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Simultaneous analysis of *trans*- and *cis*-isomers of 2-glucosyloxycinnamic acids and coumarin derivatives in *Dendrobium thyrsiflorum* by high-performance liquid chromatography (HPLC)-photodiode array detection (DAD)-electrospray ionization (ESI)-tandem mass spectrometry (MS)

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Abstract

A novel method has been developed for simultaneous analysis for three pairs of *trans*- and *cis*-isomers of 2-glucosyloxycinnamic acids, along with their biogenic metabolites (three coumarin derivatives including scopoletin, scoparone and ayapin) in a Chinese medicinal herb *Dendrobium thyrsiflorum* by using high-performance liquid chromatography (HPLC)-photodiode array detection (DAD)-electrospray ionization (ESI)-tandem mass spectrometry (MS). The method was carried out by using a Polaris C_{18} column with a gradient solvent system of 0.5% acetic acid aqueous solution–acetonitrile. Seven target analytes including isodensifloside, isothyrsifloside, densifloside, thyrsifloside, scoparone, ayapin and scopoletin were exclusively identified by comparing their retention behaviors, UV and MS spectra with the authentic standards, and their contents in *D. thyrsiflorum* were simultaneous determined by employing UV detection at 342 nm. In addition, another pair of isomers of 2-glucosyloxycinnamic acids was putatively elucidated mainly based on the MS fragmentation. The method was validated and found to be satisfactorily linear, selective and robust. Recoveries ranged from 95.56 to 97.94% for all compounds at three different spiking levels. The limits of detection (LOD) and quantitation (LOQ) ranged, respectively, from 0.02 to 0.13 $\mu g\,mL^{-1}$ and 0.07 to 0.39 $\mu g\,mL^{-1}$ depending on various compounds. The established quality evaluation method was successfully used for evaluating the quality of *D. thyrsiflorum* samples of different organs and collections.

Keywords: *Dendrobium thyrsiflorum*; 2-Glucosyloxycinnamic acid; Coumarin; High-performance liquid chromatography (HPLC)-photodiode array detection (DAD)-electrospray ionization (ESI)-tandem mass spectrometry (MS); Quality evaluation

1. Introduction

The genus *Dendrobium* (Ochidaceae) is a highly evolutive and divers group represented by more than 1100 species widely distributed throughout Asia, Europe and Australia, and there are 74 species and 2 variations of *Dendrobium* plants in China. The dried or fresh stems of *Dendrobium* plants, known as Caulis Dendrobii (Shihu or Huang Cao in Chinese) have been used in traditional or folk medication as a Yin tonic to nourish the stomach, promote the production of body fluid and reduce fever. Recent pharmacological studies have shown that some of the components and extractives of *Dendrobium* species displayed anti-tumor [1], anti-angiogenic [2], anti-platelet aggregation [3], anti-inflammation [4] and immunoregulatory activities [5], which are partly responsible for its actions and indications of the herb in traditional remedies.

Phytochemical investigation disclosed that *Dendrobium* plants mainly contain phenolic derivatives as bibenzyls, phenanthrenes, fluorenones, flavones, coumarins, as well as alkloids [6–10]. *Dendrobium thyrsiflorum* Rchb.f. is one of the main

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sources of commercial Caulis *Dendrobium* contributed by its wide distribution and exuberance growing in the nature. Moreover, tissue cultural and cultivating techniques have been successively developed for the growth of *D. thyrsiflorum* to meet the increasing demand for its utilization in medication and other health products preparing purposes, and to protect the natural source of *D. thyrsiflorum*. In our previous investigation on the chemical components, 33 natural products including 6 coumarin, 3 bibenzyls, 4 fluorenones, 5 phenanthrenes, 5 flavones and 3 anthraquinones were isolated from this plant [11–12].

Among the isolated compounds, coumarins have been shown to act as the main and characteristic markers for *D. thyrsiflorum*. In our recent chemical investigation on the flower of *Dendrobium*, two new 2-glucosyloxycinnamic acid derivatives, along with two known ones, were isolated and identified as isodensifloside, isothyrsifloside, densifloside and thyrsifloside (to be reported separately). These four 2-glucosyloxycinnamic acids derivatives were presumed to be biogenic precursor of the corresponding coumarins of ayapin and scoparone (Fig. 1), the latter has been extensively investigated for its vascular relaxant and hypolipidaemic activities [13].

Nevertheless, as a group of potential natural products, the distribution and determination of these sorts of *trans*- and *cis*-isomers of 2-glucosyloxycinnamic acids and their biogenic metabolites in natural sources were rarely reported. Only few publications dealt with the determination of scoparone in some *Dendrobium* herbs by using high-performance liquid chromatography (HPLC) [14] and thin-layer chromatographic densitometry [15]. Recently we detected the distribution of total coumarins in different organs of this plant by a technique of laser scanning confocal microscopy (LSCM) [16].

HPLC-DAD-ESI-MS has been proven to be a powerful approach for the rapid identification and determination of the ingredients in botanic extracts and TCM (traditional Chinese medicine) products, advanced in trace quantity detection capability, high specificity and providing molecular mass information [17–19]. In this paper, a HPLC-DAD-ESI-MS method was developed firstly for the validated qualitative and quantitative analyses of two pairs of *trans*- and *cis*-isomers of 2-glucosyloxycinnamic acids and their biogenic metabolites coumarin derivatives in *D. thyrsiflorum* for the purpose of quality evaluation of Caulis Dendrobii and other related medicinal plants containing the above mentioned coumarins and their biogenic precursors.

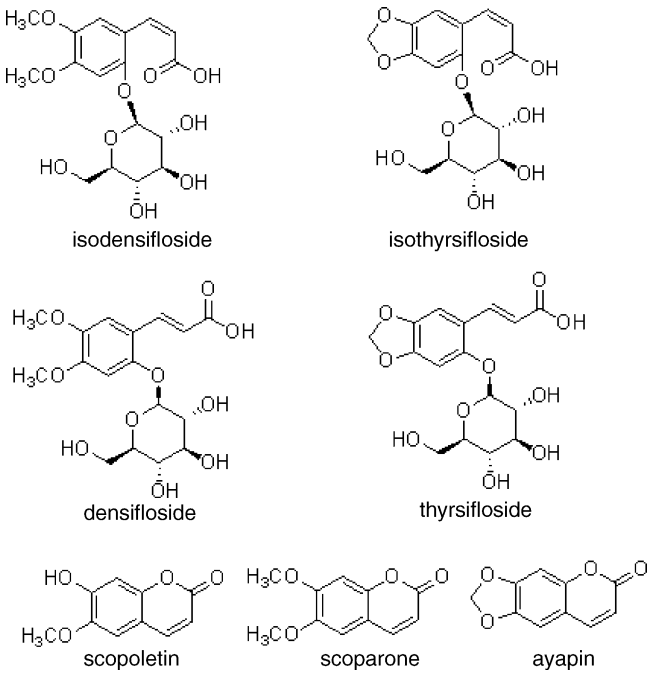

Fig. 1. Chemical structures of the target constituents.

2. Experimental

2.1. Instrumentation and chromatographic conditions

Analyses were performed on an Agilent HPLC system model HP-1100 (Agilent, Palo Alto, CA, USA), consisting of a quaternary pump, a diode array spectrophotometric detector (DAD), a thermostatted column compartment and an autosampler, controlled by HP Chemstation software. The HPLC column was a Polaris (Metachem, Switzerland) C_{18} column (250 mm × 4.6 mm i.d., 5 μm), and a linear gradient elution of A (0.5% acetic acid aqueous solution) and B (CH_3CN) was used. The composition of the eluent was varied from 10 to 20% B in 20 min, the flow rate was kept at 0.8 mL min^{-1}; 20–30% B from 20 to 35 min while simultaneously the flow rate was changed from 0.8 to 1.0 mL min^{-1}; 30–90% B from 35 to 45 min, the flow rate was kept at 1.0 mL min^{-1}. Column temperature was kept constant at 40 °C and the injection volume was 20 μL.

For HPLC-ESI-MS analysis, all experiments were carried out in the positive ion mode by using a Surveyor LC system (ThermoFinnigan, San Jose, USA) equipped with a quaternary pump with on-line degasser, an automatic sampler, a photodiode array detector and the LCQ DECA XP^{plus} mass spectrometer (ThermoFinnigan) with an ESI interface. The raw data were acquired and processed by ThermoFinnigan $Xcalibur_{1.3}$ workstation. The LC initial flow rate was 1.0 mL min^{-1} and was introduced with a splitter to provide a 150 μL min^{-1} flow to the MS system. The operating conditions for the ESI interface were as follows: positive ionization mode; temperature of the capillary, 300 °C; spray voltage, 4.8 kV; capillary voltage, 5 V; tube lens offset, −5 V; sheath gas (N_2) flow, 40 A.U.; auxiliary gas (N_2) flow, 38 A.U.

^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra of the target analytes were recorded with a Brucker AMX-500 spectrometer in DMSO-d_6 (**1**–**4**) and $CDCl_3$ (**5**–**7**), and the chemical shifts are reported in ppm by referencing to the center peak of the residual solvent signal at δ 2.50 (**1**–**4**) and 7.27 (**5**–**7**) for ^1H NMR and at δ 40.0 (**1**–**4**) and (**5**–**7**) for ^{13}C NMR.

2.2. Reagents and materials

HPLC grade acetonitrile was purchased from Sigma. Analytical grade acetic acid and methanol were purchased in Shanghai Chemical Reagent Co. Ltd. (Shanghai, China). Water was double distilled and subsequently filtered through a 0.45 μm membrane (Millipore, Bedford, MA, USA).