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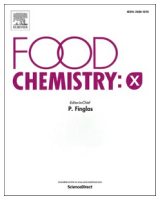

Analytical Methods

Variation in the contents of four flavonoid glycosides in edible *Dendrobium officinale* leaves during different harvesting periods and optimization of the extraction process

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A R T I C L E I N F O

Chemical compounds studied in this article:
Vicenin 2 (Pubchem CID: 442664)
Stellarin 2 (Pubchem CID: 44258167)
Vicenin 1 (Pubchem CID: 56843190)
Schaftoside (Pubchem CID: 442658)
Vicenin 3 (Pubchem CID: 185958)
Apigenin 6-*C*-*α*-L-arabinopyranosyl-8-*C*-*β*-D-xylopyranoside (Pubchem CID: 46240560)
Rutin (Pubchem CID: 5280805)
Isoviolanthin (Pubchem CID: 101422758)
More information is available at: http://www.elsevier.com/Pubchem.

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A B S T R A C T

Despite being recognized as a specialty food in four Chinese provinces with established safety standards, *Dendrobium officinale* Kimura et Migo (Orchidaceae) leaf physicochemical indices overlook vital flavonoid glycoside components. Given the inconsistency in flavonoid quality under current standards, we devised a quantitative analysis targeting vicenin 2, vicenin 3, rutin, and isoviolanthin. Our analysis revealed significant seasonal variations, with harvests yielding the highest total content of these four flavonoid glycosides in February (1.6378 mg/g) and July (2.0642 mg/g). This finding provides a scientific basis for optimal collection timing, enhancing quality control and utilization. Furthermore, we optimized the extraction conditions (59.63 °C, 22.44 min, 1:37.91 g/mL solid-to-liquid ratio, 43.24 % ethanol) to maximize the flavonoid glycoside content. This study lays a foundation for refining *D. officinale* leaf quality standards and advancing related product development in China.

1. Introduction

Dendrobium officinale Kimura et Migo is a member of the order Orchidaceae. *D. officinale* in China is widely distributed in southern provinces and regions (Yuan et al., 2020). This plant has been planted on a large scale in the southern provinces of China (Wang et al., 2022). The dry stem of *D. officinale* (the traditional medicinal part of *D. officinale*) has a long history in medicine and food in China. *D. officinale* leaves and flowers are used as local food ingredients in China. During harvest, many leaves (byproducts) remain on the stems (Zhang et al., 2017), and fresh *D. officinale* leaves account for approximately 50 % of the total biomass (Zhang et al., 2013). Previous studies have confirmed the medicinal and edible value of *D. officinale* leaves, which exhibit antihyperlipidemic, antihypertensive, antihyperuricemic, anti-inflammatory, antioxidant, cytotoxic and antitumor, hepatoprotective, hypoglycemic, immunomodulatory, lipase-inhibitory, and tyrosinase-inhibitory activities (Fang et al., 2022; Wang, 2021). However, the quality of *D. officinale* leaves in Chinese markets is uneven (Huang et al.,

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2022). Although the four provinces in China, namely, Yunnan, Guizhou, Fujian, and Zhejiang, have classified *D. officinale* leaves as local characteristic foods and established local food safety standards, the existing physicochemical indices only include water content, total ash, and crude polysaccharide and lack attention to other active chemical constituents, such as flavonoids (Qian et al., 2023; Zhou et al., 2023).

Previous studies revealed that *D. officinale* leaves are rich in flavonoids and are more abundant than *D. officinale* stems are (Cao et al., 2019; G. Zhou & Lv, 2012a). The flavonoids in *D. officinale* leaves are mainly apigenin and quercetin glycosides, such as vicenin 1, vicenin 2, vicenin 3, rutin, schaftoside, violanthin, isoviolanthin, and apigenin 6-*C*-*β*-D-glucopyranosyl-(1 → 2)-*α*-L-arabinopyranoside (Zhang et al., 2017; Chen et al., 2023; Feng et al., 2022; Tao et al., 2015; Williams, 1979; G. Zhou & Lv, 2012b; Zhu et al., 2019). Vicenin 2 from *D. officinale* leaves inhibited cancer metastasis by inhibiting transforming growth factor (TGF)-*β*1-induced epithelial–mesenchymal transformation (EMT) (Luo et al., 2019). Isoviolanthin inhibited the TGF-*β*1-induced migration and invasion of hepatocellular carcinoma cells (Xing et al., 2018). Apigenin 6-*C*-*β*-D-glucopyranosyl-(1 → 2)-*α*-L-arabinopyranoside has been shown to inhibit tyrosinase (Chen et al., 2023). Vicenin 1 was shown to have protective effects against ultraviolet B (UVB)-induced damage in a 3D skin model in which human keratinocytes were used (Chen et al., 2022).

Given the defects of the existing evaluation system, a content determination method based on flavonoid glycosides in *D. officinale* leaves as the main index is urgently needed to provide a stable and reliable quality control standard for the development and utilization of *D. officinale* leaves and to establish a quality control foundation for subsequent resource development. However, the literature reveals a lack of comprehensive and systematic descriptions of quantitative analysis methods for determining the contents of several flavonoid glycosides in *D. officinale* leaves. Moreover, in recent years, research on the leaves of *D. officinale* has focused mainly on its pharmacological components, while relevant research on the harvest period of *D. officinale* leaves is still lacking in the literature. In addition, determining the optimal extraction parameters for *D. officinale* leaves is crucial for ensuring the retention of their pharmacological activity. However, methods for extracting effective components from *D. officinale* leaves based on flavonoid glycosides have not yet been reported.

In this study, based on the LC–MS/MS results (Fig. 1 and Table 1), we selected three major flavonoid *C*-glycosides, namely, vicenin 2 (peak 1), vicenin 3 (peak 5), and isoviolanthin (peak 8), along with one flavonoid *O*-glycoside, namely, rutin (peak 7) (for chemical structures of these four glycosides, see Fig. S1), to determine their contents in *D. officinale* leaves at different harvesting periods. Additionally, ultrasound-assisted extraction conditions were optimized to maximize the contents of these four flavonoid glycosides in *D. officinale* leaves via response surface methodology.

2. Experimental methods

2.1. Materials, instruments, and reagents

2.1.1. Plant material

Various harvest times were used to collect plant material from a cultivated base in Pu'er City, Yunnan Province, China. The botanical samples were identified and characterized as *Dendrobium officinale* Kimura et Migo by Associate Professor Shu-Yun Li at Kunming Institute of Botany, Chinese Academy of Sciences. The voucher specimen (No. PE1912) was stored in the Key Laboratory of Resource Plants and Biotechnology, Institute of Botany, Chinese Academy of Sciences.

After the sludge removal operation, the leaves were sent to an oven at 50 °C to dry, polished into powder and screened for use with 60 mesh. Information on the *D. officinale* leaf samples is given in Table S1.

2.1.2. Chemicals and reagents

Isoviolanthin (≥ 95 % pure) was obtained from Chengdu Alfa Biotechnology Co., Ltd. (Chengdu, China). Rutin (≥ 97 % pure) was obtained from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai,

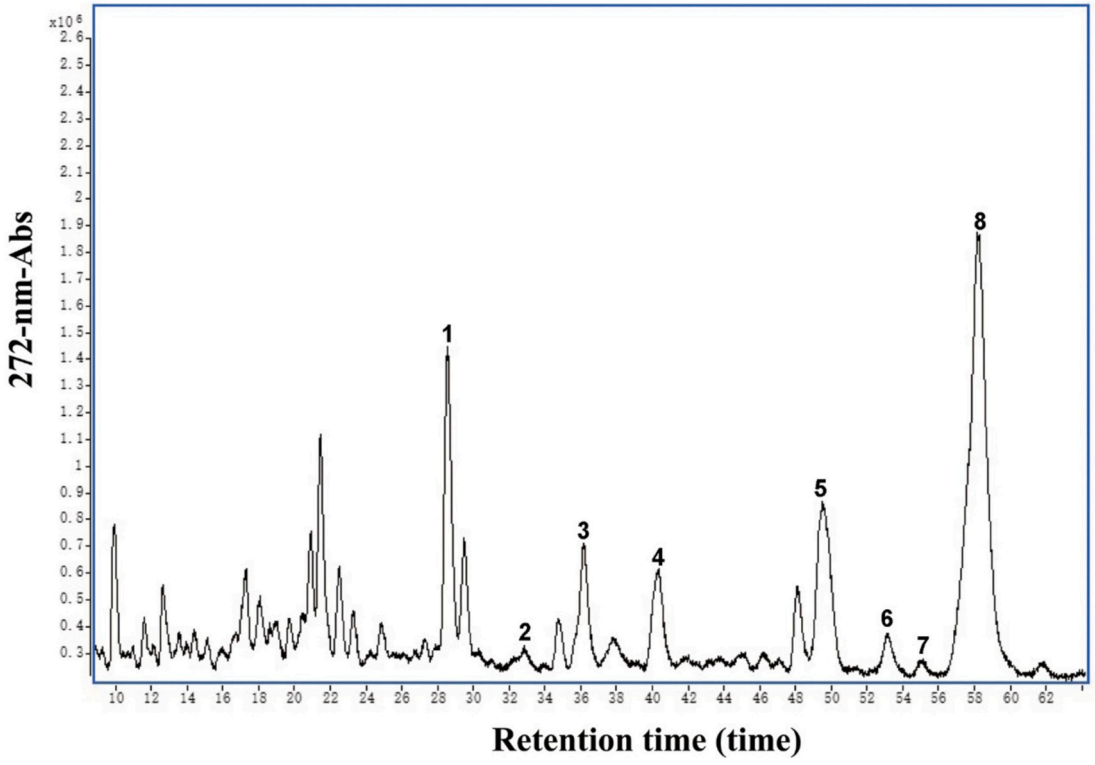

Fig. 1. Chromatogram of the flavonoid glycosides detected in *Dendrobium officinale* leaves. Peak 1, vicenin 2; peak 2, stellarin 2; peak 3, vicenin 1; peak 4, schaftoside; peak 5, vicenin 3; peak 6, apigenin 6-*C*-*α*-L-arabinopyranosyl-8-*C*-*β*-D-xylopyranoside; peak 7, rutin; peak 8, isoviolanthin.

Table 1
Identification of the main flavonoid glycosides in *Dendrobium officinale* leaves via LC–MS/MS.

Peak No.	RT (min)	Observed ion mass(*m/z*)	Theoreticalion mass (*m/z*)	Error (ppm)	MS fragments (*m/z*)	Compound identification	Ref.
1	28.227	594.1643	594.1584	0.0010	593.16, 503.13, 383.09, 353.08, 325.08, 297.08	Vicenin 2	(Y. Wang et al., 2019)
2	32.821	624.1758	624.1690	0.0011	503.13, 533.14, 383.09, 413.10, 355.09, 312.08	Stellarin 2	(Y. Wang et al., 2019)
3	36.107	564.1531	564.1479	0.0009	565.15, 503.13, 473.12, 443.11, 425.10, 383.09, 353.08, 325.08, 297.08	Vicenin 1	(Y. Wang et al., 2019)
4	39.797	564.1531	564.1479	0.0009	563.15, 473.12, 443.11, 383.09, 353.08, 325.08, 297.08	Schaftoside	(Y. Wang et al., 2019)
5	49.238	564.1516	564.1479	0.0006	563.15, 503.12, 473.12, 443.11, 383.09, 353.07, 325.08	Vicenin 3	(Y. Wang et al., 2019)
6	52.773	534.1423	534.1373	0.0009	473.12, 443.11, 383.09, 353.08	Apigenin 6-*C*-*α*-L-arabinopyranosyl-8-*C*-*β*-D-xylopyranoside	(Sui et al., 2023)
7	54.740	610.1586	610.1534	0.0009	609.16, 300.04, 271.03	Rutin	(Sui et al., 2023)
8	58.526	578.1692	578.1635	0.0010	503.13, 473.12, 457.13, 383.09, 353.08	Isoviolanthin	(Sui et al., 2023)

China). Vicenin 3 and vicenin 2 (≥ 98 % pure) were obtained from Sichuan Vicchi Biotechnology Co., Ltd. (Chengdu, China). Chromatographically pure methanol and acetonitrile were obtained from Shanghai Xingke Biochemical Co., Ltd. (Shanghai, China). Sodium nitrite, aluminum nitrate nonahydrate, and sodium hydroxide were analytically pure and purchased from Sichuan Xilong Scientific Co., Ltd. (Chongqing, China). Deionized water was prepared via the use of an ultrapure water purifier ZYJDI-20 L/H by Dongguan Zhongjie Water Purification Environmental Protection Technology Co., Ltd. (Dongguan, China). The remaining reagents were of analytical grade. Information on the experimental reagents is given in Table S2.

2.1.3. Instruments

To conduct our analyses, we utilized an Agilent 1260 Infinity II liquid chromatography system (Agilent, USA), which allowed us to conduct the necessary assessments with precision and accuracy. Then, we procured an ultrasonic cleaner, specifically the SK7200H model, from Shanghai Kedao Ultrasonic Instrument Co., Ltd., situated in Shanghai, China. Additionally, an electronic analytical balance, model AR1140, was obtained from Mettler Toledo in Switzerland. A rotary evaporator OSB-2200 and an electric thermostatic water bath ZYJDI-20 L/H were purchased from Tokyo Rikakikai Co., Ltd. (Tokyo, Japan). The TDL-40B centrifuge was obtained from Shanghai Anting Scientific Instrument Factory (Shanghai, China). The Tecan Infinite 200 Pro multifunctional enzyme marker was obtained from Tecan Trading AG (Männedorf, Switzerland).

2.2. Methods

2.2.1. Solution preparation

Standard solutions. The standard stock solutions of vicenin 2, vicenin 3, rutin, and isoviolanthin were diluted with 60 % ethanol to concentrations of 110.0 μg/mL (vicenin 2), 109.6 μg/mL (vicenin 3), 101.2 μg/mL (rutin), and 133.2 μg/mL (isoviolanthin). The standard products vicenin 2 (7.08 mg), vicenin 3 (4.15 mg), rutin (8.18 mg), and isoviolanthin (4.02 mg) were accurately measured and placed in the same 25 mL bottle with 60 % ethanol to obtain a mixed standard reserve solution. The concentrations of vicenin 2, vicenin 3, rutin, and isoviolanthin in the mixed standard solution were 283.2, 166.0, 327.2, and 160.8 μg/mL, respectively.

Sample solutions. The *D. officinale* leaf powder was accurately weighed to 1.0000 g via an analytical balance and then transferred into a screw-capped plastic centrifuge tube with a flat bottom, and 40 mL of a 60 % ethanol aqueous solution was added. The centrifuge tube was placed at 30 °C for ultrasonic treatment (power consumption of 350 W, working frequency of 53 kHz) for 40 min, removed, cooled, and then centrifuged at low speed (3000 rpm) for 20 min. The supernatant was subsequently filtered, and the solvent was removed from the filtrate under vacuum. The dry matter was quantified and dissolved in 60 % ethanol, and the volume was adjusted to 25 mL. All sample solutions were refrigerated at 4 °C.

All requisite solutions must be preserved at 4 °C and subjected to a purification process using either a 0.45 μm pore size nylon membrane or a comparable filtering medium. After purification, they are introduced into an HPLC apparatus for examination. Ethanol (60 %) was used as a blank control.

2.2.2. Chromatographic condition optimization

All analyses were performed in an Agilent 1260 Infinity II liquid chromatography system (Agilent, USA). In this study, an Agilent Zorbax SB-C_{18} column (4.6 mm × 250 mm, 5 μm) was selected for the rapid analysis of four flavonoid glycosides. This column exhibited an intact and symmetric peak shape under the chromatographic conditions used and offered high detection sensitivity. Chromatography separation was successful when the products were eluted via the aforementioned column, with the mobile phase composed of acetonitrile (A) and aqueous 0.1 % (*v/v*) formic acid (B).

The elution gradient was carefully optimized to achieve efficient separation of the target compounds. Specifically, the elution program was set as follows: from 0 to 15 min, 7.5–10.5 % A; from 15 to 25 min, 10.5–11.5 % A; from 25 to 35 min, 11.5–12.5 % A; from 35 to 45 min, 12.50–13.5 % A; and from 45 to 65 min, 13.50–14.5 % A. Following a preequilibration of five minutes, the samples underwent injection for further analysis. The timeline of the elution conditions is shown in Table S3.

The detection wavelength was 272 nm, and the fluid flow velocity was 1.0 mL/min. The volume injected was 8 μL, and the temperature of the column enclosure was steadily held at 35 °C.

2.2.3. Target identification by LC–MS/MS

Utilizing the capabilities of the Agilent Mass Hunter Workstation Software, we conducted an in-depth data analysis. We utilized 2 scanning modes based on our established procedure: (1) the initial scan event was held in full scan mode covering a range from *m/z* 100 to 1700 Da; (2) the subsequent scan event was configured as Auto MS/MS. The pressure for atomization was adjusted to 30 pounds per square inch, with a dry gas flow velocity of 8 L/min. The drying temperature was maintained at 350 °C, and the ion source potential was set at 3500 V. The capillary voltage was configured to 175 V, the cone aperture bias voltage was adjusted to 65 V, and the quadrupole lens potential was established at 750 V.

2.2.4. Data processing and statistical analysis

The initial processing of the raw HPLC data involved using Agilent Chem Station software, followed by exporting the data as AIA files to the professional software Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (version 2004 A) to calibrate peak retention times and conduct comparative analyses.

2.2.5. System suitability

Prior to commencing the analysis, the HPLC system was given time to equilibrate on one occasion. A blank solution of 60 % ethanol, along with the standard and sample solutions (No. S1), was sequentially injected for analysis, and the suitability of the system was analyzed according to the chromatographic conditions in Table S4. The raw data of all the experiments involved in the methodology validation were processed via Microsoft Office 2016 software. The analysis of variance and quadratic polynomial equations involved in response surface design are automatically processed via Design Expert 13.0 software.

2.2.6. Method validation

During the verification process for the quantification methodology of vicenin 2, vicenin 3, rutin, and isoviolanthin in *D. officinale* leaves, diverse parameters were assessed, including linearity assessment, limit of detection (LOD) and limit of quantification (LOQ) tests, precision evaluation, reproducibility analysis, stability tests, and recovery efficiency determination. The completed methodology validation process conforms to the requirements of the International Conference on Harmonization (ICH) guidelines.

2.2.6.1. Linearity. Ethanol (60 %) was consistently included in varying volumes to produce standard solutions with different concentrations. The mixed standard solution was accurately removed into a 10 mL volumetric bottle. A consistent volume of ethanol (60 %) was added to produce a solution with a reduced concentration. This solution was diluted 2, 4, 8, 16, and 32 times, and the volume was fixed to obtain six parts of a mixed standard solution. After filtration with an organic membrane, the remaining filtrate was preserved. The peak area was determined according to the specified method, as detailed in Section 2.2.2. The peak area data corresponding to the chromatographic peak of each concentration gradient of the standard product were collected as the ordinate, and the final mass concentration of the gradient dilution of each standard product was set as the horizontal coordinate. A standard curve was drawn, the regression equation was fitted, and the slope and *y*-intercept were determined.

2.2.6.2. LOD and LOQ tests. LOD and LOQ were utilized to evaluate methodological sensitivity. Control solutions were prepared for the four flavonoid glycosides—vicenin 2, vicenin 3, rutin, and isoviolanthin. These control solutions were then diluted with a 60 % ethanol solution multiple times for chromatographic peak height recording and signal-to-noise ratio (S/N) calculation. LOD and LOQ were determined by S/N ratios of 3:1 and 10:1.

2.2.6.3. Precision. The system precision was investigated by analyzing six replicate injections of different concentrations of mixed standard solution, and the percentage relative standard deviation was calculated for the area responses of all the known analyte peaks. The mixed standard solution at three concentrations (high, medium, and low) was injected into the HPLC system and tested in sequence. *D. officinale* leaves from batch S1 were prepared via the procedure described in Section 2.2.1. Each sample was repeatedly injected 6 times, with 8 μL each time. The peak area was determined according to the protocol detailed in Section 2.2.2, and the relative standard deviation of the corresponding experimental group was calculated.

2.2.6.4. Repeatability. Six samples of *D. officinale* leaf powder (1.0005 g) from the same batch (batch S1) were prepared via the procedure outlined in Section 2.2.1. The peak area of each target component was determined based on the chromatographic conditions outlined in in Section 2.2.2, and the content of each flavonoid glycoside and the corresponding relative standard deviation were calculated algebraically.

2.2.6.5. Stability. A sample solution of *D. officinale* leaves (from batch S1) was prepared via the procedure outlined in Section 2.2.1. The sample solution was promptly analyzed via HPLC, following the procedure outlined in Section 2.2.2, to measure the peak areas of the four target flavonoid glycosides, which were then stored at room temperature in the dark. The peak area of each flavonoid glycoside was determined again at 2, 4, 8, 12, and 24 h after sample preparation, and the relative standard deviation was calculated.

2.2.6.6. Recovery rate. *D. officinale* leaf powder (from batch S1) with known contents of vicenin 2, vicenin 3, rutin, and isoviolanthin was precisely weighed to 0.25 g, and then a single reference solution with 100 % content of each flavonoid glycoside was precisely added. The solution sample was subsequently created in accordance with the procedure outlined in Section 2.2.1, and six samples were consistently generated. The peak areas of each flavonoid glycoside were determined under chromatographic conditions as detailed in Section 2.2.2, and the recoveries and relative standard deviations were calculated.

2.2.7. Determination of flavonoid glycosides in D. officinale leaves after different harvesting months

From January 2021 to December 2021, leaves of *D. officinale* plants, which were more than 15 cm above the ground, were randomly collected every other month, dried at 50 °C and crushed through a 60-mesh screen. Each batch of dry *D. officinale* leaf powder was accurately weighed to 1.0000 g, and the sample mixture was prepared via the method described in Section 2.2.1. Under the chromatographic conditions outlined in Section 2.2.2, this analytical methodology was employed for the concurrent examination of four flavonoid glycosides (vicenin 2, vicenin 3, rutin, and isoviolanthin) in *D. officinale* leaf samples (No. M1–M12). The samples were used to quantify the levels of four different analytes. Table S11 contains all the results, including the average content from three repeated analyses.

2.2.8. Optimization of the ultrasonic-assisted extraction parameters

Flavonoid glycosides, namely, vicenin 2, vicenin 3, rutin, and isoviolanthin, were extracted via ultrasonic-assisted extraction, and the following parameters, including the extraction temperature (50–70 °C), extraction time (10–30 min), sample-to-liquid ratio (1:30–1:50 g/mL), and ethanol concentration (30–60 %), were originally examined via a single-factor experimental design. RSM was employed to increase the extraction conditions to achieve the maximum concentration of flavonoid glycosides. A Box–Behnken experimental design was utilized to assess the impact of extraction temperature (X_1), extraction duration (X_2), sample-to-solvent ratio (X_3), and ethanol strength (X_4) at three different levels. According to the outcomes of the experiments involving a single factor, Table S12 presents the coded and actual levels of the extraction process for the four independent variables. The experimental data and model coefficients were subjected to analysis of variance (ANOVA) to identify any notable distinctions among the treatment combinations. Furthermore, visual representations in the form of 3D-surface plots were generated to observe the combined impacts of the important factors on the response variable (Y: the yield of the total contents of the four ingredients). The total contents of the four ingredients were quantified via the method described in Section 2.2.2.

3. Results and discussion

3.1. LC/MS–MS analysis

In a previous study, flavonoid glycosides, such as apigenin-6-*C*-*α*-L-arabinosyl-8-*C*-*β*-D-xyloside (G. Zhou & Lv, 2012c), isovitexin, narcissin, and apigenin-6-*C*-*β*-D-glucopyranosyl-(1 → 2)-*α*-L-arabinopyranoside (Wang et al., 2021), were reported as the main compounds in *D. officinale* leaves. As shown in Table 1 and Fig. 1, eight flavonoid glycosides were identified via LC–MS/MS analysis. The presence of phytochemical constituents within the leaves of *D. officinale* is visually discernible via the LC–MS/MS spectroscopic profiles presented in Fig. 1. The peaks of *D. officinale* leaves, including vicenin 2, vicenin 3, rutin, and isoviolanthin, were further characterized via comparison with the fragmentation ions of reference standard solutions. The identification of other flavonoid compounds was executed via comparison with those paternal compounds and their fragment ion information. The potential elucidation of peaks 2, 3, 4, and 6 is depicted in Figs. S6, S7, S8, and S10, respectively. We matched the relevant data with the existing fragmentary information in the online database and compared it with the data reported in the past literature for further confirmation. Peak 1, with *m/z* 593, was identified as vicenin 2. The fragment ions of $[M-H-CH_2O]^-$, $[M-H-C_3H_6O_3]^-$, and $[M-H-C_4H_8O_4]^-$ in flavonoid glycosides are the 30, 90, and 120 (*m/z*) ions formed by the removal of glycoxyl fragments from the molecular ion peak, respectively. Flavonoids are mainly substituted by hydroxy and oxymethyl groups in different quantities and at different positions, resulting in $[M-H]^-$ and continuous CH_3·removal signals. Generally, several oxymethyl groups can be used to remove several CH_3 molecules, and the retention time of hydroxy groups significantly increases after being replaced by methyl groups. The findings from LC–MS/MS analysis revealed that the primary compounds identified in *D. officinale* leaves were isoviolanthin, vicenin 2, and vicenin 3 (Fig. 1).

As mentioned previously, the flavonoid class exhibits a diverse array of biological activities. Moreover, rutin effectively inhibits cell proliferation, inflammation (Wang, Ma, et al., 2023), ROS production and NLRP3 inflammasome activation (Wu et al., 2023). Rutin was identified as the primary antioxidant component in the leaves of *D. officinale* (Zhang et al., 2017). The leaves of *D. officinale* were phytochemically studied to yield isoviolanthin, which exhibited inhibitory potency against *α*-glucosidase and was characterized by an IC_{50} of 1.09 ± 0.46 *μ*M (Feng et al., 2022). Isoviolanthin reduces skin damage caused by active oxides, such as hydrogen peroxide, and it has been confirmed that isoviolanthin can act as a skin-protective agent (Wang, Yin, et al., 2023). Vicenin 2 had scavenging effects on both OH free radicals and DPPH free radicals when vitamin C was used as a positive control, with IC_{50} values of 17 and 28 *μ*M, respectively (Duan et al., 2019). Vicenin 2 can absorb ultraviolet photons, and the SPF value was 10.68 compared with that of the standard sunscreen, *p*-aminobenzoic acid (Duan et al., 2019). Vicenin 1 has been reported to exhibit potent protective effects against radiation, in addition to its notable antioxidant properties and anti-inflammatory actions, all of which are considered highly effective. (Uma Devi, 2001; Uma Devi & Satyamitra, 2004). In summary, three major *C*-glycosides, namely, vicenin 2 (peak 1), vicenin 3 (peak 5), and isoviolanthin (peak 8), as well as one *O*-glycoside, namely, rutin (peak 7), in *D. officinale* leaves (Fig. 1) were selected as the quantitative indices for a follow-up study. In view of this, we also speculated that the *D. officinale* leaf flavonoid extracts obtained with vicenin 2, vicenin 3, rutin, and isoviolanthin, which are quality evaluation indices, have a variety of potential application values and might be used in the development of functional health foods or skin care products in the future.

3.2. Investigation of the HPLC methodology

The HPLC method was established after various parameters, including the column, mobile phase composition, elution mode, gradient conditions, and other chromatographic conditions, were investigated. This HPLC method was developed as a content determination method that can stably indicate the target component and better separate and eluate various polars and nonpolar substances, with simplicity, rapidity, effectiveness, and acceptable accuracy.

3.2.1. Detection wavelength

The physical and chemical properties of the target components, such as the properties of the sample (polar or nonpolar), molecular weight, UV absorption, etc., were analyzed during the development of the preliminary method. In this experiment, the mixed standard solution was injected into the HPLC system, and then full-wavelength scanning was carried out in the range of 200 nm to 400 nm. At 272 nm, the four flavonoid glycosides presented greater ultraviolet absorption (Fig. S2). Thus, the wavelength of 272 nm was selected as the optimal detection point for quantifying the content in this experimental setup.

3.2.2. Solvent for standard products

After taking into account the solubility and stability of the analyte, a ratio of 60:40 (*v/v*) of ethanol to water was finally used as the diluent for the standard.

3.2.3. Column

Flavonoid glycosides and their related flavonoids have different affinities for different stationary phases. Therefore, it is essential to identify the most suitable stationary phase that can maintain excellent stability under the final chromatographic conditions while ensuring effective retention and separation during analysis. Most flavonoids in *D. officinale* leaves have moderate polarity and are typically separated via reversed-phase chromatographic columns. An Agilent Zorbax SB-AQ column (4.6 × 250 mm, 5 *μ*m), a Chiral CD-Ph column (4.6 × 250 mm, 5 *μ*m), and an Agilent Zorbax SB-C_{18} column (4.6 mm × 250 mm, 5 *μ*m) were used to separate the chromatographic peaks of the target components and their associated impurities. The peak shape of the target component was suboptimal with either the AQ or phenyl stationary phases, resulting in inadequate retention of chromatographic peaks and insufficient separation between known impurities. The results showed that an Agilent Zorbax SB-C_{18} column was used for the rapid analysis of these four flavonoid glycosides because the peak shapes were intact and symmetrical, and the detection sensitivity was high under chromatographic conditions.

3.2.4. Mobile phase

Because the chemical structures of flavonoid glycosides contain multiple phenolic hydroxy groups, a negative peak effect on a chromatographic peak can easily occur, or a more severe tailing phenomenon can be produced. Volatile acid regulators, such as formic acid, phosphoric acid, and trifluoroacetic acid, can be added. Hence, aqueous mobile phases consist of water and buffers (such as phosphate and formic acid buffer systems), whereas the organic mobile phase comprises methanol and acetonitrile. Given the ultimate detection wavelength of 272 nm, an aqueous mobile phase containing 0.1 % formic acid in water was concluded to be optimal for effectively carrying the target component and maximizing absorption. Furthermore, taking into account the elution capacity, column back pressure, and UV absorption at 272 nm, we identified acetonitrile as a suitable organic mobile phase.

3.2.5. Elution model

Although we tested the isocratic elution model due to the problems of a low theoretical plate number, insufficient separation degree, and peak broadening, the model could not achieve proper retention of the analyte in the specified time. Therefore, the HPLC elution procedure was finally performed in gradient elution mode.

3.2.6. Column temperature

The temperature of the column oven was maintained at 35 °C to

increase the consistency of future experiments, minimize the baseline column pressure, and facilitate the separation of components.

3.2.7. Velocity of the mobile phase

The velocity of the mobile phase also had a certain effect on chromatographic peak separation. When the flow rate was set to 1 mL/min, the four flavonoid glycosides could be effectively separated within 65 min, and the time required for analysis and testing decreased to a certain extent.

3.3. Method validation

3.3.1. System suitability

To verify the effectiveness of the developed method for the required separation, the suitability characteristics of the system were researched. The experimental findings indicated that the use of 60 % ethanol as the solvent did not interfere with the determination, and the theoretical column number exceeded 10,000. Furthermore, the chromatographic peaks of each individual component were fully resolved, with a separation ratio exceeding 2 compared with adjacent peaks (Table S4). The assessment of the baseline separation between chromatographic peaks for the analytes confirmed that the system's suitability criteria were within acceptable limits. (Fig. 2). The column efficiency met the experimental requirements, and the components had good resolution, meeting the requirements of subsequent HPLC analysis.

3.3.2. Linearity

Consequently, the flavonoid glycosides in the leaves of *D. officinale* exhibited favorable linearity across the pertinent concentration spectrum, with a correlation coefficient exceeding 0.9999 ($R^2 > 0.9999$). Each of the calibration curves pertaining to the four constituents demonstrated strong linearity across their respective ranges, and all the standard curves were greater than 0.9990. The results are shown in Table S5 and Fig. S3.

3.3.3. LOD and LOQ tests

The result showed that when the concentration of vicenin 2 was reduced to 0.2270 μg/mL, the S/N ratio was approximately 3.0, and when the concentration of vicenin 2 was reduced to 1.1352 μg/mL, the S/N ratio was approximately 10.0. Thus, the LOD and LOQ values for vicenin 2 were 0.2270 and 1.1362 μg/mL, respectively. The limits of detection and quantification for the other three components are shown in Table S6.

3.3.4. Precision test

The results of the precision tests are shown in Table S7, Table S8, Table S9, and Table S10, and the relative standard deviations of the peak areas of the four flavonoid glycosides met the requirements for subsequent content determination.

The information in Table 2 reveals that an assessment was conducted on the variability of peak areas for vicenin 2, vicenin 3, rutin, and isoviolanthin, measured by RSD (relative standard deviation). The peak areas are less than 2 % in the concentration ranges of the four different mixed solutions containing these compounds, which indicates that liquid chromatography exhibits good precision in measuring the peak areas within the specified concentration ranges.

3.3.5. Repeatability test

The average mass concentrations of vicenin 2, vicenin 3, rutin, and isoviolanthin in the same batch of *D. officinale* leaves (batch S1) were 3.29 mg/g, 0.48 mg/g, 0.13 mg/g, and 0.21 mg/g, respectively. The total content of the four flavonoid glycosides in dry *D. officinale* leaves was 4.11 mg/g. In *D. officinale* leaves, the content of isoviolanthin was the highest, the content of vicenin 2 was the second highest, and the content of vicenin 3 was the third highest.

As shown in Table 3, the relative standard deviations of the peak areas obtained by repeated determination of the four target components were all less than 1 % ($n = 6$), indicating that the method has good repeatability.

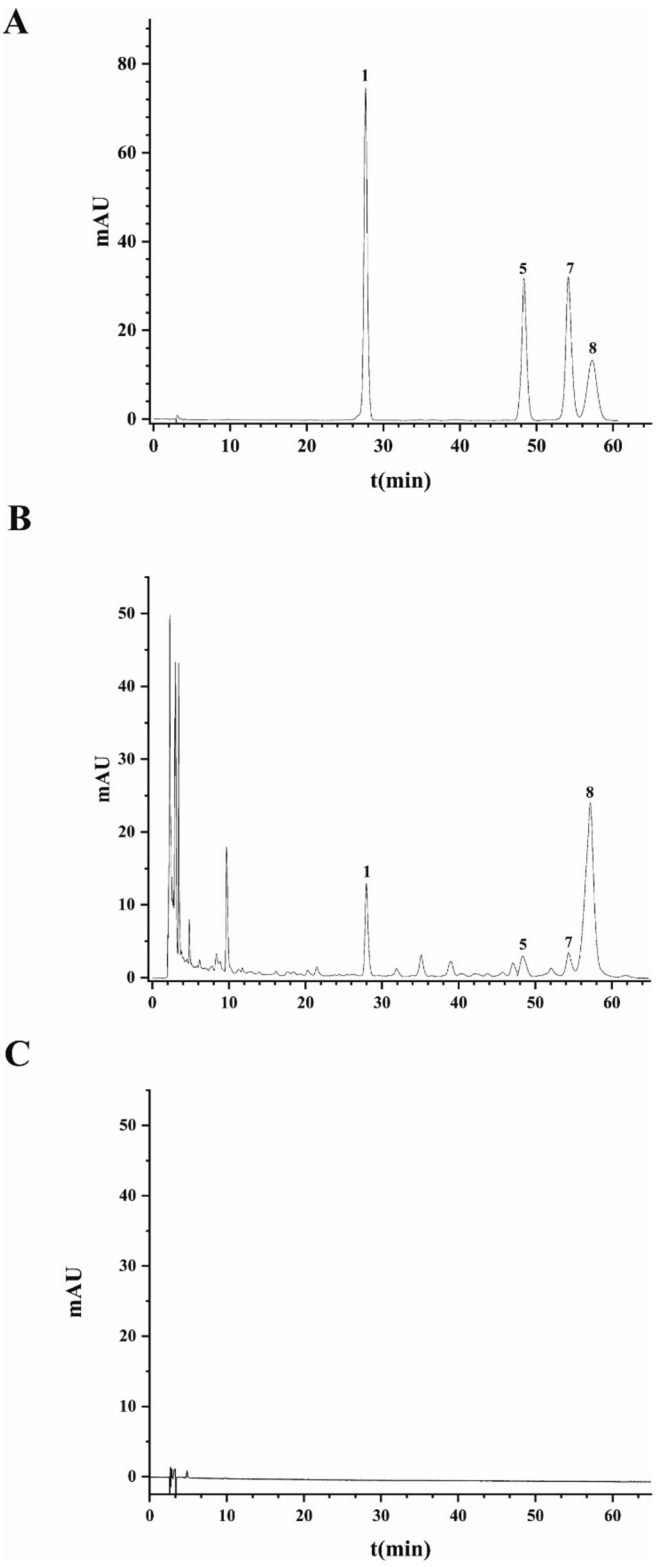

Fig. 2. HPLC chromatograms of the reference substances (A), samples S1 (B), and blank control (C). Peak 1, vicenin 2; peak 5, vicenin 3; peak 7, rutin; peak 8, isoviolanthin.

Table 2
Precision of the four flavonoid glycosides in *Dendrobium officinale* leaves.

Compound	Concentration (μg/mL)	Mean ± SD (mAu)	RSD (%)
Vicenin 2	283.200	4902.60 ± 54.81	1.12
	35.400	633.60 ± 3.89	0.61
	2.594	83.90 ± 1.60	1.90
Vicenin 3	166.000	2967.40 ± 9.76	0.33
	20.750	394.10 ± 0.96	0.24
	2.594	52.95 ± 0.99	1.88
Rutin	327.200	3526.80 ± 24.28	0.69
	40.900	449.27 ± 0.60	0.13
	5.113	53.95 ± 0.55	1.03
Isoviolanthin	160.800	2474.10 ± 12.10	0.49
	20.100	299.70 ± 2.61	0.87
	2.513	37.30 ± 0.52	1.39

3.3.6. Stability test

The results (Table 4) showed that the relative standard deviations of the peak areas of the four indices were all less than 1 % ($n = 6$), which proved that the test solution prepared by this method could maintain good stability at room temperature for 24 h and that the four flavonoid glycosides in the sample did not decompose.

3.3.7. Recovery rate test

The results of addition recovery are often used to characterize the accuracy of content determination methods in methodological verification (Table 5). The experimental findings revealed that the RSD of the peak area of the four flavonoid glycosides was less than 2 % ($n = 6$), indicating that the recovery rate of addition under these chromatographic conditions met the requirements.

3.4. Determination of the sample content

The validated analytical approach was effectively utilized for the concurrent assessment of four flavonoid glycosides, namely, vicenin 2, vicenin 3, rutin, and isoviolanthin, in *D. officinale* leaf samples (Nos. M1–M12). The concentrations of the four indices in the samples were measured, and the findings are presented in Table S11, along with the mean concentrations from three repeated analyses ($n = 3$).

Compared with existing studies (Huang et al., 2015; Zhang et al., 2019), our study included a more comprehensive investigation of peak purity, system stability, LOD and LOQ, and other indicators of standard products. Moreover, the theoretical plate number of each index component, the separation degree of each peak from adjacent peaks, and the sensitivity even at low concentrations also demonstrated the robustness and reliability of the present HPLC-DAD method.

The content and abundance of flavonoids in different parts of *D. officinale* differed and were affected by the harvest period. The contents of the four flavonoid glycosides in *D. officinale* leaves collected from January to December are shown in Table S11. According to Fig. S13, Fig. 3, and Table S11, the color of the *D. officinale* leaf extract significantly differed among the harvest periods, the contents of the four flavonoid glycosides in the *D. officinale* leaves differed, and the total contents of the four components in the *D. officinale* leaves clearly fluctuated among the same growth years and from the same origin. The color of the extract solutions from March to August seemed red, whereas the color of those from September to February seemed orange (Fig. S13), which may be caused by the variation in the content of these flavonoid glycosides (Table S11). However, quantitative experiments are needed in the future.

The content of vicenin 2 in *D. officinale* leaves harvested in January and July was the highest (0.3753 mg/g and 0.3779 mg/g, respectively) and reached the lowest value in May (0.1348 mg/g). The content of vicenin 3 in *D. officinale* leaves harvested in January was the highest (0.5141 mg/g) and decreased, with the lowest point occurring in June (0.0207 mg/g). The content of rutin in *D. officinale* leaves harvested in March was the highest (0.7681 mg/g) and notably decreased, reaching the minimum value in May (0.0156 mg/g). The content of isoviolanthin in *D. officinale* leaves harvested in July was the highest (1.3911 mg/g) and significantly decreased, reaching its nadir in November (0.0707 mg/g). The content of isoviolanthin fluctuated relatively greatly, and the overall trend increased and then decreased. The overall content of these four flavonoid glycosides in *D. officinale* leaves at various times of the year was greatest in July (2.0642 mg/g), reaching its nadir in May (0.6244 mg/g). The total contents were greater in late winter and early spring (1.1820 mg/g in January, 1.6378 mg/g in February, 1.5193 mg/g in March, and 1.2718 mg/g in April).

Therefore, if the total content of the four flavonoid glycosides was used as an indicator of the harvest time of *D. officinale* leaves, the optimal harvest time for the leaves was February or July. However, *D. officinale* ground strips generally reach the harvest age requirement after one and a half years of growth. Farmers typically harvest *D. officinale* stems from November to March of the following year, and

Table 3
Repeatability of the four flavonoid glycosides in *Dendrobium officinale* leaves.

Sample name	Vicenin 2		Vicenin 3		Rutin		Isoviolanthin	
	Peak area (mAu)	RSD (%)	Peak area (mAu)	RSD (%)	Peak area (mAu)	RSD (%)	Peak area (mAu)	RSD (%)
R-1	343.50	0.66	108.99	0.99	96.67	0.83	1850.20	0.65
R-2	343.80		106.59		95.31		1850.30	
R-3	343.80		109.21		96.47		1861.50	
R-4	339.70		107.34		97.02		1830.20	
R-5	338.80		107.39		95.44		1855.50	
R-6	340.60		107.15		97.20		1863.60	

Table 4
Stability of the four flavonoid glycosides in *Dendrobium officinale* leaves.

Injection time (h)	Vicenin 2		Vicenin 3		Rutin		Isoviolanthin	
	Peak area (mAu)	RSD (%)	Peak area (mAu)	RSD (%)	Peak area (mAu)	RSD (%)	Peak area (mAu)	RSD (%)
0	285.90	0.79	108.56	0.89	105.10	0.64	1576.9	0.69
2	286.60		109.15		105.60		1582.3	
4	287.40		109.31		106.78		1570.6	
6	288.20		110.98		105.70		1579.2	
8	287.40		110.54		105.60		1590.9	
10	285.90		109.94		104.70		1593.6	
12	292.90		109.88		104.70		1603.1	
24	286.60		108.09		105.70		1595.4	

Table 5
Test results of the sample recovery ($n = 6$).

Compound	Mass (sample, g)	Mass (original, μg)	Mass (added, μg)	Mass (found, μg)	Recovery (%)	Average recovery (%)	RSD (%)
Vicenin 2	0.25	119.34	118.25	225.36	94.85	95.49	0.81
	0.25	119.34	118.25	225.48	94.90		
	0.25	119.34	118.25	227.02	95.55		
	0.25	119.34	118.25	225.19	94.78		
	0.25	119.34	118.25	229.16	96.45		
	0.25	119.34	118.25	229.01	96.39		
Vicenin 3	0.25	33.36	32.33	68.09	103.65	103.03	1.83
	0.25	33.36	32.33	68.01	103.53		
	0.25	33.36	32.33	66.40	101.08		
	0.25	33.36	32.33	69.78	106.23		
	0.25	33.36	32.33	67.11	102.16		
	0.25	33.36	32.33	66.69	101.51		
Rutin	0.25	51.35	50.09	106.49	104.98	104.01	1.88
	0.25	51.35	50.09	106.49	104.98		
	0.25	51.35	50.09	104.28	102.80		
	0.25	51.35	50.09	104.28	102.80		
	0.25	51.35	50.09	108.46	106.92		
	0.25	51.35	50.09	103.05	101.58		
Isoviolanthin	0.25	822.91	821.44	1595.49	97.03	96.69	1.91
	0.25	822.91	821.44	1537.73	93.52		
	0.25	822.91	821.44	1581.23	96.16		
	0.25	822.91	821.44	1586.76	96.50		
	0.25	822.91	821.44	1623.84	98.75		
	0.25	822.91	821.44	1614.39	98.18		

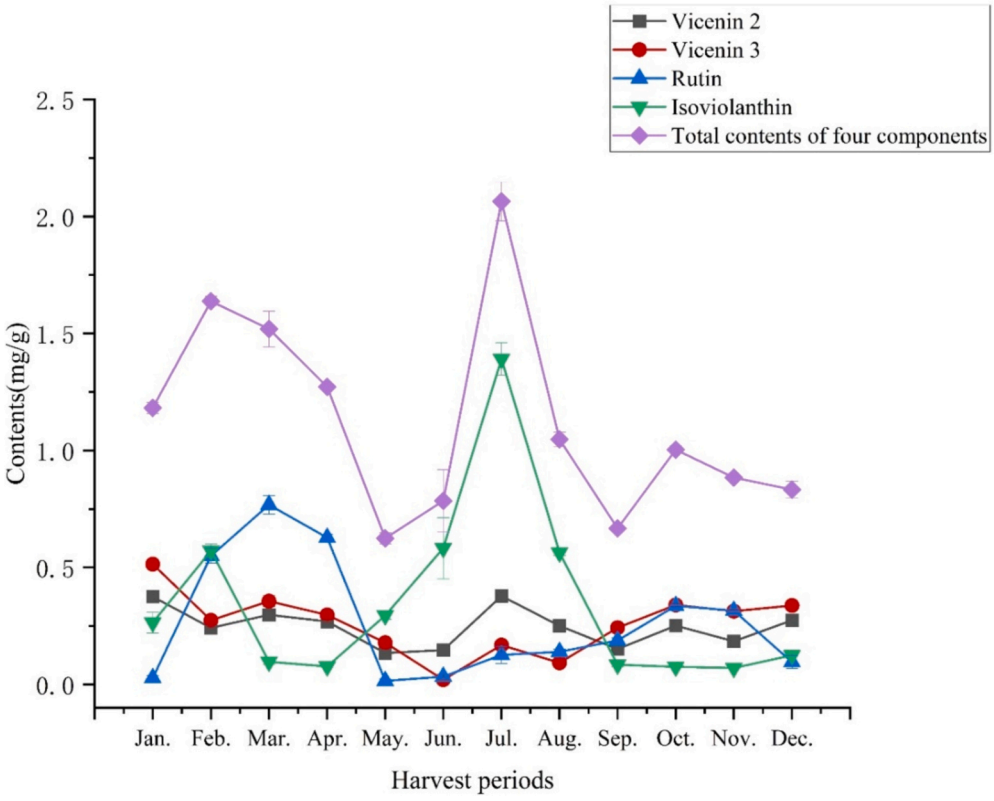

Fig. 3. Dynamic changes in the contents of four flavonoid glycosides in *Dendrobium officinale* leaves during different harvesting periods.

D. officinale flowers are collected from April to June. The remaining time is the seedling maintenance period, and the base does not organize picking work. Therefore, it can be inferred that the best harvest time for *D. officinale* leaves is February, when the total content of the four flavonoid glycosides is used as the evaluation index.

Combined with the results of previous reports on the effects of harvest month on the stem physicochemical index of *D. officinale*, we infer that harvesting from January to March has no adverse effects on the effective components or medicinal value of stems (DB33/T, 2021; Zang, 2020; Zhang et al., 2015). Therefore, it is possible to reach an agreement on the optimum harvesting time for *D. officinale* leaves and stems.

However, significant differences exist in the composition and proportions of secondary metabolites among samples from different origins (Huang et al., 2015). Undoubtedly, the differences in flavonoid glycosides in *D. officinale* leaves across various cultivation regions are worthy of further study to facilitate a more accurate assessment of the impact of

environmental factors on the synthesis of these compounds in *D. officinale* leaves and provide a more scientific basis for the development and utilization of this plant resource.

3.5. Enhancement of the extraction procedure

3.5.1. Consequences of the single-factor experiments

We studied four factors influencing the ultrasonic-assisted extraction of flavonoid glycosides from *D. officinale* leaves, namely, extraction temperature, extraction time, sample-to-liquid ratio, and ethanol concentration through single-factor experiments. As indicated in Fig. S4A, the yield of the total contents of the four ingredients increased when the extraction temperature increased from 40 °C to 60 °C, resulting in a decrease in yield when the extraction temperature exceeded 60 °C. The findings revealed that the optimal extraction temperature for extracting the maximum yield of the total contents of the four ingredients was within 50–70 °C. With respect to the duration of extraction (Fig. S4B), there was an increase in the yields of the total contents of the four constituents as the time increased from 10 to 20 min. However, subsequent enhancements in the extraction time led to a decrease in this yield. Therefore, the most suitable extraction time was in the range of 10–30 min for the highest yield of the total contents of the four ingredients. The overall extraction yield of the four components was also notably impacted by the sample-to-liquid ratio. We observed that a higher total yield of the four components was achieved when the sample-to-liquid ratio approached 1:40 g/mL. (Fig. S4C). Nevertheless, a higher sample-to-liquid ratio did not result in enhanced extraction of the total contents of the four ingredients, as it resulted in a decreased yield. Therefore, the best sample-to-liquid ratio for the highest total contents of the four ingredients was suggested to be in the range of 1:30 to 1:50 g/mL. Like other variables, the yield significantly increased as the ethanol concentration increased from 10 to 45 %. Nevertheless, the persistent increase in the ethanol concentration was unable to further increase the overall yield of the four ingredients (Fig. S4D). The most appropriate ethanol concentration ranged from 30 to 60 % (*v/v*) to attain the maximum yield of the total contents of the four ingredients.

3.5.2. Outcomes derived from the response surface analysis

The extraction temperature, extraction time, sample-to-liquid ratio, and ethanol concentration were further optimized via the Box–Behnken model to achieve optimal ultrasonic-assisted extraction conditions for obtaining the highest yield of the total contents of the four ingredients from *D. officinale* leaves.

Using the Design-Expert software, we designed 29 groups of experiments and collected the total contents of the actual yields of the four ingredients acquired from all the extraction schemes in accordance with the experimental design (Table S13). The data from the experiment concerning the yield of the total contents of the four ingredients were inspected, and details of the *p*-values and correlation coefficients can be found in Table S14.

Then, we eliminated the nonsignificant factors and acquired the ultimate predictive formula for calculating the maximum total levels of the four components in *D. officinale* leaves as follows:

$$Y_B^{'} = -30.91606 + 0.8369728X_3 + 0.364432X_4 - 0.002631X_1X_2 - 0.001926X_2X_3 - 0.0026967X_3X_4 - 0.002768X_1^2 - 0.004801X_2^2 - 0.008145X_3^2 - 0.003810X_4^2$$

In the equation, Y represents the total yield of the four ingredients, and X_1, X_2, X_3, and X_4 represent the extraction temperature, extraction time, sample-to-liquid ratio, and ethanol concentration, respectively.

The significance of the model equation was assessed through analysis of variance (ANOVA), and the findings are detailed in Table S14. The significance levels associated with the quadratic model of the coefficient of determination (R^2) and the adjusted coefficient of determination are both greater than 0.9, indicating that the quadratic model fits the experimental values well. The R-squared value was ascertained to be 0.9869, indicating a robust fit between the predicted and experimental values. Additionally, the coefficient of variation (C.V. = 0.94 %) implies low variability within the fitted model, confirming the accuracy and dependability of the experimental values.

The results indicated that the influence of the independent variables, particularly the extraction time ($P < 0.0001$) and the ratio of sample to solvent ($P < 0.0001$), had the most profound effects on the cumulative yield of the four components, whereas the concentration of ethanol ($P = 0.0002$) held a subsequent position in terms of significance; nevertheless, the extraction temperature ($P = 0.3186$) had no substantial effect on the total contents of the four ingredients. Fig. 4 displays the response surface plot of the interaction effect of the independent variables on the yield of the total contents of the four ingredients to demonstrate t how the independent variables collectively influence the response values. Maintaining constant levels of both the sample-to-solvent ratio and ethanol concentration, our investigation revealed an interplay between the extraction temperature and duration, significantly influencing ($P = 0.0025$) the overall yield of the four primary components, as shown in Fig. 4A. The interaction effects of the extraction temperature and sample-to-liquid ratio on the yield of the total contents of the four ingredients ($P = 0.8604$) are shown in Fig. 4B. In Fig. 4C, the response surface plot shows that the interactive effect of extraction temperature and ethanol concentration on the response value was not obvious ($P = 0.4337$). Under constant extraction temperature and ethanol concentration conditions, as depicted in Fig. 4D, the peak yield of the four components was attained at a sample-to-solvent ratio of 1:40 (g/mL) and an extraction period of 20 min. The interaction effect of the extraction time and ethanol concentration on the yield of the total contents of the four ingredients was significant ($P = 0.003$), as shown in Fig. 4E. Ultimately, the data presented in Fig. 4F indicate that the combined impact of varying sample-to-liquid ratios and ethanol concentrations significantly affected the overall yield of the total contents of the four ingredients ($P < 0.0001$). The above experimental results imply that it is necessary to optimize the polarity of the aqueous ethanol solution and other four variables to ensure the effective extraction of the four index components.

3.5.3. Determination of the optimal process parameters

By employing a Box–Behnken design, the extraction of flavonoid glycosides from *D. officinale* leaves was optimized. The optimal parameters for this process were determined to be an ultrasonic temperature of 59.626 °C, an ultrasonic extraction duration of 22.436 min, an ethanol concentration of 43.244 % (*v/v*), and a solid-to-liquid ratio of 1:37.914 (g/mL). The optimal predicted yield of the four flavonoid glycosides was 5.298 ± 0.05 mg/g.

To further verify the accuracy and reliability of the optimal extraction scheme, 5 parallel repeated experiments were performed while maintaining the above extraction conditions. After algebraic calculation, the average yield of the four flavonoid glycosides in *D. officinale* leaves under the optimal ultrasonic-assisted extraction conditions was 5.23 ± 0.05 mg/g, while the predicted value was 5.298 mg/g. The ratio between the actual value and the predicted value of the four flavonoid glycosides was 5.23/5.298 = 0.9871, which proved that the predicted value of the model was in good agreement with the experimental results. Therefore, the optimal process design of response surface design can be considered scientific, stable, and reliable.

4. Conclusion

A specific, sensitive, robust, and fast HPLC-DAD method was established and verified for the quantification of four flavonoid glycosides in *D. officinale* leaves at various harvest times. The results of the present study indicate that spring (from January to February) is the optimal harvesting period for *D. officinale* leaves and stems. This approach offers a robust scientific foundation for ensuring the consistency of the quality

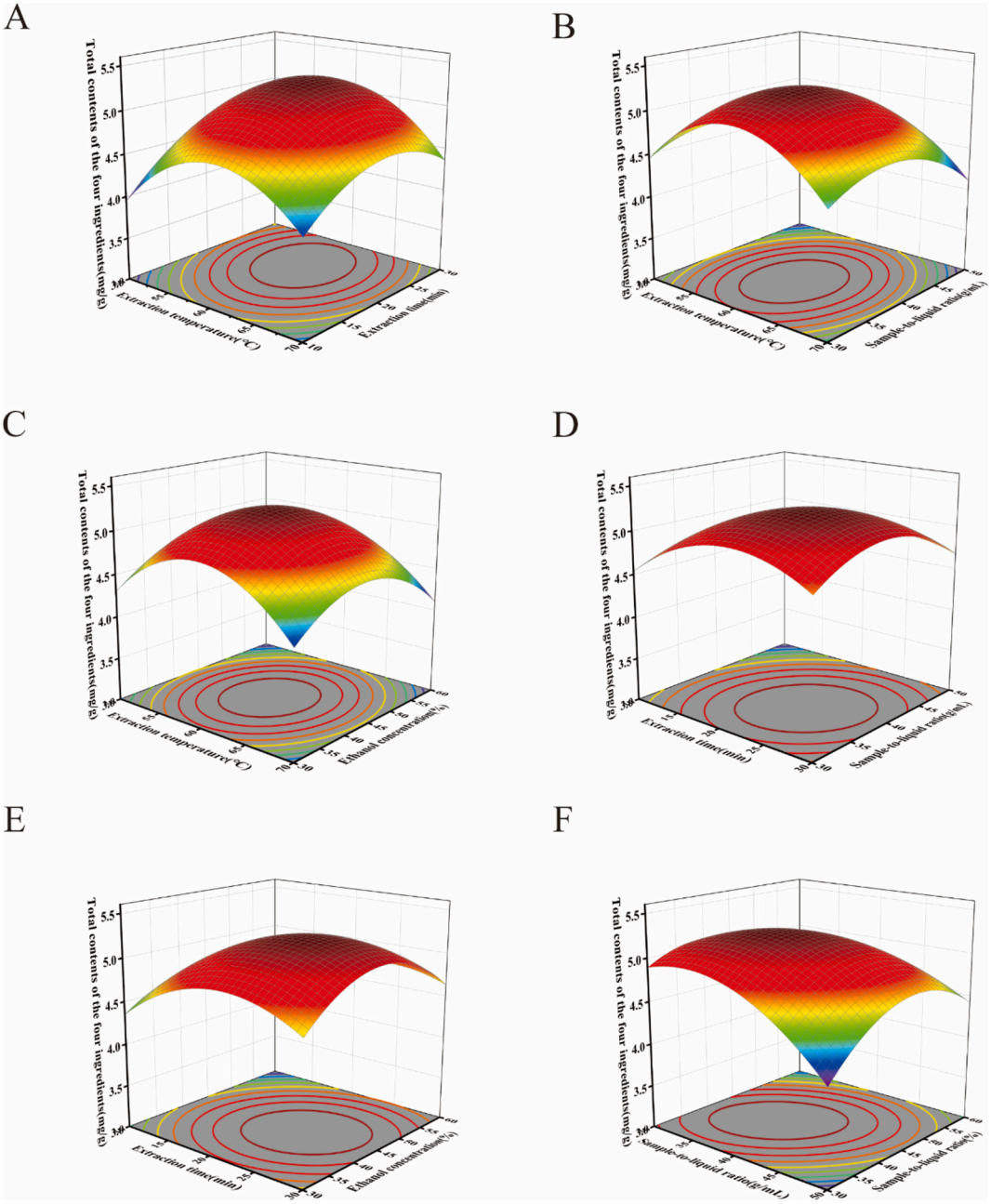

Fig. 4. Response surface analysis of the yield of the total contents of the four ingredients from *Dendrobium officinale* leaves. (A) Interaction between the extraction temperature and extraction time; (B) interaction between the extraction temperature and sample-to-liquid ratio; (C) interaction between the extraction temperature and ethanol concentration; (D) interaction between the extraction time and sample-to-liquid ratio; (E) interaction between the extraction time and ethanol concentration; (F) interaction between the sample-to-liquid ratio and ethanol concentration.

of *D. officinale* leaves, thereby facilitating their effective quality control.

The present investigation revealed the efficacy of RSM in refining the conditions for *D. officinale* leaf extraction and investigated the intricate interplay between independent and response variables. Furthermore, the model's validity was statistically substantiated through ANOVA. Moreover, the use of these flavonoid glycosides, which are obtained at low cost from the nonmedicinal parts of *D. officinale*, presents an interesting option for improving the profitability of these related industries and promoting the establishment of high-value-added industrial chains.

CRediT authorship contribution statement

Yangwenqing Deng: Writing – original draft, Software, Methodology, Formal analysis, Data curation. **Si Li:** Software, Methodology, Investigation, Conceptualization. **Yu-Ru Shi:** Software, Formal analysis. **Dong-Bao Hu:** Software, Formal analysis. **Ji-Feng Luo:** Resources. **Pei-Ji Zhao:** Writing – review & editing, Conceptualization. **Wen-Juan Yuan:** Writing – review & editing, Visualization, Methodology, Investigation, Conceptualization. **Yue-Hu Wang:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors affirm the absence of any declared or potential conflicts of interest that might have biased the reported research outcomes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101933.

Data availability

Data will be made available on request.

References

Cao, H., Ji, Y., Li, S., Lu, L., Tian, M., Yang, W., & Li, H. (2019). Extensive metabolic profiles of leaves and stems from the medicinal plant *Dendrobium officinale* kimura et migo. *Metabolites, 9*(10), 215. https://doi.org/10.3390/metabo9100215

Chen, Y.-F., Luo, J.-F., Zhang, Y.-N., & Wang, Y.-H. (2023). Flavonoid diglycosides from *Dendrobium officinale* leaves and their tyrosinase inhibitory activity. *Chemistry of Natural Compounds, 59*(6), 1067–1074. https://doi.org/10.1007/s10600-023-04198-y

Chen, Y.-F., Zhang, D.-D., Hu, D.-B., Li, X.-N., Luo, J.-F., Duan, X.-Y., et al. (2022). Alkaloids and flavonoids exert protective effects against UVB-induced damage in a 3D skin model using human keratinocytes. *Results in Chemistry, 4*, Article 100298. https://doi.org/10.1016/j.rechem.2022.100298

Vol. DB33/T. (2021). *Technical regulations for Dendrobium officinale. Quality and technology supervision of Zhejiang Province* (pp. 635–2021). https://bz.zjamr.zj.gov.cn/public/std/db/view/0c05c50714f14c048a8c400e8ad09d93.html

Duan, X., Wu, T., Liu, T., Yang, H., Ding, X., Chen, Y., & Mu, Y. (2019). Vicenin-2 ameliorates oxidative damage and photoaging via modulation of MAPKs and MMPs signaling in UVB radiation exposed human skin cells. *Journal of Photochemistry And Photobiology B-Biology, 190*, 76–85. https://doi.org/10.1016/j.jphotobiol.2018.11.018

Fang, J., Lin, Y., Xie, H., Farag, M. A., Feng, S., Li, J., & Shao, P. (2022). *Dendrobium officinale* leaf polysaccharides ameliorated hyperglycemia and promoted gut bacterial associated SCFAs to alleviate type 2 diabetes in adult mice. *Food Chemistry: X, 13*, Article 100207. https://doi.org/10.1016/j.fochx.2022.100207

Feng, H., Peng, C., & Li, X. (2022). Study on the separation and identification of flavone *C*-glycosides from the leaves of *Dendrobium officinale* and their inhibitory activities to α-gulcosidase. *China Pharmacy, 33*(18), 2187–2191. http://lib.cqvip.com/Qikan/Article/Detail?id=7108051907&from=Qikan_Search_Index.

Huang, H.-Q., Wu, Y.-R., Li, P., Song, Z.-P., Liang, Z.-W., Qian, L., & Gao, R.-F. (2022). Identification and analysis of *Dendrobium candidum* in retail market. *China Port Science and Technology, 4*(8), 67–72. http://lib.cqvip.com/Qikan/Article/Detail?id=7108068398&from=Qikan_Search_Index.

Huang, Y.-C., Xie, Z.-S., Ren, J., Ye, J.-H., & Wei, G. (2015). Comparison of HPLC characteristic spectrum of flavonoids on leaves of *Dendrobium officinale* from three provenance sources. *Chinese Journal of Experimental Traditional Medical Formulae, 21*(24), 37–40. https://doi.org/10.13422/j.cnki.syfjx.2015240037

Luo, Y., Ren, Z., Du, B., Xing, S., Huang, S., Li, Y., et al. (2019). Structure identification of viceninII extracted from *Dendrobium officinale* and the reversal of TGF-β1-induced epithelial-mesenchymal transition in lung adenocarcinoma cells through TGF-β/Smad and PI3K/Akt/mTOR signaling pathways. *Molecules, 24*(1), 144. https://doi.org/10.3390/molecules24010144

Qian, Y.-C., Lu, Q.-J., Liang, D.-G., & Mao, B.-Z. (2023). Research progress on nutrient components and resource development of flowers and leaves of *Dendrobium officinale*. *Journal of Zhejiang Agricultural Sciences, 64*(12), 2891–2896. https://doi.org/10.16178/j.issn.0528-9017.20221072

Sui, M., Feng, S., Liu, G., Chen, B., Li, Z., & Shao, P. (2023). Deep eutectic solvent on extraction of flavonoid glycosides from *Dendrobium officinale* and rapid identification with UPLC-triple-TOF/MS. *Food Chemistry, 401*, Article 134054. https://doi.org/10.1016/j.foodchem.2022.134054

Tao, Y., Cai, H., Li, W., & Cai, B. (2015). Ultrafiltration coupled with high-performance liquid chromatography and quadrupole-time-of-flight mass spectrometry for screening lipase binders from different extracts of *Dendrobium officinale*. *Analytical and Bioanalytical Chemistry, 407*(20), 6081–6093. https://doi.org/10.1007/s00216-015-8781-4

Uma Devi, P. (2001). Radioprotective, anticarcinogenic and antioxidant properties of the Indian holy basil, *Ocimum sanctum* (Tulasi). *Indian Journal of Experimental Biology, 39*(3), 185–190. https://europepmc.org/article/MED/11495275.

Uma Devi, P., & Satyamitra, M. (2004). Protection against prenatal irradiation-induced genomic instability and its consequences in adult mice by *Ocimum* flavonoids, orientin and vicenin. *Indian Journal of Experimental Biology, 80*(9), 653–662. https://doi.org/10.1080/09553000400005494

Wang, J., Yin, H., Zhu, W., He, Q., Zhang, H., Sun, L., et al. (2023). Research on the resistance of isoviolanthin to hydrogen peroxide-triggered injury of skin keratinocytes based on transcriptome sequencing and molecular docking. *Medicine (Baltimore), 102*(47), Article e36119. https://doi.org/10.1097/md.0000000000036119

Wang, J.-Q., Tian, L.-X., Chen, X.-M., & Guo, S.-X. (2022). Comparison and health risk assessment of mineral elements in stems and leaves of *Dendrobium officinale* cultivated with conventional method and mycorrhizal fungi. *China Journal of Chinese Materia Medica, 47*(21), 5824–5831. https://doi.org/10.19540/j.cnki.cjcmm.20220623.101

Wang, J.-W., Fan, L., Peng, Y., Peng, C.-S., & Li, X.-B. (2021). Protective effects of *Dendrobium officinale* leaf and flower on hydrogen peroxide-in duced oxidative damage of HaCaT cells and LC-MS analysis. *Lishizhen Medicine and Materia Medica Research, 32*(2), 288–292. http://lib.cqvip.com/Qikan/Article/Detail?id=7104921179&from=Qikan_Search_Index.

Wang, M., Ma, X., Gao, C., Luo, Y., Fei, X., Zheng, Q., et al. (2023). Rutin attenuates inflammation by downregulating AGE-RAGE signaling pathway in psoriasis: Network pharmacology analysis and experimental evidence. *International Immunopharmacology, 125*, Article 111033. https://doi.org/10.1016/j.intimp.2023.111033. Part A.

Wang, Y., Liang, Z., Liao, X., Zhou, C., Xie, Z., Zhu, S., et al. (2019). Identification of *C*-glycosyl flavones by high performance liquid chromatography electrospray ionization mass spectrometry and quantification of five main *C*-glycosyl flavones in *Flickingeria fimbriata*. *BMC Chemistry, 13*, 94. https://doi.org/10.1186/s13065-019-0616-5

Wang, Y.-H. (2021). Traditional uses, chemical constituents, pharmacological activities, and toxicological effects of *Dendrobium* leaves: A review. *Journal of Ethnopharmacology, 270*, Article 113851. https://doi.org/10.1016/j.jep.2021.113851

Williams, C. A. (1979). The leaf flavonoids of the orchidaceae. *Phytochemistry, 18*(5), 803–813. https://doi.org/10.1016/0031-9422(79)80019-9

Wu, H., Wang, Y., Huang, J., Li, Y., Lin, Z., & Zhang, B. (2023). Rutin ameliorates gout via reducing XOD activity, inhibiting ROS production and NLRP3 inflammasome activation in quail. *Biomedicine & Pharmacotherapy, 158*, Article 114175. https://doi.org/10.1016/j.biopha.2022.114175

Xing, S., Yu, W., Zhang, X., Luo, Y., Lei, Z., Huang, D., et al. (2018). Isoviolanthin extracted from *Dendrobium officinale* reverses TGF-β1-mediated epithelial-mesenchymal transition in hepatocellular carcinoma cells via deactivating the TGF-β/Smad and PI3K/Akt/mTOR signaling pathways. *International Journal of Molecular Sciences, 19*(6), 1556. https://doi.org/10.3390/ijms19061556

Yuan, Y., Tang, X., Liu, S., & Zhang, J. (2020). The major factors influencing distribution of three species of *Dendrobium*: Analysis of potential ecologically suitable distributions. *Journal of Applied Research on Medicinal and Aromatic Plants, 19*, Article 100275. https://doi.org/10.1016/j.jarmap.2020.100275

Zang, H.-J. (2020). Changes of effective components in *Dendrobium officinale* with different varieties and harvesting time. *Journal of Zhejiang Agricultural Sciences, 61*(8), 1539–1541. https://doi.org/10.16178/j.issn.0528-9017.20200818

Zhang, S.-B., Zhou, K.-J., Zhang, Z., Lu, R.-R., Li, X., & Li, X.-H. (2015). Study on the suitable harvesting time of *Dendrobium officinale* in Yunnan Province. *China Journal of Chinese Materia Medica, 40*(18), 3549–3552. http://lib.cqvip.com/Qikan/Article/Detail?id=666328250&from=Qikan_Search_Index.

Zhang, X.-F., Zhou, C.-H., Zhang, L.-K., Jiang, M., Xie, Z.-S., Yuan, Y., 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*(1), 29–34. https://doi.org/10.13422/j.cnki.syfjx.20182417

Zhang, X.-L., Si, J.-P., Wu, L.-S., Guo, Y.-Y., Yu, J., & Wang, L.-H. (2013). Field experiment of F1 generation and superior families selection of *Dendrobium officinale*. *China Journal of Chinese Materia Medica, 38*(22), 3861–3865. http://lib.cqvip.com/Qikan/Article/Detail?id=47860641&from=Qikan_Search_Index.

Zhang, Y., Zhang, L., Liu, J., Liang, J., Si, J., & Wu, S. (2017). *Dendrobium officinale* leaves as a new antioxidant source. *Journal of Functional Foods, 37*, 400–415. https://doi.org/10.1016/j.jff.2017.08.006

Zhou, G.-F., & Lv, G.-Y. (2012a). Comparative studies on scavenging DPPH free radicals activity of flavone *C*-glycosides from different parts of *Dendrobium officinale*. *China Journal of Chinese Materia Medica, 37*(11), 1536–1540. http://lib.cqvip.com/Qikan/Article/Detail?id=42240080&from=Qikan_Search_Index.

Zhou, G.-F., & Lv, G.-Y. (2012b). Study on eight flavone *C*-glycosides in *Dendrobium officinale* leaves and their fragmentation pattern by HPLC-DAD-ESI-MS^n. *Chinese Pharmaceutical Journal, 47*(1), 13–19. http://lib.cqvip.com/Qikan/Article/Detail?id=40433285&from=Qikan_Search_Index.

Zhou, G.-F., & Lv, G.-Y. (2012c). Study on HPLC fingerprints of flavone *C*-glycosides in *Dendrobium officinale* leaves and determination of index component. *Chinese Pharmaceutical Journal, 47*(11), 889–893. https://doi.org/10.3969/j.issn.1008-0805.2021.02.09

Zhou, M., Zhang, Z., Zheng, X., Liang, J., & Zhang, C. (2023). Application value and development and utilization of *Dendrobium officinale* leaves. *Farm Products Processing, 9*, 77–80. https://doi.org/10.16693/j.cnki.1671-9646(X).2023.09.019

Zhu, Y., Yu, J., Jiao, C., Tong, J., Zhang, L., Chang, Y., et al. (2019). Optimization of quercetin extraction method in *Dendrobium officinale* by response surface methodology. *Heliyon, 5*(9), Article e02374. https://doi.org/10.1016/j.heliyon.2019.e02374