biomimetic Synthesis of Novel α -Pyrone Polyketides of an Endophytic *Penicillium* sp. in *Catharanthus roseus*." *Organic Letters* 15, no. 5: 1020–1023.
- Assaf, Z., A. P. Larsen, R. Venskutnyte, et al. 2013. "Chemoenzymatic Synthesis of New 2, 4-Syn-Functionalized (S)-Glutamate Analogues and Structure–Activity Relationship Studies at Ionotropic Glutamate Receptors and Excitatory Amino Acid Transporters." *Journal of Medicinal Chemistry* 56, no. 4: 1614–1628.
- Ballini, R., and G. Bosica. 1996. "Nitroalkanes as Alkyl Anion Synthons—A New Approach to the Synthesis of 2-Substituted N-Ethyl Succinimides and 2-Substituted Succinate Diesters via Nitroalkanes." *Liebigs Annalen* 1996, no. 12: 2087–2089.
- Becchi, M., and D. Fraisse. 1989. "Fast Atom Bombardment and Fast Atom Bombardment Collision-Activated Dissociation/Mass-Analysed Ion Kinetic Energy Analysis of C-Glycosidic Flavonoids." *Biomedical & Environmental Mass Spectrometry* 18, no. 2: 122–130.
- Bhandari Neupane, J., R. P. Neupane, Y. Luo, W. Y. Yoshida, R. Sun, and P. G. Williams. 2019. "Characterization of Leptazolines A–D, Polar Oxazolines From the Cyanobacterium *Leptolyngbya* sp., Reveals a Glitch With the 'Willoughby–Hoye' Scripts for Calculating NMR Chemical Shifts." *Organic Letters* 21, no. 20: 8449–8453.
- Brunetti, L., A. Chiavaroli, A. Cocco, et al. 2013. "Synthesis and Neuromodulatory Effects of TRH-Related Peptides: Inhibitory Activity on Catecholamine Release In Vitro." *Pharmacological Reports* 65, no. 4: 823–835.
- Cantrell, C. L., B. P. Case, E. E. Mena, T. M. Kniffin, S. O. Duke, and D. E. Wedge. 2008. "Isolation and Identification of Antifungal Fatty Acids From the Basidiomycete *Gomphus floccosus*." *Journal of Agricultural and Food Chemistry* 56, no. 13: 5062–5068.
- Chattopadhyay, S., S. Biswas, and B. Pal. 2006. "Efficient Construction of a Doubly Functionalized Trioxazole Derivative Relevant to the Synthesis of the Novel Telomerase Inhibitor Telomestatin and Its Analogues." *Synthesis* 2006, no. 08: 1289–1294.
- Chen, Y., X. Yang, L. Zhang, et al. 2023. "Tryptophan-Centered Metabolic Alterations Coincides With Lipid-Mediated Fungal Response to Cold Stress." *Heliyon* 9: e13066.
- Cui, S., J. Wang, L. Yang, J. Wu, and X. Wang. 2015. "Qualitative and Quantitative Analysis on Aroma Characteristics of Ginseng at Different Ages Using E-Nose and GC–MS Combined With Chemometrics." *Journal of Pharmaceutical and Biomedical Analysis* 102: 64–77.
- Diallo, T., Y. Makni, A. Lerebours, H. Thomas, T. Guérin, and J. Parinet. 2023. "Wide-Scope Screening of Multi-Class Contaminants in Seafood Using a Novel Sample Preparation (QuEChUP) Procedure Coupled With UHPLC-Q-TOF-MS: Application for Semi-Quantitation of Real Seafood Samples." *Food Chemistry* 426: 136572.
- Farooq Biabani, M. A., M. Baake, B. Lovisetto, H. Laatsch, E. Helmke, and H. Weyland. 1998. "Anthranilamides: New Antimicrobial Active Substances From a Marine *Streptomyces* sp." *Journal of Antibiotics* 51, no. 3: 333–340.
- Fujimoto, H., Y. Satoh, K. Yamaguchi, and M. Yamazaki. 1998. "Monoamine Oxidase Inhibitory Constituents From *Anixiella micropertusa*." *Chemical and Pharmaceutical Bulletin* 46, no. 10: 1506–1510.
- Gao, H., T. Zhu, D. Li, Q. Gu, and W. Liu. 2013. "Prenylated Indole Diketopiperazine Alkaloids From a Mangrove Rhizosphere Soil Derived Fungus *Aspergillus effuses* H1-1." *Archives of Pharmacol Research* 36: 952–956.
- Ghosal, S., D. K. Jaiswal, S. K. Singh, and R. S. Srivastava. 1985. "Dichotosin and Dichotosinin, Two Adaptogenic Glucosyloxy Flavans From *Hoppea dichotoma*." *Phytochemistry* 24, no. 4: 831–833.
- Guo, J.-P., J.-L. Tan, Y.-L. Wang, et al. 2011. "Isolation of Talathermophilins From the Thermophilic Fungus *Talaromyces thermophilus* YM3-4." *Journal of Natural Products* 74, no. 10: 2278–2281.
- Haruyama, H., T. Takayama, T. Kinoshita, M. Kondo, M. Nakajima, and T. Haneishi. 1991. "Structural Elucidation and Solution Conformation of the Novel Herbicide Hydantocidin." *Journal of the Chemical Society, Perkin Transactions 1* 1, no. 7: 1637–1640.
- Hensens, O. D., M. A. Goetz, J. M. Liesch, et al. 1995. "Isolation and Structure of Flutimide, a Novel Endonuclease Inhibitor of Influenza Virus." *Tetrahedron Letters* 36, no. 12: 2005–2008.
- Hernández, I. L. C., M. L. Macedo, R. G. S. Berlinck, A. G. Ferreira, and M. J. L. Godinho. 2004. "Dipeptide Metabolites From the Marine Derived Bacterium *Streptomyces acrymicini*." *Journal of the Brazilian Chemical Society* 15: 441–444.
- Hu, L., C. Wang, X. Guo, et al. 2021. "Flavonoid Levels and Antioxidant Capacity of Mulberry Leaves: Effects of Growth Period and Drying Methods." *Frontiers in Plant Science* 12: 684974.
- Itabashi, T., N. Matsuishi, T. Hosoe, et al. 2006. "Two New Dioxopiperazine Derivatives, Arestrictins A and B, Isolated From *Aspergillus restrictus* and *Aspergillus penicilloides*." *Chemical and Pharmaceutical Bulletin* 54, no. 12: 1639–1641.
- Jaderson, M., and J.-H. Park. 2020. "Effect of Storage Temperature and Duration on Concentrations of 27 Fungal Secondary Metabolites Spiked Into Floor Dust From an Office Building." *Journal of Occupational and Environmental Hygiene* 17, no. 5: 220–230.
- Jaramillo, K., C. Dawid, T. Hofmann, Y. Fujimoto, and C. Osorio. 2011. "Identification of Antioxidative Flavonols and Anthocyanins in *Sicana odorifera* Fruit Peel." *Journal of Agricultural and Food Chemistry* 59, no. 3: 975–983.
- Jaroszewski, J. W., T. Strøm-Hansen, and L. L. Hansen. 1992. "Optical Stability of Gossypol." *Chirality* 4: 216–221.
- Jin, Q., C. Jiao, S. Sun, et al. 2016. "Metabolic Analysis of Medicinal *Dendrobium officinale* and *Dendrobium huoshanense* During Different Growth Years." *PLoS One* 11, no. 1: e0146607.
- Ju, Y., H. Liu, S. Niu, et al. 2025. "Optimizing Geographical Traceability Models of Chinese *Lycium barbarum*: Investigating Effects of Region, Cultivar, and Harvest Year on Nutrients, Bioactives, Elements and Stable Isotope Composition." *Food Chemistry* 467: 142286.
- Kim, J.-Y., E. E. Woo, L. S. Ha, D.-W. Ki, I.-K. Lee, and B.-S. Yun. 2019. "Three New Meroterpenoids From Culture Broth of *Perenniporia medulla-panis* and Their Antioxidant Activities." *Journal of Antibiotics* 72, no. 8: 625–628.
- Kosemura, S., S. Kojima, and S. Yamamura. 1997. "Citreopyrones, New Metabolites of Two Hybrid Strains, KO 0092 and KO 0141, Derived From the *Penicillium* Species." *Chemistry Letters* 26, no. 1: 33–34.
- Le Goff, G., M.-T. Martin, B. I. Iorga, et al. 2013. "Isolation and Characterization of Unusual Hydrazides From *Streptomyces* sp. Impact of the Cultivation Support and Extraction Procedure." *Journal of Natural Products* 76, no. 2: 142–149.

- Li, Z., L. Feng, H. Wang, et al. 2023. "The Impact of Growth Years on the Medicinal Material Characteristics and Metabolites of *Stellaria dichotoma* L. Var. *lanceolata* Bge. Reveals the Optimal Harvest Age." *Plants* 12, no. 12: 2286.
- Li, Y., D. Kong, Y. Fu, M. R. Sussman, and H. Wu. 2020. "The Effect of Developmental and Environmental Factors on Secondary Metabolites in Medicinal Plants." *Plant Physiology and Biochemistry* 148: 80–89.
- Li, D. L., X. M. Li, T. G. Li, H. Y. Dang, and B. G. Wang. 2008. "Dioxopiperazine Alkaloids Produced by the Marine Mangrove Derived Endophytic Fungus *Eurotium rubrum*." *Helvetica Chimica Acta* 91, no. 10: 1888–1893.
- Li, Y.-Y., M.-Z. Wang, Y.-J. Huang, and Y.-M. Shen. 2010. "Secondary Metabolites From *Phomopsis* sp. A123." *Mycology* 1, no. 4: 254–261.
- Liang, Z., Z. Xie, Y. Huang, et al. 2019. "HPLC Characteristic Spectrum Optimization of Flavonoid Glycosides on *Dendrobium officinale* and Characteristics Analysis of Different Provenances." *Chinese Journal of Experimental Traditional Medical Formulae* 25, no. 1: 22–28.
- Li, X. B., Y. H. Zhou, R. X. Zhu, et al. 2015. "Identification and Biological Evaluation of Secondary Metabolites From the Endolichenic Fungus *Aspergillus versicolor*." *Chemistry & Biodiversity* 12, no. 4: 575–592.
- Lin, T., X. Chen, L. Du, et al. 2023. "Traceability Research on *Dendrobium devonianum* Based on SWATHtoMRM." *Foods* 12, no. 19: 3608.
- LingShang, W. U., S. I. JinPing, Y. XiaoQing, and S. H. I. XueRong. 2009. "Quantitive Variation of Flavonoids in *Houttuynia cordata* From Different Geographic Origins in China." *Chinese Journal of Natural Medicines* 7, no. 1: 40–46.
- Liu, C., Y. Cheng, D. Guo, et al. 2017. "A New Concept on Quality Marker for Quality Assessment and Process Control of Chinese Medicines." *Chinese Herbal Medicines* 9, no. 1: 3–13.
- Liu, J., Z. Li, M. Zeng, et al. 2023. "Rapid Comparison of Antitumor Chemical Constituents and Mechanisms Between *Dendrobium nobile* and *Dendrobium officinale* by UPLC-IT-TOF, Network Pharmacology and Experimental Verification." *Acta Chromatographica* 35, no. 2: 123–138.
- Luo, Z., L. Liu, Q. Nie, et al. 2023. "HPLC-Based Metabolomics of *Dendrobium officinale* Revealing Its Antioxidant Ability." *Frontiers in Plant Science* 14: 1060242.
- Lv, M., Q. Liang, X. He, et al. 2023. "Hypoglycemic Effects of *Dendrobium officinale* Leaves." *Frontiers in Pharmacology* 14: 1163028.
- Maldonado-Reina, A. J. s., R. López-Ruiz, R. Romero-González, J. L. Martínez Vidal, and A. Garrido-Frenich. 2022. "Assessment of Co-Formulants in Marketed Plant Protection Products by LC-Q-Orbitrap-MS: Application of a Hybrid Data Treatment Strategy Combining Suspect Screening and Unknown Analysis." *Journal of Agricultural and Food Chemistry* 70, no. 23: 7302–7313.
- Marchelli, R., A. Dossena, A. Pochini, and E. Dradi. 1977. "The Structures of Five New Didehydropeptides Related to Neoechinulin, Isolated From *Aspergillus amstelodami*." *Journal of the Chemical Society, Perkin Transactions 1* 1, no. 7: 713–717.
- Moon, K.-J., H.-K. Shim, K.-S. Lee, J. Zieba, and P. N. Prasad. 1996. "Synthesis, Characterization, and Second-Order Optical Nonlinearity of a Polyurethane Structure Functionalized With a Hemicyanine Dye." *Macromolecules* 29, no. 3: 861–867.
- Nakai, R., H. Ogawa, A. Asai, et al. 2000. "UCS1025A, a Novel Antibiotic Produced by *Acremonium* sp." *Journal of Antibiotics* 53, no. 3: 294–296.
- Nie, L. J., J. Liang, F. Shan, et al. 2021. "A UPLC-MS/MS Method for Determination of Endogenous L-Carnitine and Acetyl-L-Carnitine in Serum of Patients With Depression." *Biomedical Chromatography* 35, no. 3: e4991.
- Nies, J., and S.-M. Li. 2020. "Prenylation and Dehydrogenation of a C2-Reversely Prenylated Diketopiperazine as a Branching Point in the Biosynthesis of Echinulin Family Alkaloids in *Aspergillus ruber*." *ACS Chemical Biology* 16, no. 1: 185–192.
- Offor, B. C., M. I. Mhlongo, P. A. Steenkamp, I. A. Dubery, and L. A. Piater. 2022. "Untargeted Metabolomics Profiling of *Arabidopsis* WT, *lbr2-2* and *bak1-4* Mutants Following Treatment With Two LPS Chemotypes." *Metabolites* 12, no. 5: 379.
- Opala, B., S. Mielcarek, and Z. Łowicki, et al. 2013. "Determination of Lotaustralin in *Rhodiola* Species." *Herba Polonica* 59, no. 2: 13–23.
- Ortori, C. A., J.-F. Dubern, S. R. Chhabra, et al. 2011. "Simultaneous Quantitative Profiling of N-Acyl-L-Homoserine Lactone and 2-Alkyl-4 (1H)-Quinolone Families of Quorum-Sensing Signaling Molecules Using LC-MS/MS." *Analytical and Bioanalytical Chemistry* 399: 839–850.
- Ostermeier, M., and R. Schobert. 2014. "Total Synthesis of (+)-Chloriolide." *Journal of Organic Chemistry* 79, no. 9: 4038–4042.
- Ou, Y., Y. Li, X. Qian, and Y. Shen. 2012. "Guanacastane-Type Diterpenoids From *Coprinus radians*." *Phytochemistry* 78: 190–196.
- Pant, P., S. Pandey, and S. Dall'acqua. 2021. "The Influence of Environmental Conditions on Secondary Metabolites in Medicinal Plants: A Literature Review." *Chemistry & Biodiversity* 18, no. 11: e2100345.
- Patel, A. A., H. B. Lad, K. R. Pandya, C. V. Patel, and D. I. Brahmabhatt. 2013. "Synthesis of a New Series of 2-(2-Oxo-2H-Chromen-3-Yl)-5H-Chromeno [4, 3-b] Pyridin-5-Ones by Two Facile Methods and Evaluation of Their Antimicrobial Activity." *Medicinal Chemistry Research* 22: 4745–4754.
- Peng, M.-J., S.-Y. Shi, L. Chen, S.-H. Zhang, P. Cai, and X.-Q. Chen. 2016. "Online Coupling Solid-Phase Ligand-Fishing With High-Performance Liquid Chromatography–Diode Array Detector—Tandem Mass Spectrometry for Rapid Screening and Identification of Xanthine Oxidase Inhibitors in Natural Products." *Analytical and Bioanalytical Chemistry* 408: 6693–6701.
- Peng, D., W. Tian, M. An, et al. 2023. "Characterization of Antidiabetic Effects of *Dendrobium officinale* Derivatives in a Mouse Model of Type 2 Diabetes Mellitus." *Food Chemistry* 399: 133974.
- Piispanen, J., U. Bergmann, J. Karhu, T. Kauppila, and J. Kaitera. 2023. "Variation of Compounds in Leaves of Susceptible and Resistant Alternate Hosts of *Cronartium pini* and *C. ribicola*." *European Journal of Plant Pathology* 165, no. 4: 677–692.
- Rafi, M. M., B. C. Vastano, N. Zhu, et al. 2002. "Novel Polyphenol Molecule Isolated From Licorice Root (*Glycyrrhiza Glabra*) Induces Apoptosis, G2/M Cell Cycle Arrest, and Bcl-2 Phosphorylation in Tumor Cell Lines." *Journal of Agricultural and Food Chemistry* 50, no. 4: 677–684.
- Refaat, J., S. Y. Desoukey, M. A. Ramadan, and M. S. Kamel. 2015. "Rhoifolin: A Review of Sources and Biological Activities." *International Journal of Pharmacognosy* 2: 102–109.
- She, J., Y. Chen, Y. Ye, et al. 2022. "New Carboxamides and a New Polyketide From the Sponge-Derived Fungus *Arthrimum* sp. SCSIO 41421." *Marine Drugs* 20, no. 8: 475.
- Tawfike, A., G. Abbott, L. Young, and R. Edrada-Ebel. 2018. "Metabolomic-Guided Isolation of Bioactive Natural Products From *Curvularia* sp., an Endophytic Fungus of *Terminalia laxiflora*." *Planta Medica* 84, no. 3: 182–190.
- Toshima, H., H. Sato, and A. Ichihara. 1999. "Total Synthesis of (2S, 3R, 5S)-(–)-2, 3-dihydroxytetradecan-5-olide, a New Biologically Active δ -Lactone Produced by *Seiridium unicorne*." *Tetrahedron* 55, no. 9: 2581–2590.
- Verma, N., and S. Shukla. 2015. "Impact of Various Factors Responsible for Fluctuation in Plant Secondary Metabolites." *Journal of Applied Research on Medicinal and Aromatic Plants* 2, no. 4: 105–113.
- Wang, Y.-H. 2021. "Traditional Uses, Chemical Constituents, Pharmacological Activities, and Toxicological Effects of *Dendrobium* Leaves: A Review." *Journal of Ethnopharmacology* 270: 113851.

- Wang, Y.-N., L. Tian, H.-M. Hua, et al. 2009. "Two New Compounds From the Broth of the Marine Fungus *Penicillium griseofulvum* Y19-07." *Journal of Asian Natural Products Research* 11, no. 11: 912–917.
- Wang, W.-L., Z.-Y. Lu, H.-W. Tao, et al. 2007. "Isoechinulin-Type Alkaloids, Variecolorins A–L, From Halotolerant *Aspergillus varicolor*." *Journal of Natural Products* 70, no. 10: 1558–1564.
- Wang, X.-D., Z.-J. Xu, S. Zhu, Y. Wu, and Y.-J. Hou. 2016. "Synthesis and Configurations of YF-0200R A and B." *Tetrahedron* 72, no. 23: 3177–3184.
- Wang, Y., S. Qi, Y. Zhan, et al. 2015. "Aspertetranones A–D, Putative Meroterpenoids From the Marine Algal-Associated Fungus *Aspergillus* sp. ZL0-1b14." *Journal of Natural Products* 78, no. 10: 2405–2410.
- Williams, D. E., J. Davies, B. O. Patrick, et al. 2008. "Cladoniamides A– G, Tryptophan-Derived Alkaloids Produced in Culture by *Streptomyces uncialis*." *Organic Letters* 10, no. 16: 3501–3504.
- Wohlgemuth, V., F. Kindinger, X. Xie, B.-G. Wang, and S.-M. Li. 2017. "Two Prenyltransferases Govern a Consecutive Prenylation Cascade in the Biosynthesis of Echinulin and Neoechinulin." *Organic Letters* 19, no. 21: 5928–5931.
- Wu, C., M. H. Medema, R. M. Läkamp, et al. 2016. "Leucanicidin and Endophenazines Result From Methyl-Rhamnosylation by the Same Tailoring Enzymes in *Kitasatospora* sp. MBT66." *ACS Chemical Biology* 11, no. 2: 478–490.
- Yoo, T.-K., W. T. Jeong, J. G. Kim, et al. 2021. "UPLC-ESI-Q-TOF-MS-Based Metabolite Profiling, Antioxidant and Anti-Inflammatory Properties of Different Organ Extracts of *Abeliophyllum distichum*." *Antioxidants* 10, no. 1: 70.
- Yuan, Y., J. Zuo, H. Zhang, M. Zu, M. Yu, and S. Liu. 2022. "Transcriptome and Metabolome Profiling Unveil the Accumulation of Flavonoids in *Dendrobium officinale*." *Genomics* 114, no. 3: 110324.
- Zeng, H., S. Su, X. Xiang, et al. 2017. "Comparative Analysis of the Major Chemical Constituents in *Salvia miltiorrhiza* Roots, Stems, Leaves and Flowers During Different Growth Periods by UPLC-TQ-MS/MS and HPLC-ELSD Methods." *Molecules* 22, no. 5: 771.
- Zhang, W.-F., J.-K. Ma, X.-X. Zhang, Y.-N. Qian, and J. Xu. 2021. "Immunosuppressive Polyketides From the Mangrove Endophytic Fungus *Pestalotiopsis* sp. HHL-14." *Chemistry of Natural Compounds* 57, no. 6: 1130–1133.
- Zhang, T., W. Song, J. Zhao, and J. Liu. 2017. "Full Solution-Phase Synthesis of Acetyl Hexapeptide-3 by Fragments Coupling Strategy." *Industrial & Engineering Chemistry Research* 56, no. 41: 11697–11704.
- Zhang, W., S. Wei, J. Zhang, and W. Wu. 2013. "Antibacterial Activity Composition of the Fermentation Broth of *Streptomyces djakartensis* NW35." *Molecules* 18, no. 3: 2763–2768.
- Zhang, X., C. Zhou, L. Zhang, et al. 2019. "Isolation and Identification of Main Flavonoid Glycosides of *Dendrobium officinale* From Danxia Species and Yunnan Guangnan Species." *Chinese Journal of Experimental Traditional Medical Formulae* 25, no. 1: 29–34.
- Zhao, M., J. Fan, Q. Liu, et al. 2021. "Phytochemical Profiles of Edible Flowers of Medicinal Plants of *Dendrobium officinale* and *Dendrobium devonianum*." *Food Science & Nutrition* 9, no. 12: 6575–6586.
- Zhao, P., N. Zhu, and Y. Shen. 2004. "Identification of Chemical Constituent of Seed Crusts From *Trewia nudiflora* L." *Chinese Journal of Medicinal Chemistry* 14: 287–290.
- Zhou, F., R. Huang, T. Cao, et al. 2022. "4-Phenylcoumarins From *Mesua ferrea* With Selective CYP1B1 Inhibitory Activity." *Medicinal Chemistry Research* 31, no. 12: 2172–2181.
- Zhou, C., Z. Xie, Z. Lei, Y. Huang, and G. Wei. 2018. "Simultaneous Identification and Determination of Flavonoids in *Dendrobium officinale*." *Chemistry Central Journal* 12: 40.
- Zhou, S., X. Yan, J. Yang, et al. 2021. "Variations in Flavonoid Metabolites Along Altitudinal Gradient in a Desert Medicinal Plant *Agriophyllum squarrosum*." *Frontiers in Plant Science* 12: 683265.
- Zhou, Q.-Y., X.-Q. Yang, Z.-X. Zhang, et al. 2018. "New Azaphilones and Tremulane Sesquiterpene From Endophytic *Nigrospora Oryzae* Cocultured With *Irpex lacteus*." *Fitoterapia* 130: 26–30.