Study on HPLC Fingerprint of *Hedyotis hedyotidea*

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Abstract [**Objectives**] To establish a high-performance liquid chromatography (HPLC) fingerprint analysis method for *Hedyotis hedyotidea*, providing a scientific basis for its quality evaluation. [**Methods**] An Agilent ZORBAX SB-C_{18} column (5 μm, 4.6 mm × 250 mm) was employed with 0.1% phosphoric acid-acetonitrile solution as the mobile phase for gradient elution. The column temperature was maintained at 40 ℃, detection wavelength at 240 nm, flow rate at 0.8 mL/min, and injection volume at 10 μL. The *Traditional Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System* (2004A edition) was used to analyze the HPLC fingerprints of 10 batches of *H. hedyotidea* samples. [**Results**] An HPLC fingerprint with ursolic acid as the reference standard was established, identifying 17 common characteristic peaks. The similarity of the 10 batches of *H. hedyotidea* samples ranged from 0.718 to 0.993. [**Conclusions**] This method demonstrates good systematic performance, with precision (*RSD* < 1.5%), repeatability (*RSD* < 2.0%), and 24-h stability (*RSD* < 2.5%) meeting established requirements. It can provide data support for quality control and standard formulation of *H. hedyotidea* medicinal materials.

Key words *Hedyotis hedyotidea*, High-performance liquid chromatography (HPLC), Similarity evaluation, Quality control

1 Introduction

Hedyotis hedyotidea is the dried whole herb of *Hedyotis hedyotidea* (DC.) Merr., a plant belonging to the genus *Hedyotis* in the Rubiaceae family[1-2]. It is characterized by year-round harvestability. As a traditional medicinal material in the Lingnan region of China, it exhibits therapeutic effects such as dispelling wind and dampness, promoting tendon and bone regeneration, and clearing heat to relieve summer heat. Clinically, it is widely used for treating conditions like acute gouty arthritis and acute infectious hepatitis[3-5]. Modern pharmacological studies have further revealed its multi-target bioactivities, including analgesic and anti-inflammatory, immunomodulatory, antitumor (with anti-leukemic effects), antibacterial, anti-inflammatory, and anti-genotoxic properties[6]. This herb is broadly distributed in southern China, primarily in Guangdong, Guangxi, Fujian, Guizhou, and Yunnan provinces. Notably, despite its wide distribution, no systematic studies have been reported on the quality variations of *H. hedyotidea* from different geographic origins, urgently requiring in-depth investigation. Given the complexity of natural medicinal chemical components and inherent quality variability, this study employs RP-HPLC fingerprinting technology to establish a multidimensional quality evaluation system for *H. hedyotidea* for the first time. By systematically calibrating characteristic common peaks and integrating digital similarity analysis models, this approach achieves precise characterization of the herb's quality attributes. The research enhances the methodological framework for quality evaluation of *H. hedyotidea* and provides important data support for developing scientific and standardized quality control system.

Received: March 20, 2025 Accepted: May 12, 2025

Supported by Guangxi University of Chinese Medicine 2019 - 2021 Guangxi First-class Discipline Construction Open Project (2019XK096).

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2 Materials and methods

2.1 Instrument and equipment High performance liquid chromatograph (model: 1200, Agilent, USA); analytical balance (model: SQP, Sartorius Scientific Instruments (Beijing) Co., Ltd.); ultrasonic cleaner (model: KQ5200B, Kunshan Ultrasonic Instrument Co., Ltd.); laboratory ultrapure water device (model: UPR-I-5/10/15TNP, Sichuan Ulupure Technology Co., Ltd.); constant temperature water bath (model: HH-S, Jiangsu Jintan Medical Instrument Factory); centrifuge (model: TGL-16G, Shanghai Anting Scientific Instrument Factory).

2.2 Reagents and drugs Methanol and acetonitrile (LC grade, Thermo Fisher Scientific (China) Co., Ltd.); methanol (AR grade, Xilong Scientific Co., Ltd.); ethanol (AR grade, Sinopharm Chemical Reagent Co., Ltd.); phosphoric acid (AR grade, Tianjin Kemiou Chemical Reagent Co., Ltd); ursolic acid (HPLC reference standard, purchased from National Institutes for Food and Drug Control, purity ≥99.0%, batch No.: 110742-201823); ultrapure water. The collection details of 10 batches of *H. hedyotidea* (DC.) Merr. samples from different origins are listed in Table 1. All samples were authenticated by Senior Experimentalist Lu Hailin from the College of Pharmacy, Guangxi University of Chinese Medicine, as the whole herb of *H. hedyotidea*.

2.2 Methods

2.2.1 Chromatographic conditions. The chromatographic separation was carried out on an Agilent ZORBAX SB-C_{18} column (4.6 mm × 250 mm, 5 μm) using a mobile phase consisting of acetonitrile (A) and 0.1% phosphoric acd (B). A gradient elution program was implemented as follows: from 0 to 65 min, the proportion of acetonitrile (A) increased linearly from 5% to 28%; from 65 to 95 min, the acetonitrile concentration rose sharply from 28% to 100%; and from 95 to 110 min, the mobile phase was maintained at 100% acetonitrile (A). The analysis was performed under controlled conditions with a flow rate of 1.0 mL/min, a detection wavelength of 210 nm, a column temperature of 40 ℃, and an injection volume of 10 μL.

Table 1 Production areas of *Hedyotis hedyotidea* samples

No.	Production areas	Collection date
NBT-1	Dadeng Town, Bobai County, Yulin City, Guangxi	2021 – 12 – 15
NBT-2	Green Villa, Qingxiu District, Nanning, Guangxi	2021 – 12 – 20
NBT-3	Gaofeng Forest Farm, Yongwu Road, Xingning District, Nanning, Guangxi	2022 – 01 – 04
NBT-4	Pingji Town, Qinbei District, Qinzhou, Guangxi	2021 – 12 – 15
NBT-5	Shuitan Village, Liujiang District, Liuzhou, Guangxi	2021 – 12 – 15
NBT-6	Sanjiao Township, Jinxiu County, Jinxiu Town, Laibin City, Guangxi	2021 – 12 – 16
NBT-7	Xinping Village, Guancheng Town, Pingnan County, Guigang City, Guangxi	2021 – 12 – 18
NBT-8	Pingjiang Village, Longmen Town, Pubei County, Qinzhou City, Guangxi	2021 – 12 – 15
NBT-9	Tangba Village, Longtan Town, Jiexi County, Jieyang City, Guangdong	2021 – 12 – 15
NBT-10	Gukeng Village, Tingxi Town, Tongan District, Xiamen, Fujian	2021 – 12 – 18

2.2.2 Sample determination. (i) Preparation of reference substance. Accurately weighed 2 mg of ursolic acid reference substance and transferred it into a 10 mL brown volumetric flask. Added methanol to the mark, mix thoroughly to prepare a 0.2 mg/mL ursolic acid reference solution, and stored for later use.

(ii) Preparation of test sample. Dried *H. hedyotidea* samples from different origins were further dried in an oven at 55 ℃, pulverized, passed through a 60-mesh sieve, and stored in sealed bags. Accurately weighed 2 g of each powdered sample, added 10 mL of 75% ethanol using a pipette, and recorded the total weight. Reflux extraction was performed for 1.5 h. After cooling, the mixture was re-weighed, and any weight loss was compensated by adding 75% ethanol. The solution was then filtered, and the filtrate was collected. Transferred 1.5 mL of each extract into a 1.5 mL centrifuge tube, centrifuged at 12 000 rpm for 10 min, and collected the supernatant into HPLC vials for subsequent analysis.

3 Results and analysis

3.1 Methodological investigation

3.1.1 Precision test. The same *H. hedyotidea* test sample solution prepared using the method described in Section **2.2.2** was injected six consecutive times under parallel conditions. Using the ursolic acid chromatographic peak in the chromatogram as the internal reference peak (S), the relative standard deviations (*RSD*) of the relative retention times (RRT) for all chromatographic peaks of the *H. hedyotidea* test samples were less than 0.50%, and the *RSD* values of the relative peak areas were below 3.00%. The similarity of the chromatographic profiles was calculated using the *Traditional Chinese Medicine Fingerprint Similarity Evaluation System* (2004A edition), yielding a similarity score of ≥0.993. These results demonstrate excellent precision of the analytical instrument employed in this experiment.

3.1.2 Stability test. The same test sample solution prepared according to the method in Section **2.2.2** was analyzed at 0, 2, 4, 8, 12, and 24 h to evaluate the stability of *H. hedyotidea*. The *RSD* values of the RRT for all chromatographic peaks were less than 0.50%, and the *RSD* values of the relative peak areas were below 3.00%. The similarity of the chromatographic profiles, calculated using the *Traditional Chinese Medicine Fingerprint Similarity Evaluation System* (2004A edition), was ≥0.991. These results confirm that the *H. hedyotidea* test samples remained stable within the 24-h testing period.

3.1.3 Repeatability test. Six replicates of the same batch of *H. hedyotidea* samples were prepared according to the test sample preparation method outlined in Section **2.2.2**. Each replicate was individually injected and analyzed under the chromatographic conditions specified in Section **2.2.1**. The *RSD* values of the RRT and relative peak areas (RPA) for all characteristic chromatographic peaks of the *H. hedyotidea* test samples were calculated. The *RSD* values for the chromatographic peaks and relative retention times were all less than 0.50%, while the *RSD* values for the relative peak areas were below 3.00%. The similarity score, determined using the *Traditional Chinese Medicine Fingerprint Similarity Evaluation System* (2004A edition), was ≥0.943. These results confirm that the experimental methodology exhibits excellent repeatability.

3.2 Fingerprint construction of *H. hedyotidea*

3.2.1 Identification of common peaks. By comparing the chromatographic fingerprints of 10 batches of *H. hedyotidea* samples from different geographical origins, 17 common characteristic peaks (accounting for over 90% of the total peak area) were identified as consistently present in all tested samples. A reference fingerprint for *H. hedyotidea* was generated using the *Traditional Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System* (2004A edition). Peak No. 12, corresponding to ursolic acid, was designated as the reference peak (S), as illustrated in Fig. 1. The relative peak areas of the 10 batches were calculated individually. The chromatographic fingerprints of all 10 batches are shown in Fig. 2, and the relative peak areas of the common characteristic peaks are summarized in Table 2.

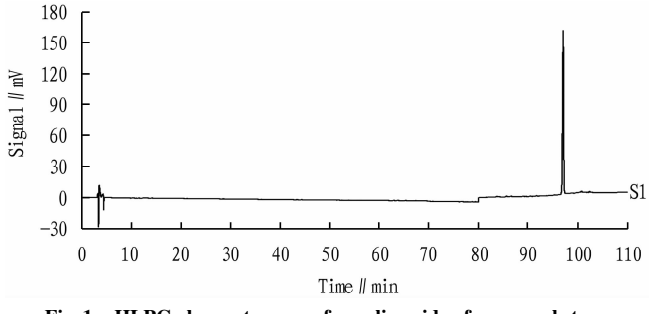

Fig. 1 HLPC chromatogram of ursolic acid reference substance

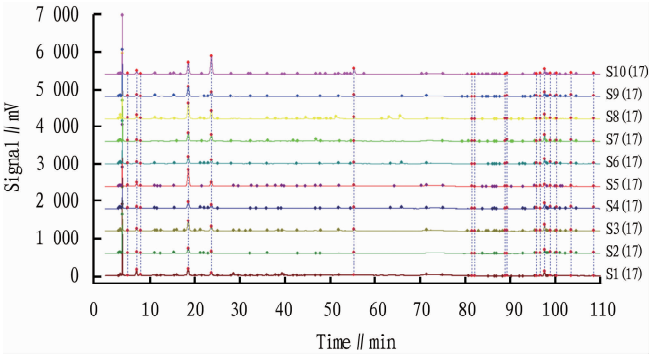

Fig. 2 Fingerprints of *Hedyotis hedyotidea* from different producing areas

3.2.2 Fingerprint similarity analysis. The similarity of test samples was calculated using the *Traditional Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System* (2004A Edition). With the fingerprint of the NBT-1 *H. hedyotidea* sample as the reference fingerprint, the median method was employed with a time window width of 0.10. Using 17 common characteristic peaks of *H. hedyotidea* samples as calibration points, a reference fingerprint for samples from different origins was generated (Fig. 2). The calculated similarity results of fingerprints from different origins of *H. hedyotidea* are shown in Table 3. The HPLC fingerprint similarity of 10 batches of *H. hedyotidea* medicinal materials from different origins ranged from 0.718 to 0.993.

Table 2 Retention time and relative peak area of common characteristic peaks in the fingerprints of *Hedyotis hedyotidea* from different producing area

No.	Time	Relative peak area of common characteristic peak									
		NBT-1	NBT-2	NBT-3	NBT-4	NBT-5	NBT-6	NBT-7	NBT-8	NBT-9	NBT-10
1	5.009	0.723	1.186	0.862	1.453	1.199	1.395	0.672	2.642	0.986	0.845
2	7.056	10.162	4.612	4.285	5.540	5.495	4.577	2.188	14.176	2.857	4.181
3	7.868	2.878	1.435	1.272	1.753	1.077	1.514	0.898	3.308	0.860	1.010
4	18.512	13.995	14.333	22.961	21.982	30.142	21.812	13.641	63.530	18.707	17.581
5	23.639	6.633	6.744	12.519	13.076	7.786	12.569	12.505	34.426	9.092	28.978
6	55.291	1.798	2.876	3.068	3.351	1.393	3.365	0.608	6.666	3.045	7.549
7	81.996	0.294	0.241	0.203	0.344	0.265	0.275	0.483	0.272	0.113	0.124
8	82.763	0.276	0.212	0.471	0.335	0.176	0.337	0.642	0.226	0.219	0.200
9	89.057	0.590	0.525	0.932	0.961	0.463	0.848	0.570	1.581	0.422	0.348
10	89.294	1.122	0.721	1.285	1.660	0.623	1.750	1.908	1.225	1.242	1.080
11	95.764	0.506	0.921	0.671	1.529	0.386	1.359	0.623	0.937	0.762	0.371
12	96.648	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
13	97.638	7.470	8.180	9.936	12.052	2.929	10.955	8.365	12.368	6.370	5.427
14	98.745	0.835	2.954	1.464	3.153	1.668	2.787	0.839	1.939	2.128	1.266
15	100.277	0.209	1.397	0.859	1.755	0.582	0.697	0.548	0.806	1.135	0.740
16	103.547	0.488	4.346	1.649	4.477	0.899	2.234	1.759	2.784	2.180	1.505
17	108.555	0.418	3.322	1.146	4.276	0.629	2.571	1.022	2.373	2.036	1.170

Table 3 Results of similarity evaluation of the chromatograms of *Hedyotis hedyotidea* from different producing areas

No.	NBT-1	NBT-2	NBT-3	NBT-4	NBT-5	NBT-6	NBT-7	NBT-8	NBT-9	NBT-10
NBT-1	1.000	0.920	0.920	0.920	0.865	0.923	0.873	0.904	0.900	0.751
NBT-2	0.920	1.000	0.958	0.989	0.875	0.975	0.923	0.914	0.965	0.783
NBT-3	0.920	0.958	1.000	0.984	0.935	0.995	0.969	0.98	0.993	0.856
NBT-4	0.920	0.989	0.984	1.000	0.890	0.995	0.966	0.946	0.982	0.845
NBT-5	0.865	0.875	0.935	0.890	1.000	0.912	0.838	0.968	0.950	0.718
NBT-6	0.923	0.975	0.995	0.995	0.912	1.000	0.972	0.964	0.990	0.855
NBT-7	0.873	0.923	0.969	0.966	0.838	0.972	1.000	0.933	0.948	0.912
NBT-8	0.904	0.914	0.980	0.946	0.968	0.964	0.933	1.000	0.980	0.864
NBT-9	0.900	0.965	0.993	0.982	0.950	0.990	0.948	0.98	1.000	0.841
NBT-10	0.751	0.783	0.856	0.845	0.718	0.855	0.912	0.864	0.841	1.000

4 Discussion and conclusions

H. hedyotidea is the primary medicinal component in Zhonghua Dieda Pills, as listed in the *Chinese Pharmacopoeia*[2]. It also serves as a core ingredient in traditional Chinese patent medicines such as Sanshedan Chuanbei Drops[7], Bairong Zhike Syrup[8], and Shedan Chuanbei Syrup[9]. Furthermore, it represents a key medicinal resource in the Zhuang and Yao ethnic medicine systems[10-11]. Notably, its roots and stems exhibit dual medicinal and culinary value in Guangxi Zhuang Autonomous Region due to their natural sweetness: they are utilized both as raw materials for heat-clearing herbal teas and to exert traditional therapeutic effects such as lung-moistening, heat-clearing, detoxification, and anti-

inflammatory actions[12]. This multidimensional applicability highlights its significant industrial potential in pharmaceutical development (novel TCM formulations) and health-related industries (functional foods).

This study systematically analyzed *H. hedyotidea* samples from different geographical origins using HPLC fingerprinting technology and the *Traditional Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System* (2004A Edition). Through digital evaluation of parameters including retention times, relative peak areas of common characteristic peaks, and similarity indices, we conducted the first chemical component group-level quality control investigation. While most samples demonstrated high consistency, certain origin-specific samples exhibited similarity indices below 0.80, revealing substantial variations in chemical component composition that may influence the herb's quality.

The established HPLC fingerprint-based quality evaluation system effectively assesses both the consistency and intrinsic variability of *H. hedyotidea*, providing critical data support for quality control standard formulation and development of this medicinal material.

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anti-diarrheal application remains confined to few folk practices. Therefore, this study investigated the anti-diarrheal effects of *C. sclerophylla* fruit from a pharmacodynamic perspective.

This study evaluated the anti-diarrheal effects of *C. sclerophylla* fruit using a castor oil-induced diarrhea model. Castor oil, an irritant laxative, exerts its diarrheic mechanism by releasing ricinoleic acid in the gastrointestinal tract, which stimulates intestinal mucosa to produce prostaglandins and other endocrine substances that induce inflammation, enhance intestinal motility, and inhibit water-electrolyte absorption, ultimately leading to exudative diarrhea[5]. Experimental results demonstrated that *C. sclerophylla* fruit significantly delayed initial diarrhea onset time, reduced loose stool frequency, and alleviated diarrhea symptoms in a dose-dependent manner, with the high-dose group showing comparable efficacy to the positive control drug loperamide. The charcoal propulsion test revealed that *C. sclerophylla* fruit markedly inhibited intestinal propulsion in castor oil-induced diarrheic mice. Medium- and high-dose groups showed no significant difference in propulsion rate compared to the positive control group, indicating its inhibitory effect on small intestinal motility.

In conclusion, the anti-diarrheal mechanism of *C. sclerophylla* fruit may involve suppression of intestinal peristalsis. This study provides experimental evidence for further investigation into its anti-diarrheal mechanisms and offers references for its potential clinical application.

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